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# Comparative Acute Toxicity of Mercury to Air Breathing Fish, *Channa gachua* (Ham.) and Non-Air Breathing Fish *Cyprinus carpio* (Linn.): Ethological and Haematological Consideration

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***Abstract***: In the present investigation, a comparative study on acute toxicity of mercury to air breathing fish, *Channa gachua* (Ham.) and non-air breathing fish, *Cyprinus carpio* (Linn.) was undertaken. The 96h median lethal concentration (LC50) for *C. gachua* (0.188 mg $l^{-1}$) was higher than that of *C. carpio* (0.105 mg $l^{-1}$). From the result, the non-air breathing fish, *C. carpio* was 1.79 times more susceptible than *C. gachua* to mercury toxicity. Both the fishes showed changes in their ethological responses with the progress of time of exposure and increasing concentration of the toxicant. An alteration in the haematological parameters also recorded when the fishes exposed to mercury stress. The haemoglobin content (Hb), total erythrocyte count (TEC) and haematocrit (Hct) for both fishes significantly reduced) during their acute exposure. The level of total protein and serum globulin significantly decreased) at 96h in both fishes compared to the initial exposure at 0h, whereas, total glucose, triglyceride, cholesterol and albumin increased significantly in both fishes at 96h. The degree of change for each parameter was similar in both the fishes. This comparative study may help to determine the degree of toxicity of mercury as well as to understand its mode of action in the light of ethological and haematological responses to the fishes belong to different physiological nature.

***Keywords***: *Channa gachua*, *Cyprinus carpio*, Mercury, Acute toxicity, Ethological responses, Haematological changes

Anthropogenic activities resulting in the release of chemicals, salts and metals in the environment are a major problem to aquatic organisms, human and wildlife. Many of these chemicals and salts used in industry and agriculture have a final destiny in the aquatic environment (Coimbra and Reis-Henriques 2007; Zheng et al 2021). Mercury, a non-essential heavy metal, is rarely found free in nature and is usually discharged into environment through the effluents from thermal power stations, paper and pulp industries, electronic wastes, batteries, electroplating, metal industries and fossil fuel combustion (Morcillo et al 2017). Most of the mercury in surface waters is typically inorganic in nature (Hoffman et al 2000) and mercuric chloride, a simple inorganic salt form of the toxicant, is predominant in many surfaces water bodies. The inorganic mercury is a global concern due to its high toxicity at low concentration and bioavailability (Vázquez-Núñe et al 2007, Zheng et al 2021). In the aquatic ecosystem, inorganic mercury can be microbiologically converted into the highly toxic form, methyl mercury (Baughman 2006, Gutiérrez et al 2020). This organometallic compound is a highly lipophilic environmental contaminant derived from inorganic mercury by bacterial activity which easily crosses the blood barrier and disrupts cellular ion transport processes (Authman et al 2015; Thangam et al 2016; Zheng et al 2021). Fish exposed to different concentrations of mercuric chloride in the laboratory experiments showed alterations in hematological parameters including changes in blood metabolite levels (Kalaivani et al 2008, Maheswaran et al 2008, Gupta and Banerjee 2011, Pathak and Jha 2011, Morcillo et al 2017).

An acute toxicity test is the most accepted technique in the assessment of biological effects of any toxicant and is used to detect and evaluate the potential toxicological effects of any toxicant on the organisms (Parasuraman 2011). It can be evaluated by several quantitative parameters like survival and mortality of the test organisms and their sensitivity to the toxicant (Banavathu et al 2016, Zheng et al 2021). The present investigation was undertaken to determine the degree of acute toxicity of mercury to air-breathing fish, *Channa gachua* Ham. And non-air breathing fish, *Cyprinus carpio* Linn.

## MATERIAL AND METHODS

Test organisms used in the bioassay comprised of adult male *Channa gachua*, Ham. (Length 8.1 ± 1.20 cm, weight 9.23 ± 1.7 g) and *Cyprinus carpio* Linn. (Length 9.5 ± 1.15 cm, weight 18.58 ± 2.5 g). The fish was acclimatized to the laboratory condition for ten days before their use, maintaining the photoperiod of 12h light and 12h darkness. Fishes were fed ad libitum with dried organic mini sinking pellet (manufactured by SPF India Pvt. Ltd.). Analytical grade mercuric chloride, HgCl2 (purity 98%, molecular weight 271.52 g/mol; E. Merck, made in Germany) was used as the test chemical. Static replacement bioassays were used for both 96h acute toxicity tests following the methods outlined by American Public Health Association (APHA 2012). The physicochemical properties of water used during the experiment were: temperature (26.4 ± 1.42 °C, pH 7.2 ± 0.56, free CO2 10.0 ± 1.28 mg $l^{-1}$, DO 6.2 ± 1.36 mg $l^{-1}$, total alkalinity 170 ± 5.36 mg $l^{-1}$ as CaCO3 and total hardness 131 ± 4.82 mg $l^{-1}$ as CaCO3). Acute toxicity tests for fish were conducted in 15 l glass aquaria holding 10 l of water in the laboratory. The selected test concentrations of mercuric chloride for *C. gachua* were 0.0, 0.10, 0.15, 0.20, 0.25 and 0.30 mg $l^{-1}$ and for *C. carpio*, were 0.098, 0.100, 0.105, 0.110, 0.115 and 0.120 mg $l^{-1}$ based on rough range finding tests to determine their 96h lethal concentrations (LC1, 5, 10, 15, 50, 85, 90, 95, 99). Each concentration was accompanied by three replicates. Ten organisms were used in each replicate. The fishes were not fed 24h before and during the bioassays. The number of dead fishes was counted every 24h and removed immediately from the test medium to avoid any organic decomposition and oxygen depletion. Mortality rate at different concentrations of mercuric chloride to fish was analyzed using the computer software R version 2.14.0 (US EPA, 1999) and Probit analysis (Finney 1971) for determining 96h lethal concentrations with 95% confidence limits. On the basis of median lethal concentration (LC50) values, the degree of fish susceptibility to the toxicant was determined as susceptibility factor (SF) which was calculated after Otitolojo and Don-Pedro (2002) by the following formula:

Susceptibility factor (SF) = (96h LC50 value of other test organism) / (96h LC50 value of the most sensitive test organism)

The safe level estimation was calculated for both the fish by multiplying their 96h LC50 with different application factors (AF) based on Sprague (1971), Committee on Water Quality Criteria (CWQC 1972), National Academy of Sciences/ National Academy of Engineering (NAS/NAE 1973), International Joint Commission (IJC 1977) and Canadian Council of Resources and Environmental Ministry (CCREM 1991). The ethological changes in respect of body balance, loss of reflex, swimming rate, erratic swimming, jumping movement, fin movement, opercular movement, air gulping and mucous secretion of the test organisms exposed to the toxicant were recorded systematically by naked eye observation during 96h bioassay following the method of Mukherjee and Saha (2014). The observations were done once a day at a distance of 2 m from the aquarium in order to avoid interruption of fish behaviour. The number of fish that exhibited the different types of behavior was counted. The total duration of observation was two minutes followed by two minutes of interval for each aquarium.

Hematological analyses were carried out by standard methods suggested by Mostakim et al (2015). For the haematological study, a sublethal dose of 1/10$^{th}$ of LC50 value (0.02 mg $l^{-1}$ and 0.01 mg $l^{-1}$) of mercuric chloride was used for *C. gachua* and *C. carpio* respectively. Blood was drawn by caudal venipuncture of fish using a sterile 5 ml 22-gauge needle syringe (Dispovan, India), quickly transferred to EDTA vacutainer tubes (Becton Dickinson, USA) and tapped with fingers to prevent clotting of blood. Haemoglobin concentrations (Hb) were determined with commercial kit by the cyanohemoglobin method at 540 nm (Larsen and Snieszko 1961). Haematocrit (Hct) were determined by using microhaematocrit heparinized capillary tubes and a microhaematocrit centrifuge (12,000 rpm for 3 min) (Haider and Rauf 2014). Total erythrocyte count (TEC) was made under light microscope with an improved Neubauer haemocytometer using Hayem's fluid (Mgbenka et al 2003). The haematological indices like mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were calculated using standard formulae (Mostakim et al 2015).

MCHC (g $dl^{-1}$) = [Hb (g dl-1) × 100] / Hct (%)

MCH (pg $cell^{-1}$) = [Hb (g dl-1) × 10] / TEC (millions $mm^{-3}$)

MCV (fl $cell^{-1}$) = [Hct (%) × 10] / TEC (millions $mm^{-3}$)

Serum albumin, Serum globulin, cholesterol and triglyceride levels were measured using standardized kits. Total protein was estimated using the method of Lowry et al (1951). Total glucose content in control and treated fish blood without EDTA was estimated by the assay kit following Enzymatic-Colorimetric method (Trinder 1969).

**Statistical analysis:** The data on behaviour was organized and analyzed using Kruskal Wallis one way analysis of variance on ranks. The comparison of means was done by Dunnett's method. The values of haematological parameters of fish were subjected to Duncan's Multiple Range Test (DMRT) for determining significant differences among the means.

## RESULTS AND DISCUSSIONS

No mortality was observed in the control group during the experiment. In both *C. gachua* and *C. carpio*, significant relationship between mortality rate and exposure times were observed. The relationship between mortality rate and exposure concentrations was significant at different exposure times for both the fish. However, their susceptibility factor values showed that *C. carpio* are 1.79 times more sensitive to mercury than *C. gachua* (Table 1).

Toxicity test in fish is useful in assessing possible ecotoxicological risks of contaminants (Prusty et al 2011). It is the culmination of a series of events involving various physical, chemical and biological processes (Banavathu et al 2016). $LC_{50}$ value, as an effective tool of toxicity test, is the level of resistance of population response to metals (Bakr et al 2010). In the present study, 96h $LC_{50}$ value of mercury to *Channa gachua* (0.188 mg/l) and *Cyprinus carpio* (0.105 mg $l^{-1}$) (Table 1) almost corresponds with the findings of Shyong and Chen (2000) on the freshwater fish, *Zacco barbata* (0.161 mg $l^{-1}$) and *Variocorhinus barbatulus* (0.168 mg/l) and Gupta and Banerjee (2011) on air-breathing fish *Heteropneustes fossilis* (0.12 mg $l^{-1}$). The result of the present study is also in agreement with the findings of Mansouri and Baramaki (2011) on the acute toxicity (96h $LC_{50}$) of mercury to the cyprinid fish, *Capoeta fusca* (0.118, 0.154 and 0.180 mg $l^{-1}$ at the soft, hard and very hard water respectively). Similar observation was also recorded by different earlier workers to other fish groups, such as *Ptychocheilus lucius* (0.168 mg $l^{-1}$), *Gila elegans* (0.108 mg $l^{-1}$), *Xyrauchen taxanus* (0.090 mg $l^{-1}$) (Brandt et al 2021), *Misgurnus anguillicaudatus* (0.150 mg $l^{-1}$), *Fundulus diaphanous* (0.110 mg $l^{-1}$) (Gheorghe et al 2017) and even to the similar fish species (*C. carpio*) as 0.180 mg $l^{-1}$ (Gheorghe et al 2017) and 0.100 mg/l (Mondal et al 2018). Such type of variation in acute toxicity level even in a single species and single toxicant may be attributed to variability in their size, age, sex and condition of the test species along with experimental factors (Farah et al 2004, Kumar and Gupta 2006, Ebrahimpour et al 2010, Mansouri and Baramaki 2011, Dhara et al 2013, 2014, Sadeghi and Peery 2018). Comparatively higher median lethal value for *C. gachua*, as recorded in the present study, probably due to their hardy nature as air-breathing fish and activeness to cope up with the stress condition in the environment (Pal et al 2018). Further, Hedayati et al (2013) and Sadeghi and Peery (2018) opined that the metal which is highly toxic to a fish species at low concentration may be less or even nontoxic to other species at the same or even higher concentration, which corroborates with the present study.

The susceptibility of fish species to a particular heavy metal is a very important factor for assessing acute toxicity. Tolerance is a crucial mechanism by which an organism reacts to adverse environment. The degree of tolerance or susceptibility in fish to each heavy metal is species dependent and it may be attributed to the degree of altered physiological responses (Enuneku and Ezemonye 2012, Sadeghi and Peery 2018). In the present study, *C. carpio* was found 1.79 times more susceptible to mercury than *C.*

**Table 1.** Lethal concentrations of mercuric chloride to *Channa gachua* and *Cyprinus carpio*

| Name of the fish | Lethality | 96h Lethal Concentration value (mg $l^{-1}$) with 95% confidence limits | Slope ± SE | Intercept ± SE | Susceptibility factor value at 96h $LC_{50}$ |
|---|---|---|---|---|---|
| *Channa gachua* | $LC_1$ | 0.044 (0.003-0.080) | 3.688±1.201 | 7.679±0.885 | 1.79 |
| | $LC_5$ | 0.067 (0.010-0.105) | | | |
| | $LC_{10}$ | 0.084 (0.019-0.122) | | | |
| | $LC_{15}$ | 0.098 (0.029-0.135) | | | |
| | $LC_{50}$ | 0.188 (0.137-0.251) | | | |
| | $LC_{85}$ | 0.358 (0.263-1.166) | | | |
| | $LC_{90}$ | 0.418 (0.292-1.760) | | | |
| | $LC_{95}$ | 0.524 (0.339-1.259) | | | |
| | $LC_{99}$ | 0.802 (0.445-1.442) | | | |
| *Cyprinus carpio* | $LC_1$ | 0.082 (0.059-0.090) | 21.237±5.951 | 25.764±5.782 | 1.00 |
| | $LC_5$ | 0.088 (0.069-0.095) | | | |
| | $LC_{10}$ | 0.092 (0.075-0.098) | | | |
| | $LC_{15}$ | 0.094 (0.080-0.099) | | | |
| | $LC_{50}$ | 0.105 (0.100-0.110) | | | |
| | $LC_{85}$ | 0.118 (0.112-0.134) | | | |
| | $LC_{90}$ | 0.121 (0.115-0.142) | | | |
| | $LC_{95}$ | 0.125 (0.118-0.155) | | | |
| | $LC_{99}$ | 0.135 (0.124-0.182) | | | |

*gachua* (Table 1). Differences in their sensitivity to mercury may be related to the differences in decreased uptake, increased excretion and redistribution of metal to less sensitive target sites in both species (Enuneku and Ezemonye 2012). The estimated possible safe level of mercuric chloride for both the fish as calculated by multiplying their 96h $LC_{50}$ values with different application factors are recorded in Table 2. The safe level estimated for the toxicant is varied from 0.0188-0.00000188 mg/l and 0.0105-0.00000105 mg/l for *C. gachua* and *C. carpio* respectively.

The estimated probable safe mercury level of the present investigation not only exhibited species-specific variation, but also differed for each species (Table 2). However, the wide range of acceptable levels established by diverse methodologies has sparked debate about its appropriateness (Pandey et al 2005). The reliance on the LC50 number in calculating the application factor (AF) is a serious flaw (Yasmeen et al 2019). As a result, extrapolating laboratory findings to the field as an acceptable concentration as "safe" for the toxicant is problematic (Yasmeen et al 2019). Several previous researchers had made similar observations in several fish species exposed to various contaminants (Nwani et al 2010, 2013, Odo et al 2017, Ani et al 2018, Soni and Verma 2019).

No changes were observed in the control group of fish that showed normal behaviour throughout the experiment. With the progress of time and increasing concentration, fish also showed the signs of stress like loss of body balance and reflex, erratic swimming, air gulping, hyperactivity in fin movement, operculum movement and jumping (Table 3 and 4). Body balance and swimming rate were gradually decreased in both the fish with the progress of time of exposure and increasing concentration. Similarly, loss of reflex and fin movement was also increased in both the treated fish. With the advent of time and increasing concentration, operculum movement was gradually decreased in *C. gachua* but increased in *C. carpio*. Jumping movement and air gulping were significantly increased in *C. gachua* but it was not so pronounced in *C. carpio*. Excessive mucus secretion from the body surface as avoidance reactions from the toxicant was also noticed at higher concentrations in *C. gachua* (0.30 mg $l^{-1}$ at 96h) and in *C. carpio* (0.115 and 0.120 mg $l^{-1}$ at 72h and 96h) (Table 3 and 4).

Ethological changes are the most sensitive indicator of potential heavy metal toxicity (Dhara et al 2020, Saha et al 2020, Saha et al 2021). In the present study, the changes in ethological responses in the treated fish were probably an indicative of internal disturbances of the body functions such as inhibition of enzymes functions, impairment in neural transmission, and disturbances in metabolic pathways (Dhara et al 2021). At 24 hours after being exposed to a higher dose, both treated fish demonstrated decreased body balance and swimming rate, as well as increased fin movement. This was most likely an early sign of their toxicant avoidance reflex. The avoidance reflex could be due to narcotic effects or a change in chemo receptor sensitivity (Dhara et al 2019).

The neurotoxic effects of heavy metals may also be attributed to the fish's ethological alterations (Tiwari et al 2011). Exposed fish displayed indicators of stress such as loss of reflex, erratic swimming, air gulping, changes in fin movement, operculum movement, and jumping as time went on, especially at higher concentrations, and eventually sank into a sleepy state after 96 hours. Such reactions were most likely caused by mercury's severe and irreversible effects on the central nervous system, which included demyelination, autonomic dysfunction, sensory nerve conduction delay, and neurotoxic buildup of serotonin, aspartate, and glutamate, among other things (Venugopal 2008). Similar ethological changes were also recorded by various workers in *Anabas*

**Table 2.** Estimate of safe levels of mercuric chloride to *Channa gachua* and *Cyprinus carpio* at 96h exposure time

| Name of the test fish | 96h $LC_{50}$ (mg $l^{-1}$) | Method | Application factor (AF) | Safe level (mg $l^{-1}$) |
|---|---|---|---|---|
| *Channa gachua* | 0.188 | Sprague (1971) | 0.1 | 0.0188 |
| | | CWQC (1972) | 0.01 | 0.00188 |
| | | NAS/NAE (1973) | 0.1-0.00001 | 0.0188-0.00000188 |
| | | IJC (1977) | 5% of 96h $LC_{50}$ | 0.0094 |
| | | CCREM (1991) | 0.05 | 0.0094 |
| *Cyprinus carpio* | 0.105 | Sprague (1971) | 0.1 | 0.0105 |
| | | CWQC (1972) | 0.01 | 0.00105 |
| | | NAS/NAE (1973) | 0.1-0.00001 | 0.0105- 0.00000105 |
| | | IJC (1977) | 5% of 96h $LC_{50}$ | 0.00525 |
| | | CCREM (1991) | 0.05 | 0.00525 |

*testudineus*, *Danio rerio*, *Clarias batrachus*, *Clarias gariepinus* and *Labeo rohita* exposed to mercury (Vutukuru and Basani 2013, Dhara 2014, Banavathu et al 2016, Bhise 2018). Accumulation of acetylcholine at synaptic junctions due to inactivation of acetylcholinesterase probably leads to hyperactivity and erratic swimming in the fish (Pandey et al 2005). Air gulping might be acting as a compensatory action in the exposed fish to cope up with the oxygen deficiency. Excess mucous secretion in the fish exposed to mercury probably prevents the entry of metal ions into the body as the

**Table 3.** Impact of mercury on the ethological responses of *Channa gachua* at various doses

| Parameters | Doses (mg $l^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | 0.00 | 0.10 | 0.15 | 0.20 | 0.25 | 0.30 |
| 24h | | | | | | |
| Body balance | 0 | 0 | 0 | 0 | 0 | 4±1 |
| Loss of reflex | 0 | 0 | 0 | 0 | 0 | 0 |
| Swimming rate | 0 | 0 | 0 | 0 | 0 | 0 |
| Erratic swimming | 0 | 2±1 | 3±1 | 4±1* | 4±1* | 4±1* |
| Jumping movement | 0 | 0 | 0 | 0 | 0 | 3±1 |
| Fin movement | 0 | 0 | 0 | 0 | 0 | 2±1 |
| Opercular movement | 0 | 0 | 0 | 0 | 0 | 5±1 |
| Air gulping | 0 | 2±1* | 2±1 | 2±1* | 3±1* | 3±1* |
| Mucus secretion | 0 | 0 | 0 | 0 | 0 | 0 |
| 48h | | | | | | |
| Body balance | 0 | 0 | 5±1 | 6±1 | 5±1 | 9±1* |
| Loss of reflex | 0 | 0 | 3±1* | 3±1* | 3±1* | 5±1* |
| Swimming rate | 0 | 0 | 5±1 | 5±1 | 6±1* | 7±1* |
| Erratic swimming | 0 | 2±0 | 3±1 | 4±1 | 5±1* | 7±1* |
| Jumping movement | 0 | 0 | 0 | 2±1 | 4±1 | 6±1* |
| Fin movement | 0 | 0 | 3±1 | 5±1 | 6±1* | 6±1* |
| Opercular movement | 0 | 0 | 0 | 0 | 6±1 | 9±1* |
| Air gulping | 0 | 2±0 | 2±1 | 3±0 | 4±1* | 6±1* |
| Mucus secretion | 0 | 0 | 0 | 0 | 0 | 0 |
| 72h | | | | | | |
| Body balance | 0 | 0 | 5±1* | 6±1* | 9±1* | 4±1* |
| Loss of reflex | 0 | 2±0 | 6±1 | 7±1* | 7±1* | 9±0* |
| Swimming rate | 0 | 0 | 5±1* | 8±1* | 7±1* | 3±1* |
| Erratic swimming | 0 | 2±1* | 3±0* | 3±1* | 6±1* | 3±1* |
| Jumping movement | 0 | 0 | 6±1 | 6±1 | 7±1* | 8±1* |
| Fin movement | 0 | 0 | 6±1 | 7±1* | 9±1* | 4±1 |
| Opercular movement | 0 | 0 | 3±1* | 7±1* | 6±1* | 9±1* |
| Air gulping | 0 | 3±1 | 3±1 | 4±0 | 7±1* | 7±1* |
| Mucus secretion | 0 | 0 | 0 | 0 | 3±1 | 4±1* |
| 96h | | | | | | |
| Body balance | 0 | 6±1 | 6±1* | 7±1* | 9±0* | 4±1 |
| Loss of reflex | 0 | 6±1* | 6±1* | 9±1* | 10±1* | 9±1* |
| Swimming rate | 0 | 6±1* | 6±1* | 8±1* | 8±1* | 4±1* |
| Erratic swimming | 0 | 3±1 | 3±1 | 3±1 | 6±1* | 4±1 |
| Jumping movement | 0 | 0 | 6±1 | 7±1* | 9±1* | 4±1 |
| Fin movement | 0 | 4±1* | 6±1* | 8±1* | 9±1* | 4±1* |
| Opercular movement | 0 | 6±1* | 6±1* | 7±1* | 7±1* | 9±1* |
| Air gulping | 0 | 3±1* | 3±1* | 6±1* | 9±1* | 3±1* |
| Mucus secretion | 0 | 3±0 | 4±1 | 6±0* | 6±1* | 10±1* |

**Table 4.** Impact of mercury on the ethological responses of *Cyprinus carpio* at various doses

| Parameters | Doses (mg $l^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.00 | 0.098 | 0.100 | 0.105 | 0.110 | 0.115 | 0.120 |
| 24h | | | | | | | |
| Body balance | 0 | 0 | 0 | 0 | 5±1 | 6±1* | 6±1* |
| Loss of reflex | 0 | 0 | 0 | 0 | 0 | 0 | 3±1 |
| Swimming rate | 0 | 0 | 0 | 0 | 5±1 | 6±1 | 6±1* |
| Erratic swimming | 0 | 2±1 | 3±1 | 3±1 | 4±1* | 4±1* | 4±1* |
| Jumping movement | 0 | 2±1 | 2±0 | 3±1* | 3±1* | 3±1* | 4±1* |
| Fin movement | 0 | 0 | 3±1 | 3±0 | 4±1* | 4±1* | 6±1* |
| Opercular movement | 0 | 0 | 0 | 2±1 | 3±0* | 3±1 | 3±1* |
| Air gulping | 0 | 2±1* | 2±1* | 2±0* | 3±1* | 3±1* | 3±1* |
| Mucus secretion | 0 | 0 | 0 | 0 | 0 | 0 | 3±1 |
| 48h | | | | | | | |
| Body balance | 0 | 0 | 0 | 5±1 | 6±1 | 7±1* | 9±1* |
| Loss of reflex | 0 | 0 | 0 | 0 | 0 | 0 | 6±1 |
| Swimming rate | 0 | 0 | 0 | 5±1 | 5±1 | 8±1* | 9±1* |
| Erratic swimming | 0 | 2±1 | 3±1 | 3±0 | 4±1* | 5±1* | 6±1* |
| Jumping movement | 0 | 2±0 | 2±1 | 3±1 | 3±1* | 4±0* | 4±1* |
| Fin movement | 0 | 0 | 3±0* | 6±1* | 7±1* | 7±1* | 9±1* |
| Opercular movement | 0 | 0 | 0 | 5±1* | 5±1* | 8±1* | 8±1* |
| Air gulping | 0 | 2±1* | 2±1* | 2±0* | 3±1* | 3±1* | 3±1* |
| Mucus secretion | 0 | 0 | 0 | 0 | 0 | 2±1 | 3±1* |
| 72h | | | | | | | |
| Body balance | 0 | 0 | 3±0 | 6±1 | 8±1* | 9±1* | 4±0 |
| Loss of reflex | 0 | 0 | 0 | 0 | 3±1 | 6±1 | 8±1* |
| Swimming rate | 0 | 0 | 3±1 | 6±1 | 8±1* | 9±1* | 9±1* |
| Erratic swimming | 0 | 2±1* | 3±1* | 3±1* | 3±1* | 7±1* | 8±1* |
| Jumping movement | 0 | 2±1 | 3±1 | 3±1 | 4±0* | 4±1* | 4±1* |
| Fin movement | 0 | 0 | 3±1* | 5±1* | 8±1* | 9±1* | 4±0* |
| Opercular movement | 0 | 0 | 0 | 4±1 | 5±0 | 7±1* | 8±1* |
| Air gulping | 0 | 3±0* | 3±1* | 3±1* | 4±1* | 4±0* | 5±1* |
| Mucus secretion | 0 | 0 | 0 | 0 | 4±1 | 6±1 | 8±1* |
| 96h | | | | | | | |
| Body balance | 0 | 0 | 2±1* | 6±1* | 9±1* | 3±1* | 3±1* |
| Loss of reflex | 0 | 0 | 0 | 0 | 6±1 | 8±0 | 10±0* |
| Swimming rate | 0 | 0 | 3±0 | 5±0 | 9±1* | 8±1* | 4±0 |
| Erratic swimming | 0 | 2±0 | 3±1 | 3±1 | 6±1* | 6±1* | 4±1 |
| Jumping movement | 0 | 3±1 | 4±1 | 3±0 | 4±1 | 4±1 | 5±1* |
| Fin movement | 0 | 0 | 5±1 | 9±1* | 8±1* | 9±1* | 4±1 |
| Opercular movement | 0 | 0 | 0 | 6±1 | 9±1 | 10±0* | 10±0* |
| Air gulping | | 3±1 | 4±0 | 4±1 | 3±1 | 5±1* | 9±1* |
| Mucus secretion | 0 | 0 | 0 | 4±1 | 6±1 | 10±0* | 10±0* |

thiol (–SH) groups present in the mucous acts as protective ion trap (Jayakumar and Paul 2006).

Fish haematological parameters are linked to their habits, behavioural physiology, and environmental physico-chemical conditions (Malathi et al 2012). The higher values for haematological indices such as Hb content, Hct, TEC, MCV, MCH, and MCHC in *C. gachua*, as shown in Table 4, are likely related to their hardy nature, active movement, and ability to breathe air (Hidayati et al 2020). It may be as a part of their physiological mechanism of compensation to combat for oxygen demand generally required in their natural habitat i.e., in low land, swampy places etc. Similarly, comparatively lesser value for cholesterol and higher values for total protein, triglyceride, albumin and globulin in *C. gachua*may be attributed to their activeness for being predatory behaviour (Malathi et al 2012). The Hb content, Hct, TEC for *C. gachua* and *C. carpio* significantly reduced in their mean values compared to the initial exposure (0h) during their acute exposure (Table 5).

MCH also showed a significant decrease ($p<0.05$) for *C. carpio* compared to 0h but was found to be insignificant ($p>0.05$) for *C. gachua* at 96h. Similarly, MCHC showed a significant decrease ($p<0.05$) at 96h for both the fishes compared to 0h. MCV too showed a significant variation ($p<0.05$) at 96h compared to the initial exposure for both the fish species. Similar changes in haematological values in teleosts were also recorded due to physiological stress and toxic environmental conditions indicating anaemia and erythropenia (Lal Shah 2010, Pathak and Jha 2011, Zaki et al 2011, Thangam et al 2016, Odedeyi and Odo 2017).

Haemoglobin is the oxygen-carrying component in the blood of fish and its significant decrease can be used as a good indicator of anaemia. Such decline trend in Hb content may be due to decreased rate of production of erythrocytes (Pathak and Jha 2011). The decrease in Hb concentration, TEC, and Hct is thought to represent decreased erythropoiesis as a result of metal's direct effect on the haematopoietic centre (kidney/spleen) or rapid erythroclasia due to membrane permeability changes and/or faulty Fe metabolism (Khattab et al 2021). This illustration depicts the pathophysiological failure of the haemopoietic system in mercury-exposed fish (Thangam et al 2016). Furthermore, Ramesh and Saravanan (2008) suggested that the decrease in Hb content in fish exposed to toxicants could be due to fast oxidation of haemoglobin to methaemoglobin or the production of free $O_2$ radicals. The decrease in MCH and MCHC values was linked to the promotion of erythropoiesis as a defence mechanism against the harmful effects of mercury. The decrease in RBCs, Hb, and Hct due to exacerbated disruptions in both metabolic and haemopoietic processes of fish exposed to the pollution could also be linked to the decrease in RBCs, Hb, and Hct (Kori-Siakpereand Ikomi 2011).

Total protein and serum globulin levels in both fishes fell

**Table 5.** Haematological parameters of *Channa gachua* and *Cyprinus carpio* exposed to mercuric chloride

| Parameters | *Channa gachua* | | | | | *Cyprinus carpio* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0h | 24h | 48h | 72h | 96h | 0h | 24h | 48h | 72h | 96h |
| Hb (gm $dl^{-1}$) | $11.31^{a}$ | $10.17^{a}$ | $9.35^{b}$ | $9.20^{b}$ | $8.78^{c}$ | $5.78^{a}$ | $4.86^{b}$ | $4.35^{b}$ | $3.66^{c}$ | $3.10^{c}$ |
| Hct (%) | $39.80^{a}$ | $35.24^{b}$ | $34.67^{c}$ | $34.18^{c}$ | $33.56^{c}$ | $28.20^{a}$ | $24.58^{b}$ | $21.15^{c}$ | $20.75^{d}$ | $19.93^{d}$ |
| TEC ($10^{6}$ $mm^{-3}$) | $3.58^{a}$ | $3.14^{b}$ | $3.01^{b}$ | $2.97^{c}$ | $2.78^{c}$ | $3.32^{a}$ | $2.87^{b}$ | $2.56^{b}$ | $2.44^{c}$ | $2.13^{d}$ |
| MCV ($\mu m^{3}$) | $111.17^{a}$ | $112.23^{b}$ | $115.18^{c}$ | $115.08^{c}$ | $120.72^{d}$ | $84.93^{b}$ | $85.64^{c}$ | $82.62^{a}$ | $85.04^{c}$ | $93.57^{d}$ |
| MCH (pg) | $31.59^{b}$ | $32.39^{c}$ | $31.06^{b}$ | $30.97^{a}$ | $31.58^{b}$ | $17.41^{d}$ | $16.93^{c}$ | $16.99^{c}$ | $15.00^{b}$ | $14.55^{a}$ |
| MCHC (%) | $28.42^{b}$ | $28.86^{b}$ | $26.97^{a}$ | $26.92^{a}$ | $26.16^{a}$ | $20.50^{d}$ | $19.77^{c}$ | $20.57^{d}$ | $17.64^{b}$ | $15.55^{a}$ |
| Total protein (gm $dl^{-1}$) | $4.98^{a}$ | $3.76^{b}$ | $3.33^{bc}$ | $3.10^{c}$ | $2.85^{d}$ | $3.87^{c}$ | $3.65^{c}$ | $2.59^{a}$ | $2.78^{ab}$ | $2.90^{b}$ |
| Glucose (mg $dl^{-1}$) | $78.41^{a}$ | $134.27^{d}$ | $135.44^{d}$ | $130.10^{c}$ | $121.98^{b}$ | $87.67^{a}$ | $110.31^{b}$ | $123.55^{d}$ | $129.90^{e}$ | $117.66^{c}$ |
| Triglyceride (mg $dl^{-1}$) | $132.14^{a}$ | $139.75^{b}$ | $145.39^{c}$ | $154.23^{cd}$ | $158.79^{d}$ | $114.77^{a}$ | $124.20^{b}$ | $127.36^{c}$ | $130.12^{d}$ | $135.87^{e}$ |
| Cholesterol (gm $dl^{-1}$) | $135.43^{a}$ | $148.13^{b}$ | $151.68^{c}$ | $167.71^{d}$ | $170.85^{e}$ | $225.10^{a}$ | $230.10^{b}$ | $231.76^{bc}$ | $239.51^{c}$ | $240.20^{d}$ |
| Albumin (gm $dl^{-1}$) | $2.6^{a}$ | $2.8^{a}$ | $3.2^{b}$ | $3.4^{b}$ | $3.7^{c}$ | $1.9^{a}$ | $2.1^{b}$ | $2.4^{c}$ | $2.2^{b}$ | $2.7^{d}$ |
| Globulin (gm $dl^{-1}$) | $3.2^{c}$ | $3.0^{c}$ | $2.7^{b}$ | $2.5^{b}$ | $2.2^{a}$ | $2.6^{c}$ | $2.2^{b}$ | $2.1^{b}$ | $1.8^{a}$ | $1.7^{a}$ |

The Hb content, TEC and Hct for both *C. gachua* and *C. carpio* showed a significant reduction in their mean values compared to the initial exposure (0h). MCV too showed a significant variation at 96h compared to the initial exposure for both the fish species. At 96h, MCH showed a significant decrease for *C. carpio* compared to 0h but was found to be insignificant for *C. gachua*. MCHC showed a significant decrease at 96h for both the fishes compared to 0h. The level of total protein also showed a significant decrease at 96h in both *C. gachua* and *C. carpio* compared to the initial exposure at 0h. On the other hand, total glucose showed a significant increase at 96h exposure compared to 0h for both the fishes. The mean values of blood triglyceride and cholesterol for both the fishes increased significantly at 96h compared to 0h exposure. A significant increase was observed in the mean values of serum albumin at 96h compared to 0h for both *Channa gachua* and *Cyprinus carpio* whereas serum globulin for both the fishes showed a significant reduction in their mean values compared to the initial exposure.

considerably after 96 hours as compared to the first exposure at 0 hours, however total glucose, triglyceride, cholesterol, and albumin levels increased significantly in both fishes after 96 hours (Table 4). Under the toxicity of mercuric chloride, the considerable fall in protein levels could be due to decreased or inhibited protein synthesis and/or proteolysis, or even liver disease. Previous researchers have reported on hypoproteinaemia investigations (Martin et al 2008). It could possibly be due to their role in cell repair and tissue structure through the creation of lipoproteins, which are key biological elements of cell membranes and cytoplasmic organelles (Vutukuru et al 2007, Vinodhini et al 2008, Squadrone et al 2013). Fish blood glucose levels have long been utilised as stress indicators (Ramesh and Saravanan 2008). Öner et al (2008) reported that heavy metals modulate the metabolism of carbohydrates, causing hyperglycemia by stimulating glycogenolysis in some marine and fresh water fish species. Heavy metals increase the glucose content in blood, because of intensive glycogenolysis due to induced increase in circulatory catecholamines and the synthesis of glucose from extra hepatic tissue proteins and amino acids (Cicik et al 2005, Santos and Fernandes 2008). Similar trend with characteristic hyperglycemia due to heavy metal intoxication was observed in *C. carpio* by Vinodhini and Narayanan (2009). The elevated triglyceride and cholesterol levels for all exposed fish blood in the present study were also corroborated in the blood, muscle and liver tissues of various other fishes exposed to different toxicants (Mohamed and Gad 2008).

Jaheed (2021) opined that hypercholesterolemia, as observed in the present study, may be due to inhibition of enzymatic function which converts cholesterol into bile acid. The present elevation of serum cholesterol level in the exposed fish might also be attributed to the utilization of the volatile fatty acids by the damaged liver parenchyma and thereby entry into the circulation or to decreased excretion of cholesterol by the damaged liver in fish under stress (Arvind et al 2019). Firat et al (2010) and Javed et al (2017) referred that alteration in total protein levels in fish under stress condition alone may be misleading if the changes in its constituent fractions like albumin and globulin are not taken into account. The decrease in serum albumin in both of the mercury-exposed fish in this study could be due to a need to meet immediate energy demands in order to overcome the toxic stress. Similarly, significant decrease of globulin levels in the treated fish was probably to cope up with the immunotoxic stress due to mercury toxicity.

## CONCLUSION

The present finding highlights the high toxic nature of mercury to fish. The fish are very sensitive to the presence of mercury even at minute concentration and short time exposure. The non-air breathing carp fish, *C. carpio* is more susceptible to the toxicant than theair-breathing fish, *C. gachua* during their acute exposure. Present experiment demonstrates a relationship between heavy metal stress and behavioural changes in the fish. Such responses are the most sensitive indicator for rapid measuring the heavy metal toxicity in the exposed fish which can be used as a tool for creating awareness among the local farmers. On the basis of present haematological study, it can be stated that mercury poses a serious threat to the blood physiology of fish during their acute exposure. The results indicate that the changes in the haematological parameters including blood metabolites exhibited in different magnitude in both of the fishes but their responses were similar when exposed to mercury. Therefore, from the data, it would be possible to forecast the patho-physiological state of fish in natural water bodies as well as the possible mechanism of action of a toxicant to the fish body. This information may provide useful data to set up national and local water quality criteria (WQC) for mercury.

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# Estimation of Fish Production in India using ARIMA, Holt's Linear, BATS and TBATS Models

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***Abstract*:** The main aim of this study was to estimate fish production in India and this study is linked to time series data. The fish production forecasts are based on BATS and TBATS, Holt's Linear Trend and ARIMA models and tried to compare the above methods with actual data. Following the development of the best-in-class model, forecasts are carried out for marine, domestic and total fish production areas, between 1980 and 2012 (80%) as in-sample model validation forecasts, and between 2013 to 2020 (20%) as out-sample forecast which confirms that all best selected models are effective. The prediction is considered between 2021 and 2025 for all series of data; marine, domestic and total fish production, Linear Holt's Trend Model is regarded as the best-fit model in the production range of marine areas, based on the lower value of fitness criteria. Similarly, ARIMA (2,2,1) and ARIMA (3,2,0) are chosen as the best model in inland areas and overall production based on the minimum fitness value of the criteria. From the predicted values of the best fit models, the trend is increasing for fish production in all areas in the coming years. The fish production in marine areas can be estimated at 126337 tons by 2025. Similarly, 169466tons can be gained for inland fish production by 2025. For total fish production, the order was constantly rising to 295792 tonnes in 2025. These findings are extremely important to obtain the information in advance, so that can fully understand and develop strategies for investing in it in the future and is important to develop a strategic plan for fish supply, fish demand and fish prices by Indian policy makers to improve oversight and future planning strategies.

***Keywords*:** BATS, TBATS, Holt's Linear Trend, ARIMA, Forecasting, Fish production, Time series

Inland fishing and marine fishing are both important systems of food production that can contribute to food safety and nutrition safety. Due to the nutritional features of fish, fisheries are far more than a source of protein. They provide vital micronutrients-vitamins, minerals and omega 3 fatty acids that are essential for ending malnutrition and reducing global burden of communicable and transmissible diseases (Expert Market Research 2017). However, the contribution of fisheries could be undermined by threats such as overfishing, climate change, pollution and competing fresh water uses. Global fish use grew by 3.1 percent between 1961 and 2017; the population rates doubled (1.6 percent) as well as the growth rate in the consumption of other animal protein products (2.1 percent for meat, dairy, milk etc.). The per capita consumption of per capita fish has more than doubled from 9.0 kg (live weight equivalent) by 1961 to 20.5 kg by 2018 by the State of the World Fisheries Report 2020. India continues to be Asia's second largest fish producer and one of the top five fish-producing countries in the world. India has been the fourth biggest exporter since 2017, with a sharp increase in shrimp production. According to Expert Market Research, Indian fish markets reached almost 1.232 billion INR in 2020. The industry is also expected to expand by 10.5 percent between 2021 and 2026 to nearly INR 2.243 billion by 2026 (Sharma et al. 2018). The growing per capita consumption of the product in recent years has brought an increasing awareness on the Indian fish market of the health benefits of fish consumption. Tsitsika et al (2007) used ARIMA models to model and predict monthly pelagic fish production in the Mediterranean Sea. This study aims to meet future fish production needs in India.

## MATERIAL AND METHODS

The secondary data for fish production in India for marine, Inlands and total have been procured from 1980 to 2020 cumulative yearly data (Department of Animal Husbandry Dairying & Fisheries). The study compares ARIMA, Holt's linear, BATS, and TBATS forecasting models using data from 1980 to 2012 (80%) for training and the last 8 years (20%) from (2013) to (2016) for testing (2020).

**Autoregressive integrated moving average (ARIMA) models:** ARIMA (p, d, q) models are created by first taking the differences of the data from d degree for the stabilization process and then adding the ARMA (p, q) model. In the ARIMA (p, d, q) models, *p* shows the degree of the AR model, *q* represents the degree of theMA model and *d* shows the number of differences to be taken tostabilize the data (Mishra et al 2017).

The ARIMA (*p, d, q*) model can be defined as follows:

$Y_t = \phi_1 Y_{t-1} + \phi_2 Y_{t-2} + \ldots + \phi_p Y_{t-p} + \alpha_1 - \theta_1\alpha_{t-1} - \alpha_2 - \theta_2\alpha_{t-2} - \ldots - \alpha_q - \theta_q\alpha_{t-q}$ (1)

Here: $\phi_p$ represent the parameter values concerning the AR operator, $\alpha_q$ are the error term coefficient, $\theta_q$ show the parameter values relating to MA operator, $Y_t$ is the data with the difference of d degree from the original data (Brockwell et al 2016, Mishra et al 2021).

**Holt's linear trend method:** Mishra et al (2021) defined forecast, level, and trend in three equations, and Forecast Equation

$$\hat{\chi}_{t+p\backslash t} = M_t + \rho\theta_t \qquad (2)$$

Level Equation

$$M_t = \upsilon\chi_t + (1-\omega)(M_{t-1} + \theta_{t-1}) \qquad (3)$$

Trend Equation

$$b_t = \gamma^*(M_t - M_{t-1}) + (1-\gamma^*)\theta_{t-1} \qquad (4)$$

**Bats and TBATS Models**

Equation (1) is a Box-Cox transformation

$$Y_t^{(\omega)} = \begin{cases} \dfrac{y_t^{(\omega)} - 1}{\omega} \omega \neq 0 \\ \log\ y_t \omega = 0 \end{cases} \qquad (1)$$

Equation (2) represents the seasonal M pattern

$$Y_t^{(\omega)} = l_{t-1} + \phi b_{t-1} + \sum_{i=1}^{T} s_{t-m_i}^{(i)} + d_t \qquad (2)$$

Equations (3), (4) and (5) are global trends and local trends

$l_t = l_{t-1} + \phi b_{t-1} + \alpha a d_t$ (3)

$b_t = \phi b_{t-1} + \beta d_t$ (4)

$$s_t^{(i)} = s_{t-m_i}^{(i)} + \gamma_i d_t \qquad (5)$$

Equation (6) is the error modelled by ARMA

$$d_t = \sum_{i=1}^{p} \varphi_i d_{t-i} + \sum_{i=1}^{q} \theta_i \varepsilon_{t-i} + \varepsilon_t \qquad (6)$$

Where

$m_1, \ldots, m_T$ denote that seasonal period

$l_t$ and $b_t$ represent to the level and trend of components of the time series at time t

$S_t^{(i)}$ represents the ith seasonal component at time t

$d_t$ denotes an ARMA (p, q) process

$\varepsilon_t$ Gaussian white noise process when the mean equal zero and constant variance $\sigma^2$

The smoothing parameters are given by *α, β,* $\gamma_i$ for *i=1, T* and $\phi$ is the dampening parameter, which gives more control over trend extrapolation when the trend component is damped.

Trigonometric exponential smoothing models for seasonal data

$$s_t^{(i)} = \sum_{j=1}^{k_i} a_{j,t}^{(i)} \cos(\lambda_j^{(i)} t) \qquad (7)$$

$$a_{j,t}^{(i)} = a_{j,t-1}^{(i)} + a_1^{(i)} d_t \qquad (8)$$

$$\beta_{j,t}^{(i)} = \beta_{j,t-1}^{(i)} + k_2^{(i)} d_t \qquad (9)$$

Where

$k_1^{(i)}$ and $k_2^{(i)}$ are the smoothing parameters

$\lambda_j^{(i)} = 2\pi_j/m_j$ This is an extended, modified single source of error version of a single seasonal multiple source of error presentation suggested by Anwar, and Cooray (2015), Mishra et al (2021) and Hyndman and Athanasopoulos (2018).

$$a_{j,t}^{(i)} = s_{j,t}^{(i)} \cos + (\lambda_j^{(i)} t) - s_{j,t}^{(i)} \sin(\lambda_j^{(i)} t) \qquad (10)$$

$$\beta_{j,t}^{(i)} = s_{j,t}^{(i)} \sin + (\lambda_j^{(i)} t) - s_{j,t}^{(i)} \cos(\lambda_j^{(i)} t) \qquad (11)$$

$$s_t^{(i)} = \sum_{j=1}^{k_i} s_{j,t}^{(i)} \qquad (12)$$

Where

$$s_{j,t}^{(i)} = s_{j,t-1}^{(i)} \cos \lambda_j^i + s_{j,t-1}^{*(i)} \sin \lambda_j^{(i)} + \left[k_1^{(i)} \cos(\lambda_j^{(i)} t + k_2^{(i)} \sin(\lambda_j^{(i)} t\right] d_t \qquad (13)$$

$$s_{j,t}^{*(i)} = -s_{j,t-1} \sin \lambda_j^{(i)} + s_{j,t-1}^{*(i)} \cos \lambda_j^{(i)} + \left[k_2^{(i)} \sin(\lambda_j t + k_1^{(i)} \sin(\lambda_j t\right] d_t \ (14) \text{Chen et al } (2007)$$

$$s_t^{(i)} = \sum_{j=1}^{k_i} s_{j,t}^{(i)} \qquad (15)$$

Equation (16) and (17) are seasonal patterns modelled by the Fourier model. (16-17)

$$s_{j,t}^{(i)} = s_{j,t+1}^{(i)} \cos \lambda_j^{(i)} + s_{j,t-1}^{*(i)} \sin \lambda_j^{(i)} + \gamma_1^{(i)} d_t \qquad (16)$$

$$s_{j,t}^{*(i)} = -s_{j,t-1} \sin \lambda_j^{(i)} + s_{j,t-1}^{*(i)} \cos \lambda_j^{(i)} + \gamma_{21}^{(i)} d_t \qquad (17)$$

The information criterion and the error criterion are as follows: The most frequently used criteria are those described in (Pablo, 2016), information criterion; Bayesian Information Criterion (Mishra et al 2021) , MAE stands for Mean Absolute Error, RMSE stands for Root Mean Squared Error, and MAPE stands for Mean Absolute Percent Error (MAPE).

## RESULTS AND DISCUSSION

The performance of fish production in India from 1980 to 2020. The production of marine fish increased from 1555 to

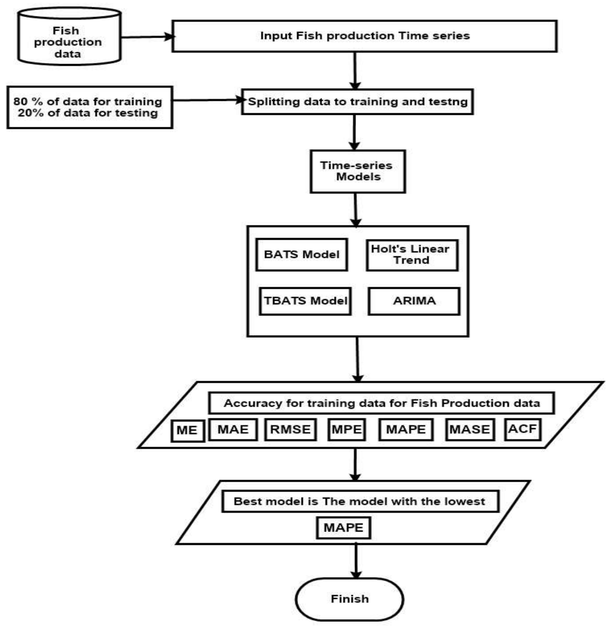


**Fig. A.** Schema of fish production modelling

108169 with an average of 47791. The positive skewness value (0.249) between -0.5 and 0.5 confirms the approximate symmetrical distribution. Kurtosis value (1824) refers to the platykurtic nature, which shows a thinner tail than the normal distribution, and also the number of outliers cannot be estimated to be large. Inland fish production rose from 887to 142780 with average of 46272. The positive value of skewness (0.819) indicates that fish production in these areas is increasingly likely. The kurtosis value (2.580) allows us to estimate that the data follow a platykurtic distribution and not the large number of outliers. Overall fish production increased over the period between 2442.and 250836with an average of 94060. The positive skewness value (0.560) indicates that fish production in these regions is increasingly likely. The kurtosis value (2.174) shows that data are based on a platykurtic distribution and the number of outliers is not high (Table 1).

BATS (1, 0, 0), where Box-Cox transformation = 1, order of ARMA error = (0, 0), damping parameter = 1. (essentially doing nothing). This model has Box-Cox transformation = 1,

**Table 1.** *Per se* performance of fish production in India

| Source | Mean | Minimum | Maximum | Skewness | Kurtosis |
|---|---|---|---|---|---|
| Marine | 47791 | 1555 | 108169 | 0.249 | 1.824 |
| Inland | 46272 | 887 | 142780 | 0.819 | 2.58 |
| Total | 94060 | 2442 | 250836 | 0.56 | 2.174 |

order of ARMA error = (0, 0), damping parameter = 1 and is best for inland fish production data series (essentially doing nothing). The best BATS model for total fish production is BATS (0.645, 0, 0, 1, -), with Box-Cox transformation = 0.645, ARMA error = 0, 0, and damping parameter = 1. (essentially doing nothing) in (Table 2).

the fish production for all data series *i.e.* marine areas, inland areas and total production, best suited TBATS model is TBATS(1, {0,0}, 1, {<6,2>}), in this model, Box-Cox transformation = 1 (doing nothing), the order of ARMA error = (0,0), the damping parameter = 1 (essentially doing nothing).Decomposition of time series data allows to draw an inferences about the pattern of changes over time, by separating different constituent components. Decomposition obtained by BATS model is represented in three parts. Observed data shows in the first part, second and third part shows level and slope respectively. Similarly decomposition generated by TBATS model is displayed four parts. First part shows observed data. Second and third part shows level and slope respectively and the fourth part define the seasonality of the fish production data series (Table 3). Seasonality pattern obtained from TBATS model shows relatively more stable (Fig. 1).

Holt's Linear Trend models for fish production in India for training data are depicted, where Box-Cox transformation for marine areas, inland areas and total production are 0.748, 0.556 and 0.644 respectively. It is observed that the forecasting accuracy by Holt's Linear Trend model is very high for all the data series, because of the lower values of sigma and AIC, comparatively to the other BATS and TBATS models from (Table-2-4).

The best fitted ARIMA models for marine, inland, and total production are ARIMA (0,2,0), ARIMA (2,2,1), and ARIMA (0,2,0) (3,2,0) (Table 5). As shown in the residual plot, the individual autocorrelation coefficient at lag 11 is within the

**Table 2.** BATS model fitted for fish production in India on training data

| Source | Model | *Box-Cox transformation (Lambda) | Smoothing parameter | | Damping Parameter For trend | ARMA Coefficients | | Prediction error | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Alpha | Beta | | AR coefficients | MA coefficients | Sigma | AIC |
| Marine | BATS (1, {0,0}, 1, -) | 1 | 1.13 | 1.186 | 1 | - | - | 171.561 | 450.18 |
| Inland | BATS (1, {0,0}, 1, -) | 1 | 0.676 | 1.736 | 1 | - | - | 152.088 | 440.469 |
| Total | BATS (0.645, {0,0}, 1, -) | 0.645 | 0.875 | 0.201 | 1 | - | - | 12.012 | 522.503 |

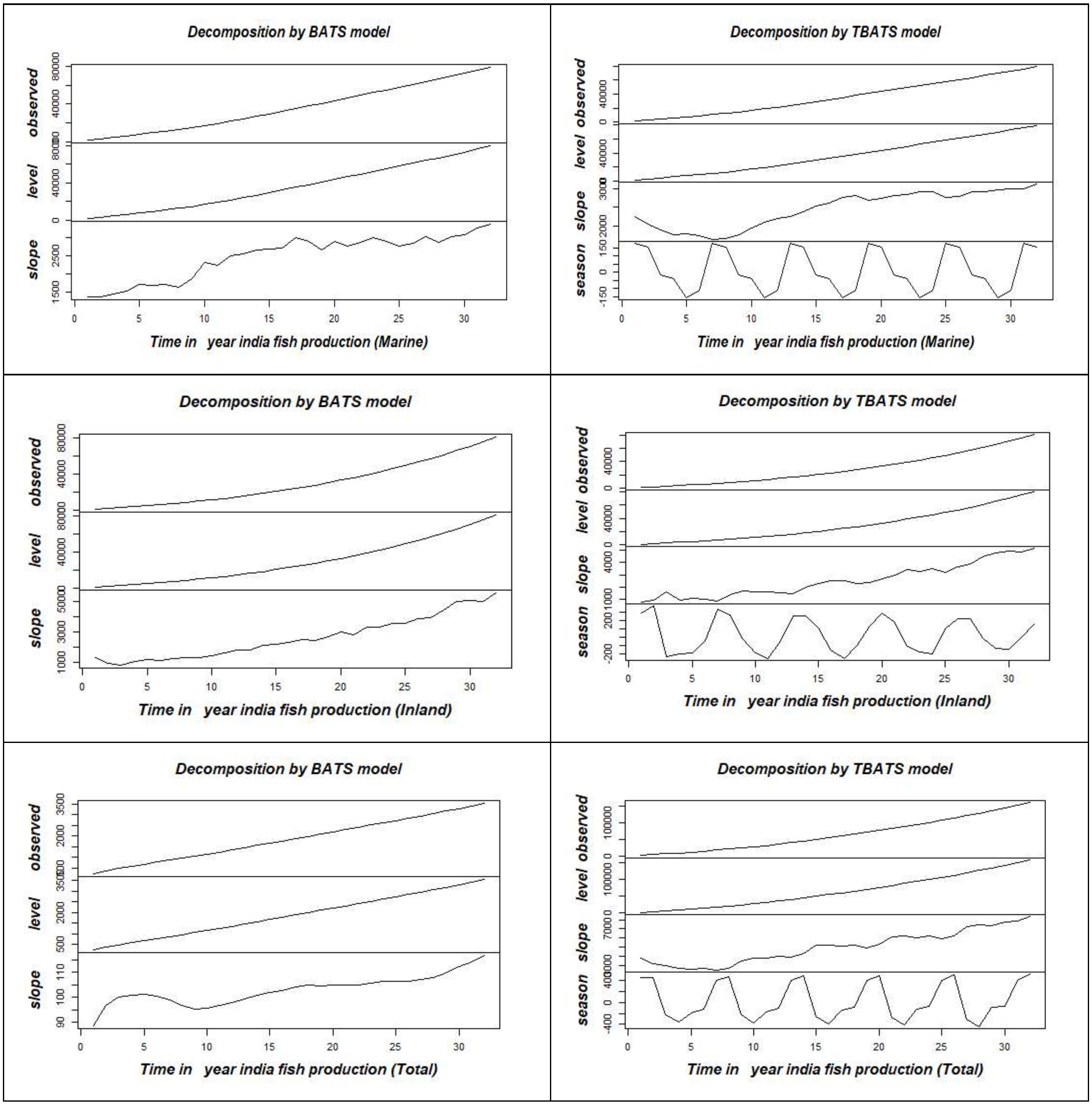


**Fig. 1.** Decomposition of data

**Table 3.** TBATS model fitted for fish production in India on training data

| Source | Model | *Box-Cox transformation (Lambda) | Smoothing parameter | | | | Damping Parameter For trend | Prediction error | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Alpha | Beta | Gamma-1 Values | Gamma-2 values | | Sigma | AIC |
| Marine | TBATS (1, {0,0}, 1, {<6,2>}) | 1 | 0.947 | 0.326 | 0 | 0 | 1 | 546.419 | 534.32 |
| Inland | TBATS (1, {0,0}, 1, {<6,2>}) | 1 | 0.971 | 1.036 | 0.107 | -0.024 | 1 | 296.85 | 495.27 |
| Total | TBATS (1, {0,0}, 1, {<6,2>}) | 1 | 0.835 | 0.66 | -0.009 | 0.008 | 1 | 742.499 | 553.945 |

**Table 4.** Holt's Linear Trend Model fitted for fish production in India on training data

| Country | Box-Cox transformation (Lambda) | Smoothing parameters | | Initial states | | Sigma | AIC |
|---|---|---|---|---|---|---|---|
| | | Alpha | Beta | L | B | | |
| Marine | 0.748 | 0.995 | 0.955 | 118.752 | 206.262 | 11.609 | 273.546 |
| Inland | 0.556 | 1 | 1 | 33.482 | 47.665 | 2.52 | 175.783 |
| Total | 0.644 | 0.838 | 0.838 | 111.605 | 125.593 | 6.628 | 237.672 |

**Table 5.** ARIMA model fitted for fish production in India on training data

| Source | Model | AR (1) | AR (2) | AR (3) | MA (1) |
|---|---|---|---|---|---|
| Marine | ARIMA (0,2,0) | - | - | - | - |
| Inland | ARIMA (2,2,1) | 0.7405 | 0.2459 | - | -0.6823 |
| Total | ARIMA (3,2,0) | 0.2276 | 0.2518 | 0.3936 | - |

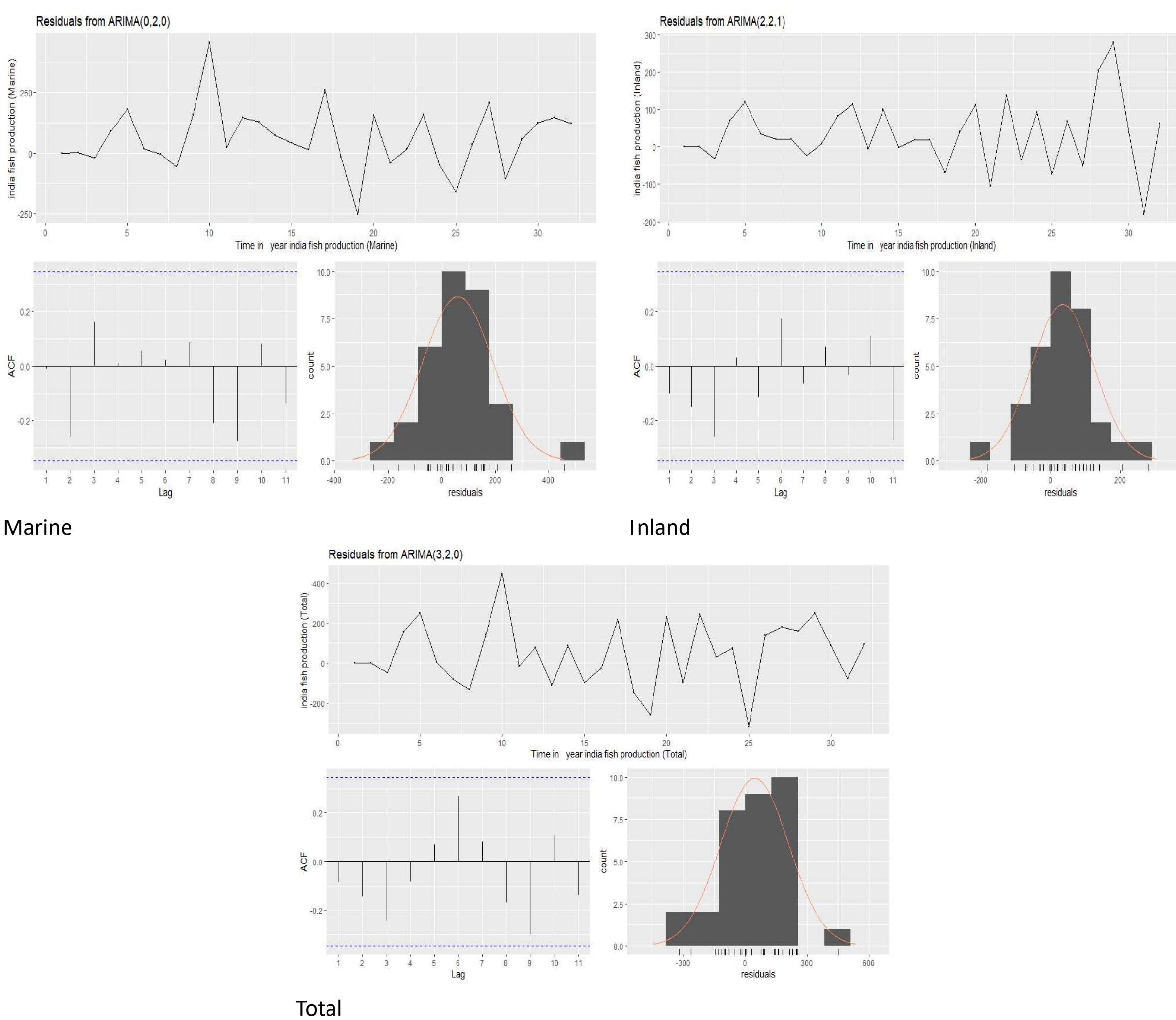


**Fig. 2.** ACF and residuals for best fitted ARIMA model

95 percent confidence limit, confirming "good fit" of the selected models (Fig. 2).

The minimum value of ME, RMSE, MAE, MPE, MAPE, MASE, and ACF1 among the chosen models is selected as best-fitted model. From Holt's Linear Trend model is considered as a best fitted model for marine areas fish production series based on the lower value of goodness of fit criteria (Table 6). Similarly ARIMA (2,2,1) and ARIMA (3,2,0) is selected as a best fitted model for inland areas production and total production respectively based on the minimum value of goodness of fit criteria. After selecting the best fitted model from training data, minimum absolute percentage error (MAPE) is calculated from testing data with respective best fitted model (Fig. 3). Minimum absolute percentage error (MAPE) for testing data, *i.e.* last 8 years data from 2013 to 2020 confirmed that the selected models perform well for testing data also (Table 7).

After developing the best fitted time series model, forecasting is carried out for all the data series of fish production. Forecasting is examined from 2021 to 2025 for all data series; marine, inland and total fish production (Table 8). All the forecast points are lies in between the confidence interval of upper and lower 80% and 95% level. All the information about forecasting from best fitted model is given

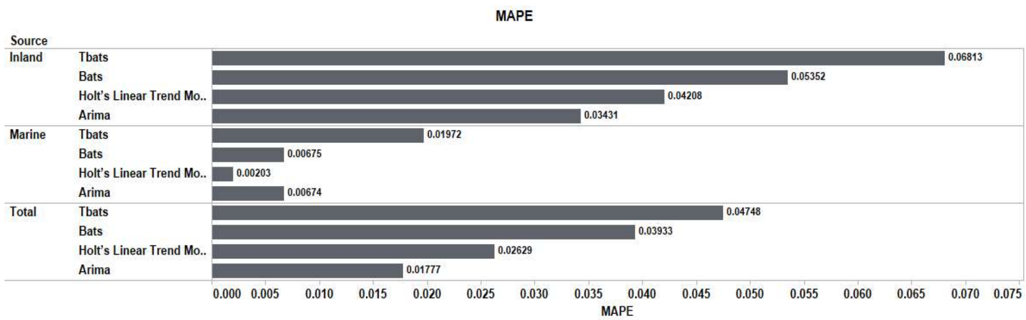


**Fig. 3.** MAPE of fish production of India using time series models

**Table 6.** BATS, TBATS, Holt's Linear Trend, and ARIMA Models fitted on training data

| Model | ME | RMSE | MAE | MPE | MAPE | MASE | ACF1 |
|---|---|---|---|---|---|---|---|
| Marine | | | | | | | |
| BATS | 71.075 | 171.561 | 129.02 | 1.484 | 1.654 | 0.051 | -0.191 |
| TBATS | 161.855 | 546.419 | 338.565 | 4.244 | 7.025 | 0.135 | -0.041 |
| Holt's linear trend | 0.176 | 134.739 | 101.381 | -0.145 | 0.556 | 0.04 | 0 |
| ARIMA (0,2,0) | 60.276 | 142.459 | 104.214 | 0.315 | 0.449 | 0.041 | -0.01 |
| Inland | | | | | | | |
| BATS | 86.662 | 152.088 | 114.588 | 1.238 | 2.138 | 0.044 | -0.254 |
| TBATS | 133.624 | 296.85 | 240.067 | 1.22 | 2.608 | 0.093 | -0.137 |
| Holt's linear trend | 16.825 | 124.138 | 92.795 | -0.927 | 1.222 | 0.036 | 0.216 |
| ARIMA (2,2,1) | 33.786 | 94.367 | 69.528 | 0.228 | 0.381 | 0.027 | -0.1 |
| Total | | | | | | | |
| BATS | 179.141 | 378.413 | 299.562 | -0.012 | 1.981 | 0.059 | 0.389 |
| TBATS | 291.906 | 742.499 | 521.746 | 2.87 | 4.971 | 0.103 | -0.071 |
| Holt's linear trend | 31.575 | 213.145 | 166.943 | -0.259 | 0.781 | 0.033 | 0.31 |
| ARIMA (3,2,0) | 46.001 | 168.412 | 134.633 | 0.147 | 0.358 | 0.026 | -0.085 |

**Table 7.** MAPE for testing data last 8 years (20 %) from (2013) to (2020) for testing models

| Source | BATS | TBATS | Holt's Linear Trend Model | ARIMA | Best Model |
|---|---|---|---|---|---|
| Marine | 0.68% | 1.97% | 0.20% | 0.67% | Holt's Linear Trend Model |
| Inland | 5.35% | 6.81% | 4.21% | 3.43% | ARIMA (2,2,1) |
| Total | 3.93% | 4.75% | 2.63% | 1.78% | ARIMA (3,2,0) |

**Table 8.** Forecasting by using the best fitted model (Tons)

| Years | Point forecast | Lo80 | Hi80 | Lo95 | Hi95 |
|---|---|---|---|---|---|
| Holt's linear trend model for marine | | | | | |
| 2021 | 111345.65 | 106839.56 | 115898.22 | 104473.33 | 118326.72 |
| 2022 | 115048.51 | 109774.85 | 120383.88 | 107008.62 | 123232.77 |
| 2023 | 118781.68 | 112694.44 | 124948.68 | 109505.05 | 128244.9 |
| 2024 | 122544.7 | 115598.97 | 129591.24 | 111963.92 | 133361.3 |
| 2025 | 126337.11 | 118488.94 | 134310.29 | 114386.34 | 138580.36 |
| ARIMA (2,2,1) model for inland | | | | | |
| 2021 | 138328.06 | 134920.93 | 141735.2 | 133117.3 | 143538.83 |
| 2022 | 145804.62 | 141628.07 | 149981.16 | 139417.14 | 152192.1 |
| 2023 | 153488.03 | 148452.3 | 158523.76 | 145786.54 | 161189.52 |
| 2024 | 161376.04 | 155388.79 | 167363.3 | 152219.33 | 170532.76 |
| 2025 | 169466.42 | 162432.89 | 176499.96 | 158709.57 | 180223.28 |
| ARIMA (3,2,0) model for total | | | | | |
| 2021 | 250345.8 | 242810.6 | 257881.1 | 238821.6 | 261870 |
| 2022 | 261457.5 | 252162.5 | 270752.6 | 247242 | 275673.1 |
| 2023 | 272742.5 | 261488.9 | 283996 | 255531.7 | 289953.3 |
| 2024 | 284191 | 270779 | 297603 | 263679.1 | 304702.9 |
| 2025 | 295792.7 | 280021.5 | 311563.8 | 271672.8 | 319912.6 |

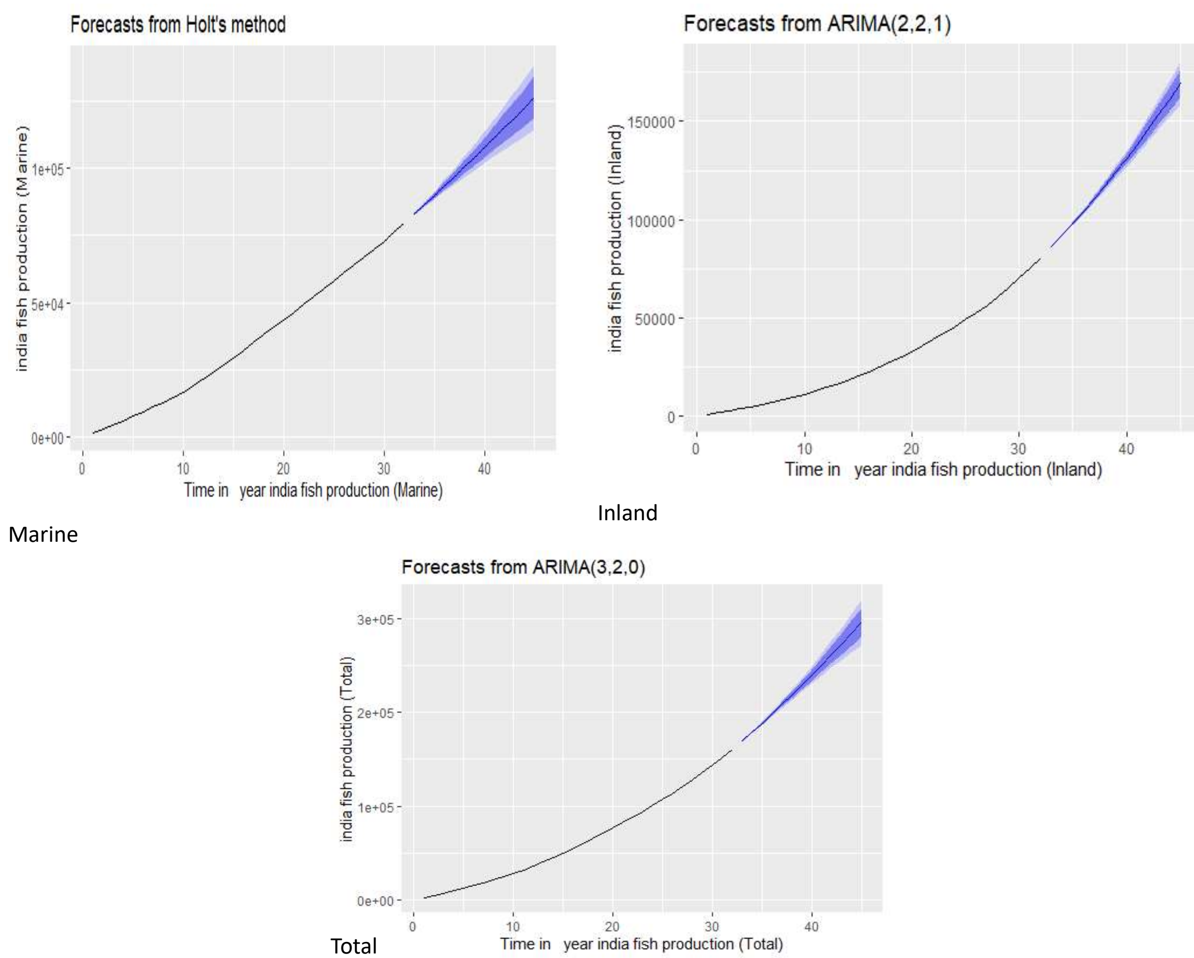


**Fig. 4.** Forecast from best fitted model

in Figure 4. The blue colour line indicate the forecast values, lies within the confidence limit of upper and lower 95% level, confirms that the good prediction of selected models. From the forecasted values, can conclude that for few coming years fish production for all areas will follow in increasing trend. For marine areas fish production can be estimated as 126337tonne in the year 2025. Similarly for inland areas fish production can be 1694662 tonne in 2025. In case of total fish production, it has been estimated as 295792.tonne in 2025 in steady increasing order.

## CONCLUSION

Holt's Linear Trend model is considered to be the best fitted model for marine area fish production series. Similarly, ARIMA (2,2,1) and ARIMA (3,2,0) are chosen as the best fitted models for inland areas and total production, respectively, based on their minimum goodness of fit values. In the coming years, the projected values of the best fit models in all areas will increase. The production of fish in marine areas is estimated at 126337 tonnes in 2025 and 169466.42 tons inland by 2025.Total fish production has been estimated to reach 295792.7 tons in 2025, growing at a steady rate. These findings are critical in obtaining information in advance in order to provide clear thoughts for understanding and developing a strategy for the future to invest in. Additionally, it is critical for policymakers in India to develop a strategic plan for fish supply, demand, and prices, which will improve oversight and future planning strategies.

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# How Does Co-management Perform in Wetland Fisheries? Fishers' Perceptions from Bangladesh

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***Abstract***: In Bangladesh, fisheries co-management emphasizes fish resources and fishers' livelihood maintenance. The role of co-management differs from fishers' diverse perceptions of socioeconomic and ecological aspects of wetland fisheries. This study conducts a survey among fishers of a north-eastern wetland area in Bangladesh to collect data for examining the effectiveness of co-management to sustain fishers' livelihood and fisheries resources. The findings demonstrate that 46% fishery users possess fishing as a main occupation while 35% users become members of the fishery users' association without having any fishing experience. Fishers earn yearly BDT 40,000 ($470; 1USD = BDT 85) from the fishery 40% fishers of which have other sources of income. Only 20% fishers capture the income of the fishery and exclude other fishers from the fishery management. The limited income and participation of fishers in fisheries management with alternative employments scarcity primarily lessen down the effectiveness of co-management. A lack of incentives to alternate fishing and mere use of local fishing knowledge and practices to foster fisheries also lessen the efficacy of co-management. Participatory management and equal benefits sharing together with alternative employments for fishers and surrounding communities may improve the success of co-management.

***Keywords:*** Socio-ecology, Fishers' evaluation, Socioecological benefits, Fisheries resources conservation, Challenges in co-management

The wetland fisheries are of rivers and estuaries, beels, lakes, and haors, which spread over 3927142 hectares of total water area with 28.45% (1216539 metric tons) share in total fish production in Bangladesh (DoF 2018). This fisheries sector provides livelihoods for rural communities, especially those who live in the floodplains over the decades (Shamsuzzaman et al 2017). The communities in low-lying areas perceive wetland fisheries resources as their key source of household income. To promote the sustainable use of wetland fisheries, the government continues to implement community-based fishery management (CBFM) in Bangladesh since 1996 (Halls et al 2017). CBFM delegates the authority to local fishery users' associations to manage fisheries in cooperation with government agencies (Department of Fisheries, Local Government), NGOs, and other stakeholders (other fishers, local communities) (Halls et al 2017). CBFM also focuses on sharing benefits from fisheries equally, training for poor fishers to alternate sources of income, and combining traditional and scientific knowledges for a sustainable fishery management (Halls et al 2017). To manage fisheries for long-term use without damaging habitats involving local fishers and government agencies initiates fisheries co-management in Bangladesh. Co-management integrates fishers and government agencies into the decision-making and implementation processes to maintain fish habitats for improving fish production, fish species variety conservation, fishers' economic benefits, and fishers' participation in wetland fisheries (Halls et al 2017). To promote participatory management for lasting use of fish habitats, co-management is considered a useful means to ensure the joint responsibility of all actors to implement decisions and plans for managing fisheries (Donda 2017, d'Armengol et al 2018). Co-management develops fishers' ability to build partnerships between and among fishers, government agencies, and other stakeholders and deal with social-ecological challenges to manage fisheries in different social settings (Nursey-Bray et al 2018). The effectiveness of co-management may be challenged by complex competitive leasing processes which are problematic to traditional fisher communities, fishers' excessive dependence on fisheries for livelihood, shortage of alternative livelihood opportunities, and conflicts between and among fishers and with the community to capture fisheries and benefits (Newaz and Rahman 2019). Additionally, local socio-politically advantaged persons tend to capture fisheries that rule out disadvantaged fishers from the management process (Quimby and Levine 2018). The lack of community support often leads to fishers' marginalization and failure in wetland fisheries co-management (Quimby and Levine 2018). Co-management, therefore, fails to ensure an income and household necessities for members of the fishery users association (Hossain and Rabby 2019).

Co-management could not bring expected socio-ecological changes (income, occupation, participation, fish species diversity) for wetland dependent fishers in Bangladesh (Hossain and Rabby 2019, Hossain and Selamat 2021). The current study focuses on the north-eastern wetland areas in Bangladesh which are surrounded by 47 big haors and over 6,000 wetland fisheries (Haque 2008). Although wetland fisheries are the main source of animal protein and employment for people in floodplain areas of Bangladesh, the contribution of this sector continues to decline mainly for overfishing, exclusion of poor fishers from fisheries management, catch decline, blocking waterflows, and habitat degradation (Shamsuzzaman et al 2017). This study aims to analyze the socioecological factors that influence the performance of co-management in wetland fisheries and fishers' livelihood. The effectiveness of co-management may be measured in terms of fishers' perceptions (Leleu et al 2012, Islam et al 2016), a body of literature has still been lacking to examine fishers' perceptions of the role of co-management in wetland fisheries (Pomeroy and Rivera-Guieb 2006). The study applies a logit regression model to examine fishers' perceptions of the efficacy of co-management in Babonpoi fishery under Sunamganj District in Bangladesh. This study intends to assess the performance and problems of co-management in wetland fisheries management and development based on fishery users existing socio-ecological advantages and disadvantages.

## MATERIAL AND METHODS

**Study area:** The location of the present study is Babonpoi wetland fishery situated within South Sunamganj Upazila of northeastern Sunamganj district in Bangladesh (Fig. 1). The climate of this area is subtropical monsoon in nature. The fishery is about 32.47 acres including two different parts, a sanctuary, and a little bush. The local fishers have created Babonpoi Beel Beboharkari Songoton (Babonpoi fishery users' association) consisting of 30 males and 4 females in 2005 to lease the fishery from the government for 2005-2012 and subsequently for 2012-2019 period. The fishery remains inundated and connects to larger waterbody of Dekhar Haor from June to October, the fishery and adjacent areas remain inundated from November to January, and water remains only in the fishery during the dry season from February to May of the year. The fish species in the fishery ranges from 100-120 in which 10-15 species are commercially important including trout, catfish, snakehead, rohu, carp fishes, chitol,

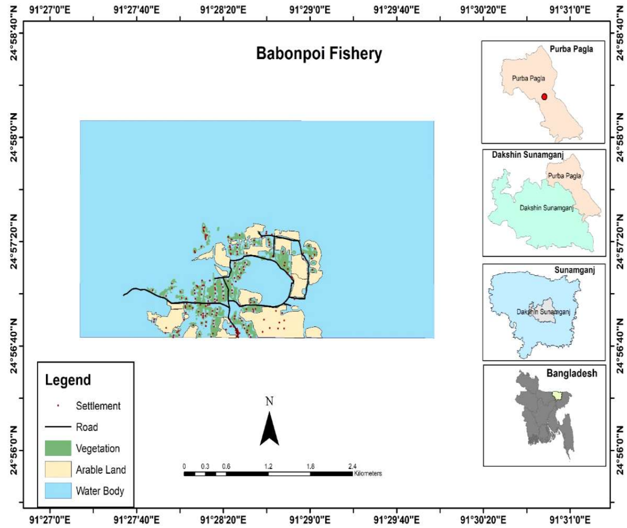


**Fig. 1.** Babonpoi fishery of South Sunamganj, Bangladesh

and stinging catfish. Fishers consider the income from the fishery as their main source of livelihood. This area is full of similar wetlands with nutritious habitats for growing fish. Agriculture provides seasonal employment for many, but alternative employments are very scarce in the area. Some fishers pursue small businesses (shopkeeping, paddy trading) whereas others pursue agricultural work and day labor to enhance their livelihood.

**Data collection:** This study uses a quantitative method to collect data. A survey is performed on all 34 Babonpoi fishery users' association members living near to the fishery area for their opinions about co-management in wetland fisheries. It administers 34 individual interviews among fishery users based on semi-structured questionnaires. The survey questionnaires focus on fishers' social and demographic aspects (age, education, income), fishing activities (methods of fishing, kind of boat and net, membership and participation in the fishery association), their fishing experience and extent of subsistence on the fishery, their opinions and feelings about the role of co-management in fishers' living and fishery's maintenance, their participation in fishery development, and their attitudes towards existing and future fishery management systems. Likert scale is deployed to assess fishers' perceptions of ecological and socioeconomic effects of the fishery.

The collection of data spans from December 2019 through February 2020. Fishers are interviewed both in their residences and the fishery area. Interviews take around one hour. Interviews' participants willingly agree to share their views and record interviews. This study sincerely maintains the secrecy of the participants and their data.

**Data analysis:** Our study applies logit regression model to assess both socioeconomic and ecological components that form fishers' perceptions and feelings about the usefulness of co-management in wetland fisheries. This model uses the below formula (Gujarati and Porter 2009) to calculate fishers' perceptions of co-management and its effects.

$$In\left(\frac{P}{1-P}\right) = a + \sum_{i=1}^{N} \beta_i X_i$$

Where p refers to the chance to happen an event (i.e., co-management is useful or useless) for several examined variables; a denotes intercept (constant); $\beta_i$ denotes the consequence of $X_i^{th}$ independent variables ($n$=7) such as education, yearly income from the fishery, fishing experience, secondary occupation, participation, government assistance, and fishing method.

## RESULTS AND DISCUSSION

**Fishers' socioeconomic characteristics:** The average age of the fishers is 45 years (Table 1). The 79% fishers possess no formal schooling, 15% fishers possess primary schooling from class I to IV classes, and 6% possess secondary schooling from class VI to X. Only 46% fishers take fishing as their primary occupation and their average fishing experience is 8 years. Notably, 35% fishers have no fishing experience, but they have become the member of the fishery users' association. Fishers' households contain average 8 members compared to countrywide average 4.4 in 2018 (BBS 2019). The 79% of fishers own houses, some fishers (21%) who migrated from other places have no houses. About 40% fishers pursue other professions like small business (shop keeping, paddy/fish trading), agricultural works (paddy planting, watering, cutting, and processing) and day labor (rickshaw/cart pulling). Fishers' average yearly household and fishery income are BDT 60,000 ($706, 1$=BDT 85) and BDT 40,000 ($470) respectively. Fishers manually use a 23-feet long boat to patrol the fishery during the rainy season and catch fishes in the dry season. They use seine nets to catch fish. About 71% fishers receive government support for organizing the fishery association and nurturing, guarding, and harvesting fish.

**Table 1.** Fishers' socioeconomic characteristics

| Variable | Description | Mean (±SD) | Frequency (%) |
|---|---|---|---|
| Age | Age of respondent (year) | 45 (±13) | --- |
| Education | Illiterate | --- | 79 |
| | Primary school (class I to V) | --- | 15 |
| | Secondary school (class VI to X) | --- | 06 |
| Housing condition | 1→Having own houses | --- | 79 |
| | 0→ Otherwise | --- | 21 |
| Household composition | Household size (Number of members) | 8 (±3) | --- |
| Main occupation | 1→ If the main occupation is fishing | --- | 46 |
| | 0→Otherwise | --- | 54 |
| Secondary occupation | Respondents having secondary occupations | --- | 40 |
| Fishing experience | Years of fishing as a profession | 8 (±2) | --- |
| Income | Respondents' yearly income from the fishery | BDT 40000 (±4000) | --- |
| | Yearly household income | BDT 60000 (±21000) | --- |
| Fish species variety | Number of varieties in the last ten years | 29 (±6) | --- |
| | Total fish production (Kgs) in the last ten years | 3300 (±580) | --- |
| Fishing methods | Type of the boat (manual→ 1, otherwise →0) | --- | 100 |
| | Size of the boat | 23(±5) | --- |
| | Type of fishing net (Seine net→1,otherwise→0) | --- | 100 |
| Government assistance | 1→If receive government assistance | --- | 71 |
| | 0→ Otherwise | --- | 29 |
| Access to credit | 1→If respondents have access to credit | --- | 66 |
| | 0→ Otherwise | --- | 34 |
| Participation | 1→Active participation in fishers' association | --- | 20 |
| | 0→Otherwise | --- | 80 |

About 66% fishers may obtain credit from local moneylenders, NGOs, and government Krishi (Agriculture) Bank. Although fishers have membership in the fishery users' association, 20% members actively participate in the association.

**Fishers' perceptions of the role of co-management in the fishery:** Most of the fishers (54%) perceive that co-management has improved the catches of fish in the fishery (Table 2). The 10% fishers give no opinion about the role of co-management in increasing catches and 16% fishers disagree that co-management increases catches. Some interviewees describe that the production of fish in the fishery increases one year and decreases another year when jointly manage the fishery. This situation implies that co-management is not beneficial to improve the production of the fishery. Sometimes, water level recedes, and local people tend to catch fish from the fishery. Fishing is allowed in open water around the fishery during the rainy season. The fact is that the fishery may be excessively exploited without collective management.

Chi-square results describe only the association between fishers' various opinions regarding co-management. The 35% fishers agree that fish diversity and density has increased in the fishery, 29% fishers disagree with the opinion ($\chi^2$=89.63, df= 2, p=0.00). About 11% of fishers gives no opinion about fish species diversity and density. Although 43% fishers recognize the role of co-management in improving fish habitats ($\chi^2$=91.21, df=2, p=0.00), 5% fishers stay indecisive. A small number of fishers (17%) feels co-management not essential to grow and protect fish. Only 11% fishers consider co-management an ineffective way to manage wetland fisheries.

Most fishers acknowledge the advantages of co-management while others inform the adversity on their socioeconomic conditions (Table 3). Fishers provide opinions on their conflicts of interest about the benefits from the fishery: 42% expressed yes, 20% no, and 12% were indecisive ($\chi^2$=91.21, df=2, p=0.00). These results indicate worse interpersonal relationships among fishers. About 48% fishers believe that co-management mostly advantage nearby water or ecosystems of the fishery, around 14% feel disinterested and 21% differ with the opinion. Most fishers identify the total catch and income from the fishery insufficient for their household needs. Some were of opinion that aggregate catches and income from the fishery is not sufficient to maintain livelihood. Nearly, 32% fishers agree that co-management increases conflicting relations in the fishers' community ($\chi^2$=89.35, df=2, p=0.00). Approximately,

**Table 2.** Fishers' perceptions of the ecological role of the fishery

| Likert-scale elements | Response (%) | | | | | |
|---|---|---|---|---|---|---|
| | Strongly agree | Agree | Neither agree nor disagree | Disagree | Strongly disagree | Chi-square* |
| Comanagement has increased catches of fish in the fishery | 20 | 54 | 10 | 16 | 0 | 93.21 |
| Fish species diversity and density has increased in the fishery | 13 | 35 | 12 | 29 | 11 | 89.63 |
| Comanagement is essential for improving fish habitats and fish production | 26 | 43 | 5 | 17 | 9 | 91.21 |
| Comanagement is a useful means of fisheries management | 0 | 47 | 42 | 11 | 0 | 93.52 |

*p value is zero

**Table 3.** Fishers' perceptions of socioeconomic effects of the fishery

| Likert-scale elements | Response (%) | | | | | |
|---|---|---|---|---|---|---|
| | Strongly agree | Agree | Neither agree nor disagree | Disagree | Strongly disagree | Chi-square* |
| Comanagement has increased conflict among fishers | 26 | 42 | 12 | 20 | 0 | 91.21 |
| Comanagement has only improved the fishery ecosystem | 17 | 48 | 14 | 21 | 0 | 95.32 |
| Comanagement has increased conflict of interests in the fishers' community | 49 | 32 | 7 | 12 | 0 | 89.35 |
| Comanagement has adversely effected fishers' income | 0 | 53 | 0 | 18 | 29 | 89.74 |
| Comanagement has failed to secure fishers' household food consumption | 0 | 59 | 0 | 24 | 17 | 91.27 |

*p value is zero

53% fishers agree and 18% disagree with the decline in income from the fishery ($\chi^2$=89.74, df=2, p= 0.00).

About 59% fishers agree that co-management has failed to ensure their household food consumption, while 24% disagree ($\chi^2$=91.27, df=2, p=0.00). Although a few fishers earn wages from the fishery working as fishing labors (caring the fishery and catching and selling fishes), they find no support in cash or goods from the government to meet their essentials during the dry season when fishing is regulated around the fishery or adjacent areas. Co-management adversely influence fishers' income because benefits are not equally distributed. Local fishers and fish traders mostly suffer as rich fishers sell fish directly to buyers from the city for more profits.

**Fishery users' perceptions and feelings about co-management:** Although 74% fishers perceive co-management helpful, 26% fishers have active participation in developing the fishery (Table 4). Lower participation of fishers mainly relates to hierarchical management systems. About 80% of fishers are willing to involve in the development of the fishery.

About 9% fishers support government-run and 17% support fishers-government collaborative management in the fishery. Most fishers (74%) consider fishing community-based management with government assistance the better way to manage wetland fisheries ($\chi^2$=85.32, df=2, p=0.00) and consider that it is hardly possible to manage fisheries by themselves.

**Elements that form fishers' perceptions of fishery development:** Logit regression model demonstrates both desirable and undesirable effects of co-management on fishers' livelihood and fishery's development in terms of yearly income from the fishery, years of fishing as a profession, secondary occupations, participation in the fishery users' association, fishing method, government assistance, and education (Table 5). The fishers (71%) who earn yearly less than BDT 100,000 ($1176) evaluates co-management a useless attempt to protect wetland fisheries. Similarly, fishers who have no alternative occupations and less participation in the fishery management evaluate co-management a useless effort to maintain wetland fisheries. The use of seine nets to catch fish, no support in cash or goods from the government, and a lack of education are also important elements to form fishers' negative opinion on the effects of co-management.

Logit model estimates that co-management fails to improve fisheries resources and fishers' socioeconomic conditions. The findings suggest that insufficient income, limited participation in the fishery management, and lack of alternative livelihood opportunities in the region primarily

**Table 4.** Fishers' opinions on co-management

| Variable | Yes | No | Chi-square | *p*-value | *Df* |
|---|---|---|---|---|---|
| Is co-management good? | 74 | 26 | 92.21 | 0.00 | 1 |
| Fishers' participation in fishery development | 26 | 74 | 93.25 | 0.00 | 1 |
| Feelings about participation | 80 | 20 | 90.23 | 0.00 | 1 |
| Usefulness of management approaches:<br>1. Government-run management<br>2. Collaboration between fishers and government<br>3. Local community-based management | 9<br>17<br>74 | | 85.32 | 0.00 | 2 |

**Table 5.** Logit model for calculating elements that form fishers' perceptions of the effectiveness of co-management in wetland fisheries

| Variable | Mean | Coefficient | Odds ratio | SE | Z | p> z |
|---|---|---|---|---|---|---|
| Income (1→If yearly income <BDT 100,000 or USD 1,176 (1$=85BDT; 0→Otherwise) | 0.45 | 2.23 | 0.05 | 1.15 | 2.41 | 0.012 |
| Experience (1→If fishing experience <10 years; 0→Otherwise) | 0.60 | 2.33 | 0.04 | 1.24 | 2.32 | 0.016 |
| Secondary occupation (1→Having other occupation; 0→Otherwise) | 0.52 | 2.47 | 0.05 | 1.18 | 2.54 | 0.013 |
| Participation (1→Active participation in the fishery users' association; 0→Otherwise) | 0.48 | 2.54 | 0.01 | 1.14 | 2.48 | 0.014 |
| Fishing method (1→Using seine net; 0→Otherwise) | 0.57 | 2.37 | 0.03 | 1.13 | 2.54 | 0.012 |
| Government assistance (1→If receive assistance; 0→Otherwise) | 0.56 | 2.45 | 0.02 | 1.44 | 2.55 | 0.002 |
| Education (1Having education; 0Otherwise) | 0.41 | 2.31 | 0.06 | 1.32 | 2.38 | 0.003 |
| Constant | | 6.43 | 75.25 | 1.88 | 2.55 | 0.015 |

SE=Standard error; Highlighted values at the right represent statistical significance at α=0.05 level for 34 respondents lessen the effectiveness of co-management. The absence of government assistance to reduce fishers' dependence on the fishery and the mere use of fishers' local fishing knowledge and practices in the management of fisheries also lessen the efficacy of co-management.

The management of the fishery or capturing benefits by a few well-off non-fisher fishery users' association members restricts the goals of co-management. Fishers receive an average benefit from the fishery even though fish production increases. Fishery users overexploit the fishery and use seine nets to catch fish indiscriminately may lead to the degradation of fisheries resources. The conflicts between and among fishers over decision-making and profit-sharing and with surrounded village communities for illicit fish catching also restrict the performance of co-management. To address issues in participatory management (membership, benefits sharing, livelihood uncertainly, and power sharing) may keep wetland fisheries productive, persistent, and resilient in Bangladesh.

## CONCLUSION

The performance of co-management in wetland fisheries differs from fishers' opinions on their socioeconomic improvement and fisheries development. Fishers' perceived benefits from the fishery indicate that co-management hardly improves their socioeconomic condition. Fishers consider their local fishing knowledge and practices important to strengthen co-management initiatives. Their active participation in co-management may improve their capacity to protect the fishery. The participatory management and equal benefits from the fishery can only change fishers' negative attitudes towards co-management. As many fishers solely depend on the fishery for their subsistence, government support either in goods or cash should be offered during the dry season to reduce their dependence on the fishery. Moreover, development initiatives to provide alternative income-generating options for fishers and adjacent communities may improve the success of co-management.

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# Morphometric Characteristics of Some Selected Hill Stream Fishes from Hadauti Region, Rajasthan, India

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***Abstract***: The present study deals with the morphometric analysis of four fish species *i.e. Callichrous pabda*, *Esomus danrica, Channa punctatus* and *Puntius ticto*. The samples were collected from selected streams of Hadauti region during 2018-19. A total of 23 characters were taken for morphometric measurements in which some characters were calculated in percentage of total length and a few in percentage of head length. The characters having the range difference less than 10% were considered as genetically controlled, the range difference of 10-15% as intermediate characters and range difference more than 15% were regarded as environmentally controlled characters. Positive correlation has been observed between total length and external body parts. Linear relationships have been observed between all the independent and dependent characters. The value of correlation coefficient was fairly high in almost all the characters so it can be concluded that all the dependent characters increase in direct proportion to each other.

***Keywords:*** Morphometric characters, Hill stream fishes, Hadauti region

Morphometric measurements and meristic counts are considered as easiest and authentic methods for the identification of specimen which is termed as morphological systematics (Jayram 1999). Morphometric characters are important for identifying fish species and their habitat as well as ecological criteria in any stream, lake or sea. This is a powerful tool for characterizing strains/stocks of the same species, which involves detection of subtle variation of shape, independent of size. The complete set of measurements used to describe a form is a morphometric character set (Strauss and Bond 1990). The studies of morphological and meristic characters of a fish give substantial information with regard to exact identification key of the species (Dhanya et al 2004) and such identification is prerequisite for cytogenetic and molecular investigations. The difference between, maximum and minimum is used to determine genetically controlled, intermediate and environmentally controlled characters. Vladykov (1934) based on the range difference expressed in percentage of various characters, divided the characters into two categories. Those characters having the range difference less than 10% are considered as genetically controlled whereas those having range difference more than 15% are regarded as environmentally controlled characters. However, recent work on Morphometric characteristics of fishes have been done by Vikas et al (2016), Sameera et al (2017), Singh et al (2018) and Rumahlatu et al (2020). During present study different morphometric characters were studied in *Callichrous pabda*, *Esomus danrica*, *Channa punctatus* and *Puntius ticto*.

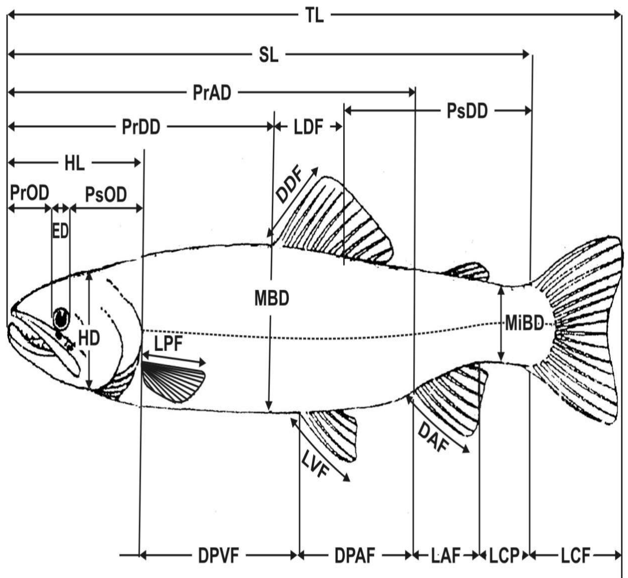
## MATERIAL AND METHODS

Fish specimens were collected on a monthly basis from selected streams (24°51' 53" N to 25°00' 23" N, 75°26' 25" E to 75°35' 52" E.) of Haduati region, Rajasthan from April 2018 to March 2019. A total of 27 specimens of *Callichrous pabda*, 9 specimens of *Esomus danrica,* 14 specimens of *Channa punctatus* and 27 specimens of *Puntius ticto* were used for the morphometric measurements. The rate of growth of different morphological body parts of fish in relation to its total length was observed. Twenty three morphometric characters were studied following the standard procedures described by Holden and Raitt (1974) and Jayaram (1981a).

## RESULTS AND DISCUSSION

***Callichrous pabda***: A total of twenty seven specimens were collected for the morphometric analysis (Table 1) and the characters were divided into genetically controlled, environmentally controlled and intermediate characters. The SL, HL, HD, Pr DD, LDF, DDF, DAF, Pr AD, LPF, LVF, Mi BD, MBD, DPVF, DPAF, LCF, LCP and LMB were genetically controlled characters as their range difference is less than 10%. Intermediate characters are those who have range difference between 10-15%, this includes PsDD, LAF and

PrOD. The environmentally controlled characters include ED, IOD, PsOD and LRB. The range difference of these characters was more than 15%. Excepting LVF and LDF the value of correlation coefficient is fairly high thus all dependent characters seems to increase in direct proportion to each other.




**Abbreviations used in morphometric studies**

| | | | |
|---|---|---|---|
| TL | : Total Length | LCP | : Length of Caudal peduncle |
| FL | : Fork Length | LDF | : Length of Dorsal fin |
| SL | : Standard Length | LPF | : Length of Pectoral fin |
| DPVF | : Distance between Pectoral fin & Ventral fin | LVF | : Length of Ventral fin |
| DPAF | : Distance between Pelvic fin & Anal fin | MBD | : Maximum body depth |
| DDF | : Depth of dorsal fin | MBD | : Minimum body depth |
| ED | : Eye diameter | PrDD | : Predorsal distance |
| HL | : Head length | PsDD | : Postdorsal distance |
| HD | : Head depth | PrAD | : Preanal distance |
| IOD | : Interorbital distance | PrOD | : Preorbital distance |
| LAF | : Length of Anal fin | PsOD | : Postorbital distance |
| LCF | : Length of Caudal fin | | |

**Table 1.** Morphometric features of *Callichrous pabda*

| Morphometric characters | Mean | Standard deviation | Correlation | Regression equation |
|---|---|---|---|---|
| Per cent of total length | | | | |
| Standard length | 86.649 | 1.666 | 0.996 | Y=0.132+0.850X |
| Head length | 16.344 | 0.968 | 0.959 | Y=0.070+0.155X |
| Head depth | 11.397 | 1.677 | 0.954 | Y=-0.304+0.152X |
| Predorsal distance | 26.156 | 1.8 | 0.982 | Y=0.441+0.207X |
| Postdorsal distance | 58.632 | 2.008 | 0.99 | Y=0.131+0.570X |
| Length of dorsal fin | 1.478 | 0.53 | 0.269 | Y= 0.066+0.007X |
| Depth of dorsal fin | 9.882 | 1.551 | 0.792 | Y= 0.269+0.065X |
| Length of anal fin | 56.153 | 2.862 | 0.971 | Y=-0.026+0.564X |
| Depth of anal fin | 8.026 | 1.716 | 0.907 | Y= -0.321+0.120X |
| Preanal distance | 29.419 | 1.504 | 0.967 | Y= 0.006+0.294X |
| Length of pectoral fin | 12.074 | 1.909 | 0.816 | Y=-0.123+0.136X |
| Length of ventral fin | 3.8 | 1.167 | 0.346 | Y= 0.225+0.010X |
| Minimum body depth | 5.214 | 0.738 | 0.815 | Y= -0.003+0.053X |
| Maximum body depth | 18.902 | 1.053 | 0.972 | Y=-0.071+0.198X |
| Distance between pectoral fin & ventral fin | 10.756 | 1.104 | 0.913 | Y=-0.092+0.119X |
| Distance between pelvic fin & anal fin | 4.495 | 0.709 | 0.785 | Y=0.002+0.045X |
| Length of caudal fin | 14.537 | 1.092 | 0.969 | Y=-0.066+0.154X |
| Length of caudal peduncle | 2.03 | 0.322 | 0.827 | Y=-0.008+0.021X |
| Per cent of head length | | | | |
| Eye diameter | 20.819 | 3.475 | 0.717 | Y=0.095+0.136X |
| Interorbital distance | 63.47 | 9.259 | 0.947 | Y=-0.268+0.840X |
| Preorbital distance | 30.225 | 3.559 | 0.856 | Y=0.008+ 0.296X |
| Postorbital distance | 47.209 | 5.539 | 0.864 | Y=-0.023+0.490X |

***Channa punctatus***: The characters HL, HD, Pr DD, Ps DD, DDF, DA F, Pr AD, LPF, LVF, MiBD, MBD, DPVF, DPAF, LCP, ED and PrOD were genetically controlled. The intermediate character includes SL, LDF, LAF, LCF, IOD and PsOD. No environmentally controlled characters were present (Table 2). The value of correlation coefficient is high in almost all the morphometric characters, thus a conclusion can be drawn that all the dependent characters increase in direct proportion to each other.

***Puntius ticto:*** Twenty-seven fishes were analysed for their morphometry (Table 3). The LAF was found negatively correlated whereas low values were documented for DDF, LCP, ED, PrOD and PsOD. All the characters in percentage of total length are genetically controlled. The ED and IOD are intermediate characters, whereas PrOD and PsOD were environmentally controlled characters.

***Esomus danrica***: All the characters in percentage of total length are genetically controlled except PrDD, PsDD and PrAD which are environmentally controlled (Table 4). All the characters in percentage of head length are genetically controlled barring IOD, which is an intermediate character. The MBD, DPAF and ED were found negatively correlated. Low values of correlation coefficient were in HL, DDF, LPF, MiBD, LCF, IOD and LRB.

In general fish demonstrate greater variances in morphological traits both within and between populations than any other vertebrates and are more susceptible to environmentally induced morphological variations. During present study, most of the characters were found genetically controlled. Further the value of correlation coefficient is high in relation to total length in almost all the morphometric characters, thus a conclusion can be drawn that all the dependent characters increase in direct proportion to each other. Engdaw (2014) studied morphometric body parameters of *Labeo barbus intermedius* in Lake Tana, Ethopia and found significant linear relation between total length and standard length and between total length and total weight. Makmur et al (2014) estimated morphometric

**Table 2.** Morphometric features of *Channa punctatus*

| Morphometric characters | Mean | Standard deviation | Correlation | Regression equation |
|---|---|---|---|---|
| Per cent of total length | | | | |
| Standard length | 82.715 | 4.08 | 0.995 | Y=-0.537+0.890X |
| Head length | 27.568 | 1.278 | 0.993 | Y=0.074+0.267X |
| Head depth | 11.435 | 2.073 | 0.885 | Y=0.311+0.079X |
| Predorsal distance | 30.386 | 1.775 | 0.993 | Y=0.498+0.246X |
| Postdorsal distance | 5.701 | 1.457 | 0.782 | Y=0.148+0.040X |
| Length of dorsal fin | 48.899 | 4.232 | 0.994 | Y=-0.956+0.602X |
| Depth of dorsal fin | 7.778 | 1.884 | 0.807 | Y=0.210+0.054X |
| Length of anal fin | 30.649 | 3.663 | 0.98 | Y=-0.513+0.368X |
| Depth of anal fin | 6.894 | 2.412 | 0.474 | Y=0.370+0.026X |
| Preanal distance | 46.156 | 2.135 | 0.991 | Y=0.366+0.420X |
| Length of pectoral fin | 15.681 | 2.215 | 0.878 | Y=0.742+0.071X |
| Length of ventral fin | 6.587 | 2.017 | 0.926 | Y=-0.241+0.095X |
| Minimum body depth | 8.696 | 1.255 | 0.914 | Y=0.179+0.067X |
| Maximum body depth | 15.12 | 1.41 | 0.983 | Y=-0.029+0.155X |
| Distance between pectoral fin & ventral fin | 4.538 | 0.684 | 0.868 | Y=0.117+0.032X |
| Distance between pelvic fin & anal fin | 14.039 | 1.13 | 0.994 | Y=-0.166+0.160X |
| Length of caudal fin | 18.979 | 2.898 | 0.884 | Y=0.780+0.100X |
| Length of caudal peduncle | 8.113 | 1.183 | 0.937 | Y=0.267+0.050X |
| Per cent of head length | | | | |
| Eye diameter | 16.36 | 2.709 | 0.911 | Y=-0.025+0.173X |
| Interorbital distance | 27.338 | 3.925 | 0.927 | Y=0.030+0.264X |
| Preorbital distance | 14.409 | 4.033 | 0.938 | Y=-0.155+0.212X |
| Postorbital distance | 66.873 | 4.006 | 0.994 | Y=-0.097+0.710X |

**Table 3.** Morphometric features of *Puntius ticto*

| Morphometric characters | Mean | Standard deviation | Correlation | Regression equation |
|---|---|---|---|---|
| Per cent of total length | | | | |
| Standard length | 82.715 | 4.08 | 0.995 | Y=-0.537+0.890X |
| Head length | 27.568 | 1.278 | 0.993 | Y=0.074+0.267X |
| Head depth | 11.435 | 2.073 | 0.885 | Y=0.311+0.079X |
| Predorsal distance | 30.386 | 1.775 | 0.993 | Y=0.498+0.246X |
| Postdorsal distance | 5.701 | 1.457 | 0.782 | Y=0.148+0.040X |
| Length of dorsal fin | 48.899 | 4.232 | 0.994 | Y=-0.956+0.602X |
| Depth of dorsal fin | 7.778 | 1.884 | 0.807 | Y=0.210+0.054X |
| Length of anal fin | 30.649 | 3.663 | 0.98 | Y=-0.513+0.368X |
| Depth of anal fin | 6.894 | 2.412 | 0.474 | Y=0.370+0.026X |
| Preanal distance | 46.156 | 2.135 | 0.991 | Y=0.366+0.420X |
| Length of pectoral fin | 15.681 | 2.215 | 0.878 | Y=0.742+0.071X |
| Length of ventral fin | 6.587 | 2.017 | 0.926 | Y=-0.241+0.095X |
| Minimum body depth | 8.696 | 1.255 | 0.914 | Y=0.179+0.067X |
| Maximum body depth | 15.12 | 1.41 | 0.983 | Y=-0.029+0.155X |
| Distance between pectoral fin & ventral fin | 4.538 | 0.684 | 0.868 | Y=0.117+0.032X |
| Distance between pelvic fin & anal fin | 14.039 | 1.13 | 0.994 | Y=-0.166+0.160X |
| Length of caudal fin | 18.979 | 2.898 | 0.884 | Y=0.780+0.100X |
| Length of caudal peduncle | 8.113 | 1.183 | 0.937 | Y=0.267+0.050X |
| Per cent of head length | | | | |
| Eye diameter | 16.36 | 2.709 | 0.911 | Y=-0.025+0.173X |
| Interorbital distance | 27.338 | 3.925 | 0.927 | Y=0.030+0.264X |
| Preorbital distance | 14.409 | 4.033 | 0.938 | Y=-0.155+0.212X |
| Postorbital distance | 66.873 | 4.006 | 0.994 | Y=-0.097+0.710X |

**Table 4.** Morphometric features of *Esomus danrica*

| Morphometric characters | Mean | Standard deviation | Correlation | Regression equation |
|---|---|---|---|---|
| Per cent of total length | | | | |
| Standard length | 80.376 | 2.92 | 0.992 | Y=-1.111+1.010X |
| Head length | 20.994 | 2.479 | 0.473 | Y=0.806+0.060X |
| Head depth | 9.82 | 0.979 | 0.708 | Y=0.133+0.073X |
| Predorsal distance | 48.032 | 8.491 | 0.711 | Y=-1.358+0.732X |
| Postdorsal distance | 18.281 | 9.686 | 0.716 | Y=-3.046+0.748X |
| Length of dorsal fin | 4.655 | 0.976 | 0.687 | Y=-0.143+0.073X |
| Depth of dorsal fin | 12.932 | 1.65 | 0.529 | Y=0.334+0.067X |
| Length of anal fin | 4.655 | 0.976 | 0.687 | Y=-0.143+0.073X |
| Depth of anal fin | 13.948 | 1.35 | 0.687 | Y=0.357+0.073X |
| Preanal distance | 50.404 | 5.426 | 0.65 | Y=1.028+0.313X |
| Length of pectoral fin | 23.458 | 2.771 | 0.452 | Y=0.905+0.066X |
| Length of ventral fin | 13.177 | 1.404 | 0.684 | Y=0.529+0.033X |
| Minimum body depth | 7.19 | 1.181 | 0.383 | Y=0.205+0.034X |
| Maximum body depth | 17.139 | 2.26 | -0.005 | Y=0.924+0.000X |
| Distance between pectoral fin & ventral fin | 21.572 | 2.089 | 0.67 | Y=0.703+0.085X |
| Distance between pelvic fin & anal fin | 16.534 | 2.277 | -0.134 | Y=0.925+-0.007X |
| Length of caudal fin | 22.276 | 2.729 | 0.367 | Y=1.053+0.027X |
| Length of caudal peduncle | 11.318 | 1.182 | 0.684 | Y=0.429+0.033X |
| Per cent of head length | | | | |
| Eye diameter | 26.908 | 2.281 | -0.144 | Y=0.322+-0.017X |
| Interorbital distance | 29.425 | 5.655 | 0.408 | Y=-0.044+0.333X |
| Preorbital distance | 18.591 | 1.948 | 0.722 | Y=-0.104+0.278X |
| Postorbital distance | 47.902 | 3.132 | 0.927 | Y=-0.337+0.778X |

parameters of Hampala fish (*Hampala macrolepidota*) from Ranau Lake, Indonesia and observed that all the morphometric measurements showed significant positive correlation. Kaur et al (2019) also observed a positive correlation between total length and external body parts in *Labeo rohita* (Hamilton) from pond near Kalayat, Kaithal, Haryana.

## CONCLUSION

The morphometric measurements confirmed that the selected fish species have very little impact of environment because this area is still undisturbed from environmental degradation point of view. Eighteen characters have been studied in percentage of total fish length from which most of characters were genetically controlled, some characters were intermediate and environmentally controlled. Positive correlation has been observed between total length and external body parts. Linear relationships have been observed between all the independent and dependent characters.

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# Prediction of Effects of Climate Change Scenarios on Water Quality of Tungabhadra River Using Historical Data

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***Abstract:*** Climate change is a major concern for many researchers since many years. One of the major effects of climate change that the researchers are worried about is the impact of climate change on surface water quality. Two most important parameters affecting the water quality and quantity are changes in rainfall and temperature patterns. In this paper, an attempt is made to study the impact of climate change on 200 km stretch of river Tungabhadra. Historical data indicates a decreasing trend in rainfall and increasing trend in temperature. Sensitivity analysis is carried out with QUAL2K software to investigate the changing behaviour of water quality (dissolved oxygen and biochemical oxygen demand) with the changing climate trends. The result quantifies as to how much impairment is caused by these changing scenarios (stream flow and temperature) on the water quality and the critical stretches in the river that are severely affected. The quality and quantity of the wastewater effluents entering the river stream through different sources are kept same throughout the analysis.

***Keywords***: Climate change, Surface water quality, Rainfall, temperature, Dissolved oxygen, Biochemical oxygen demand

Climate change being the burning issue since many years and impacts of change are reported in literature using different approaches. Water being one of the main natural and important resources has grabbed a lot of attention due to the changing climatic trends. The ground water is not directly affected by the changes but surface water is affected (Yang Nan et al 2011). Many research works have been done on as to what all properties of the surface water will be affected by the climate change but not much work has been done to quantify these changes. Many studies reported the impacts of wastewater effluents on water quality (Basavaraj et al 2014, Garg et al 2013, Gilman et al 2010). A large number of Global Circulation Models and Regional Circulation models (GCM and RCM) have been developed to study the climatic projections but due to the uncertainties of these projections, hypothetical scenarios may be used to predict the climate change and their impacts on the water quality (Rehana and Majumdar 2011, Garg et al 2013). In this paper, QUAL2K software is used to carry out a sensitivity analysis on the water quality due to the changing climate (US EPA 1985). Temperature and rainfall are the main factors contributing to changes in the surface water quality. Historical data suggests that there has been a decrease of approximately 12.5% in the rainfall pattern in the study area in last 50 years and the temperature has increased at an average rate of $0.02^0$ C per year (Gilman et al 2010). Based on this data, 8 scenarios are used to determine the sensitivity analysis of the river water quality. The direct impact of these scenarios is predicted based on changes in dissolved oxygen and biochemical oxygen demand. An attempt is made in this paper to quantify the impact by giving the changed values of stream flows and temperature as the input to the model. The present stream flows are assumed to decrease by 10, 12.5 and 15% in the next 50 years and 20, 25 and 30% for the next 100 years due to decrease in rainfall trends as suggested by historical data. Similarly, temperature increase by $1^0$C in the next 50 years and $2^0$ C in the next 100 years are assumed. However, the other variables are assumed to remain constant. A time series plot of the climatically affected stream flows and temperature are plotted for all the scenarios.

## MATERIAL AND METHODS

**Study area:** Tungabhadra River is an Indian river that flows through the state of Karnataka for most of its course and then through the border of Karnataka and Andhra Pradesh before finally joining river Krishna (Krishna Basin Report 2014). This river is formed by the confluence of Rivers Bhadra and Tunga. It has two tributaries namely Haridra River and Kumudavati River that join the river at a distance of 124 km (from right) and 84 km (from left) respectively from the junction point of Bhadra and Tunga. This river receives many effluents from different industries and residential areas through its course. Tungabhadra River is known to be one of the major polluted rivers of the country due to the direct discharge of sewage from some cities such as Shimoga and Devengare (Fig. 1). Many industrial effluents are also discharged into it such as Mysore Paper Mill, Harihar Polyfibres and Visvesvraya Iron and Steel limited (Karnataka State Pollution Control Board 2011).

To study the impacts of climate change on the river, historical data of rainfall and temperature is analysed and then it is seen how the surface variables will be affected in the future by the changes in climate (Fowler et al 2017). A simple linear regression is also used to see the relation between the air temperature and the water temperature. QUAL2K Model is used for the sensitivity analysis and then obtained values are compared with the available base line values.

**Data analysis: Rainfall** and temperature data of the past 60 years was collected and analysed. The rainfall pattern seemed to decrease by 12.5% in the past 50 years and expected to fluctuate in the range of 10-15% for the next 50 years period and 20-30% for the next 100 years period and hence 6 scenarios are developed to test the sensitivity of the water quality of the river (Fig. 2). The temperature on the is expected to increase at a rate of around 0.02$^{0}$C per year and hence, temperature may increase by around 1$^{0}$C in the next 50 years and 2$^{0}$C in the next 100 years (Table 1).

Through the decreasing trend of the rainfall pattern and the increasing trend of the temperature, 6 hypothetical scenarios are used to stimulate the climatic conditions (3 for the next 50 years and 3 for the next 100 years). The details of the climate scenarios considered are given in Table 2.

**Configurations of the river:** The 200 km stretch of river Tungabhadra under study is divided into 15 stretches. The selected stretch receives effluent from 8 major sources namely Mysore paper mill, Bhadravathi city sewage, Vishveshwaraya Iron and Steel Limited, Shimoga city sewage, Honnali city sewage, Harihar city sewage, Harihar polyfibres and Davengere city sewage. The main sources of data are KSPCB (Karnataka State Pollution Control Board) and CWC (Central Water Commission). The annual average flow values of Tunga, Bhagra, Kumudavathi and Haridra are 162.93, 17.76, 10.19 and 11.54 m3/s, respectively. Water quality simulation model, QUAL2K is use to analyse the water quality data, which a modified version of QUAL2E.

QUAL2K model was used for sensitivity analysis for the existing and the six hypothetical scenarios. The results of the climate change scenarios were then compared with the present water quality variables.

## RESULTS AND DISCUSSION

The impact of climate change on water quality of the river under study is compared with the present water quality scenario.

**Effect on dissolved oxygen:** Five critical locations are selected and the percentage decrease in the DO level for the

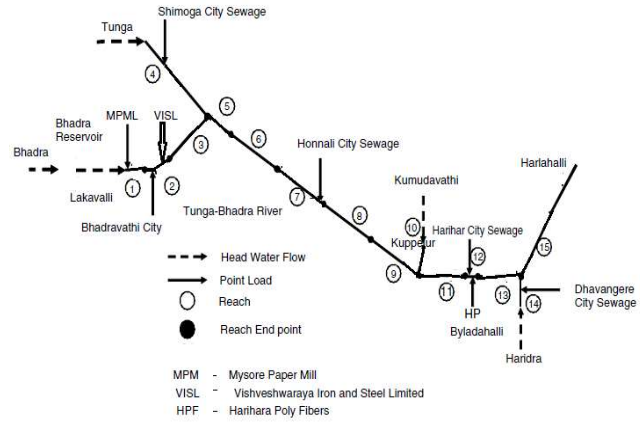
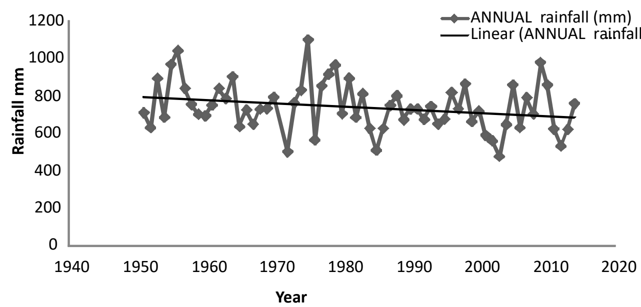



**Fig. 1.** Schematic diagram of river Tungabhadra




**Source:** Open Govt. Data on Web Site

**Fig. 2.** Rainfall pattern in North Karnataka Region

**Table 1.** Average temperature increase in the last 50 years per year in Karnataka

| Month | Temperature increase/ year ($^{0}$C) |
|---|---|
| January | 0.02 |
| February | 0.02 |
| March | 0.01 |
| April | 0.02 |
| May | 0.02 |
| June | 0.02 |
| July | 0.02 |
| August | 0.02 |
| September | 0.02 |
| October | 0.02 |
| November | 0.02 |
| December | 0.02 |
| Average increase | 0.02 |

**Source:** State Level Climate Change Trends in India Report (2013)

**Table 2.** Hypothetical scenarios

| Scenario No. | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Change in temperature (°C) | 1 | 1 | 1 | 2 | 2 | 2 |
| Change in discharge (%) | -10 | -12.5 | -15 | -20 | -25 | -30 |

six scenarios (Fig. 4 and 5). The selected locations lie immediately downstream to the wastewater outfall where the quality is expected to degrade drastically. The most severely affected location on the river was at the point just downstream of outfall from Mysore paper mill. The DO level decreased by 12% for scenario 6 (from 5.39 mg $l^{-1}$ to 4.78 mg $l^{-1}$) and 5 % for scenario 3. For effluents from Visvevaraya iron and steel limited where the degradation is found to be around 10% for scenario 6 and 4.5% for scenario 3, followed by Honnali city sewage, Harihar Polyfibres and Devengere city sewage.

In all the cases the degradation was more for scenarios 4, 5 and 6 as compared to scenarios 1, 2 and 3. This shows that increase in temperature will lead to degradation of the water quality in terms of DO.

**Effect on BOD:** BOD values of the effluents released by Mysore paper Mill and Visvesvaraya Iron and steel limited as given in Fig. 7 are 399 and 297 mg $l^{-1}$ which are very high (Fig. 6), however the BOD values are reduced to 14.10 and 11.42 mg $l^{-1}$ respectively at the downstream locations due to dilution

**Table 3.** Hydraulic variables used in water quality stimulations

| Reach no. | Bed width (M) | Manning's Co-efficient | Longitudinal slope X $10^{-3}$ |
|---|---|---|---|
| 1 | 61.85 | 0.0492 | 1.66 |
| 2 | 61.85 | 0.0492 | 1.66 |
| 3 | 61.85 | 0.0492 | 1.66 |
| 4 | 138.04 | 0.0492 | 0.27 |
| 5 | 162.15 | 0.0905 | 0.062 |
| 6 | 162.15 | 0.0905 | 0.062 |
| 7 | 162.15 | 0.0905 | 0.062 |
| 8 | 162.15 | 0.0905 | 0.062 |
| 9 | 162.15 | 0.0905 | 0.062 |
| 10 | 23.28 | 0.1235 | 0.124 |
| 11 | 162.15 | 0.0905 | 0.062 |
| 12 | 162.15 | 0.0905 | 0.062 |
| 13 | 162.15 | 0.0905 | 0.062 |
| 14 | 162.15 | 0.0905 | 0.062 |
| 15 | 162.15 | 0.0905 | 0.062 |

**Source**: Rehana and Mujumdar (2011)

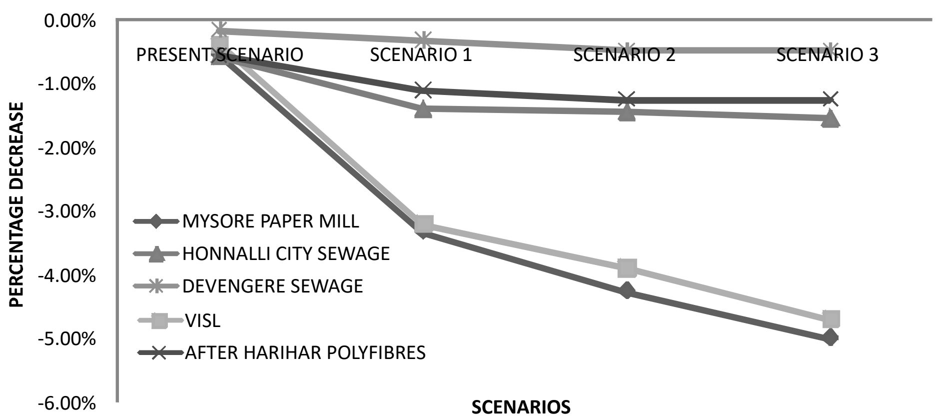



**Fig. 4.** Decrease in DO due to increase in temperature of 1°C

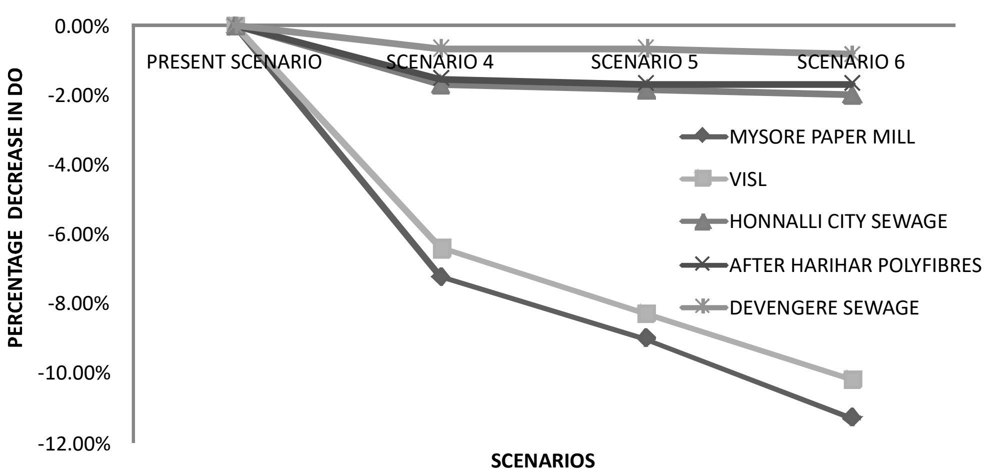



**Fig. 5.** Decrease in DO due to increase in temperature of 2°C

**Table 4.** Details of wastewater effluents

| Discharge | BOD (mg $l^{-1}$) | Temperature (°C) | Discharge ($m^3$/s) | Dissolved oxygen (mg $l^{-1}$) |
|---|---|---|---|---|
| Mysore paper mill | 399 | 24.63 | 0.868 | 7.3 |
| Bhadravathi city sewage | 15 | 25.43 | 0.308 | 5.6 |
| Vishveshwaraya iron and steel limited | 279 | 25.4 | 0.058 | 5.55 |
| Shimoga city sewage | 15 | 30.85 | 0.436 | 2 |
| Honnalli city sewage | 15 | 31.13 | 0.024 | 2 |
| Harihar city sewage | 15 | 31.09 | 0.129 | 6.76 |
| Harihar poly fibres | 15 | 27.44 | 0.5093 | 6.76 |
| Davangere city sewage | 15 | 31.88 | 0.867 | 2 |

**Source:** Karnataka State Pollution Control Board, KSPCB

in the river. These stretches may be considered as polluted stretches and the water quality lies in class D or E as stated by CPCB.

The increase in the BOD of river water at two critical locations due to the effluents from Mysore paper Mill and VISL for different climate change scenarios. The BOD value at the downstream location of Mysore paper Mill is expected to rise to 18.15 mg $l^{-1}$ due to scenario 6 and for VISL it is expected to rise till 13.96 mg $l^{-1}$ for scenario 6 (Fig. 7). Results indicate significant impact of climate change for both the cases considered during the study. Climate change will affect the water quality adversely causing a significant rise in the BOD of river water that results in water quality degradation.

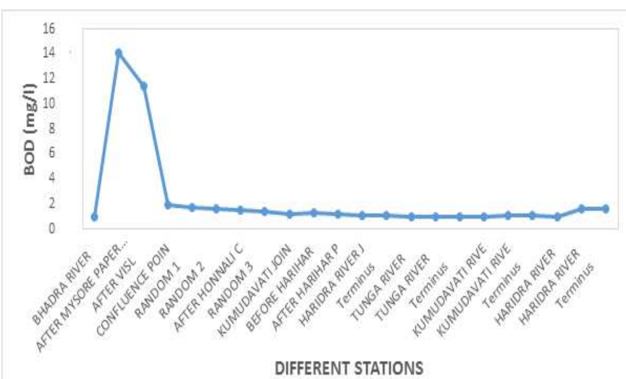



**Fig. 6.** Variation in BOD of River water at different locations

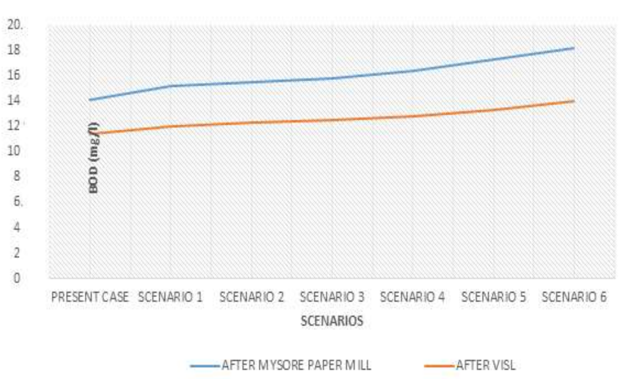



**Fig. 7.** Increase in BOD of river water for different climate change scenarios

## CONCLUSIONS

The results obtained during this study on Tungabhadra River indicate significant water quality degradation of river water due to predicted climate change scenarios. The historical data indicates likely decrease in the stream flow and increase in the temperature in this region in the next 50-100 years. The decrease in stream flow reduces the dilution effect of the river and the increase in temperature will lead to lower DO levels in river water. Both the consequences result in water quality degradation. On comparison, the results indicate that Scenario 6 (decrease in the discharge by 30% and increase in the temperature by 2°C) results in significant impact of climate change on water quality of the river. Though the projections of the climate change obtained during this study are for the hypothetical climate change scenarios, the results indicate the significant impact of climate change on water quality of the river. Furthermore, detailed study incorporating all the sources of pollution can give comprehensive impact of climate change on water quality of the river. The probable trend in river water quality degradation of the river stretch under study, demands for stringent effluent standards to mitigate the impact of climate change on river water quality. A suitable strategy for the effective water quality management of rivers is needed in perceiving persistent trends of climate change in the future.

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# Studies on Heavy Metal Concentration and Evaluation of Water Quality Index in Selected Sites of River Tamiraparani, Tamil Nadu, India

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***Abstract*:** The study sites were chosen based on the appropriate utility of river water for the purpose and practically cover the full length of the river. Water quality index has been developed for the water samples by an inclusive analysis for physicochemical parameters which comprises temperature, pH, electrical conductivity, total dissolved solids, total hardness, alkalinity, dissolved oxygen, phosphate and nitrate. Correlations between the physicochemical parameters were carried out. Trace metal concentrations such as lead (0.19 to 0.30 µg/g), Chromium (1.41 to 1.72 µg/g), Mercury (0.10 to 0.70 µg/g) and Cadmium (1.00 to1.35 in µg/g) were analysed near the urban areas indicating the moderate metal pollution in the study areas. The PCA with variance matrix rotation was operated to the heavy metals data set to construct a correlation matrix of different variables and to support in the identification of causes of several impurities. The strong factor loadings of Cd (0.947) and Pb (0.893) are governed by PCA, which accounts for 78.83 % of the total variance. The loading effects are proportional to the concentration of ions in water. The higher index value indicates the quality of river water is depriving in S5 (Vannarapettai) and S6 (Vallanadu) due to anthropogenic activities. If this situation continues, the water will become insecure for human consumption; consequently river water quality must be monitored on a regular basis.

**Keywords:** River Tamiraparani, Water quality index, Pearson correlation, Heavy metal examination

In existing circumstances the most significant concerns of our environment are the rising temperature and water crisis, where both the problems are interconnected. All the other vital issues affecting the environment would result out of them. The openness of freshwater for irrigation in the Indian agricultural segment is anticipated to turn down over the upcoming years. This will provide an adverse outcome on food production in India, with consequent effects on diets and health. Human health is under risk by excessive application of fertilizers and also by unhygienic conditions as a result of the injudicious discharge of waste. The pollution of freshwaters with a wide range of pollutants has become a matter of concern over the last few decades. Evaluating the chemical parameter of water gives us a suggestion about the water suitability for human consumption, domestic, agricultural and industrial use. Globalisation process disturbs the accessibility and nature of surface and groundwater table by inappropriate waste disposal (Sinha et al 2019) Water environments are often affected by means of effluents generated through various human activities. In addition to other pollutants some heavy metals may be naturally present in low concentration, however when it go beyond the permissible limit it will show the toxic effect over the environment including human, aquatic organisms (Sekabira et al 2010). For the purpose of evaluation of water chemistry and to measure the status of water quality in the river many methods have been used. The aptness of water sources for human utilization has articulated the necessity of Water quality index (WQI), which is one among the best efficient methods to elucidate the status of water (Tyagi et al 2013). Water quality indices are proposed for the comprehensive understanding and accessibility of the water resource to human welfare. The index value is a numerical figure that represents the stream's overall quality over time (Shah et al 2017). Anthropogenic effluents ensure their substantial impact of the occurrence of heavy metals in the water course (Mohanakavitha et al 2019). The intention of this work is to detect the quality of the Tamiraparani River water based on the index method and also to analysis the heavy metals concentration (Lead- Pb, Chromium- Cr, Mercury-Hg, Cadmium- Cd).

## MATERIAL AND METHODS

**Study Area:** The Tamiraparani river originates in the eastern slopes of Western Ghats (famous Agastyarkoodam peak of Podhigai hills 1,725 meters above the sea-level) in Tirunelveli district which is positioned between latitudes 8°30'N and 9°18'N and longitudes 77°07'30"E and 78°15'. The river stretch is about 125 km, passing through Tirunelveli and Tuticorin districts and confluences with the Gulf of Mannar region (near Punnaikkayal in Tiruchendhur Taluk) of the Bay of Bengal. This unique river is one among the Indian perennial rivers, where it receives the benefits of both the monsoons (south-west and north-east). The Tamiraparani is the predominant enriching river of Southern Districts of Tamil Nadu (India). The sampling locations were chosen based on factors, including pollution risk and the area's used by drinking, agriculture. The stations closest to the river source were supposed to be less influenced by urban contamination, whereas the stations further downstream were supposed to be more affected (Souilmi 2021). The geographical position of the study areas are given as Thalaianai (S1) 8°42'31.2''N 77°22'05.81'' (near Papanasam), Aladiyur (S2) 8°41'00.1'' N 77°25'44.6'' E, Cheranmadevi (S3) 8°42'03.0'' N 77°34'02.1''E, Kokkirakulam (S4) 8°43'30.0'' N 77°42'41.9'' E**,** Vannarapettai (S5) 8°43'57.7'' N 77°43'03.1'' E, Vallanadu (S6) 8°42'46.5'' N 77°50'11.7'' E (Fig. 1). Thalaianai is the downstream of the river below Pothigai hills, Aladiyur is a village, Cheranmadevi is semi-urban area, Kokkirakulam and Vannarpettai is in Tirunelveli corporation regions, whereas Vallanadu is the another village region of the river course.

**Sample collection and analysis:** The water samples were collected during summer season (low flow time) of May 2020. Because the pollutant concentration would be diluted during the rainy season so that samples were taken during low flow periods to analyse the pollutant range, and only then the river's status could be thoroughly assessed. Water samples from the study area were collected in 2 liters polyethylene bottles. The collected samples reserved in an ice box and then stored to a refrigerator at 4 °C, until delivery to the laboratory. The physicochemical parameter examination of sample comprises the measurements of pH, Temperature,

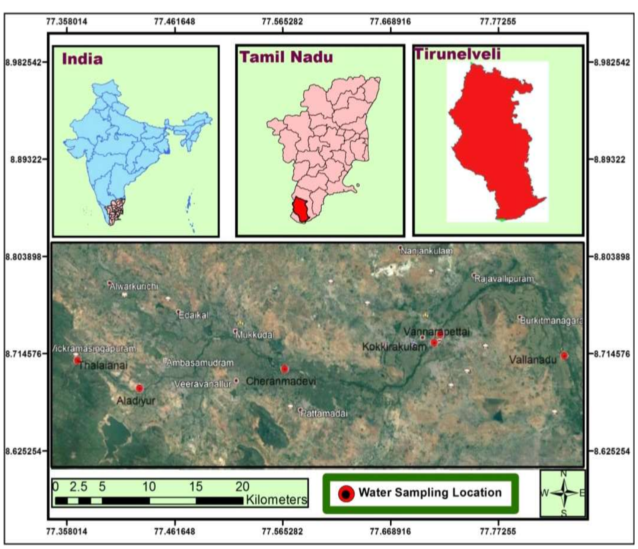


**Fig. 1.** Study area map locating the sampling sites

Electrical conductivity (EC) which was carried out in situ. Modified Winkler's method for dissolved oxygen (DO) analysis, total dissolved solids by Gravimetric method, Nitrate ($NO_3$) by Ultraviolet Screening method, Phosphate ($PO_4^{3-}$) by Spectrophotometric method, biological oxygen demand (BOD) by 5 days incubation at 20°C followed by titration, chemical oxygen demand (COD) by closed reflux method (APHA 2012).

Water quality index was evaluated by National Sanitation Foundation Water Quality Index (NSFWQI) method. Weightage were assigned for the parameters from 5 (significant effect on water quality) to 1 (minimum effect on water quality) based on their comparative consequence in the drinking water excellence (Yidana et al 2015). The assumption of weightage varies from one site to another depending on their surroundings, usage of water and source of the effluents. The obtained parameters were compared to the Bureau of Indian Standards (Akhtar et al 2018) for assessing the index value. The relative weight ($W_i$) is calculated by the equation, Wi = wi/Σwei, ($W_i$ is the relative weight comprising $w_i$, $w_i$ is the individual weight of each parameter). Quality rating, Qi = (Ci/Si) * 100, $C_i$ – concentration of each parameter in mg/l, $S_i$ - standard limits (BIS) mg/l). The sub index (SI) is the product of relative weight and quality rating (Divahar 2020); Sli = Wi * $Q_{I.}$ WQI was calculated for the six study areas. Effective weight ($Ew_i$) of the parameter was given by the equation,

$Ew_i$ = ($SI_i$ /WQI) x 100. The relative weights were related with effective weights, which reveal the priority of each parameter with respect to the other parameters in WQI calculations. The summation of the sub index values gives the assessed WQI. The figured index values are categorized into subsequent classes: less than 50 means Excellent water, 50–100 states that the quality of water is Good, 100–200 reveals that the quality of water is Poor, 200–300 - Very poor water, greater than 300 indicating the fact that the water is not suitable for drinking (Kachroud et al 2019). Principal Component Analysis done with PAST version 2.14 is shown in Figure 2 for the study of percentage of variance (Sahaya Vasanthi et al 2019). The PCA technique is used to aid in the detection of significant changes in physicochemical characteristics. Heavy metal concentrations were analyzed by Atomic Absorption Spectroscopy (AAS). The concentration of Lead (Pb), Hexavalent Chromium (Cr-VI), Mercury (Hg), Cadmium (Cd) in each water sample were examined by means of an Atomic Absorption Spectrometer (AAS: AAS: LABINDIA: AA- 7000). The prerequisite for AAS analysis was the acid ($HNO_3$) digested sample to destroy the matrix if formed and to avoid any interference during atomization.

## RESULTS AND DISCUSSION

**Physicochemical analysis:** The pH of water indicates whether the samples are acidic or basic in nature. The pH is a significant parameter classifying the usage of water for drinking and irrigation purpose. It has reflective properties on water quality, arousing affect in the solubility of metals, alkalinity and hardness of water (Osibanjo et al 2011). The pH ranged between 7.35 mg $l^{-1}$ to 7.54 mg $l^{-1}$, signifying that the water is suitable for consumption. Dissolved oxygen was high at the all sites especially in Thalaianai (S1) site as (8.97). The site Thalaianai (S1) with the elevated dissolved oxygen indicates the good aeration of the river. The high value of electrical conductivity at Aladiyur (S2) site as (509.9 µs/cm) and Cheranmadevi (S3) site as (482.7 µs/cm) indicates the presence of charged ions (Abhishek Nandal et al 2020). Total hardness and conductivity values were under permissive limits at all the sites. The total hardness of Cheranmadevi (S3) (94.8 mg/l) Kokkirakulam (S4) (98.4 mg/l) Vannarapettai (S5) (100.32 mg/l) suggested that these sites possess slightly hard water. The maximum COD at Cheranmadevi (S3), Kokkirakulam (S4), Vannarapettai (S5) and Vallanadu (S6) sites were reported. The recommended permissible limit for TDS is 500 mg/l. The TDS value ranges between 85.41 to 313.76 mg/l. The higher TDS was in Cheranmadevi (S3). The high COD value indicates that the dissolved oxygen was consumed in excess by the decomposing organic matter, which prevails to be a threat to the aquatic ecosystem in the particular sites of the river. The increasing BOD and decreasing DO was noted at Cheranmadevi (S3), Kokkirakulam (S4), Vannarapettai (S5) and Vallanadu (S6) sites clearly indicate the dumping of pollution load into the river system. Cheranmadevi (S3) possess higher alkalinity value as (251.7 mg/l). High conductivity recorded at Aladiyur (S2) as (509.9 µs/cm) and Cheranmadevi (S3) as (482.7 µs/cm) sites apparently reveals the inclusion of household waste and industrial drainage from the nearby areas.

**Water Quality Index:** For assessing WQI, initially the weight values for each parameter were assigned agreeing with their comparative importance in inclusive value of water for consumption purposes. Individual weightage assumptions were carried out in each site depending upon the variation in physicochemical parameters. The WQI of each sites are given in the (Table 2). Despite the fact that site S1 has a low index value (76.438) when compared to other study areas, it is still a cause of concern because the water quality begins to deteriorate as a result of anthropogenic disturbances. Sites S5 and S6 had the highest index values among the six study areas, indicating that the water is in deprived condition by anthropogenic activities.

**Pearson correlation analysis:** The physicochemical parameters are interpreted by Pearson Correlation (Table 3). A high correlation coefficient indicates a positive association between two variables, while a correlation coefficient of zero indicates no such relationship. A positive "r" indicates a positive link, whereas a negative value indicates an inverse relationship. The correlation reveals that the pH exhibit a positive correlation with EC/ TDS; electrical conductivity shows a positive association with TDS/ TH; total dissolved solids exhibit a positive correlation with TH; total hardness shows a positive correlation with alkalinity; alkalinity exist a positive association with nitrate.

**Principal component analysis (PCA):** The PCA (Fig. 2) with variance matrix rotation was operated to the heavy metals data set to construct a correlation matrix of different variables and to support in the identification of causes of several impurities.

**Table 1.** Physicochemical parameters of the water samples in the study area (Mean ±SD)

| Parameters | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | $S_6$ |
|---|---|---|---|---|---|---|
| Air temperature | 25±5.29 | 26.33± 3.06 | 30.64± 3.51 | 29.67± 3.51 | 31.33± 4.52 | 27.0± 2.65 |
| Water temperature | 23.67± 3.21 | 23.67± 3.21 | 25.56 ± 3.79 | 25.33± 3.06 | 25.9 ± 2.0 | 24.0± 2.65 |
| $p^H$ | 7.35 ± 0.20 | 7.68 ± 0.25 | 7.98 ± 1.6 | 7.63 ± 0.48 | 7.67 ± 0.58 | 7.54 ± 0.34 |
| EC (µs $cm^{-1}$) | 131.4 ± 35.4 | 509.9 ± 237.1 | 482.7 ± 95.7 | 463.6 ± 62.1 | 477.1 ±32.56 | 413.2 ± 10.32 |
| TDS (mg $l^{-1}$) | 85.41± 41.5 | 331.44±148.7 | 313.76 ± 65.3 | 301.34 ± 35.8 | 310.12 ±18.4 | 268.58 ± 52.9 |
| Hardness (mg $l^{-1}$) | 69.7 ± 51.0 | 83.8 ± 9.4 | 94.8 ± 16.4 | 98.4 ± 6.08 | 100.32 ±5.16 | 87.6 ± 4.25 |
| Alkalinity (mg $l^{-1}$) | 189.2 ± 20.6 | 212.2 ± 10.4 | 251.7 ± 29.08 | 245.6 ± 36.52 | 239.24±32.72 | 234.98 ± 17.65 |
| DO (mg $l^{-1}$) | 8.97 ± 1.05 | 6.31± 2.65 | 5.23 ± 0.97 | 5.07 ± 1.87 | 4.98 ± 1.16 | 6.13 ± 0.74 |
| COD (mg $l^{-1}$) | 10.67 ± 1.15 | 17.76 ± 5.43 | 21.68 ± 4.73 | 28.67 ± 13.43 | 33.94 ± 6.43 | 24.2 ± 3.56 |
| BOD (mg $l^{-1}$) | 2.43 ± 0.59 | 3.80 ± 1.13 | 3.87 ± 0.83 | 4.30 ± 1.35 | 4.67 ±1.20 | 4.61 ± 1.27 |
| Phosphate (mg $l^{-1}$) | 0.027 ±0.27 | 0.036 ±3.56 | 0.044 ±2.15 | 0.058 ±1.37 | 0.073±0.97 | 0.069 ±18.51 |
| Nitrate (mg $l^{-1}$) | 0.9±1.12 | 14.3±2.78 | 32.7±1.24 | 34.5±24.7 | 37.2±12.9 | 39.4 ±42.37 |

**Heavy metal analysis:** The heavy metals are also able to form metal complexes in higher concentrations, which are highly toxic. Trace metal concentrations (Table 4) analysed in the sample expresses the existence of anthropogenic activities. It impinges on social well-being if they comprise harmful chemicals (Kelly et al 2015). A strong positive correlation (Table 5) was observedbetween Pb-Hg (r = 0.893), Cd-Pb (r = 0.851), Cd-Cr-VI (r = 0.909).

The heavy metals data were compared by using basic statistics analysis which exposes a common association of the four elements approaching the normal distribution with their lower skewness (Table 6). Mercury displayed positively moderate skewness. The negative skewed values of Pb, Cr-VI and Cd showed that most of their values fall under higher right side of the frequency distribution curve. Higher positive Kurtosis values were observed for Cr-VI and Cd, indicating the outliers. Comparable inclinations can be detected in the values indicates that moderately polluting the particular river system of Tamiraparani basins by those metals components. The multivariate statistical techniques, like principal component analysis (PCA), factor analysis and agglomerative hierarchal cluster studies were performed to define the sources of heavy metals. Bigplot for the

**Table 4.** Heavy metals concentration (µg $g^{-1}$) from the different locations of river Tamiraparani

| Element | S1 | S2 | S3 | S4 | S5 | S6 |
|---|---|---|---|---|---|---|
| Pb | Below<0.05 | 0.19 | 0.23 | 0.48 | 0.45 | 0.30 |
| Cr | ND | 1.72 | 1.70 | 1.48 | 1.50 | 1.41 |
| Hg | ND | ND | 0.10 | 0.49 | 0.70 | 0.45 |
| Cd | ND | 1.00 | 1.00 | 1.20 | 1.35 | 1.10 |

ND – Not detectable

**Table 5.** Correlations between the heavy metals

| Element | Pb | Cr | Hg | Cd |
|---|---|---|---|---|
| Pb | 1 | 0.592 | 0.893** | 0.851** |
| Cr | | 1 | 0.327 | 0.909** |
| Hg | | | 1 | 0.689** |
| Cd | | | | 1 |

** Correlation is significant at P ≤ 0.01

**Table 2.** Water Quality Index of the selected sites

| Parameters | BIS | Thalaianai (S1) | | Aladiyur (S2) | | Cheranmadevi (S3) | | Kokkirakulam (S4) | | Vannarapettai (S5) | | Vallanadu (S6) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $w_i$ | Effective weight (%) | $w_i$ | Effective weight (%) | $w_i$ | Effective weight (%) | $w_i$ | Effective weight (%) | $w_i$ | Effective weight (%) | $w_i$ | Effective weight (%) |
| $p^H$ | 6.5-8.5 | 4 | 21.543 | 3 | 12.067 | 3 | 11.689 | 2 | 7.488 | 2 | 7.761 | 2 | 6.842 |
| EC (µs $cm^{-1}$) | 750 | 3 | 2.63 | 4 | 9.422 | 3 | 8.043 | 3 | 7.736 | 3 | 8.205 | 3 | 6.374 |
| TDS (mg $l^{-1}$) | 500 | 3 | 2.565 | 4 | 11.833 | 3 | 7.844 | 3 | 7.542 | 2 | 5.33 | 3 | 6.215 |
| TH (mg $l^{-1}$) | 300 | 3 | 3.495 | 3 | 3.727 | 3 | 3.946 | 3 | 4.105 | 2 | 2.878 | 2 | 2.253 |
| Alkalinity (mg $l^{-1}$) | 200 | 4 | 19.055 | 5 | 23.311 | 5 | 25.85 | 5 | 25.614 | 4 | 20.714 | 5 | 22.658 |
| DO (mg $l^{-1}$) | 5 | 5 | 45.1 | 5 | 27.727 | 5 | 21.486 | 4 | 16.920 | 3 | 12.908 | 4 | 18.914 |
| Phosphate (mg $l^{-}$) | 0.05 | 2 | 5.440 | 2 | 6.381 | 4 | 14.689 | 5 | 24.196 | 5 | 31.484 | 5 | 26.613 |
| Nitrate (mg $l^{-1}$) | 45 | 2 | 0.202 | 2 | 2.816 | 2 | 6.353 | 2 | 6.397 | 3 | 10.710 | 3 | 10.130 |
| WQI $=\sum W_iQ_n = \sum SI_{in}$ | | 76.438 | | 80.107 | | 85.67 | | 88.692 | | 96.455 | | 95.932 | |

**Table 3.** Correlation matrix of water quality parameter (Pearson correlation coefficients (r)

| | pH | EC | TDS | TH | Alkalinity | DO | Phosphate | Nitrate |
|---|---|---|---|---|---|---|---|---|
| pH | 1 | | | | | | | |
| EC | 0.909* | 1 | | | | | | |
| TDS | 0.925* | 0.989* | 1 | | | | | |
| TH | 0.798** | 0.807** | 0.808** | 1 | | | | |
| Alkalinity | 0.844 | 0.756** | 0.756** | 0.927* | 1 | | | |
| DO | -0.891 | -0.911 | -0.912 | -0.976 | -0.931 | 1 | | |
| Phosphate | 0.484 | 0.503 | 0.504 | 0.749** | 0.686 | -0.698 | 1 | |
| Nitrate | 0.695 | 0.692 | 0.692 | 0.878 | 0.924* | -0.872 | 0.897 | 1 |

*Correlation is significant at P ≤ 0.05 ** correlation is significant at P ≤ 0.10

**Table 6.** Basic statistics for the heavy metals determined

| Element | Mean | Median | SD | Skewness | Kurtosis | Variance |
|---|---|---|---|---|---|---|
| Pb | 0.28 | 0.27 | 0.16 | -0.09 | -0.97 | 0.027 |
| Cr | 1.30 | 1.49 | 0.65 | -2.25 | 5.25 | 0.422 |
| Hg | 0.29 | 0.28 | 0.29 | 0.29 | -2.06 | 0.088 |
| Cd | 0.94 | 1.05 | 0.48 | -2.02 | 4.55 | 0.230 |

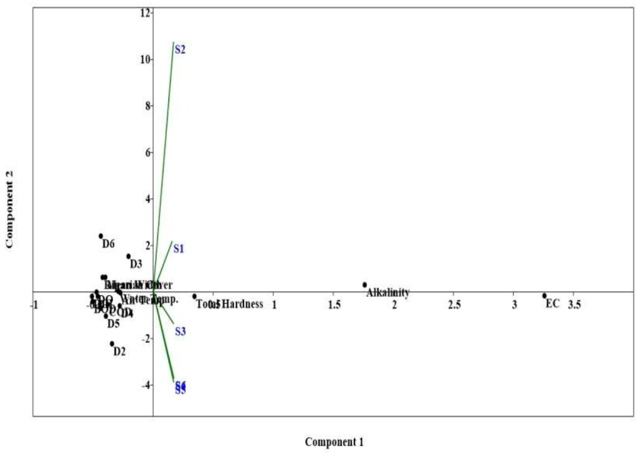


**Fig. 2.** Principal Component Analysis (PCA) based on the physic-chemical parameters in the selected six study sites of river Tamiraparani, Tamil Nadu

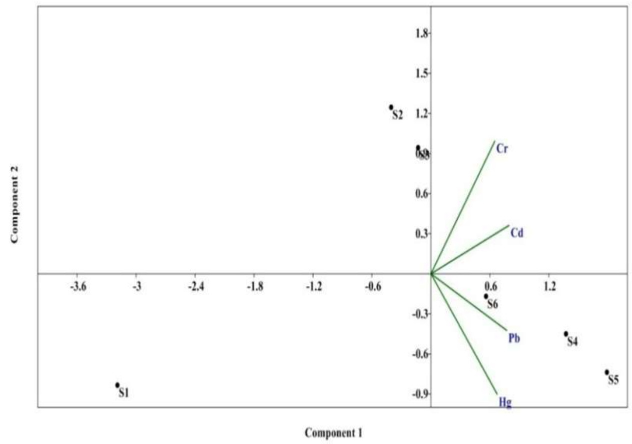


**Fig. 3.** Biplot for the components in rotated space

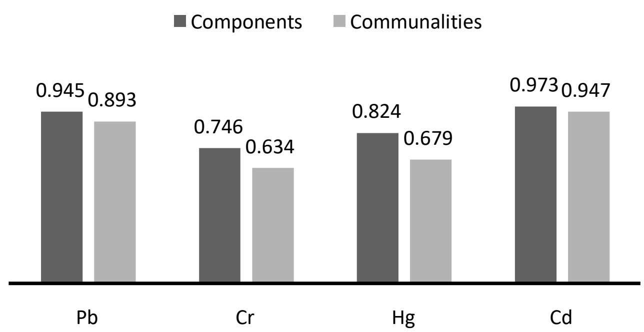


**Fig. 4.** Factor loading of the components extracted

**Table 7.** Total variance explained (one component extracted)

| Component | Initial eigenvalues | | | Extraction sums of squared loadings | | |
|---|---|---|---|---|---|---|
| | Total | Per cent of variance | Cumulative (%) | Total | Per cent of variance | Cumulative (%) |
| 1 | 3.153 | 78.829 | 78.829 | 3.153 | 78.829 | 78.829 |
| 2 | 0.782 | 19.552 | 98.381 | | | |
| 3 | 0.064 | 1.595 | 99.976 | | | |
| 4 | 0.001 | .024 | 100.000 | | | |

Extraction Method: Principal Component Analysis

components in rotated space is shown in Figure 3. The KMO value 0.504, says that it is an acceptable measure of sampling appropriateness. Barlett's test of sphericity shows a highly significant value indicating that correlation was not an identity matrix and there exist a significant association between the variables. The above mentioned investigations confirmed the adequacy of water quality data for the PCA.

Percentage of variance by different components extracted was demonstrated by PCA. The factor loadings of the different variables have I and Hg demonstrated the low factor loadings in this study revealing their independency (Fig.4) which shows that communalities correlate with the components. The most significant component possesses the highest Eigen value. The two principal components such as PCA 1 and PCA 2 were acquired by applying PCA on the water heavy metals showing a cumulative variance of 78.83% (Table 7). PCA 1 accounts for 78.83% of the entire variance and is ruled through the strong factor loadings of Cd (0.947) and Pb (0.893). The main sources of heavy metal pollution in freshwater are air deposits and habitat changes caused by chemical and metal production processes, as well as urban and domestic garbage disposal. Heavy metals may form complexes with amino acids and protein carboxylic acids, disrupting the transport mechanism through the cell wall and causing toxicity. If this trend continues, the water will become unsafe for human consumption, necessitating continuous monitoring and generating awareness in public about the river water quality.

## CONCLUSION

Physicochemical analysis results specify that the water samples of site 3,4,5,6 contain alkaline properties due to adequate human usage of washing clothes, discharge of fertilizer from the nearby fields. Among the six sites, site 5 possesses lower dissolved oxygen value by the excess growth of hydrophytes. The main sources of lead, chromium are from the influence of industrialized events and also through liberations of unprocessed local sewage discharges. The anthropogenic pollutants govern over points of sampling among certain locations of Kokkirakulam, Vannarapettai, Cheranmahadevi and Vallanadu. The factor loading coefficient of lead and cadmium indicates the strong relation between the variable and factor. The river Tamiraparani has good water status but clearly points out that the increasing WQI value states that the quality of the river is declining mainly in the Vannarapettai (S5) and Vallanadu (S6). The most important pollutant source of the Tamiraparani River are wastewater discharging from improper disposal of domestic sewage, municipal and hospital waste, paper mill, cottage industries near the river banks, agricultural activities. If the same environmental circumstances persists for a certain period the river passing through these sites (S5 and S6) will loss its trait for drinking purpose. In general, the water quality of the stream is generally good but it is deteriorating owing to human activities like neighbouring farming and the engineering activities. Therefore, required fortification actions should be taken to protect the river must be interrelated to the deliberate and appropriate utility of the perennial river.

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# Temporal Changes in Physicochemical Parameters of Water of Gharana Wetland Reserve (J&K) and Assessment of its Pollution Status Using Comprehensive Pollution Index

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***Abstract:*** Gharana wetland conservation reserve, located in the outskirts of Jammu region of Jammu and Kashmir UT has gained global recognition in the past few years since it harbours a number of migratory birds, including some rare and endangered species which migrate here during winters every year. Owing to its international recognition, an attempt has been made to assess the water quality of the wetland using Comprehensive Pollution Index (CPI) wherein various physicochemical parameters were considered. For this, the water samples were collected seasonally for a period of one year i.e. 2018-19 and analysed for various physicochemical parameters by following established procedures. The results of the present study revealed that due to encroachment of wetland area by the villagers, weed growth, siltation and various anthropogenic stresses like bathing of livestock, defecation, drainage from households and run off of insecticides as well as pesticides from the adjoining fields into the wetland, its water quality is severely deteriorating day by day. On the basis of CPI score, it was observed that this waterbody was moderately polluted. It can therefore be concluded that this continued exploitation of the Reserve will further lead to deterioration of its water quality and shrinkage of the area. It is recommended that proper maintenance of waterbody is necessary. Thus, the present study will help in formulating certain strategies to combat the pollution threat to this waterbody and convert it from a dying wetland to a thriving ecosystem.

***Keywords*:** Wetland, Eutrophication, Migratory birds

Wetlands are transitional zone between terrestrial and aquatic ecosystem, where the water table is usually at or near the land surface or flooded by water, either permanently or seasonally (Cowardin et al 1979), covering 4-6% of the earth's ice free land surface (Ramsar 2006). Serving as home to a wide range of plant and animal life, they are considered the most productive, biologically diverse and ecologically sensitive of all ecosystems on earth (Ghermandi et al 2008). They hold great ecological significance for more than one reason, most notably because they regulate biological cycles and nutrient recycling, maintain water quality by filtering and cleaning water, hold flood waters, recharge groundwater, provide shelter to countless species of birds, mammals, reptiles, amphibians, fishes and invertebrates, support fishing, hunting and recreational activities etc. (Ramsar 2006). India has been bestowed with a number of wetland ecosystems having wide range of varied climatic conditions, extending from cold Jammu and Kashmir to hot and humid Peninsula, thus exhibiting immense diversity. However, in consequence of exponential growth in human demands, unplanned urbanization and industrialization, resource exploitation and public ignorance, natural wetlands have become one of the most threatened habitats globally and are shrinking rapidly (Kadlec and Wallace 2008). They are increasingly facing several anthropogenic pressures not only in India but all over the world. It is a well-known fact that discharges from domestic sewage and industrial effluents usually lead to changes in water quality and eutrophication of wetlands, adversely affecting the microflora and fauna dwelling therein (Muwanga and Banfago 2006). Since wetlands support cultivation of rice, a staple diet of half of the world's population, most of them are being converted into agricultural land.

Interestingly, wetlands cover a tiny portion of earth's surface but due to their unique nature and the numerous ecosystem services they provide benefitting humanity, it becomes very important to protect and conserve them. As a result, this study is geared towards assessing the water quality of Gharana wetland using Comprehensive Pollution Index (CPI) in order to ascertain its pollution status.

**Study Area**

Gharana Wetland Reserve is situated in Jammu region of J&K at a small village on Indo-Pak International border near Ranbir Singh Pura Sector at about 35 km from Jammu city (Fig. 1 and 2). Located along the Central Asian Flyway, it is a notified Wetland Conservation Reserve and has been internationally recognised as one of the IBAs (Important Bird Areas) in the world declared by Birdlife International (UK) and

**Fig. 1.** Satellite view of Gharana wetland

**Fig. 2.** Sampling site

Bombay Natural History Society (BNHS). Covering an area of approximately 200 acres of marshy land, it supports more than 170 resident and migratory bird species that come there all the way from Siberia, Central Asia, China, East Europe and New Zealand during winters. Thousands of migratory birds flock to this scenic area every year in search of food and to escape the harsh winter of their native habitat, turning it into a paradise for bird-watchers.

## MATERIAL AND METHODS

**Sample collection and analysis:** The water samples were collected seasonally for a period of one year i.e. 2018-19 and analysed for various physicochemical parameters using established procedures (APHA 2012). The parameters viz. air and water temperature, pH, DO, $FCO_2$, total alkalinity, calcium, magnesium and chlorides were assessed at the sampling onsite. While for rest of the parameters like nitrate, sulphate, phosphate and BOD, water samples were collected in pre-cleaned plastic bottles and transported in ice box to the laboratory for analysis. These parameters were tested within 6 to 8 hours of sample collection.

**Statistical analysis:** Comprehensive Pollution Index (CPI): It is used to depict the overall water quality through simple numerical number and classified into different categories as proposed by Zhao (2012). It was calculated using following mathematical expression:

$$PI = \frac{\text{Measured concentration of individual parameter}}{\text{Standard permissible concentration of parameter}} \quad (1)$$

$$CPI = \frac{1}{n}\sum_{i=0}^{n} PI \quad (2)$$

where,

PI represents the pollution index of considered specific water quality parameter indicates the number of selected parameters

The calculation of CPI was done by considering the standard permissible limits of different government agencies (ICMR 1975, WHO 2011 and BIS 2012) (Table 1). CPI ranges from 0 to 2 which classify water quality as: Clean (≤ 0.20 CPI); Subclean (0.21 - 0.40 CPI); slightly polluted (0.41 – 1.00 CPI); moderately polluted (1.01 – 2.0 CPI) and severely polluted (≥ 2.01 CPI) (Mishra 2015).

## RESULTS AND DISCUSSION

**Physicochemical parameters:** The observed physicochemical parameters of water samples of Gharana wetland is shown in Table 2. During the present study, the air temperature showed a characteristic annual cycle with higher values during the summer (34°C) and lower in the winter (18°C). Water temperature was closely correlated to atmospheric conditions. For this reason the surface water of selected site was directly influenced by air temperature and fluctuated from 17.5°C (winter) to 35°C (summer). The waterbody remained mildly neutral to alkaline during the study period with its pH values varying from 7.5 (monsoon) to 9 (spring). Lowest pH during monsoon may be due to dilution of rain water. A similar monsoon minimum was also reported

**Table 1**. Guideline values as per various recommending agencies

| Parameters | Standards | Recommending agency |
|---|---|---|
| pH | 6.5-8.5 | ICMR/BIS |
| DO (mg $l^{-1}$) | 5 | ICMR/BIS |
| FCO2 (mg $l^{-1}$) | 2 | WHO |
| Total alkalinity (mg $l^{-1}$) | 120 | ICMR |
| Chlorides (mg $l^{-1}$) | 250 | ICMR |
| Calcium (mg $l^{-1}$) | 75 | ICMR/BIS |
| Magnesium (mg $l^{-1}$) | 30 | ICMR/BIS |
| BOD (mg $l^{-1}$) | 5 | ICMR/BIS |
| Nitrates (mg $l^{-1}$) | 45 | ICMR/BIS |
| Phosphates (mg $l^{-1}$) | 5 | WHO |
| Sulphates (mg $l^{-1}$) | 150 | ICMR/BIS |

by Das and Dey (2020). Increased photosynthesis by aquatic plants during growing season (spring) as a consequence of increased day length, removes carbon dioxide from the water, thus significantly increasing pH.

The low value during monsoon season may be due to the dilution of rain water

DO concentration peaked in spring (9 mg $l^{-1}$) due to an increment in photosynthesis by increased algal biomass and slightly dropped in summer (7.8 mg $l^{-1}$) because of high temperature that decreases the oxygen holding capacity of water. The free carbon dioxide ranged from nil to 30.8 mg $l^{-1}$ throughout the year. The absence of free $CO_2$ in spring and summer season may be due to its increased uptake by phytoplankton and macrophytes and also due to presence of appreciable amount of carbonates. Highest free $CO_2$ in monsoon could be the result of decomposition of large amount of sewage entering into the wetland from the catchment area that seems to enhance its carbon dioxide content (Matta et al 2018).

During the present course of study, carbonates remained absent during monsoon and winter seasons due to the presence of free $CO_2$ thus confirming an inverse relationship between the two. On the contrary, maximum level of carbonates (142 mg $l^{-1}$) present during summers may possibly be due to increased photosynthetic uptake of carbon dioxide (Zuber 2007). Bicarbonates ranged from 170 - 510 mg $l^{-1}$ showing a spike in its value during winters, owing to reduction in photosynthetic activity leading to a drop in the uptake of bicarbonates as a source of carbon thus causing increase in its value. However, minimum bicarbonates were observed in monsoon due to dilution effect of rains as also suggested by Sawhney (2004).

High concentration of chlorides in a waterbody is an indicator of pollution caused due to organic wastes which are harmful to aquatic life (Rajkumar et al 2004). Perusal of Table 2 reveals that chlorides recorded a unimodal peak in summers followed by a decline in its concentration from monsoon to spring through winters. Summer maxima (120 mg $l^{-1}$) might be attributable to increase in rate of decomposition and low water level due to high temperature. Similar findings were reported by Saxena and Saksena (2012), Lianthamluaia et al (2013) and Sharma et al (2014) that support the above results. Conversely, uptake of chlorides by growing biomass of algae and macrophytes (Singh 2004) could be a plausible reason for its spring minima (18.9 mg $l^{-1}$).

Hardness of water primarily depends on the presence of $Ca^{2+}$ and $Mg^{2+}$ ions. In the present study, the values of calcium hardness varied from 9.67 to 74.01 mg $l^{-1}$ and that of magnesium varied from 8.82-68.04 mg $l^{-1}$. Both Ca and Mg recorded highest values during winter and a decline in summer. Winter maxima in their concentration may be due to decrease in utilization of Ca and Mg by the algae and macrophytes owing to reduction in their population at low temperature (Jan 2005, Chandrakiran 2011); decrease in water level and decomposition of organic matter and increased solubility of calcium at low temperature (Garg et al 2009). The probable reasons behind summer minima in their

**Table 2**. Seasonal variations in various physicochemical parameters of water of Gharana Wetland during the period of one year (2018-19)

| Parameter | Units | Monsoon | Winter | Spring | Summer |
|---|---|---|---|---|---|
| Air temperature | °C | 29 | 18 | 25.5 | 34 |
| Water temperature | °C | 27 | 17.5 | 25 | 35 |
| pH | | 7.5 | 8.5 | 9 | 8.7 |
| DO | mg $l^{-1}$ | 8 | 8.8 | 9 | 7.8 |
| FCO2 | mg $l^{-1}$ | 30.8 | 16.72 | 0 | 0 |
| Carbonates | mg $l^{-1}$ | 0 | 0 | 88 | 142 |
| Bicarbonates | mg $l^{-1}$ | 170 | 510 | 352 | 458 |
| Total alkalinity | mg $l^{-1}$ | 170 | 510 | 440 | 600 |
| Chlorides | mg $l^{-1}$ | 67.9 | 32 | 18.9 | 120 |
| Calcium | mg $l^{-1}$ | 14.29 | 74.01 | 33.64 | 9.67 |
| Magnesium | mg $l^{-1}$ | 11.25 | 68.04 | 17.25 | 8.82 |
| BOD | mg $l^{-1}$ | 5.2 | 2.4 | 2.2 | 6.2 |
| Nitrates | mg $l^{-1}$ | 0.5765 | 0.5761 | 0.3819 | 0.5778 |
| Phosphates | mg $l^{-1}$ | 0.1639 | 0.0984 | 0.0425 | 0.2681 |
| Sulphates | mg $l^{-1}$ | 2.893 | 1.735 | 2.082 | 2.166 |

values could be because of prolific growth of photosynthetically active algae and macrophytes thereby causing an increase in uptake of Ca and Mg; high DO content due to increased use of free carbon dioxide during photosynthesis which leads to conversion of soluble calcium bicarbonate into insoluble carbonates that precipitate and settle at the bottom as marls (Singh 2004, Zuber 2007). The above observation was contrary to the findings of Jana (1973) who reported high values of Ca and Mg in summers.

Since BOD is a measure of the amount of oxygen consumed by the micro-organisms for the decomposition of organic matter, therefore it is an important water quality parameter indicating the pollution status of a water body (Zuber 2007). BOD values oscillated from 6.2 to 2.2 mg $l^{-1}$ during the investigative period of one year (2018-19). High concentration of BOD was observed in summer as a consequence of high temperature that leads to increased decomposition accompanied by high microbial activity (Chandrakiran 2011) and low DOES In contrary to this, high DO content due to increase in photosynthetic activity by abundantly growing macrophytes and phytoplankton caused a decline in BOD value in spring season (Zuber 2007).

The biological productivity of a waterbody is determined by assessing its nitrate and phosphate content, therefore, any increase in their concentration drives a waterbody towards eutrophication (Rao 2004). The level of nitrates remained high during most of the study period. However, its value ranged from minima of 0.3819 mg/l in spring to maxima of 0.5778 mg $l^{-1}$ in summer. This summer maxima could be due to high rate of decomposition of organic matter (Paulose and Maheshwari 2007) and discharge of sewage into the wetland (Kennedy and Hain 2002). Also, second peak in nitrate concentration witnessed during monsoon could be the result of surface run off of sewage and domestic waste from human habitations and influx of nitrogenous fertilizers from agricultural fields surrounding the wetland (Slathia and Dutta 2009, Yousuf et al 2015 and Zuber 2015). As the wetland is a preferred abode for hundreds of migratory aquatic birds that come to this area from far off places during winter, so their excreta might have contributed to the increased nutrient load in water. In contrary to this, fall in nitrates during spring may have resulted from active uptake of nitrogen by flourishing phytoplankton and macrophytes from water and bottom sediments (Shinde et al 2011).

During the investigation period, phosphates fluctuated from 0.0425 mg $l^{-1}$ (spring) to 0.2681 mg $l^{-1}$ (summer). High value of phosphates noted during summer can be attributed to decrease in water level due to high temperature thus concentrating the nutrients and raising their concentration (Ojutiku and Kolo 2011), increase in decomposition of organic matter (Harney et al 2013). This summer maxima were also observed by Chandrakiran (2011), Sharma (2015) and Anthal (2019) in their respective waterbodies. Since phosphate acts as a nutrient for the luxuriant growth of phytoplankton and macrophytes, so it's increased utilization by them could be a probable reason for fall in phosphate content of water in spring season. This is in conformity with the outcomes of Zuber (2007), Slathia and Dutta (2009), Patra et al (2010), Venkateshraju et al (2010) and Chandrakiran (2011). The value of sulphates fluctuated between 2.893 mg/ and 1.735 mg $l^{-1}$ with highest concentration recorded during monsoon owing to its addition in water from catchment area along with surface run off carrying domestic waste, sewage, animal waste and influx from agricultural field (Sharma 2018). However, a decline in its concentration was observed in winters due to low temperature that reduces the process of decomposition of organic matter and also due to conversion of sulphates into sulphides which get oxidized rapidly. The above observations are in concordance with the findings of Anthal (2019).

**Comprehensive Pollution Index (CPI):** The Pollution index (PI) values of 10 important water quality parameters of Gharana wetland reserve have been represented in Table 3. Based on these values, Comprehensive pollution index of the

**Table 3**. PI values of Gharana wetland during different seasons (2018-19)

| Parameters/ Seasons | Monsoon | Winter | Spring | Summer |
|---|---|---|---|---|
| DO | 1.36 | 1.76 | 1.8 | 1.36 |
| $FCO_2$ | 8.36 | 15.4 | 0 | 0 |
| Chlorides | 0.2716 | 0.128 | 0.0756 | 0.48 |
| Calcium | 0.19 | 0.9868 | 0.448 | 0.128 |
| Magnesium | 0.375 | 0.201 | 0.575 | 0.294 |
| Total alkalinity | 1.416 | 4.25 | 3.666 | 5 |
| BOD | 1.04 | 0.48 | 0.44 | 1.24 |
| Nitrates | 0.0128 | 0.0128 | 0.0084 | 0.01284 |
| Phosphates | 0.0327 | 0.0196 | 0.0085 | 0.0536 |
| Sulphates | 0.0192 | 0.0115 | 0.0138 | 0.0144 |

**Table 4.** Pollution status of Gharana wetland based on CPI score (2018-19)

| Seasons | CPI Score | Pollution status |
|---|---|---|
| Monsoon | 1.33 | Moderately polluted |
| Winter | 2.32 | Severely polluted |
| Spring | 0.77 | Slightly polluted |
| Summer | 0.85 | Slightly polluted |
| Mean | 1.31 | Moderately polluted |

study area was calculated so as to depict its pollution status in different seasons. The CPI of Gharana Wetland varied from 0.77 to 2.32 indicating slight to severe pollution. A look at Table 4 revealed that the water quality of the study area was slightly polluted in spring and summer season with CPI value of 0.77 and 0.85 respectively, moderately polluted during monsoon having a CPI score of 1.33 and severely polluted in winters with a CPI score of 2.32. However, during the present investigative period of one year (2018-19), the overall CPI score of 1.31 designated that the wetland was moderately contaminated.

## CONCLUSION

The present study summarizes great seasonality in various physicochemical parameters of Gharana Wetland. Based on CPI score, Gharana Wetland falls under the category of moderately polluted. The various factors responsible for this pollution are run off rich in insecticides and pesticides from adjoining agricultural fields, luxuriant macrophytic growth due to enrichment with various nutrients, drainage from nearby village, encroachment of the wetland area by the villagers and its conversion into agricultural land for cultivation of crops etc. It can therefore be inferred that if the anthropogenic pressure on the wetland continues to increase in the coming years it could lead to eutrophication with its complete shrinkage. In near future, this will also pose a threat to the resident as well as migratory birds coming from all over the world by delimiting their activities due to habitat loss and poor food and water quality. So, in conclusion, this wetland is suffering and is on the verge of extinction. Hence, adequate steps must be taken to conserve the wetland to make it an important tourist destination and promote eco-tourism.

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# Analysis of Physico-chemical Factors and Estimation of Total Chromium in Water Samples Collected in and around TCCL Industry, Ranipet, Vellore District, Tamil Nadu, India

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***Abstract***: Tamil Nadu Chromate Chemicals Limited (TCCL), Ranipet, Vellore, Tamil Nadu, India was closed in the year 1997. The unused raw materials inside the industry leaches out during rain and contaminate the ground water. The Physico-chemical analysis of the water samples in and around the industry reveals that the total chromium content ranges from 701.311 to 849.569 mg $L^{-1}$ exceeding the maximum permissible limit in three stations and trace amount of Chromium in other stations. There is an increase in various parameters like pH ranging from 6.5 to 9.54, EC ranged from 474 to 4900 µmho $cm^{-1}$, TDS ranged from 332 to 3430 mg $L^{-1}$, BOD fluctuates from 9 to 36 mg $L^{-1}$, COD varies from 128 to 576 mg $L^{-1}$, chlorides vary from 22 to 1000 mg $L^{-1}$, sulphate varies from 14 to 1285 mg $L^{-1}$, calcium ranged from 18 to 363.8 mg $L^{-1}$, magnesium varies from 11 to 192 mg $L^{-1}$ and free ammonia ranged from 3.64 to 5.68 mg $L^{-1}$. There is a decrease in fluoride and phosphate concentration. It is evidenced that the chemicals dumped inside the industry has deteriorated the quality of water in and around the Industry.

***Keywords***: TCCL, Ranipet, Physico-chemicals factors, Chromium

The discharge of huge amounts of metal-contaminated wastewater containing heavy metals like Cd, Cr, Cu, Ni, As, Pb, and Zn, are the most hazardous among the chemical industries (Adeyeye et al 2002). Because of their high solubility in the aquatic environments, heavy metals can be absorbed by living organisms. Once they enter the food chain, large concentrations of heavy metals may accumulate in the human body. If the metals are ingested beyond the permitted concentration, they can cause serious health disorders (Babel et al 2004). Water pollution due to development in technology, continues to be of great concern. With increasing generation of heavy metals from technological activities, many aquatic environments face metal concentrations that exceed water quality criteria (Sanjay Kumar et al 2011). Metal production have decreased in many countries due to strict legislation, improved purification technology and altered industrial activities, in the recent years (Mridul et al 2013). Chromium is the common toxic contaminant in wastewater from electroplating, leather tanning and metal-finishing industries (Prabhu et al 2019). The physiological effects of chromium on the biological system depend upon its oxidation state. Increase in the levels of metal ions and organic pollutants in the environment due to lack of strict enforcement by the regulatory authorities. Tamil Nadu Chromate Chemicals Limited is an industry located in the Chennai Bangalore national highway NH4 near Ranipet Industrial area, Vellore District which produced sodium di chromate for chrome tanning purpose. The industry was supplying sodium chromate to all chrome tanning industries distributed in Pernambut, Ambur, Vaniyambadi and Ranipet of Vellore District. Then the industry was closed in the year 1997, due to the raise of pollution in that area. The raw materials which were needed for the production were left unattended till now. The chemicals from that raw ore were getting leached out by rain water and seeps into the ground water and affects the quality of the ground water (Devi et al 2012), (Nirmala et al 2013). Hence the present work was carried out to estimate the extend of contamination in and around the TCCL industry.

## MATERIAL AND METHODS

Study Area is TCCL Industry which is located 3km north east of Ranipet, on the Chennai Bangalore national highways NH4. Water Samples were collected from in and around the TCCL Industry, Ranipet. The study area chosen for the research includes nine stations, three station within the factory and six stations around the factory. Empty distilled water cans were taken, washed and sterilized for the purpose of collection of the water samples. Water Samples were collected from different stations and checked for pH using an electronic pH meter at each station. Station 1, 2, 3, 7, 8 and 9 are Borewell water, Station 4 and 5 are water inside the TCCL industry and Station 6 was collected from outside the TCCL industry.

The water samples were analyzed for physico-chemical factors using APHA (1998) standard and APHA 23rd edition (2017) standard in the laboratory for physical examination and chemical examination (Edwin et al 2018). It was then compared with WHO standard, IS 3025 1983:2009, BIS 10500: 2012 at CVR labs Chennai, Tamil Nadu, India. For the analysis of Total Chromium the samples were kept in small vials and sent for Atomic Absorption. Spectrometry (Smith 1983) using Varian AAS Model 240 at Vellore Institute of Tehnology - TBI lab, Vellore, Tamil nadu. The present study was carried out to study the physico- chemical characteristics (Lokhande et al 2011).

## RESULTS AND DISCUSSION

Overall, all the parameters were found to be higher than the IS permissible limit, (Yashoda Saini et al 2010) and the pollution levels. Hence proper treatment methods are advised before the discharge of the industrial effluents into the sewage (Veeragandham et al 2012). The appearance of samples 1, 2, 3, 7, 8, and 9 were clear but the sample 4,5 and 6 were turbid in nature, this is because of contamination. The colour of samples 4, 5 and 6 which were collected from TCCL were yellow in colour due to the presence of metals but the samples from other stations were colourless because of absence of matals. Temperature affects chemical, biological reactions in water. The temperature of the samples varies from 28 to 30.5 $^{0}$C. The pH values were in the range 6.5-8. This is in accordance with the WHO permissible limit (6.0-8.5). But three samples Sample 5, Sample 6 and Sample 7 show very high (pH 8.32 to 9.54). The extreme pH of wastewater is generally not acceptable, as lower pH cause problems to survival of aquatic life. It also interferes with the optimum operation of wastewater treatment facilities (Sara Parwin et al 2001). Water with high or low pH is not suitable for irrigation and drinking purpose. At low pH most of the metals become soluble in water and therefore could be hazardous to the environment. At high pH most of the metals become insoluble and accumulate in the sludge and sediments. The toxicity of heavy metals also gets enhanced at pH 6. In present study, only three samples Sample 3, Sample 6 and Sample 9 shows EC exceeding the limits prescribed by WHO (1400µS/cm) and other samples are within limits, and hence these were unfit for irrigation and drinking purpose. Thus, it is a threat for the agriculture and quality of water in the adjoining villages and the lakes located near to TCCL. Hence suitable treatments are required before they are released to the sewage. The effluents with high TDS value may cause salinity problem if discharged to irrigation water. The total dissolved solids in various samples ranged from 332- 3430 mg $L^{-1}$. The sample 3 and Sample 9 show higher TDS values compared to WHO and hence treatments are needed. In the present study BOD and COD values were found in the range of 9-36 and 128 – 576 mg $L^{-1}$ respectively which goes higher side than the limit prescribed by WHO. Concentration of chloride varied from 22-1000 mg $L^{-1}$ and that of sulphate varied from 14-1285 mg $L^{-1}$. samples 3 show higher amounts of chlorides and Sample 3 and Sample 9 shows high sulphates compared to WHO limits. (250 mg $L^{-1}$). High concentration of chlorides and sulphates may be due to use chlorine compounds, like hydrochloric acid, hypochloric acid, chlorine gas and sulphate compounds like sulphuric acid, Sodium sulphate, Aluminum sulphate etc. which are used as raw materials in various process. Calcium is present in the range of 18 to 363.8 mg $L^{-1}$ which exceeds the limit set by WHO. The concentration of calcium in the samples may be due to use large amount calcium compounds used in various manufacturing process. Magnesium concentration varies in the range of 11 to 192 mg $L^{-1}$. Samples 3 and 9 exceeds the standard limit of WHO. Fluoride and phosphate

**Fig. 1.** Chromium Ore remains – TCCL, Vellore, Ranipet, Tamil Nadu, India

**Table 1.** Water sample collection sites

| Station | Name of the sites |
|---|---|
| Station 1 | MGR Nagar, Ranipet, Vellore, Tamil Nadu,India |
| Station 2 | VOC Nagar, Ranipet, Vellore, Tamil Nadu, India |
| Station 3 | Agravaram, Ranipet, Vellore, Tamil Nadu, India |
| Station 4 | Stagnant water inside the TCCL, Ranipet, Vellore, Tamil Nadu, India |
| Station 5 | Run off water from the TCCL, Ranipet, Vellore, Tamil Nadu, India |
| Station 6 | Stagnant water outside the TCCL, Ranipet, Vellore, Tamil Nadu, India |
| Station 7 | Petrol bunk backside, Opposite to TCCL, Ranipet, Vellore, Tamil Nadu, India |
| Station 8 | Pullainkannu Village, Ranipet, Vellore, Tamil Nadu, India |
| Station 9 | Karai Village, Ranipet, Vellore, Tamil Nadu, India |

**Table 2.** Analysis of physicochemical factors of water samples collected in and around TCCL

| Parameters | Acceptable limit | Maximum permissible limit | Sample -1 | Sample -2 | Sample -3 | Sample -4 | Sample -5 | Sample -6 | Sample -7 | Sample -8 | Sample -9 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Physical Examination | | | | | | | | | | | |
| Appearance | | | Clear | Clear | Clear | Turbid | Turbid | Turbid | clear | Clear | Clear |
| Colour | | | Color less | Color less | Color less | Yellowish | Yellowish | Yellowish | Hazen | Hazen | Hazen |
| Odour | Agreeable | Agreeable | None | None | None | Odoursome | Odoursome | Odoursome | Agreeable | Agreeable | Agreeable |
| Turbidity | 1 | 5 | 1 | 1 | 1 | 2 | 2 | 2 | 0.1 | 0.1 | 0.1 |
| Total Dissolved Solids (TDS) | 500 | 2000 | 785 | 916 | 3430 | 332 | 923 | 1233 | 759.6 | 695 | 3050 |
| Electrical conductivity | | | 1121 | 1309 | 4900 | 474 | 1319 | 1762 | 1114 | 1193 | 4700 |
| Chemical Examination | | | | | | | | | | | |
| pH | 6.5-8.5 | 6.5-8.5 | 8.21 | 8.02 | 7.97 | 7.36 | 9.23 | 9.54 | 8.32 | 7.66 | 7.86 |
| Total alkalinity | 200 | 600 | 148 | 296 | 116 | 132 | 380 | 536 | 194.4 | 306 | 226.8 |
| Total hardness | 200 | 600 | 296 | 328 | 1600 | 90 | 148 | 96 | 244.8 | 255 | 1642.2 |
| Calcium | 75 | 200 | 59 | 66 | 320 | 18 | 29 | 19 | 65.41 | 57.2 | 363.8 |
| Magnesium | 30 | 100 | 36 | 39 | 192 | 11 | 18 | 12 | 19.84 | 27.3 | 178.6 |
| Iron | 0.3 | 1.0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | BDL | BDL | 0.79 |
| Manganese | 0.1 | 0.3 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Free ammonia | 0.5 | 0.5 | 0.00 | 0.00 | 5.68 | 3.64 | 4.78 | 4.54 | 0.00 | 0.00 | 5.70 |
| Nitrite | | | 0.00 | 0.00 | 0.24 | 0.12 | 0.21 | 0.28 | 0.00 | 0.00 | 0.26 |
| Nitrate | 45 | 45 | 20 | 17 | 42 | 11 | 17 | 20 | 22 | 19 | 45 |
| Chloride | 250 | 1000 | 208 | 168 | 1000 | 22 | 48 | 54 | 185.89 | 148.8 | 949.1 |
| Fluoride | 1.0 | 1.5 | 0.8 | 0.8 | 1.0 | 1.2 | 1.2 | 1.2 | 0.7 | 0.9 | 1.0 |
| Sulphate | 200 | 400 | 77 | 131 | 465 | 14 | 31 | 31 | 105 | 54 | 1285 |
| Phosphate | | | 0.00 | 0.00 | 0.31 | 0.13 | 0.24 | 0.31 | 0.11 | 0.21 | 0.35 |
| Chromium | | | 0.001 | 0.002 | 0.005 | 701.31 | 782.04 | 849.56 | 0.003 | 0.002 | 0.009 |
| BOD | | 20 | 9 | 9 | 12 | 21 | 36 | 18 | 9 | 9 | 11 |
| COD | | 250 | 128 | 128 | 208 | 192 | 576 | 176 | 126 | 126 | 201 |

**Note:** All values are expressed in mg $L^{-1}$, Temperature in degree and EC in µS $cm^{-1}$

are less than maximum permissible limit. Nitrate concentration ranges from 11 to 45 mg $L^{-1}$ equal to the maximum permissible limit. Free ammonia ranges from 3.64 to 5.68 mg $L^{-1}$ which is high when compared to the maximum permissible limit. The presence of ammonia and nitrate could be because the organic decomposition by microorganisms. Chromium is highly toxic and the maximum concentration permissible is 158.6 mg $L^{-1}$. The total chromium content ranges from 701.311 to 849.569 mg $L^{-1}$ exceeding the maximum permissible limit in samples 4, 5 and 6 and trace amount in the other samples.

## CONCLUSION

The heavy metal chromium which is dumped in the form of ore inside the factory is silent slow toxin, leaching out and gets accumulated into the underground water aquifers. It is advised to take necessary actions to remove the ore from the abandoned industry and to control the leaching of the chemicals to the surroundings. It is a mandate to remediate the polluted water using physical, chemical and biological methods.

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# Riverbank Erosion Induced Problems and Farmers Migration: A Case Study of Dhubri and Dhemaji District of Assam

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***Abstract:*** The present study observed the extent of migration of farmers influenced by riverbank induced problems and the factors that force the farmers to migrate. Primary data were collected from a household survey of two highly eroded districts of Assam namely Dhubri and Dhemaji. Applying multi stage random sampling technique sample households were selected from three categories of village i.e. high, medium and low eroded villages. Moreover, households from four non-eroded villages, two from each districts has also been interviewed. The survey was conducted during January-March, 2017 with the help of a semi-structure schedule. Altogether 255 farmers including 210 farmers from eroded and 45 farmers from non eroded villages surveyed in the study districts. The basic statistics of the variables reveals that the socio economic characteristics of households among the different categories of villages are almost same. In some variables variation observed between the different categories of eroded villages. The logistic result reveals that problems arise due to riverbank erosion such as loss of cropland, loss of livestock assets, riverbank erosion induced education and transportation problem influence the migration decision of the farmers. This indicates that riverbank erosion act as an important factor in farmer's migration.

***Keywords*:** Environmental degradation, Riverbank erosion, Migration, Logistic model

Environmental change or environmental degradation that has led to population migration becomes a common concern in some regions of the world. The environmental events such as land degradation, soil erosion, and droughts have severe effects on farmers that force the farmers to migrate from rural agricultural area to urban industrial area for employment (Larson et al 2004). Dry land degradation is an important form of land degradation due to climate change and it is the central cause of population migration in Gujarat, India (Shah 2005). Similarly, flood and riverbank erosion becomes a common problem in some parts of India (Das et al 2013) and Bangladesh due to climate change (Uddin and Basak 2012).

The environmental events as mentioned, severely affect the economics of farmers (Bhuyan et al 2017). Therefore, farmers adopt various forms of coping mechanisms including migration in order to reduce the risks of natural disasters. These mechanisms vary according to the type of disasters. However, in all categories of disasters farmers adopt migration as risk coping strategy. Collecting the data on the migration patterns of individuals over a twenty-year period and temperature degree days to proxy agricultural risk the coping strategies of Nigerians were examined. They studied the migratory response of the farmers due to agricultural risk in Ethiopia. Moreover, in north-western Baluchistan, various type of coping strategies adopted by the farmers to mitigate the impact of drought on the agro-based practices (Ashraf and Rautry 2013). Among them, the important coping strategies were crop management, adjustment in agriculture inputs, water management, income diversification, asset depletion, expenditure adjustment and migration. Crop diversification is one of the well accepted adaptive strategies used by farmers to reduce risk (Rahman and Islam 2018). Further, in the semi-arid landscapes communal practice of sharing is an important one. Boafo et al (2016) investigated how the communal practice of sharing is being used by Ghanaian poor rural communities as a coping and adaptation strategy in semi-arid landscape of West-Africa on the basis of primary data. There is no single coping or adaptation strategy found that can sufficiently enhance the resilience of households and communities that are vulnerable to the effects of climate and ecosystem changes. Rahut (2017) argued that the poor farm household in the rural areas is highly vulnerable because of the inadequate ex-ante risk management and ex-post shock coping capability. They used five types of climate risk coping strategy: drought-resistant varieties, participation in non-farm activities, adjustment in the sowing time, planting trees and crop-livestock interaction. Migration is used as a core response to environmental pressure, both as an ex-ante measure of risk mitigation strategy to cope with the uncertain agricultural production and as an ex-post coping mechanism in the wake of environmental shocks.

Studies in Assam show that riverbank erosion becomes a severe disaster in the flood-plain areas of the Brahmaputra river and its tributaries (Baishya 2014, Ahmed 2016, Kalita 2016). As per the information of Water Resource Department of Assam, around 17 riverine districts of Assam are affected by riverbank erosion and lost large plots of land. Due to riverbank erosion, the victims' loss their homestead and crop land as well as their survival strategy in the eroded areas. The statistics of Water Resource Department reveals that in 2003 and 2004 value of property lost was Rs. 9885 and Rs. 8337 crore, respectively. In 2014, more than 12 thousand ha of land eroded and more than 78 thousand families displaced. Moreover, farmers largely affected due to riverbank erosion as they loss their sources of income. This forces farmers to migrate to another place for their survival. In this context, the paper tries to see the extent of migration of farmers influenced by riverbank induced problems and the factors that force the farmers to migrate. The paper has been structured into five broad sections. The second section discusses the methodology of the study. The third section presents the results of the study and fourth section presents discussion throws light on the extent of migration and the factors influencing migration. Finally, the fifth section concludes with a summary of findings and recommendations.

## MATERIAL AND METHODS

**Data sources**: Both secondary and primary data have been used in this study to show the effect of riverbank erosion on farmers. At first secondary information has been used to see the status of riverbank erosion. The secondary information has been collected from the Water Resource Department; revenue and Disaster Management Department, Govt. of Assam and circle offices of each selected district. The primary data collected from a field study conducted in Dhubri and Dhemaji district during January and February month of 2017.

**Study area**: Two highly erosion affected districts of Assam, namely Dhubri and Dhemaji, are covered under the study. Geographically, both the districts located in two different locations. Dhubri is located between $90^0 15'$ E to $90^0$ $20'$ E longitude and $26^0$ $15'$ N to $26^0$ $26'$ N latitude and it is situated at 30 meters above the sea level on average. Total geographical area of the district is 2176 sq km. Mighty river Brahmaputra is flowing through this district from east to west with its tributaries like Champabati, Gourang, Gadadhar, Gangadhar, Tipkai, Sankosh, Silai, Jinjiram etc. Dhemaji district on the other hand emerges from the foothills and stretches to the Brahmaputra river with Subansiri one side and the river Siang on the other. Geographically, Dhemaji is located between $94^0$ 12' 18'' E and $95^0$41' 32'' E longitudes and $27^0$ 05' 27'' N and $27^0$ 57' 16'' N latitudes, the district cover an area of 3237 Sq. Km and is a basically plain area lying at an altitude of 104 m above the Mean Sea Level. Both the districts are highly eroded as per the information of Revenue and Disaster Management Department, Assam Secretariat, Government of Assam (2014). The total area eroded due to riverbank erosion in Assam in 2014 was 12,579.13 ha, which is 0.16 percent of the total area of Assam. In Dhemaji and Dhubri district total area eroded in 2014 was 8636 ha (2.67 % of district area) and 1608 ha (0.74% of district area) respectively. This erosion displaced 9194 and 12,863 families in Dhubri and Dhemaji district respectively. The districts under study are also highly rural populated as per 2011 census. Out of 2.63 million total population 1.35 million are male and 1.29 million are female and the sex ratio is 953 per 1000. Population in both the districts belongs to different religious communities. The majority of the population in Dhemaji district is Hindu (95.47 percent), while that of in Dhubri district is Islam (79.67 percent). The average literacy rate is considerable, i.e. 65.52 percent and the density of population are 485 per sq. km (both the districts combined). The rural population in the study districts is very high, due to which agricultural workers also high in the study area (Table 1).

Since, the agricultural worker is very high and hence a large area falls under cropped area (Table 2). However, the irrigation process is not so much in the studied districts. Against this, the workers involved in the industrial sector (MSME) are also lower than the agricultural sector. Due to this backwardness, the poverty rate in the study districts is very high. Population below poverty line in Dhemaji district was 69.53 percent and in Dhubri district was 28.66 percent in 2007-08 (Assam Development Report, undated).

**Research design**: Multi stage random sampling method has been used in this study to collect primary data. First, based on district Circle Office information in 2014 about the degree of erosion, one highly eroded Development Block (DB) from each study district has been selected. Since, all the villages of the selected DB have not been equally eroded hence 2 villages of 3 types based on the degree of erosion: high, medium and low from each DB has been selected randomly. Along with these, 2 non eroded villages from each of the two districts considering similar socio-economic and demographic characteristics have also been chosen randomly. In each village 10 percent total households have been selected randomly. In this way 194 households from Sissiborgaon DB of Dhemaji district are selected for interview. Similarly for South Salmara DB (DB is under newly constructed South-Salmara district) of Dhubri district, 242 households are selected for interview. Thus, altogether 437 households are interviewed. However, out of these 437 households, 255 households have been selected for this study; because their occupation is agricultural farming. Interviews are conducted with the head of the households when available and otherwise with any other adult member of the household. Focus group discussions (FGD) are also conducted in the villages to get some village level information. A semi-structured questionnaire comprising status of river bank erosion, short term and long term migration with their reasons as well as socioeconomic and demographic characteristics of households have been used and it is translated into local languages for better understanding of the respondents. The survey was conducted with the help of a local data enumerator during the month of January and March 2017.

Chi square and t-test has been used to see the differences among high eroded and other category of eroded villages. Analysis of data has been conducted using SPSS version 16.0. For migration of farmers' logistic regression analysis method was used. To examine the cause and effect relationship between binary dependent variable and qualitative as well as quantitative independent variables, logistic regression is a useful method. The dependent variable, migration is taken as binary dependent, i.e. whether people migrate or not (1 represents at least one household member migrate and 0 represents no migration). Thus, to see the effect of both qualitative and quantitative independent variables on the binary dependent variable; the logistic regression model is used. The model is formulated as.

$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \ldots + \beta_n X_n + \mu_i \ldots\ldots\ldots\ldots$ (1)

In the above model Y=1, if the migration happens, Y= 0 otherwise; $\beta_0$ is the intercept; $\beta_1 \ldots\ldots \beta_n$ are the regression coefficients associated with the explanatory variables $X_1 \ldots\ldots\ldots\ldots X_n$. The logistic form of the model explained below.

Regression Y on Xs using OLS will lead to three problems. First, the error-term, μ, obviously not normally distributed as we generally assumes, and more importantly, estimated probabilities can lie outside the range (0, 1). Furthermore, the error variance is not constant across the levels of the Xs. However, we can assume that P follows a logistic distribution.

$P = 1/ (1 - \exp [- (\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \ldots + \beta_n X_n)])$ (2)

$P/ (1- P) = \exp [- (\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \ldots + \beta_n X_n)]$ (3)

Where, P/1–P is the odds of the outcome such as migration. It is clear from the equation that the logarithm of the odds, or simply log odds, is a linear function of the explanatory variables, Xs as:

$\text{Log } (P/1- P) = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \ldots + \beta_n X_n$ (4)

Since P is assumed to follow a logistic distribution, maximum likelihood method can be used to estimate the coefficients $\beta_1 \ldots\ldots \beta_n$. Exp (β) represents the expected change in the odds of migration versus no migration per unit in the explanatory variable, other things being equal.

On the basis of the above explanations the model with the explanatory variables can be expressed as

$\text{Migr}\{\text{Ln}(P/1\text{-}P)\} = \beta_0 + \beta_1 (\text{NAM}) + \beta_2 (\text{Caste}) + \beta_3 (\text{TPL}) + \beta_4 (\text{LVS}) + \beta_5 (\text{Irr}) + \beta_6 (\text{Crr}) + \beta_7 (\text{L\_Lvs}) + \beta_8 (\text{Ed\_P}) + \beta_9 (\text{Tr\_P}) + \beta_{10} (\text{W\_NREGA}) + \beta_{11} (\text{F\_Inc}) + \mu_i \ldots\ldots\ldots\ldots\ldots$ (5)

The explanatory variables used in the above logistic regression model are explained in Table 3.

**Table 1.** Population characteristics of the study area

| Variables | Percentage of population |
|---|---|
| SC population | 4.36 |
| ST population | 12.36 |
| Average literacy | 65.52 |
| Rural population | 91.00 |
| Cultivators (out of total main workers) | 33.01 |
| Agricultural labours (out of total main workers) | 11.37 |

**Source:** Census of India (2011)

**Table 2.** Area under agricultural activities and workers in MSME's

| Variables | Area in ha | In numbers |
|---|---|---|
| Cropped area (2012-13) | 0.29 million | |
| HYV area (2012-13) | 0.14 million | |
| Total irrigated area (2014-15) | 1970 ha | |
| Minor irrigation (2014-15) | 0.03 million | |
| Livestocks | | 1.65 million |
| MSME industries | | 140 (5.33 percent of state) |
| Industrial workers involved in MSME's | | 4.01 percent |

**Source:** Statistical Handbook of Assam (2015)

## RESULTS AND DISCUSION

**Socio-economic characters and Migration:** In all the category of surveyed villages' demographic characters almost similar. In case of asset base it has seen that in the high eroded villages' present land occupied by the surveyed household is 0.55 ha on an average; while in the low eroded villages was 0.67 ha and in the non-eroded villages 0.81 ha. The number of livestock acquired by the people in the high eroded villages is 6.51 while in the low eroded villages 10.49. The irrigation facility is only in 0.09 ha in high eroded villages, while in low eroded villages it is 0.29 ha and in non-eroded villages it is 0.29 ha. People accessed to banking facility are only 22 percent in high eroded villages but more in low eroded villages (32 percent).

**Table 3.** Description of the variables

| Variables | Sign |
|---|---|
| Dependent Variable | |
| Migration ($M_i$ ) | |
| Independent Variables | |
| Number of adult members (NAM) | + |
| Caste (1= ST, 0= others) | + |
| Total present land (TPL) | - |
| Present livestock (LVS) | - |
| Irrigation (Irr) {1= yes, 0= no} | - |
| Crop land Erroded (Crr) | + |
| Loss of livestock (L_Lvs) | + |
| Education problem (Ed_P) {1= Yes, 0= No} Dummy | + |
| Transportation problem (Tr_P) {1= Yes, 0= No} | + |
| Members worked in NREGA (W_NREGA) {1= Yes, 0= No} | - |
| Farm Income (F_Inc) {In numbers} | - |

The 0.35 ha of land eroded on an average in the high eroded villages and in low eroded was only 0.14 ha. The value of loss of livestock in high eroded villages was Rs. 8939.62. The 78 percent of the surveyed households faced education problem while only 51 percent households faced the education problem in the low eroded villages. However, 100 percent of the respondents faced the transportation problem in the high eroded villages.

The 83 percent of the households in the high eroded villages report migration both short-term and long-term. Due to which 61 percent household changed their occupation from farming to non-farming and 34 percent household shifted to other location. In low eroded villages, 51 percent household reported migration and 48 percent changed their occupation, only 25 percent household reported shifting to other location. People engaged in NREGA are only 9 percent in high eroded villages.

**Logistic regression:** To see the cause and effect relationship between the farmers' migration and the various factors influencing migration, binary logistic regression analysis is used (Table 5). The variables Irrigation facility (Irr), cropped land eroded (C_err) and education problem are highly significant. The loss of livestock (L_Liv) and transportation (T_Prblm) were also significant. Percentage of correct prediction in the model was 84.2. The P-value also indicates that the model is correctly constructed.

## CONCLUSION

The riverbank erosion creates various types of problems such as loss of cropland and livestock, education problem, breakdown of road connectivity, loss of livelihood etc. and

**Table 4.** Basic statistics of the variables observed

| Category | | Demographic variables | | | | | | | Asset base and income | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sex (1=male, 0=female) | Age of the respondent in years) | Education (years of schooling) | Religion (1=Hindu, 0=Others) | Community (1=ST, 0=Others) | Family size | Adult members | Present land ( ha) | Livestock (numbers) | Income (Rs.) |
| Eroded | High | 0.99 | 44.09 | 5.12 | 0.46 | 0.30 | 5.32 | 3.21 | 0.55 | 6.51 | 39450.00 |
| | Medium | 0.95 | 48.70 | 5.29 | 0.77 | 0.71 | 5.89 | 3.80 | 0.60 | 6.87 | 40361.10 |
| | Low | 0.99 | 45.96 | 6.00 | 0.60 | 0.45 | 5.76 | 3.46 | 0.67 | 10.49 | 45823.60 |
| | All types | 0.98 | 46.25 | 5.45 | 0.61 | 0.47 | 5.65 | 3.47 | 0.59 | 8.22 | 41845.76 |
| Non-eroded | No erosion | 0.93 | 44.09 | 5.80 | 0.56 | 2.58 | 5.80 | 3.51 | 0.81 | 9.22 | 47854.44 |

| Category | | Irrigation and banking (Mean) | | | Problems faced | | | | Migration and others (in percentages) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Irrigation (in ha) | Bank Account (1=yes, 0=no) | Sources of Loan (1=relatives, 0=others) | Total Area Eroded (in mean ha) | Loss of Livestock (in Rs.) Mean values | Education Problem (1=yes, 0=no) In % | Transportation Problem (1=yes, 0=no) In % | All Migration (1= yes, 0= no) | Occupation change (1= yes, 0= no) | Worked in NREGA (1= yes, 0= no) | Survival Strategy (1= shifting, o= others) |
| Eroded | High | 0.09 | 0.22 | 0.42 | 0.35 | 8939.62 | 78.00 | 100.00 | 83.00 | 61.00 | 9.00 | 34.00 |
| | Medium | 0.13 | 0.25 | 0.34 | 0.24 | 7951.11 | 72.00 | 81.00 | 73.00 | 58.00 | 22.00 | 32.00 |
| | Low | 0.29 | 0.32 | 0.10 | 0.14 | 5343.28 | 51.00 | 75.00 | 51.00 | 48.00 | 33.00 | 25.00 |
| | All types | 0.16 | 0.26 | 0.45 | 0.23 | 7209.70 | 65.00 | 84.00 | 69.00 | 59.00 | 25.00 | 30.00 |
| Non-eroded | No erosion | 0.29 | 0.35 | 0.56 | 0 | 0 | 27.00 | 22.00 | 27.00 | 0.00 | 33.00 | 0.00 |

**Note:** Values for some variables expressed in mean and some variables (qualitative) are expressed in percentages.
**Source:** Calculated and compiled by Authors from field survey data

**Table 5.** Logistic regression results

| Variables | β | Wald | Exp (β) |
|---|---|---|---|
| Constant | 4.886 | 9.040 | 132.48 |
| NAM | 0.240 | 1.427 | 1.271 |
| TPL | -0.297 | 0.343 | 1.345 |
| Livs | -0.028 | 0.388 | 0.972 |
| Irr(1) | -4.083*** | 11.748 | 0.017 |
| F_Inc | 0.000 | 0.099 | 1.000 |
| C_err | 1.416** | 0.828 | 0.243 |
| L_Liv | 0.000* | 2.860 | 1.000 |
| Ed_Prbl(1) | 2.583*** | 22.726 | 0.076 |
| T_Prblm(1) | 1.380* | 3.139 | 1.252 |
| W_NREGA(1) | -0.192 | 0.105 | 0.825 |
| Caste(1) | -0.470 | 0.889 | 0.625 |
| Model chi square (df) | 90.106 (11) | | |
| Per cent of Correct prediction | | 84.20 | |
| -2 log likelihood | | 128.34 | |
| Psedou $R^2$ | | 0.57 | |
| P-value | | 0.99 | |

**Source:** Calculated and compiled by authors.
N.B. Number of adult members (NAM), Total Present land (TPL), Present livestock assets, Irrigation practices (Irr), Farmers' Income (F_Inc), Cropland eroded (C_err), Loss of livestock (L_lvs), Education problem (Ed_problem), Transportation problem (T_pr), Members worked in NREGA (W_NREGA), Caste and community (Caste),

these all related to the degree of erosion. All the problems simultaneously create vulnerability to poverty and insecure life for the erosion victims due to which most of the affected people mainly the famers migrated outside to earn money income as well as for better livelihood. Therefore, it can be say that riverbank erosion; act as an important factor in farmer's migration. It is therefore want to recommend that erosion control through construction of guard walls /embankments and expansion of job opportunities through the schemes like NREGA and provide financial grants for the losses occurred due to erosion are important.

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# Dendrochronological Study on *Cedrus deodara* in Kumrat Valley, Pakistan: The Relationship of Tree Age and Tree Growth

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***Abstract*:** Dendrochronology, or tree-ring analysis, is the science used to calculate the exact age of a tree species by counting the rings in a tree. This study aimed to estimate the age of *Cedrus deodara* stands and to evaluate the total growth and mean annual increment of *Cedrus deodara* forest in Kumrat valley, Hindukush range of Khyber Pakhtunkhwa, Pakistan using protocol of tree ring analysis. For the study of tree-ring analysis, 70 trees of *Cedrus deodara* were randomly selected as a sample. Increment cores were collected from each sample tree at diameter at breast point by using Swish Pressler borer and studied in the laboratory to determine the exact age, total growth, and mean annual increment of *Cedrus deodara*. Regression models were used to see the relationship between tree age and total growth and mean annual increment. It was found that the total minimum growth was 2.75 cm at 10 years and the maximum growth was 32.50 cm at the age of 60 years. There is a strong positive relationship between tree age and total growth indicated by the value of $R^2$ is 0.6810. On the other hand, the maximum mean annual increment was 0.075 cm at 10 years and the minimum mean annual increment was 0.012 cm at 60 years. There is a strong negative relationship between tree age and mean annual increment indicated by the value of $R^2$ is 0.9928. The results of this study suggest that *Cedrus deodara* is slow growing tree species, implying a long cutting rotation is required to achieve sustainable yield production.

***Keywords*:** Dendrochronology, Tree age, Total increment, Mean annual increment

The word 'Dendrochronology' is derived from three Greeks words 'dendro' means tree, 'cronos' means time and 'logos' means studies or discussion. Dendrochronology, often referred as tree-ring analysis, is the science that uses tree rings to calculate the exact age of a tree species and it relates to the counting of rings . Trees develop a ring annually which varies in thickness and in density due to a number of reasons and is highly affected by ecological factors, i.e. precipitation, temperature, latitude, altitude and even by the species type. Along with the increase of tree age, the rings are accumulated which determine the age of the tree and other valuable parameters such as stored information on past conditions. Dendrochronology is most commonly used in both old and modern sciences. It is also considered as a tool for archaeology, climatology, as an art of history, forest, and biology. Recently it had a broad range of applications such as global climate change, the carbon cycle, canopy process declines and many others. Study of tree-ring analysis is an important and effective tool which is widely applied in ecological studies for determining exact ages of tree species. Tree ring data has been widely used in forest inventory programs and the knowledge of stand age is a core component for forest management. Stand age is an important variable that is mostly measured from the counting of tree rings The knowledge of tree rings analysis can be effectively used for the determination of past, current and future growth that can also be used for ecological and forest dynamic information. The age of a tree species is an important factor for yield regulation and as well as increment determination. The age can be used in developing growth models and yield table development. Therefore, current works on tree ring studies are designed to bridge the gap between stand age, yield regulation and cutting rotation to achieve sustainable forest management. In all conifer tree species, tree rings are visible and wide, but in broad-leaved tree species, tree rings are close, dense and not clearly visible due to its compactness. The absence of clearly identifiable annual growth rings in broad-leaved tree species made it difficult for determining cutting rotation in comparison to clear and visible growth rings of conifers tree species.

Tree ring research in Pakistan is at the initial stage of development and different research has been carried out on the tree age, rate of growth and to correlate tree rings with environmental variables in the moist and dry temperate region mainly on coniferous species. Study on tree ring analysis and its scopes in Pakistan was started in 1987 and discussed about the growth rate and age of few angiosperm tree species. More recent studies on tree ring analysis on *Abies pindrow* and *Cedrus deodara* from the moist temperate forest, and *Pinus gerardiana* and *Picea smithiana* from dry temperate forest areas of the Himalayan range of Pakistan were successfully conducted . Also, dendrochronological techniques was used to evaluate the dynamics of *Cedrus deodara* from Hindukush and Himalayan Ranges, covering its distributional limit. While these works represent efforts to enhance research on dendrochronology in Pakistan, but very little work regarding the use of tree ring analysis for sustainable forest management was documented. *Cedrus deodara* or Himalayan *Cedrus deodaraa* is a conifer tree species from the family of Pinaceae that has medicinal and economic value. The wood quality of *Cedrus deodara* is good, excellent and durable which is used for construction and furniture purposes. Its wood is useful to treat urinary disorder, rheumatism, and respiratory disorder. The oil extracted from its root is useful for ulcer and skin disease in camel and goat. The bark can serve as astringent and is useful to cure fever, diarrhea, and dysentery. The oil is diaphoretic which is useful to treat skin diseases and ulcer. In Pakistan, *Cedrus deodara* forest is distributed in Dir, Chitral, Gilgit, and Swat. There was no proper scientific study has been conducted on dendrochronological of *Cedrus deodara* forest stand in Pakistan. This study aimed to estimate the exact age of natural forest of *Cedrus deodara* and to evaluate the total growth and mean annual increment of *Cedrus deodara* forest in Kumrat valley, Hindukush range of Khyber Pakhtunkhwa, Pakistan using protocol of tree ring analysis.

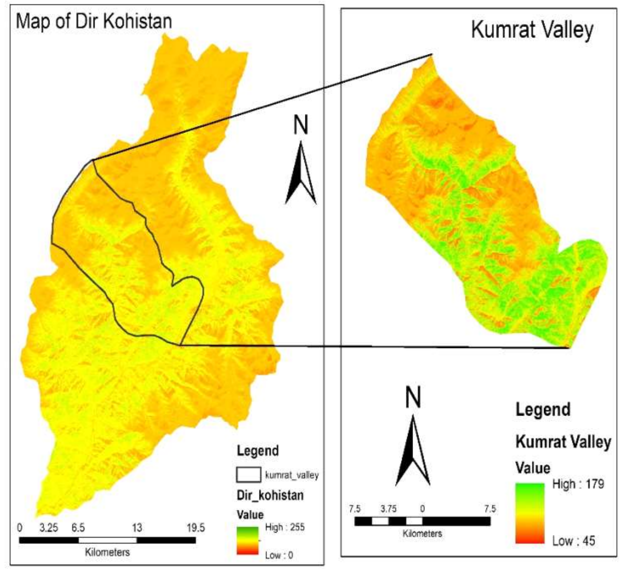



**Fig. 1.** The map of the study site (Kumrat Valley)

## MATERIAL AND METHODS

**Study site:** The present study was carried out in out in Kumrat valley, Hindukush range of Khyber Pakhtunkhwa, Pakistan. The valley is located at 35.15° N latitude and 71.22° E longitude at an elevation of 1371 meters above the sea level. The soil of the study site is shallow and acidic in nature and the major rocks of the valley include diorite, schist, and granite (Atta Ullah et.al 2013). The mean annual precipitation ranges from 750 mm to 1000 mm. The annual temperature ranges from 0.7 to 30° C. The value of soil PH is in the range of 6.5 to 7.5 while the total organic matter in the soil was 1.775% present while the total nitrogen in the soil was 1.2%.

**Sample cores collection and measurements:** In the present study trees of *Cedrus deodara* were randomly selected for collection of sample cores. There were 72 sample cores were collected using Swedish Pressler borer. The diameter of each sample tree was measured at two points at diameter at breast height (DBH) for the calculation of cylindrical volume and diameter at mid-point (DMP) for the calculation of actual volume. For the measurement of diameter at mid-point, we climbed to the tree and recorded the diameter using Caliper and diameter tape. Total tree height was measured using three different tools, depending on tree condition: by climbing and using staff-rod when the tree height was low or using Annie's level if the tree was on a steep slope at it had high height. Crown cover was measured of each sample tree from four different sides using measuring tape. Sample cores collected were then labelled with information about tree diameter, height and crown cover were written on the label. The cores were brought to the laboratory for further analysis. The analysis included calculating the exact age of each sample by counting the annual rings. Tree total growth was measured from the length of each collected sample core, while the mean annual increment (MAI) was measured from the relation of tree total increment (cm) divided by tree age (years).

**Statistical data analysis:** For data analysis, the following software were used: Microsoft Excel, PAST, and Sigma Plot. The data were arranged in Excel sheets. The univariate statistics were carried out in PAST software. The figures and graphs were developed in Sigma Plot to find out the impact of tree age on total growth and mean annual increment. Regression models were also used in Sigma Plot. The statistics univariates were used to find out the Mean, SD (Standard deviation), CV (Coefficient variance) of all sample trees based on sample cores with their respectively ages for different parameters: i.e., total growth (total increment) and mean annual increment (MAI).

## RESULTS AND DISCUSSION

**Tree age and total growth relationship:** The average total growth was 18.89 cm at age of 38.87 years with the maximum total growth was 32.50 cm recorded at the age of 60 years and the minimum was 2.75 cm recorded at the age of 10 years as shown in Table 1. To investigate the relationship between tree age and total growth, we used the regression polynomial cubic equation as shown in Figure 2. It shows that the value of $R^2$ is 0.6810, indicating a strong positive relationship. The result of the present study regarding the total growth suggests that this parameter can be compared with age. When the age of the tree increases so the annual growth and total growth will be also increased with standard deviation and the coefficient of variance is given in Table 2. The total growth increases with age of trees from age of 10 years to 60 years.

**Tree age and mean annual increment (MAI) relationship:** The average mean annual increment (MAI) was 0.024 cm achieved at age of 38.87 years with the minimum mean annual increment was 0.012 cm at the age of 60 years and the maximum mean annual increment (MAI) was 0.075 cm recorded at the age of 10 years as shown in Table 3. Using regression analysis, there is very strong negative relationship between tree age and mean annual increment with the value of $R^2$ is 0.9928 as shown in Figure 3. This is supported with the results of statistical analysis in Table 4 (i.e., a coefficient of variance and the standard deviation), suggesting that the mean annual increment decrease as the age of tree increase. It means that at the early stage of tree growth mean annual increment is more than the later stage of tree growth.

The results of this study suggest that *Cedrus deodara* is slow growing tree species, implying a long cutting rotation is required to achieve sustainable yield production. This study determined the age of the tree and found age is an important factor that affect total growth and mean annual diameter increment. Age is an independent variable and with increasing age, the total growth is increasing, but the mean annual increment is decreasing. As such, planting conifers, such as *Cedrus deodara*, can be used in afforestation and

**Table 1.** The relation between tree age (years) and total growth (cm)

| Age (Year) | Total growth (cm) | Age (Year) | Total growth (cm) | Age (Year) | Total growth (cm) |
|---|---|---|---|---|---|
| 10 | 2.75 | 35 | 15 | 45 | 26.25 |
| 11 | 3.5 | 35 | 22 | 45 | 28.75 |
| 12 | 22.5 | 36 | 15 | 45 | 22.5 |
| 13 | 18.12 | 36 | 17 | 46 | 23.25 |
| 15 | 6.25 | 36 | 11.25 | 46 | 28.37 |
| 15 | 4.62 | 37 | 22.12 | 47 | 21.25 |
| 15 | 6.25 | 37 | 18.75 | 47 | 22.5 |
| 15 | 7.5 | 37 | 17.87 | 47 | 18.75 |
| 16 | 8.75 | 37 | 20.62 | 47 | 21.25 |
| 19 | 11.37 | 37 | 19.01 | 49 | 26.25 |
| 20 | 11.25 | 37 | 18.37 | 49 | 26.25 |
| 20 | 9.375 | 38 | 30 | 50 | 25.87 |
| 20 | 8.75 | 39 | 19 | 50 | 26.25 |
| 25 | 5.75 | 39 | 17.5 | 50 | 24.37 |
| 28 | 12.12 | 40 | 25 | 51 | 22.5 |
| 28 | 15 | 40 | 21.5 | 52 | 30 |
| 29 | 12.5 | 42 | 20.5 | 53 | 26.25 |
| 30 | 15 | 43 | 16.62 | 53 | 32.5 |
| 30 | 16.62 | 43 | 21.87 | 53 | 18.75 |
| 32 | 15.62 | 43 | 25.12 | 54 | 27.5 |
| 32 | 10.25 | 43 | 23.75 | 55 | 27.5 |
| 33 | 14 | 43 | 21.25 | 55 | 22.5 |
| 33 | 15.75 | 44 | 27.5 | 60 | 32.50 |
| 34 | 28.75 | | | | |

reforestation to control deforestation in northern Pakistan because it takes a longer time for its growth. Planting conifers trees on degraded land and on steep slopes will also control soil erosion. Increasing the forest areas with proper management will give more economical, social and biological benefits to a country in term of providing habitat for wildlife, producing timber, fuelwood and other non-timber forest products, protecting watershed and good quality of water, and providing environmental services. According to previous research and its results shows that aboveground ecosystem C increased considerably during the first two decades after establishment and between the age of 30 and 65. Belowground ecosystem C remained stable during the early decades and then started to increase with stand age due to an increase in total tree root biomass. Thus, the phase of stand maturing between the age of 30 and 60 may be considered most important for C sequestration due to an increase in both the above- and belowground ecosystem carbon stock. While this research shows the tree age impact on biomass and carbon accumulation in the stand which define that when the tree age was increase with increase in biomass and carbon stocks of the trees, current value resembles with the previous results in terms of volume, basal area, biomass, and carbon stocks. In another research results have several implications for efforts to apply age-dependent relationships to understand forest carbon cycling and storage and carbon pools and fluxes were related to both stand age, implying that measures of forest age can be useful

**Table 2.** Statistical analysis of the relationship between tree age and total and annual increment

| Variable | Unit | N | Minimum | Maximum | Mean | Sum | SD | CV (%) |
|---|---|---|---|---|---|---|---|---|
| Age | Years | 70 | 10 | 60 | 38.87 | 2532 | 12.76 | 34.78 |
| Total increment | Cm | 70 | 2.75 | 32.50 | 18.89 | 1303.35 | 7.38 | 39.08 |

**Source:** Author

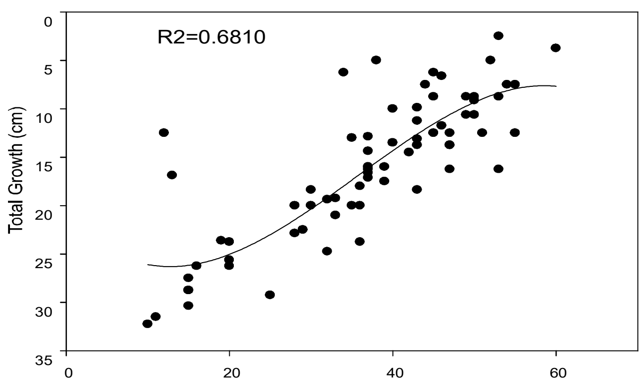
**Table 3.** Tree age (Years) and mean annual increment

| Age (Years) | MAI (cm) | Age (Years) | MAI (cm) | Age (Years) | MAI (cm) |
|---|---|---|---|---|---|
| 10 | 0.075 | 35 | 0.022 | 45 | 0.017 |
| 11 | 0.069 | 35 | 0.022 | 45 | 0.017 |
| 12 | 0.063 | 36 | 0.021 | 45 | 0.017 |
| 13 | 0.058 | 36 | 0.021 | 46 | 0.016 |
| 15 | 0.050 | 36 | 0.021 | 46 | 0.016 |
| 15 | 0.050 | 37 | 0.020 | 47 | 0.016 |
| 15 | 0.050 | 37 | 0.020 | 47 | 0.016 |
| 15 | 0.050 | 37 | 0.020 | 47 | 0.016 |
| 16 | 0.047 | 37 | 0.020 | 47 | 0.016 |
| 19 | 0.040 | 37 | 0.020 | 49 | 0.015 |
| 20 | 0.038 | 37 | 0.020 | 49 | 0.015 |
| 20 | 0.038 | 38 | 0.020 | 50 | 0.015 |
| 20 | 0.038 | 39 | 0.019 | 50 | 0.015 |
| 25 | 0.030 | 39 | 0.019 | 50 | 0.015 |
| 28 | 0.027 | 40 | 0.019 | 51 | 0.015 |
| 28 | 0.027 | 40 | 0.019 | 52 | 0.015 |
| 29 | 0.026 | 42 | 0.018 | 53 | 0.014 |
| 30 | 0.025 | 43 | 0.018 | 53 | 0.014 |
| 30 | 0.025 | 43 | 0.018 | 53 | 0.014 |
| 32 | 0.024 | 43 | 0.018 | 54 | 0.014 |
| 32 | 0.024 | 43 | 0.018 | 55 | 0.014 |
| 33 | 0.023 | 43 | 0.018 | 55 | 0.014 |
| 33 | 0.023 | 44 | 0.017 | 60 | 0.013 |
| 34 | 0.022 | | | | |

**Table 4.** The determined statistical value of age with mean annual increment

| Variable | Unit | N | Minimum | Maximum | Mean | Sum | SD | CV (%) |
|---|---|---|---|---|---|---|---|---|
| Age | Years | 70 | 10 | 60 | 38.87 | 2581 | 12.75 | 34.59 |
| Mean annual increment | cm | 70 | 0.075 | 0.013 | 0.024 | 1.739 | 0.014 | 56.89 |

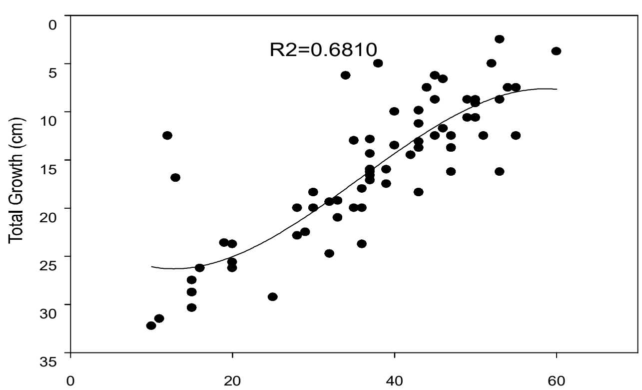
**Source:** Author




f=y0+a*x+b*x^2+c*x^3 R2=0.6810,y0=13.8309,a=-0.8687,b=0.0415, c=-0.0004, Age(years)Equation: Polynomial, Cubic

**Source:** Author

**Fig. 2.** The relation between tree age and total growth




f=y0+a*x+b*x^2+c*x^3 R2=0.6810,y0=13.8309,a=-0.8687,b=0.0415,c=-0.0004 Age(years)Equation: Polynomial, Cubic

**Source:** Author

**Fig. 3.** Relation of tree age with mean annual growth

for understanding and assessing carbon stocks and fluxes in these forests. Despite several studies that illustrate age-related patterns in forest carbon storage and cycling. Although this research resemble in terms of age of stand or history have impacts on biomass and carbon stocks accumulation.

## CONCLUSIONS

The result of the recent conducted study revealed that from all conifers trees *Cedrus deodara* is the national tree of Pakistan. It is found in northern areas. *Cedrus deodara* is light demander species and growing with the slow rate and the rotation age of deodar is long. The current research study determined the age of the tree and age is an important factor that impacts on total growth, mean annual diameter growth, total, and mean annual increment and basel area is positive. Age is an independent variable; age increases total growth and mean annual increment and basel area rate will be increased to the age. By forestation and reforestation of conifers trees on degraded land and on steep slopes areas to controls soil erosion. Increasing the forest areas with proper management that will give more economical, social, and biological benefits to a country like a forest that will provide habitat for wildlife, provide timber, fuelwood, watershed areas provide good quality of water, provides environmental services and other NTFS.

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# Macro- proliferation Cup Technique for Mass Multiplication of *Woodfordia fruticosa* (L.) Kurz (*Dhawai*) in Sub-humid Foothills of Eastern Himalaya

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***Abstract***: Vegetative propagation in *Woodfordia fruticosa* through shoot tip cuttings having 1-6 nodes with and without cutting aid (commercial growth hormone) was attempted in different seasons to analyze the effect of cutting aid, number of nodes and seasons on survival, rooting and growth of cuttings. Cuttings covered with transparent plastic cups after planting for a fortnight rooted profusely, while cuttings left uncovered dried immediately. Survival and growth performance of 2-3 node cuttings was significantly better than other cuttings. Entire cutting aid treated three node cuttings and entire untreated two node cuttings survived, while survival of others was 20-80 % lesser. Entire 2-3 node cuttings planted during monsoon survived, while survival of cuttings planted in other seasons was 7-60 % lesser. The survival of the cuttings was found significantly and positively correlated with relative humidity, while no relationship of cutting survival was found with temperature and rainfall. Healthy and uniform *Dhawai* planting materials in Terai region of West Bengal can be produced by following protocols like preparing fresh two-leaved shoot tip cuttings with two or three nodes during monsoon and post monsoon seasons, ridge planting the cuttings in rows and covered with transparent plastic cups (10 cm × 6 cm) for a fortnight and out-planting of four months healthy seedlings with root ball.

***Keywords:*** *Dhawai,* Medicinal Plant, Macro-proliferation, Cup technique, Multiplication

*Woodfordia fruticosa* (L.) Kurz, commonly known as '*Dhawai*' or Fire Flamed Bush (Family: Lythraceae) is an important medicinal gregarious shrub widely present in India and South East Asian countries. All parts of this plant possess valuable medicinal properties and hence potential for commercial cultivation. The flowers are more prized in domestic and international markets for preparation of herbal medicines (Oudhia 2003). Compounds like tannins, flavonoid, glycosides, sterols and polyphenols have been isolated from this species principally the flower and leaves possess pharmacological importance (Khushalani et al 2006). Both leaves and flowers are used as astringent, antibacterial, acrid, refrigerant, stimulant, styptic, uterine sedative, constipating, anthelminthic, emetic and stimulant. This plant is one such medicinally much used dye and gum (similar to gum tragacanth) yielding plant species. The red dye extracted from the flowers is used extensively throughout India, in perfume, leather and textile industry and believed to be superior for woolen and silk fabrics (Grover and Patni 2011). The market for medicinal plants had been rapidly growing in India with high growth potential. There is a huge scope for export of raw materials. Nevertheless, quality, traceability, and organic certification of materials are vital concern. As there is a huge demand of this species for its pharmaceutical value chances of resource depletion is likely to occur.

The species springs up naturally on landslips, abandoned cultivation, and other open places, killing out grass, binding the soil together and acting as a useful soil-improver and a most efficient nurse to tree species, including *Shorea robusta*. It is not eaten by cattle, and is frost-hardy, and thus appears often in extensive pure masses on open ground subject to grazing. It flowers profusely during the summer months and seeds are produced abundantly. However, seeds have very low germination along with poor survival of seedlings under normal conditions (Joshi et al 1992, Shankar and Rawat 2013, Mathew et al 2018). It is also unable to regenerate by seed in shady places so, as the trees grow larger this species gradually dies out. Hence to overcome this aspect propagation and conservation methods has to be followed. *In vitro* techniques have already been developed for this species as a tool for germplasm conservation (Bulle et al 2012, Meena and Satish 2015, Nautiyal et al 2017). Propagation through stem cuttings during June and October advised for better results (Joshi et al 1992, Shankar and Rawat 2013). Hence, the vegetative propagation through apical shoot cuttings has scientific advantage over sexual propagation for mass scale nursery production of quality planting materials of *Dhawai*.

The increased genetic gains through mass propagation have been obtained in several medicinal plants (Swamy et al 2002). However, root initiation in cuttings is affected by various factors like plant growth regulators, season, size of the cuttings (Hartmann et al 2002). For ayurvedic medicines, raw materials such as herbs and shrubs can be grown and harvested in a period of one year, while in perennial *Dhawai* shrub it takes few years to get ready for harvesting in conventional methods. The mature *Dhawai* plant can be utilized for vegetative multiplication either collected from wild or maintained mother plant. The length of cuttings used for vegetative propagation is commonly determined by the availability of mother plants. Earlier studies indicated that cuttings with a minimum of a single node, cuttings with two, three and more numbers can be used for propagation (Girma et al 2012). Furthermore, the use of short cuttings with 2-4 nodes is becoming prevalent (Adugna et al 2015, Namirembe-Ssonkko et al 2005, Umesha et al 2011). Vegetative propagation using leafy stem cuttings has been successful in various medicinal plants. Position on the stem also has a significant effect on the development and number of root primordia on stem cuttings (Adugna et al 2015). Application of plant growth regulators has proved useful in determining the rooting success of many species (Ky-Dembele et al 2011). Henceforth, the objective of this research is to determine influence of different growth hormones, number of nodes and season on nursery performance of *Dhawai.* Thus, it is important to develop simple, swift and farmer friendly multiplication technique of this important medicinal plant to popularize its cultivation integrating its manifold applications into agroforestry systems for the overall benefit of the rural communities, while conserving the species both *ex situ* and *in situ*.

## MATERIAL AND METHODS

A greenhouse experiment was setup during July 2018 to July 2019 under natural environmental conditions at the field of the Department of Forestry, Uttar Banga Krishi Viswavidyalaya (UBKV) campus, Pundibari, West Bengal, India, situated at an altitude of 43 m above mean sea level, intersected by 26°23'45.8” N latitude and 89°23'16.7” E Longitude. The average minimum and maximum temperature vary from 18.22°C during winter (January) to 33.19°C during summer (August). Average annual rainfall was 2000-3500 mm, most of which was recorded from April to September but very less during winter with relative humidity of 55-90 %. Overall, the area is warm and humid. The soil of the experimental plot was moist and well drained, rich in organic matter having sandy loam texture with pH ranging from strongly acidic to moderate.

The fresh cuttings *i.e.*, two-leaved shoot tip along with varying (1-6) nodes were collected (Plate 1 a) from the four-year-old *Dhawai* clonal seed orchard established in the field of the Department of Forestry, UBKV, Pundibari, West Bengal, India. The first trial was setup from July to November 2018 with 12 different treatment combinations (auxin enriched powder treated and untreated in equal proportion), five cuttings each (replicated thrice). The commercially available auxin enriched powder (cutting aid) was supplemented with indole-3 butyric acid (IBA), naphthalene acetic acid (NAA), polyhydroxy butyrate (PHB), boric acid ($H_3BO_3$), Vitamin, Surfactant, Talc power. The cut end of the cutting was quick dipped in the auxin enriched powder whereas, untreated cuttings considered as control. After treating with cutting aid these cuttings were row planted on ridges directly in nursery soil and covered with transparent plastic cups (10 cm × 6 cm) for a fortnight (then permanently removed) to control the initial micro-moisture conditions (Plate 1 b). Observations such as survival (%), shoot and root length (cm), collar diameter (mm), number of leaves, branches, nodes and roots were recorded at fortnightly interval for 120 days (Mathew et al 2018). A cutting was considered to be rooted if it had at least one primary root of about 1 mm long. For measurements, five randomly selected cuttings from each treatment were uprooted carefully to avoid damage to the roots. Experiments were laid out in randomized block design.

In the second trial randomly selected healthy and best performed four-months-old macro-proliferated seedlings (i.e., with three nodes for hormone treated cuttings and two nodes for untreated cuttings) produced in the first trial were compared with four-month-old seedlings developed from seeds collected from the clonal seed orchard. The seedlings with root ball were transferred to polybag containing soil and FYM (3:1 ratio) media and observations were recorded after four months of transplanting. A total of 30 healthy well-established eight months old seedlings were compared with three replications in each treatment. The growth and economical yield (flower) parameters were recorded. Shoot length (cm), collar diameter (mm), number of leaves, number of branches, number of nodes, flowers and roots were also recorded. This macro-proliferation cup technique (first trial) was repeated as per the Indian Meteorological Department (IMD) designated four climatological official seasons of India to standardize the ideal season for mass multiplication in the third trial. In this trial only the best performed 2-3 node cuttings (15 cuttings in each treatment) of the first trial were selected. Success rate based on survival per cent of the respective seasons were recorded at four months after planting.

**Statistics analysis:** Randomized block design (RBD) with three replications was followed in the first and third trials, while completely randomized design (CRD) with three replications was employed for the second trial. Data collected was analyzed using Analysis of Variance (ANOVA) and treatment means were compared using Least Square Difference (LSD) test at 5% level of significance. Duncan's test was performed to compare the significant difference between the mean values. The correlation among meteorological parameters with survival per cent were analyzed using spearman's correlation significant at the 0.01 level (2-tailed) using SPSS for windows version 17.0 software (2010).

## RESULTS AND DISCUSSION

**Effect of growth hormone and number of nodes on cuttings:** The number of nodes had significantly influenced the survival of cuttings ($F(5, 78) = 2.89$; $P = 0.019$), while non-significant differences were found with growth hormone on survival of the cuttings. Entire three node cuttings treated with growth hormone survived, while survival of others (i.e., cuttings with one, two, four, five and six nodes) were significantly lesser than the three node cuttings (i.e., 40-80 % lesser). However, cuttings not treated with growth hormones survival was cent per cent with two nodes, while survival of others (i.e., cuttings with one, three, four, five and six nodes) were significantly lesser than the two node cuttings (i.e., 20-80 % lesser; Plate 1 c-f). Results showed an increasing trend in mean values of cuttings survival and growth parameters as the number of nodes per cutting increased from 1 to 3. However, this trend was inverted in the cuttings with more than three numbers of nodes. There was a significant difference in the interaction effect of growth hormone and number of nodes on observed variables (Table 1, 2), except the number of branches (Table 3). Number of nodes in the cuttings irrespective of hormonal treatment or no treatment significantly influenced the shoot length (cm), collar diameter (mm), number of roots, root length (cm) and number of leaves per cuttings, however these growth parameters of hormone treated cuttings irrespective of number of nodes were statistically similar with the growth parameters of untreated cuttings, while the interaction effect was generally significant on the growth parameters of the cuttings (Table 1-3). The aerial growth parameters of the cuttings (irrespective of number of nodes) increased with hormonal treatment but these increments were non-significant compared to the untreated cuttings (Table 1, 2). The root growth parameters (number and length of roots per cuttings) also increased with hormonal treatment but the increments were only significant

**Table 1.** Main effect of number of nodes and growth hormone on shoot length (cm) and collar diameter (mm) of *Dhawai* at different days after planting (DAP)

| Number of nodes | Shoot length (cm) at different DAP | | | | Collar diameter (mm) at different DAP | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 Days | 30 Days | 60 Days | 120 Days | 0 Days | 30 Days | 60 Days | 120 Days |
| One | 3.37 $^{e}$ | 3.67 $^{f}$ | 6.11 $^{d}$ | 23.16 $^{a}$ | 1.06 $^{ab}$ | 1.07 $^{a}$ | 1.15 $^{ab}$ | 1.77 $^{a}$ |
| Two | 5.31 $^{d}$ | 5.80 $^{e}$ | 9.48 $^{c}$ | 24.28 $^{a}$ | 0.98 $^{b}$ | 1.12 $^{a}$ | 1.30 $^{ab}$ | 1.66 $^{a}$ |
| Three | 8.93 $^{c}$ | 9.04 $^{d}$ | 13.70 $^{b}$ | 26.66 $^{a}$ | 1.05 $^{ab}$ | 1.10 $^{a}$ | 1.20 $^{ab}$ | 1.53 $^{a}$ |
| Four | 10.46 $^{b}$ | 10.46 $^{c}$ | 13.98 $^{b}$ | 21.72 $^{a}$ | 1.04 $^{ab}$ | 1.02 $^{a}$ | 1.12 $^{b}$ | 1.36 $^{a}$ |
| Five | 13.34 $^{a}$ | 12.60 $^{b}$ | 19.64 $^{a}$ | 27.62 $^{a}$ | 1.18 $^{ab}$ | 1.23 $^{ab}$ | 1.29 $^{ab}$ | 1.63 $^{a}$ |
| Six | 14.01 $^{a}$ | 16.53 $^{a}$ | 19.90 $^{a}$ | 23.37 $^{a}$ | 1.31 $^{a}$ | 1.38 $^{a}$ | 1.38 $^{a}$ | 1.47 $^{a}$ |
| Mean | 9.24 | 9.69 | 13.80 | 24.47 | 1.10 | 1.15 | 1.24 | 1.57 |
| SEd | 0.20 | 0.18 | 0.29 | 1.00 | 0.09 | 0.07 | 0.08 | 0.17 |
| Growth hormone | | | | | | | | |
| Treated | 9.40 | 9.41 | 13.04 | 25.26 | 1.09 | 1.12 | 1.23 | 1.60 |
| Untreated | 9.01 | 10.00 | 14.56 | 23.68 | 1.12 | 1.18 | 1.25 | 1.54 |
| Mean | 9.24 | 9.69 | 13.80 | 24.47 | 1.10 | 1.15 | 1.24 | 1.57 |
| SEd | 0.29 | 0.26 | 0.41 | 1.42 | 0.05 | 0.04 | 0.05 | 0.10 |
| *p value* | | | | | | | | |
| GH | 0.26 | 0.09 | 0.01* | 0.44 | 0.60 | 0.37 | 0.66 | 0.67 |
| NON | 0.00* | 0.00* | 0.00* | 0.53 | 0.11 | 0.01* | 0.19 | 0.58 |
| GH *NON | 0.21 | 0.01* | 0.00* | 0.72 | 0.08 | 0.05* | 0.05* | 0.38 |

Means with the same alphabet are not significantly different from each other.
DAP= Days after planting; GH= Growth hormone; NON= Number of nodes
*= Significant ($\alpha$= 0.05)

up to 15 days of planting for root length and after 30 days of planting for number of roots compared to the untreated cuttings.

Application of growth hormones was reported to induce rooting on cuttings (Hartmann et al 2002). Position, size and length (number of nodes) of cutting significantly influence the differentiation and development of roots on stem cuttings (Adunga et al 2015). Cuttings not treated with hormones resulted to better root development, while treated cuttings were observed with more elongated roots (Table 2). Keeping the shoot tip untrimmed might have regulated the translocation of native auxin present in the apical portion of the cuttings to its basal portion initiating root differentiation. Trimming the shoot tips disturbs the auxin balance not enough to be translocated towards the base for root differentiation; stimulating only lateral bud initiation (Salisbury and Ross 1985). Cuttings (both hormonal treated and untreated) with a smaller number of nodes were observed with significantly more number of roots (Plate 1 c, e). This might be due to presence of proportionately higher concentration of auxins in the cuttings with lesser nodes (i.e., ratio of auxin concentration to relative cutting mass/volume). Interestingly, high concentrations of auxin were reported to inhibit root elongation and instead enhance adventitious root formation, while root initiation was faster and vigorous in cuttings with 2-4 nodes (Girma et al 2011, Umesha et al 2011). Tender shoots do not have sufficient food material thus pair of leaves present on tip of the shoots are retained as they help in preparing food through photosynthesis. Vegetative propagation using leafy stem cuttings has been successful in various medicinal plants. Furthermore, the use of short cuttings with 2-4 nodes is becoming prevalent (Adunga et al 2015, Namirembe-Ssonkko et al 2005, Umesha et al 2011). Hormone treatment was found effective in inducing significantly higher number of root development after 45 and 60 days of planting (Table 2). This might be due to the accumulation of metabolites at the auxin application site, cell enlargement, enhanced hydrolysis of carbohydrates, synthesis of new proteins, and cell division to support root initiation, growth and development (Strydem and Hartman 1960, Sevik and Guney 2013, Phuyal et al 2018).

**Comparative growth parameters of seedlings developed from seeds and macro-proliferation:** The comparative growth and economical yield (flower) parameters of three node treated cuttings, two node untreated cuttings and seedlings developed from seeds were illustrated in Figure 1.

**Table 2.** Main effect of number of nodes and growth hormone on number of roots and root length (cm) of *Dhawai* at different days after planting (DAP)

| Number of nodes | Number of roots at different DAP | | | | Root length (cm) at different DAP | | | |
|---|---|---|---|---|---|---|---|---|
| | 15 Days | 30 Days | 45 Days | 60 Days | 15 Days | 30 Days | 45 Days | 60 Days |
| One | 7.12 $^{a}$ | 8.53 $^{a}$ | 9.75 $^{a}$ | 10.72 $^{a}$ | 2.21 $^{b}$ | 3.23 $^{b}$ | 3.69 $^{b}$ | 3.90 $^{bc}$ |
| Two | 7.00 $^{a}$ | 8.94 $^{a}$ | 9.62 $^{a}$ | 9.96 $^{ab}$ | 3.24 $^{a}$ | 4.49 $^{a}$ | 5.13 $^{a}$ | 5.33 $^{ab}$ |
| Three | 5.31 $^{b}$ | 6.58 $^{b}$ | 7.14 $^{b}$ | 8.28 $^{bc}$ | 3.36 $^{a}$ | 4.54 $^{a}$ | 4.93 $^{a}$ | 6.15 $^{a}$ |
| Four | 3.97 $^{c}$ | 5.97 $^{bc}$ | 6.48 $^{b}$ | 8.59 $^{abc}$ | 3.05 $^{a}$ | 4.04 $^{a}$ | 4.60 $^{ab}$ | 4.99 $^{ab}$ |
| Five | 3.95 $^{c}$ | 4.91 $^{c}$ | 6.15 $^{b}$ | 7.88 $^{bc}$ | 2.31 $^{b}$ | 3.07 $^{bc}$ | 4.49 $^{ab}$ | 5.21 $^{ab}$ |
| Six | 5.50 $^{b}$ | 5.93 $^{bc}$ | 6.73 $^{b}$ | 7.39 $^{c}$ | 1.73 $^{b}$ | 2.62 $^{c}$ | 2.48 $^{c}$ | 3.41 $^{c}$ |
| Mean | 5.48 | 6.81 | 7.64 | 8.80 | 2.65 | 3.66 | 4.22 | 4.83 |
| SEd | 0.17 | 0.21 | 0.29 | 0.30 | 0.08 | 0.08 | 0.16 | 0.18 |
| Growth hormone | | | | | | | | |
| Treated | 5.43 | 7.04 | 8.71 | 10.03 | 2.37 | 3.76 | 4.38 | 4.89 |
| Untreated | 5.52 | 6.58 | 6.58 | 7.57 | 2.93 | 3.57 | 4.05 | 4.78 |
| Mean | 5.48 | 6.81 | 7.64 | 8.80 | 2.65 | 3.66 | 4.22 | 4.83 |
| SEd | 0.24 | 0.29 | 0.41 | 0.43 | 0.12 | 0.11 | 0.22 | 0.26 |
| *p value* | | | | | | | | |
| GH | 0.79 | 0.28 | 0.00* | 0.00* | 0.00* | 0.24 | 0.30 | 0.76 |
| NON | 0.00* | 0.00* | 0.00* | 0.01* | 0.00* | 0.00* | 0.00* | 0.00* |
| GH *NON | 0.00* | 0.04* | 0.19 | 0.20 | 0.00* | 0.04* | 0.36 | 0.38 |

Means with the same alphabet are not significantly different from each other.
DAP= Days after planting; GH= Growth hormone; NON= Number of nodes
*= Significant (α= 0.05)

Significant differences were observed in shoot length, collar diameter and number of nodes, leaves, branches and flowers. Maximum growth and yield parameters were recorded in treated cuttings with three nodes followed by untreated cuttings with two nodes and minimum growth and yield parameters were noted in seedlings developed from seeds. This indicates that the vegetatively propagated seedlings were significantly superior to the sexually propagated seedlings. Maximum growth parameters were observed in vegetative means of propagated plants over plants of seed origin. The productivity (flower yield) of vegetatively multiplied stock is more as compared to stock of seed origin at four months after transplanting. Sexually propagated plants have long gestation period, which results in delayed flowering and fruiting. Most of the medicinal and horticultural crops are commercially propagated by vegetative or asexual method of propagation (Girma et al 2011, Umesha et al 2011).

**Effect of season on success rate:** Seasons significantly influenced the survival of the cuttings (Table 4). Entire cuttings planted during monsoon (15$^{th}$ June) survived, while survival of cuttings planted during post-monsoon (15$^{th}$ October), winter (15$^{th}$ January) and summer (15$^{th}$ March) seasons was 93.33%, 76.67% and 40%, respectively. Spearman's correlation analysis indicates strong significant positive correlation between survival of cutting and relative humidity (Table 4). Higher the relative humidity, higher was the cutting survival. No such relationship was observed for temperature and rainfall with cutting survival as non-significant correlation was observed between these parameters and cutting survival.

Successful results were recorded when *Dhawai* propagated through stem cuttings during monsoon (Joshi et al 1992, Shankar and Rawat 2013). Two stages transplanting with root balls were found effective for successful seedling production of *Dhawai* (Mathew et al 2018). The climate of the study area is moist tropical (Anonymous 2001) with 2000-3500 mm annual rainfall might had also positive links to early and well establishment of *Dhawai* through macro-proliferation cup technique. Paul et al (2021) found that root was initiated in a better way from the cuttings in the humid region covered with transparent plastic cups (to control the initial micro-moisture conditions) for an initial fortnight. Spearman's correlation also indicates strong significant positive correlation in relative humidity with survival per cent. Nevertheless, meteorological parameters such as

**Table 3.** Main effect of number of nodes and growth hormone on number of leaves and number of branches of *Dhawai* at different days after planting (DAP)

| Number of nodes | Number of leaves | | Number of branches | |
|---|---|---|---|---|
| | 60 Days | 120 Days | 60 Days | 120 Days |
| One | 7.17 $^{a}$ | 18.00 $^{b}$ | 1.17 $^{c}$ | 2.00 $^{ab}$ |
| Two | 7.50 $^{a}$ | 17.50 $^{b}$ | 2.50 $^{bc}$ | 2.67 $^{ab}$ |
| Three | 8.33 $^{a}$ | 18.67 $^{b}$ | 3.33 $^{ab}$ | 3.33 $^{ab}$ |
| Four | 8.33 $^{a}$ | 18.17 $^{b}$ | 2.67 $^{bc}$ | 4.00 $^{a}$ |
| Five | 8.83 $^{a}$ | 26.00 $^{a}$ | 5.00 $^{a}$ | 4.17 $^{a}$ |
| Six | 9.50 $^{a}$ | 18.33 $^{b}$ | 1.67 $^{bc}$ | 1.33 $^{b}$ |
| Mean | 8.28 | 19.44 | 2.72 | 2.92 |
| SEd | 0.83 | 2.07 | 0.66 | 0.74 |
| Growth hormone | | | | |
| Treated | 8.33 | 18.39 | 2.72 | 3.22 |
| Untreated | 8.23 | 20.50 | 2.72 | 2.61 |
| Mean | 8.28 | 19.45 | 2.72 | 2.92 |
| SEd | 0.48 | 1.20 | 0.38 | 0.43 |
| *p value* | | | | |
| GH | 0.87 | 0.22 | 0.99 | 0.32 |
| NON | 0.40 | 0.06 | 0.01* | 0.08 |
| GH *NON | 0.03* | 0.05* | 0.12 | 0.28 |

Means with the same alphabet are not significantly different from each other.
DAP= Days after planting; GH= Growth hormone; NON= Number of nodes
*= Significant (α= 0.05)

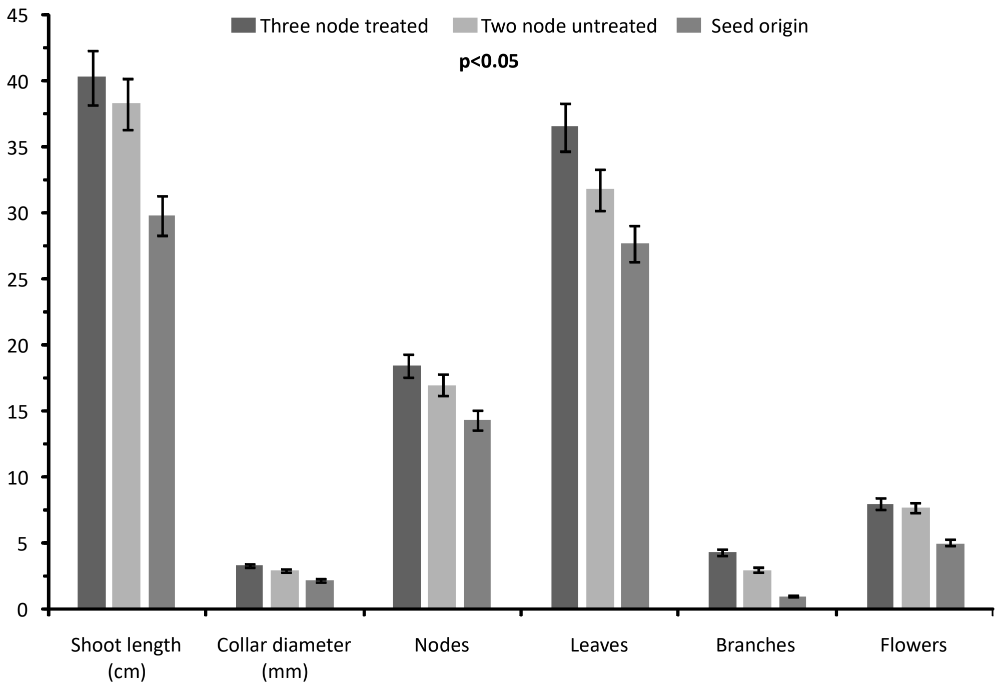


**Fig. 1.** Comparative growth parameters of three node treated cuttings, two node untreated cuttings and seedlings developed from seeds at four months after transplanting

**Table 4.** Survival per cent of macro-proliferation cup technique with two and three node cuttings under varied conditions

| Season (Date of planting) | Condition for establishment | Survival % | Correlation (Meteorological parameters with survival %) | *Dhawai* annual life cycle | Inference |
|---|---|---|---|---|---|
| Monsoon (15th June) | Placement in greenhouse conditions for 4 months and subsequent planting in the field. | 100.00 % | Relative humidity with survival (r= 1.00) ** | Young flush appearance | Suitable time for propagation |
| Post-monsoon (15th October) | | 93.33 % | Temperature with survival (r= 0.40) | | |
| Winter (15th January) | | 76.67 % | Rainfall with survival (r= 0.40) | Flowering and fruiting | Not-suitable |
| Summer (15th March) | | 40.00 % | | | |

** Correlation (Spearman's) is significant at the 0.01 level (2-tailed)

temperature and rainfall such trend was not observed as evidenced from low values of correlation coefficients. Survival of cuttings was reported higher at higher relative humidity level (Kalyoncu et al 2009, Paul et al 2021).

## CONCLUSION

Cuttings with two and three nodes planting in a garden soil are advantageous as the entire cuttings survived in the nursery. Cuttings covered with transparent plastic cups for a fortnight after planting rooted more effectively, thus is recommended in nurseries at Terai region of West Bengal for its easy, continuous and faster multiplication without ruining the mother plant. The mature *Dhawai* plant can be utilized for vegetative multiplication either collected from wild or maintained in the clonal seed orchards throughout the year but may be avoided during flowering in winter and also when seeds mature during summer. Based on the present study and observations, it is recommended to prepare fresh two-leaved shoot tip cuttings with two or three nodes throughout monsoon (June to September) and post monsoon (October to December). Ridge planting of the cuttings in rows and covered with transparent plastic cups (10 cm × 6 cm) for a fortnight is appropriate and four months old, well-established seedlings with root ball should be used to establish plantations.

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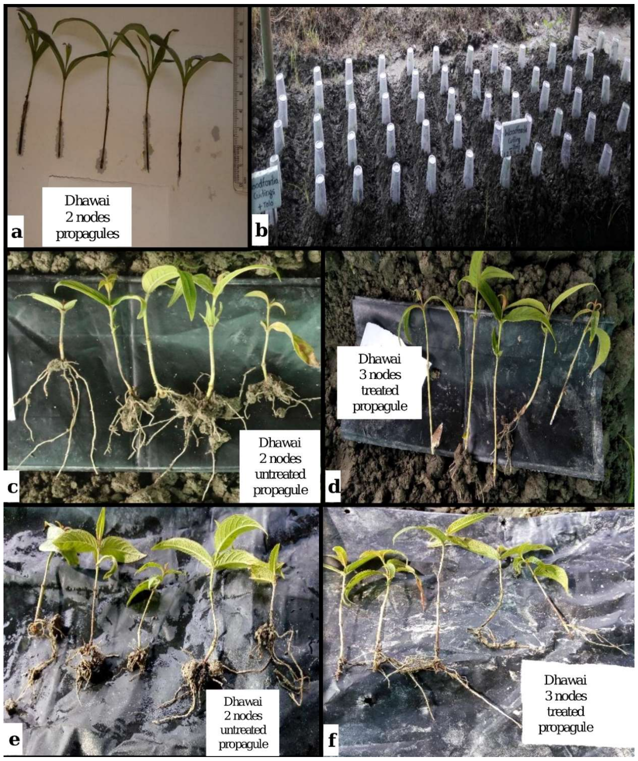


**Plate 1.** a) *Dhawai* cuttings with 2 nodes; b) *Dhawai* cuttings covered with transparent plastic cup for initial fortnight; c) Two nodes untreated cuttings at 30 Days after planting (DAP); d) Three nodes treated cuttings at 30 DAP; e) Two nodes untreated cuttings at 45 DAP; f) Three nodes treated cuttings at 45 DAP

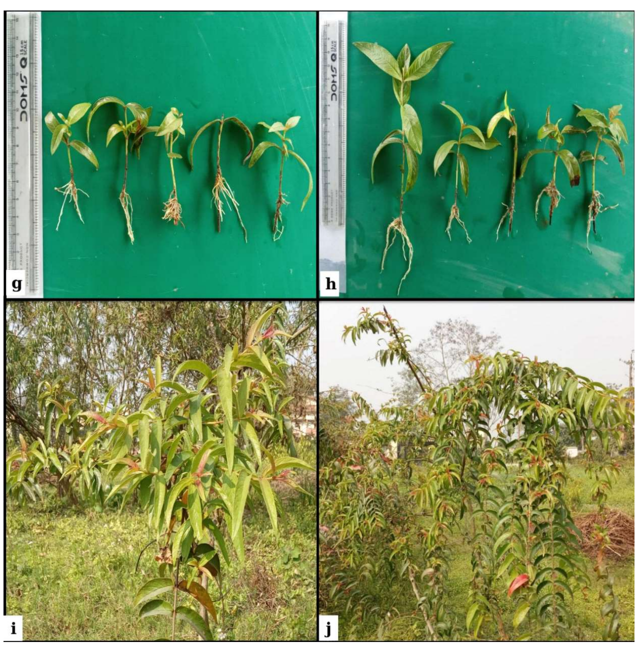


**Plate 2.** g) Cuttings with two nodes at 30 DAP during summer; h) Cuttings with three nodes at 30 DAP during summer; i) One-year-old *Dhawai* in outfield; j) Two-year-old well-established *Dhawai* in outfield

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# Studies on the Effect of Different Doses of Nitrogen on Quality Parameters of Wheat under Eucalyptus Based Agri-Silvi-Horticultural System

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***Abstract***: The study was conducted to assess the quality traits of four different wheat varieties (WH-1105, HD-2967, WH-711 and DPW-621-50) under a five year old Kinnow and Eucalyptus based agri-silvi-horticultural system with different doses of fertilizers. The experiment was laid in a split plot design with four different doses of nitrogen *viz.* Recommended dose of fertilizer (RDF), RDF + 10% extra dose of N, RDF + 20% extra dose of N, RDF + 30% extra dose of N. The whole amount of P and K and half N was applied at the time of sowing. The remaining N through urea was top dressed at crown root initiation (CRI) stage. Similarly, all the four varieties *viz.,* WH-1105, HD-2967, WH-711 and DPW-621-50 were also sown as sole crop (devoid of trees) with recommended dose of fertilizer only. The data pertaining to different quality parameters *viz.* protein content, gluten content, sedimentation value, grain hardness etc. in different wheat varieties revealed that the higher values were recorded under agri-horti and agri-silvi-horti system over sole cropping system. Further, it was observed that higher protein content, grain hardness and grain lustre were recorded in wheat variety HD- 2967 while, variety WH-711 exhibited higher gluten content and sedimentation values under both the agroforestry based systems in comparison to mono-cropping system. Similarly, it was also observed that different quality parameters increased with the increase in dose of fertilizer however, the difference between RDF+10%, RDF+20% and RDF+30% was found to be non-significant.

***Keywords:*** Fertilizer, Quality parameters, Wheat, Eucalyptus, Agri-silvi-horticultural system

In India, agroforestry has been a way of life. Our social, religious and cultural ethics have been closely linked with the planting and protection of various trees. Tree worship is still continued in many societies of our country. Planting and maintaining trees were considered noble acts. They were considered better acts than having sons, as has been said in Agnipuran during 1000 B.C. (Dwivedi 1992). However, with increase in demographic pressure resulting in over exploitation of resource base leading to degradation and vast gap between demand and supply of wood products, agroforestry has once again been identified as the most appropriate land use option. It is considered as a panacea for maladies of intensive agriculture (Pingali 1999). It is a way out to practice agriculture without deteriorating agro-ecosystem. Its role in the light of combating hunger, diseases, and environmental degradation is highly appreciable (Garrity 2004). Agroforestry acts as a bridge among the agriculture and natural resource conservation with environmental enhancement and human needs. An agroforestry system has more than two components that provide food grain, fruit, vegetables, spices and livestock food products. Among different agroforestry systems, agri-silvi-horticultural system plays vital role in food and nutritional security and fulfil the requirement fuel wood and timber.

The fundamental challenge is therefore to develop an economically viable farming system that will be adopted by the farmers. The dynamic nature of nutrient cycling is one of the obstacles in nutrient management in agroforestry systems. It dictates that soil nutrient capital useful for supplying nutrients for plant growth must be equated with short to medium-term, rolling capital (the monthly or annual salary), rather than long-term reserves (gold in the bank). The role of organics is varied and complex, the challenge is to use organics of differing quality in combination with inorganic fertilizers to optimize nutrient availability to plants which further helps to determine the quality traits in the arable crops to supplement the nutritional security. Therefore, keeping in view the vital importance of soil nutrients in supplementing quality traits in cereals under different farming systems in the present study was conducted to determine the effect of different doses of nitrogen on quality parameters of wheat under agri-silvi-horticultural system.

## MATERIAL AND METHODS

The present study was conducted during 2015-16 and 2016-17 CCS Haryana Agricultural University, Hisar (29°09'N latitude and 75°43'E longitude at an elevation of 215 m above sea level), situated in semi-arid region of North-Western

India during the winter season. The climate is subtropical-monsoonic with an average rainfall of 350-400 mm, 70-80 per cent of which occurs during July to September. A pre-established five year old plantation of Kinnow alone and Kinnow plus Eucalypts forming agri-horticultural and agri-silvi-horticulural systems, respectively were used as the basic agroforestry models in the study. Four wheat varieties viz., WH-1105, HD-2967, WH-711 and DPW-621-50 were sown during second fortnight of November during both the years under both agri-horticulture and agri-silvi-horticultural systems with four fertilizer levels i.e. recommended dose of fertilizer [RDF: 150 kg N + 60 kg $P_2O_5$ + 30 kg $K_2O$ + 25 Kg $ZnSO_4$ per hectare), RDF + 10% additional dose of N, RDF + 20% additional dose of N and RDF + 30% additional dose of N while only recommended dose of fertilizer was applied in control (sole crop). The whole amount of P and K and half N was applied at the time of sowing while the remaining N through urea was top dressed at crown root initiation (CRI) stage.

The soil samples (0-15, 15-30, 30-45, 45-60 and 60-90 cm depth) were assessed every year before sowing and after the harvesting of the wheat crop to study the various soil chemical properties *viz.* pH, electrical conductivity and organic carbon and available nutrients (N, $P_2O_5$ and $K_2O$) during the study period. Further, the wheat varieties were analyzed to determine various quality parameters *viz.* grain quality, baking properties and pasting characters under both the agroforestry based systems (AFS) in comparison to sole cropping system (control) during both the years of study

**Wheat grain quality parameters:** The wheat grain quality parameters were defined by grains protein content, grain hardness and grain luster. The grain protein content was estimated using automatic protein analyser (Infratec 1241, Foss Tecator) directly using grains to estimate the total protein content. Whereas, the Grain hardness was assessed using TAXT plus texture analyzer with 36 mm cylinder probe (CP/36) having 5 Kg load cell. The grains were kept on the platform and the force was applied to crush the grains. The force required to crush the grains was expressed as grain hardness (N). While, the grain luster characteristic were ranged from 0 (least lustrous) to 10 (most lustrous) on visual indexing basis.

**Wheat baking properties:** Baking properties namely sedimentation value and gluten content (wet basis) were assessed for all the four wheat varieties under both agri-horti and agri-silvi-horti systems in comparison to control (devoid of trees). Sedimentation value of flour was determined according to procedure standardized by American Association of Cereal Chemists (AACC 1995). 5 g flour sample was weighed and transferred to 250 ml stoppered graduated cylinder and 50 ml of distilled water was added to it and the cylinder was shaken horizontally for 15 sec. and kept for two minutes. The contents of cylinder were again shaken horizontally for 15 sec. at 2 min. and 4 min. intervals. 50 ml SDS- lactic acid reagent was added immediately after the last shake and mixed by inverting the cylinder four times. Inversion was repeated four times at 6, 8 and 10 min. interval. The contents of cylinder were allowed to settle for 20 min. and the sedimentation values were read and recorded in ml units. Whereas, the Gluten content was assessed using hand washing method. 10 g of wheat flour sample was weighed and 7 ml distilled water was added to it. A dough ball was prepared by adding additional quantity of water. Prepared dough ball was dipped in water for 1 hr. Dipped dough ball was removed and washed under the tap water gently using muslin cloth. The washed mass 'wet gluten' was weighed and was used to determine per cent wet gluten in wheat flour.

**Pasting characteristics:** Wheat flour was assessed for various pasting characteristics viz., peak viscosity, trough, break down, final viscosity, set back, peak time and pasting temperature using Rapid Visco Analyzer, (Newport Scientific Australia). 25 ml of distilled water was taken into a canister. 3.5 g sample (14% moisture basis) was weighed and transferred into canister. Paddle was placed into the canister and jogged to disperse the sample. Paddle and canister was inserted into Rapid Visco Analyzer (RVA) and waited for the command for pressing down the tower from the thermocline windows till the temperature of RVA reached 50 $^{0}$C. Pressed down the tower and wait till the test was run for 13 min. Canister was removed on completion of test. From thermocline windows various observations were recorded.

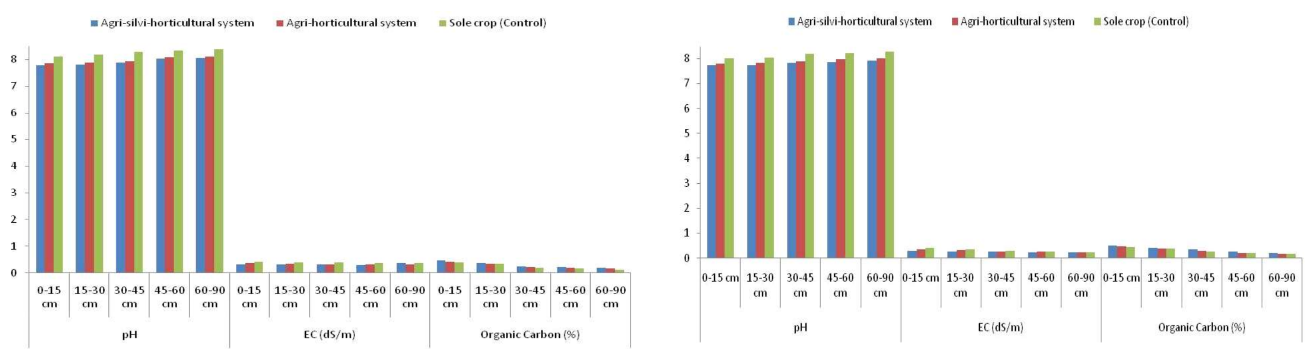
## RESULTS AND DISCUSSION

**Physio- chemical properties of soil under different agroforestry system and sole crop:** The study shows that in general agroforestry based (agri-horti and agri-silvi-horti) systems resulted in significant improvement in pH, EC, organic carbon and available nitrogen, phosphorus and potassium in soil over sole crop (control). The improvement might be attributed to increase in the soil organic matter content brought about by the incorporation of the biomass of the different components in two-tier and tier systems resulting in better aggregation properties of soil and the intercultural operations which decreased the compactness of soil, increased soil aeration under the agroforestry based systems. Similar effect of intercropping on the physio-chemical properties of soil has been reported by Swain and Patro (2007), Swain et al (2012), Swain (2014), Sangwan et al (2015), Adak et al (2016).

The Figure 1 (A and B) shows that there was a little change in soil pH from 2015-16 to 2016-17. However, after six years there was significant decrease in soil pH in comparison to initial values at the time of plantation of both the systems (2011). In agri-silvi-horti system, the soil pH value decreased upto 4.19 and 4.56 percent over the initial value at 0-15 cm soil depth during 2015-16 and 2016-17, respectively. Similarly, after 6 years the electrical conductivity at soil surface (0-15 cm) of the experimental field decreased steeply from 0.48 to 0.30 and 0.36 dSm-1 under agri-silvi-horti and agri-horti systems, respectively after crop harvest during 2016-17. However, in control (sole crop) the rate of decrease in electrical conductivity was comparatively low and it merely decreased from 0.48 to 0.40 dSm$^{-1}$. However, the soil organic carbon in agri-silvi-horti and agri-horti system increased significantly upto 30 cm soil profile depth over the initial values at the time of start of the experiment. The soil organic carbon content decreased with the increase in soil profile depth in agroforestry (agri-silvi-horti and agri-horti systems) as well as sole cropping system during both the years of observations. However, the organic carbon content of soil increased with the increase in age of eucalyptus and kinnow under both agri-horti and agri-silvi-horti systems. The increase in organic carbon of soil, pH and EC under the intercropping system and in the open could be due to the enhanced plant cover on the soil which helped in least crust formation, increased porosity and reduced bulk density. The results are in conformity with Ganeshamurthy et al (2016). There was improvement in the available nitrogen, phosphorus and potassium {Fig. 2 (A and B)} content in the soil at the end of the experiment which might be due to the increase in the humus content of soil after decomposition of biomass of intercrops and application of additional dose of nitrogen to the recommended dose fertilizer to the intercrop in the agroforestry based systems. Similar results of improvement in nutrient status of soil due to intercropping have been reported by Tiwari and Baghel (2014), Bhardwaj et al (2016), Sirohi and Bangarwa (2017).

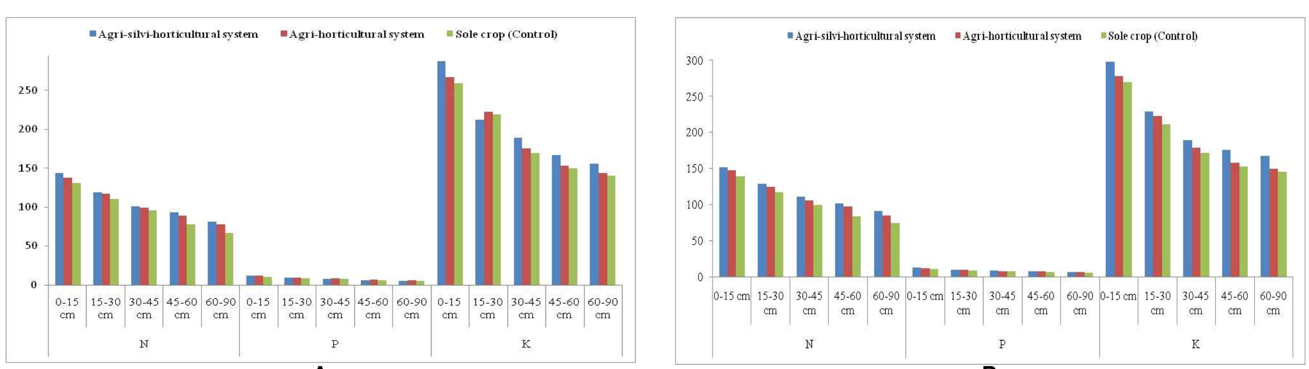
**Quality Parameters of Wheat**

**Grain quality parameters:** The average protein content of different wheat varieties in present study was minimum under monocropping system (sole crop) in comparison to both the agroforestry based systems (agri-horti and agri-silvi-horti systems) during both the years of study (Table 1). In sole cropping system (control) maximum protein content was recorded in wheat variety WH-1105 (11.3%) followed by variety HD-2967 (11.2%), DPW-621-50 (11.1%) and WH-711




A B

**Fig. 1.** pH, Electrical conductivity and organic carbon of soil before (A) and after (B) start of the experiment




A B

**Fig. 2.** N, P and K content of soil before (A) and after (B) start of the experiment

(11%) during 2015-16 (Fig. 3). However, under both the agroforestry based systems higher values of protein content was recorded in wheat variety HD-2967 (11.4 and 11.9%) followed by variety DPW-621-50 (11.3 and 11.6%), WH-1105 (11.3 and 11.5%) and WH-711 (11.2 and 11.3%) during 2015-16. Similar pattern of protein content in different wheat varieties in present study was also recorded during 2016-17. However, significantly higher protein content was observed under agri- horticultural system as compared to agri-silvi-horticultural system during both the years of study. The increase in protein content under agri-horticultural system may be due to reason that a high moisture level associated with more moderate temperature in shade that results in a faster rate of mineralization, litter breakdown and turnover of N than occurs in full sunlight (Wilson 1996, Chauhan et al 2013). Neelam et al (2012) and Dhillon et al (2016) also reported that a positive effect of shade on protein content of seed in the cereal crops. In present study, it was further observed that the protein content in different wheat varieties (WH-1105, HD-2967, WH-711 and DPW-621-50) under both the agroforestry based (agri-silvi-horti and agri-horti) systems increased with an increase in additional dose of nitrogen (10, 20 and 30 per cent) to the recommended dose of fertilizer during 2015-16 and 2016-17. The corroborative results were also reported by Pechanek et al (1997).

The varying levels of nitrogen under agri-silvi-horticultural and agri- horticulture systems (Table 2) significantly affected the grain hardness of different wheat varieties (WH-1105, HD-2967, DPW-621-50 and WH-711) over sole cropping systems (wheat varieties without trees) during 2015-16 and 2016-17 (Fig. 3). It was observed that the grain hardness in different wheat varieties in present study increased significantly with the increase in additional dose of nitrogen (10, 20 and 30 per cent) to the recommended dose of fertilizer during both the years of study. This could be due to accumulation of higher gluten content in wheat endosperm

**Table 1.** Effect of different nitrogen levels on protein content (%) of different wheat varieties under different agroforestry systems during 2015-16 (2016-17)

| Nitrogen level* | Agri-horticulture | | | | | Agri-silvi-horticulture | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Wheat varieties | | | | | Wheat varieties | | | | |
| | WH-1105 | HD-2967 | WH-711 | DPW-621-50 | Mean | WH-1105 | HD-2967 | WH-711 | DPW-621-50 | Mean |
| RDF | 11.1 (11.4) | 11.3 (11.7) | 10.6 (11.0) | 11.0 (11.7) | 11.0 (11.5) | 10.9 (11.4) | 11.1 (11.8) | 10.7 (10.9) | 11.0 (11.7) | 10.9 (11.5) |
| RDF+10% | 11.5 (11.9) | 11.9 (12.5) | 11.4 (11.6) | 11.7 (12.1) | 11.7 (12.1) | 11.3 (11.6) | 11.3 (12.1) | 11.1 (11..3) | 11.3 (11.8) | 11.3 (11.7) |
| RDF+20% | 11.6 (12.2) | 12.1 (12.6) | 11.5 (11.8) | 11.9 (12.2) | 11.8 (12.2) | 11.4 (11.6) | 11.4 (12.2) | 11.1 (11.3) | 11.3 (11.9) | 11.3 (11.8) |
| RDF+30% | 11.6 (12.3) | 12.1 (12.6) | 11.5 (11.8) | 11.9 (12.2) | 11.8 (12.2) | 11.4 (11.7) | 11.4 (12.2) | 11.3 (11.4) | 11.4 (12.1) | 11.4 (11.9) |
| Mean | 11.5 (12.0) | 11.9 (12.4) | 11.3 (11.6) | 11.6 (12.1) | | 11.3 (11.6) | 11.4 (12.1) | 11.2 (11.2) | 11.3 (11.9) | |
| CD at 1% | AFS: 0.12 (0.14) | | | Variety: 0.17 (0.18) | | | Nitrogen level: 0.21 (0.24) | | | |
| | AFS × Variety × Nitrogen level: 0.59 (0.59) | | | | | | | | | |

*RDF + 10%, RDF + 20% and RDF + 30% represents additional dose of nitrogen to the recommended dose of fertilizer under agri-horti and agri-silvi-horti systems (2016-17 values in parentheses)

**Table 2.** Effect of different nitrogen levels on grain hardness (kg/grain) of different wheat varieties under different agroforestry systems during 2015-16 (2016-17)

| Nitrogen level* | Agri-horticulture | | | | | Agri-silvi-horticulture | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Wheat varieties | | | | | Wheat varieties | | | | |
| | WH-1105 | HD-2967 | WH-711 | DPW-621-50 | Mean | WH-1105 | HD-2967 | WH-711 | DPW-621-50 | Mean |
| RDF | 9.7 (10.0) | 9.9 (10.4) | 9.4 (9.6) | 9.6 (9.9) | 9.7 (10.1) | 10.3 (10.7) | 10.4 (10.9) | 9.8 (10.4) | 10.1 (10.8) | 10.1 (10.7) |
| RDF+10% | 10.9 (11.2) | 11.0 (11.6) | 10.6 (10.9) | 10.8 (10.4) | 10.8 (11.3) | 11.2 (11.7) | 11.4 (11.9) | 10.9 (11.3) | 11.0 (11.5) | 11.3 (11.6) |
| RDF+20% | 11.5 (12.2) | 11.6 (12.7) | 11.3 (11.5) | 11.4 (12.0) | 11.5 (11.9) | 11.9 (12.5) | 12.2 (12.8) | 11.7 (11.9) | 11.4 (12.3) | 11.9 (12.4) |
| RDF+30% | 13.0 (13.3) | 13.7 (14.4) | 12.2 (12.7) | 12.7 (13.0) | 12.9 (13.1) | 13.1 (13.7) | 13.9 (14.7) | 12.5 (12.5) | 12.9 (13.0) | 13.1 (13.5) |
| Mean | 11.3 (11.6) | 11.6 (11.9) | 10.9 (11.5) | 11.1 (11.8) | | 11.6 (12.2) | 11.9 (12.6) | 11.5 (11.5) | 11.8 (11.9) | |
| CD at 1% | AFS: 0.18 (0.19) | | | Variety: 0.19 (0.12) | | | Nitrogen level: 0.19 (0.17) | | | |
| | AFS × Variety × Nitrogen level: NS (NS) | | | | | | | | | |

*RDF + 10%, RDF + 20% and RDF + 30% represents additional dose of nitrogen to the recommended dose of fertilizer under agri-horti and agri-silvi-horti systems (2016-17 values in parentheses)

that enhances the formation of higher amount of amylase and binding starch which results in the formation of harder seeds than with lower levels of nitrogen (Salmanowicz et al 2012). Comparatively harder grains were observed in different wheat varieties under study in agri-silvi-horticultural system in comparison to agri-horticulture and sole crop during both the years of study. Under agri-silvi-horticultural system harder grains were recorded in HD-2967 (11.9 kg grain$^{-1}$) closely followed by DPW-621-50 (11.8 kg grain$^{-1}$), WH-1105 (11.6 kg grain$^{-1}$) and WH-711 (11.5 kg grain$^{-1}$) during 2015-16. However, under sole cropping system (control) highest grain hardness was recorded in HD-2967 followed by WH-1105, DPW-621-50 and WH-711 during both the years of study. Similar results were also reported by Hrušková and Švec (2009) and Joanne et al (2015) for different wheat varieties sown under different growing environments.

Effect of different nitrogen levels on grain lustre of different wheat varieties under agri-horti-silvi and agri-horti systems has been presented in Table 3. It was observed that the grain lustre of different wheat varieties under study increased with the increase in the additional dose of nitrogen (10, 20 and 30 per cent) to the recommended dose of fertilizer. The highest rating of grain lustre was recorded at RDF + 30 per cent additional dose of nitrogen over recommended dose of fertilizer. However, comparatively similar ratings of grain lustre were observed under both agri-horticultural and agri-silvi-horticultural systems during both the years of study. Whereas, more lustrous grain were observed under both the agroforestry based systems as compared to the mono-cropping system (control) (Fig. 3). Under both the agroforestry based systems the most lustrous grains were observed in wheat variety HD-2967 followed by variety DPW-621-50, WH-1105 and WH-711. The results obtained are in line with the findings of Malaker et al (2009) and Pawar et al (2016).

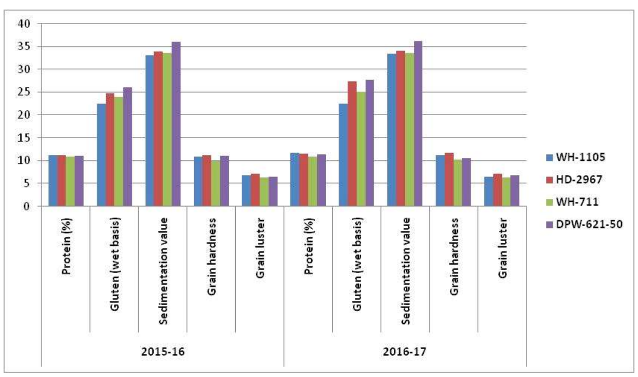



**Fig. 3.** Quality parameters of wheat varieties under sole cropping (control) system during 2015-16 and 2016-17

**Wheat baking properties:** Gluten content in different wheat varieties in present study varied significantly under both the agroforestry based (agri-silvi-horticultural and agri-horticultural) system as compared to sole crop (Table 4). It was observed that significantly higher (30.1 and 31.4 per cent) gluten content was recorded under agri-silvi-horticultural system over agri-horticultural (29.3 and 30.4 per cent) and sole cropping systems (24.4 and 25.7 per cent) during both the years of observation (Fig. 3). This may be due to the presence of more heat stress under open condition. This occurs when the crop is subjected to high temperature during grain filling, particularly in the period just before harvest. This is the critical period when post-translational glutenin polymerization proceeds rapidly (Stone and Nicolas 1994). Earlier, Randall and Moss (1990) also reported that during grain filling, dough strength (gluten) increases with the increase in daily mean temperature upto 30°C however, it decreased with further increase in temperature. The heat

**Table 3.** Effect of different nitrogen levels on grain lusture (visual indexing) of different wheat varieties under different agroforestry systems 2015-16 and 2016-17

| Nitrogen level* | Agri-horticulture | | | | | Agri-silvi-horticulture | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Wheat varieties | | | | | Wheat varieties | | | | |
| | WH-1105 | HD-2967 | WH-711 | DPW-621-50 | Mean | WH-1105 | HD-2967 | WH-711 | DPW-621-50 | Mean |
| RDF | 5.1 (5.4) | 6.4 (6.7) | 5.6 (5.9) | 6.0 (6.3) | 5.8 (6.1) | 5.7 (6.0) | 6.4 (6.7) | 5.6 (5.9) | 6.0 (6.3) | 5.9 (6.2) |
| RDF+10% | 6.3 (6.6) | 6.5 (6.8) | 6.1 (6.4) | 6.7 (7.0) | 6.4 (7.0) | 6.6 (6.9) | 6.5 (6.8) | 5.9 (6.2) | 6.4 (6.7) | 6.4 (6.7) |
| RDF+20% | 6.7 (7.0) | 6.9 (7.2) | 6.2 (6.5) | 6.7 (7.0) | 6.6 (6.8) | 6.4 (6.7) | 6.7 (7.0) | 6.4 (6.7) | 6.7 (7.0) | 6.6 (6.9) |
| RDF+30% | 7.3 (7.7) | 7.1 (7.5) | 6.9 (7.2) | 6.8 (7.1) | 7.0 (7.2) | 6.9 (7.3) | 7.1 (7.6) | 6.9 (7.2) | 7.2 (7.6) | 7.0 (7.4) |
| Mean | 6.4 (6.7) | 6.7 (7.1) | 6.4 (6.5) | 6.6 (6.9) | | 6.4 (6.7) | 6.7 (7.0) | 6.4 (6.7) | 6.6 (6.9) | |
| CD at 1% | AFS: 0.12 (0.14) | | | Variety: 0.09 (0.11) | | | Nitrogen level: 0.15 (0.18) | | | |
| | | | | AFS × Variety × Nitrogen level: 2.56 (3.01) | | | | | | |

*RDF + 10%, RDF + 20% and RDF + 30% represents additional dose of nitrogen to the recommended dose of fertilizer under agri-horti and agri-silvi-horti systems (2016-17 values in parentheses)

stress due to increased temperature causes a reduction in the size of gluten polymers (Browne et al 2006). Under sole cropping system higher gluten content was recorded in wheat variety DPW-621-50 (26.2 per cent) followed by variety HD-2967 (24.8 per cent), WH-711 (24.0 per cent) and WH-1105 (21.5 per cent) during 2015-16. However, under agri-silvi-horticultural and agri-horticultural system higher value of gluten content was observed in wheat variety WH-711 (34.2 and 33.8 per cent) followed by variety HD-2967 (28.9 and 27.9 per cent), DPW-621-50 (28.4 and 27.5) and WH-1105 (29.3 and 28.2 per cent) during 2015-16. Similar pattern was also observed during second year of observation *i.e.* 2016-17 under both the agroforestry based systems. Dalal et al (2016) also reported similar findings under *Prosopis cineraria* based agri-silvi-horticultural system in comparison to the sole crop. Further, a positive effect of additional dose of N (10, 20 and 30 per cent) to the recommended dose of fertilizer under both the agroforestry based systems was observed on the gluten content in all the wheat varieties in present study over recommended dose of fertilizer. However, the different between RDF + 10% additional dose of N, RDF + 20% additional dose of N and RDF + 30% additional dose of N was recorded to be non-significant. The increasing value of gluten content with the additional dose of nitrogen has also been proclaimed by Doekes and Wennekes (1982) and Scheromm et al (1992), respectively, in wheat.

The data depicted in Table 5 showed that significantly higher sedimentation value in different wheat varieties (WH-1105, HD-2967, WH-711 and DPW-621-50) was observed under agri-silvi-horticultural (kinnow + eucalyptus + wheat) system over agri-horticultural (kinnow + wheat) system and sole crop (devoid of trees) during both the years of experimentation (Fig. 3). Wheat variety WH-711 recorded the highest (37.8 and 38.8; 38.5 and 39.5 ml) sedimentation value followed by HD-2967 (36.8 and 37.6; 37.6 and 38.3 ml), WH-1105 (36.4 and 36.8; 36.9 and 38.0 ml) and DPW-621-50 (35.8 and 36.1; 36.7 and 37.1 ml) under both the agroforestry based systems during 2015-16 and 2016-17, respectively. However, lesser sedimentation values were recorded in sole crop (wheat crop devoid of tress) during 2015-16 and 2016-17. The possible reason for this could be that the swelling of the gluten fraction of flour in lactic acid solution affects the rate of sedimentation of a flour suspension in the lactic acid medium. Higher gluten content and better gluten quality both give rise to slower sedimentation and higher sedimentation test values (AACC, 1995). In the present study, it was further observed that the addition of extra dose of N (RDF + 10, RDF + 20 and RDF + 30 per cent) to the recommended dose of fertilizer in both agri-silvi-horticultural and agri-horticultural systems significantly increased the sedimentation value in wheat varieties over recommended dose of fertilizer. This could be due to the fact that the increasing levels of nitrogen increases the starch and gluten content in wheat flour which affects the swelling of glutamine section of the flour in sodium dodecyl sulfate sedimentation index (SDS-SI) (Aguirre et al 2002). The results are in conformity with the finding of Nagarajan and Mishra (2001) and Khattak et al (2005).

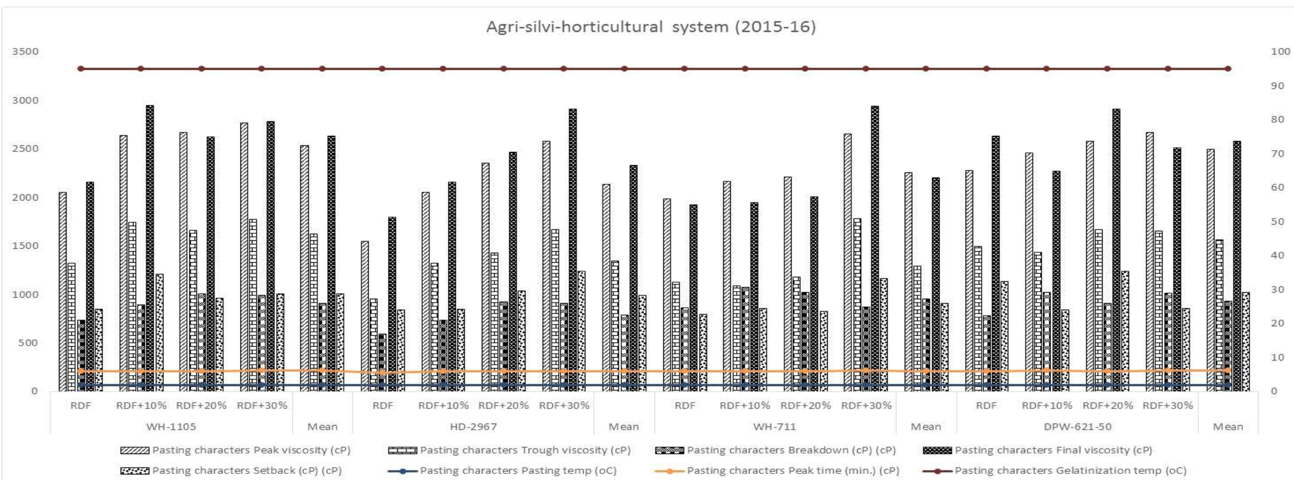
**Pasting characteristics:** Compositional and morphological properties of starch such as amylose content, phosphorus content and mean granule size play crucial role in influencing the pasting and rheological properties of starches (Liu et al 2003, Zaidul et al 2007, Abegunde et al 2013). Peak viscosity (PV) is an indicator of water binding capacity and ease with which the starch granules are disintegrated and often correlated with final product quality (Thomas and Atwell

**Table 4.** Effect of different nitrogen levels on gluten content (%) (wet basis) of different wheat varieties under different agroforestry systems during 2015-16 (2016-17)

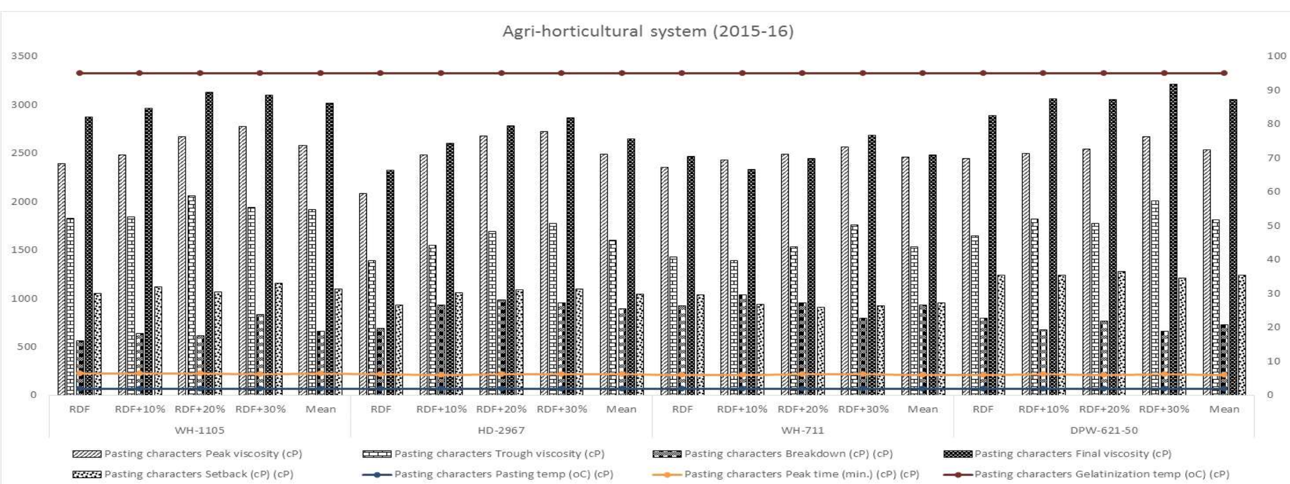
| Nitrogen level* | Agri-horticulture | | | | | Agri-silvi-horticulture | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Wheat varieties | | | | | Wheat varieties | | | | |
| | WH-1105 | HD-2967 | WH-711 | DPW-621-50 | Mean | WH-1105 | HD-2967 | WH-711 | DPW-621-50 | Mean |
| RDF | 25.6 (27.7) | 25.1 (26.9) | 30.6 (33.0) | 25.0 (26.3) | 26.6 (28.5) | 26.9 (28.3) | 26.4 (27.3) | 32.7 (34.2) | 26.0 (26.6) | 28.0 (29.1) |
| RDF+10% | 28.6 (29.6) | 28.3 (29.4) | 34.6 (35.1) | 27.9 (28.9) | 29.9 (30.8) | 29.8 (30.2) | 29.4 (29.8) | 34.6 (36.4) | 28.6 (29.4) | 30.6 (31.9) |
| RDF+20% | 28.9 (30.1) | 28.9 (29.7) | 34.9 (35.4) | 28.3 (29.2) | 30.3 (31.1) | 30.0 (30.4) | 29.7 (30.2) | 34.8 (36.4) | 29.1 (29.9) | 30.9 (32.2) |
| RDF+30% | 29.5 (30.3) | 29.2 (30.2) | 35.1 (35.4) | 28.7 (30.0) | 30.6 (31.5) | 30.3 (30.7) | 30.0 (30.6) | 34.8 (36.6) | 29.8 (30.2) | 31.2 (32.5) |
| Mean | 28.2 (29.4) | 27.9 (29.1) | 33.8 (34.7) | 27.5 (28.6) | | 29.3 (29.9) | 28.9 (29.5) | 34.2 (35.9) | 28.4 (29.0) | |
| CD at 1% | AFS: 0.24 (0.09) | | | Variety: 0.34 (0.14) AFS × Variety × Nitrogen level: 2.55 | | | Nitrogen level: 0.90 (0.71) | | | |

*RDF + 10%, RDF + 20% and RDF + 30% represents additional dose of nitrogen to the recommended dose of fertilizer under agri-horti and agri-silvi-horti systems (2016-17 values in parentheses)

1999). Amylose leaching, amylose lipid complex formation, friction between swollen granules and granule swelling has been reported as key influencing factors of peak viscosity (Zaidul et al 2007). It was observed that under agri-horticultural system higher values of peak viscosity and trough viscosity was recorded in wheat variety WH-1105 (2580.5 and 2576.8 cP; 1918.0 and 1916.7 cP) followed by variety DPW-621-50 (2538 and 2570 cP; 1812 and 1837 cP), HD-2967 (2492.5 and 2524 cP; 1600.5 and 1604.3 cP) and WH-711 (2460.8 and 2488 cP; 1531.3 and 1544.7 cP), respectively during both the years of study {Fig. 4 (A and B)}. Similar results have been reported by other workers also (Hill and Gray 1999, Jyothi et al 2005). The reduction in peak viscosity under agri-silvi-horticultural system {Fig. 5 (A and B)} could be due to break down of the glycoside linkages of the long amylopectin chains and conversion of amylose to low molecular weight chains (Abdorreza et al 2012, Babu et al 2015). During the cold treatment in RVA the maximum value of breakdown viscosity under agri-horticultural system was recorded in wheat variety WH-711 (929.5 and 927.5 cP) followed by wheat variety HD-2967 (892.0 and 903.5 cP), DPW-621-50 (727 and 727 cP) and WH-1105 (662.5 and 658.3 cP) during 2015-16 and 2016-17. However, the highest values of final viscosity and set back viscosity during both the years of investigation under agri-horticultural system were recorded in wheat variety DPW-621-50 (3055 and 3061 cP; 1244 and 1246.5 cP) followed by WH-1105 (3016.3 and 2980 cP; 1098.3 and 1086.5 cP), HD-2967 (2645 and 2650.8 cP; 1044.5 and 1057.8 cP) and WH-711 (2482.8 and 2650.8 cP; 951.5 and 951.8 cP). The higher peak viscosity exhibited by control starches in this instance was due to higher granule rigidity and integrity, contributed by the presence of amylose




A



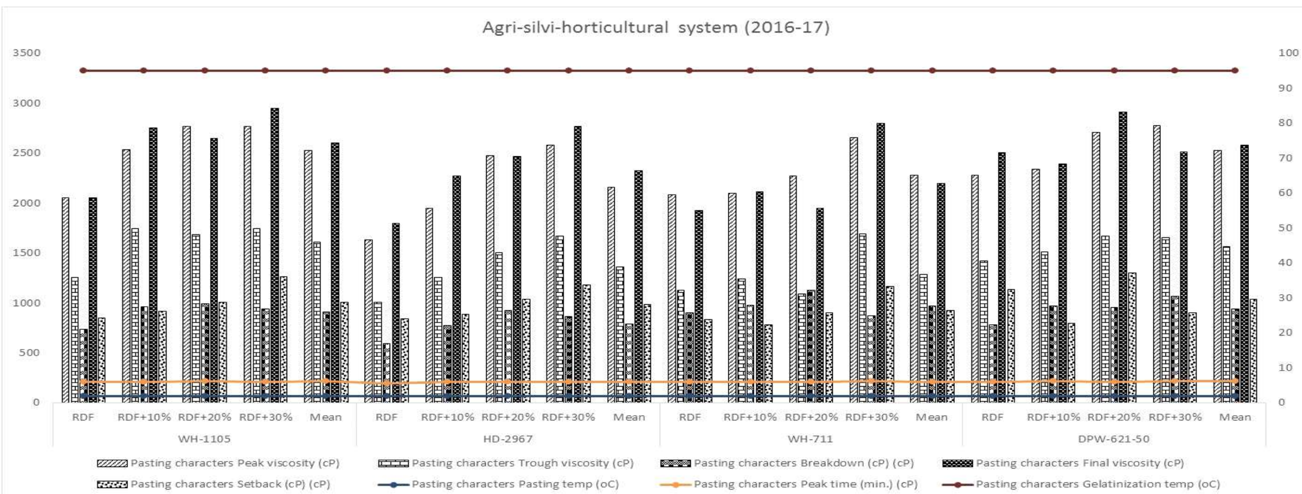

B

**Fig. 4.** Effect of different nitrogen levels on pasting characters of wheat flour of different wheat varieties under agri-silvi-horticultural (A) and agri-horticultural (B) systems during 2015-16

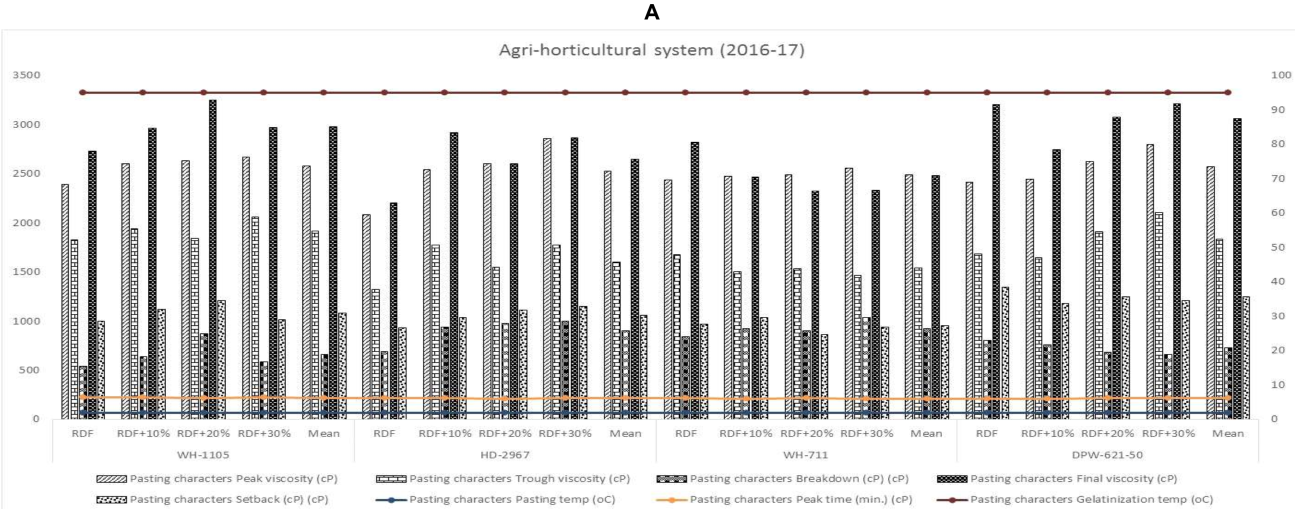
**Table 5.** Effect of different nitrogen levels on sedimentation value (ml) of wheat flour of different wheat varieties under different agroforestry systems during 2015-16 (2016-17)

| Nitrogen level* | Agri-horticulture | | | | | Agri-silvi-horticulture | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Wheat varieties | | | | | Wheat varieties | | | | |
| | WH-1105 | HD-2967 | WH-711 | DPW-621-50 | Mean | WH-1105 | HD-2967 | WH-711 | DPW-621-50 | Mean |
| RDF | 33.6 (34.7) | 34.6 (35.1) | 35.4 (36.8) | 33.3 (34.2) | 34.5 (35.2) | 34.9 (35.6) | 35.0 (36.2) | 36.2 (37.3) | 34.5 (35.0) | 35.1 (36.0) |
| RDF+10% | 36.7 (37.0) | 36.3 (37.4) | 38.0 (38.8) | 35.8 (36.0) | 36.7 (37.1) | 37.0 (38.2) | 37.8 (38.2) | 38.7 (39.5) | 36.9 (37.1) | 37.6 (38.3) |
| RDF+20% | 37.3 (37.6) | 37.9 (38.6) | 38.7 (39.4) | 36.2 (36.8) | 37.5 (37.9) | 37.7 (39.1) | 38.3 (38.9) | 39.3 (40.1) | 37.4 (37.7) | 38.2 (39.0) |
| RDF+30% | 37.9 (37.9) | 38.4 (39.2) | 39.1 (40.0) | 37.0 (37.3) | 38.1 (38.5) | 38.0 (39.6) | 39.2 (39.7) | 39.6 (40.9) | 37.9 (38.4) | 38.7 (39.7) |
| Mean | 36.4 (36.8) | 36.8 (37.6) | 37.8 (38.8) | 35.8 (36.1) | | 36.9 (38.0) | 37.6 (38.3) | 38.5 (39.5) | 36.7 (37.1) | |
| CD at 1% | AFS: 1.33 (1.37) | | Variety: 1.88 (1.79) | | | Nitrogen level: 1.21 (1.28) | | | | |
| | AFS × Variety × Nitrogen level: 5.85 (4.62) | | | | | | | | | |

*RDF + 10%, RDF + 20% and RDF + 30% represents additional dose of nitrogen to the recommended dose of fertilizer under agri-horti and agri-silvi-horti systems (2016-17 values in parentheses)




**A**




**B**

**Fig. 5.** Effect of different nitrogen levels on pasting characters of wheat flour of different wheat varieties under agri-silvi-horticultural (A) and agri-horticultural (B) systems during 2016-17

(Karim et al 2007). It was further observed that the pasting characters significantly increased with the increase in additional dose of nitrogen (10, 20 and 30 per cent) to the recommended dose of fertilizer under both the agroforestry based systems during both the years of study however, the peak time, pasting temperature and gelatinization temperature were not affected with the increase in nitrogen levels. The increase in granule swelling and viscosity of starch paste in the presence of alkali might be because of the anions ($OH^-$) that associate at the specific sites in starch granules and create a large hydration sphere (Mistry and Eckhoff 1992). Increase in final viscosity might be due to the aggregation of the amylose molecules. Similar results have already been reported by other research workers (Lim et al 1999, Debet and Gidley 2006, Dhillon et al 2010). Hence, the study clearly reveals that agroforestry based systems not only improves the soil physio-chemical properties but also supplements the quality traits in arable crops which helps to fulfils the nutrition requirements of the ever increasing population in the country.

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# Estimation of Tree Biomass in Dry Deciduous Forests of Seshachalam Hill Ranges, Southern Eastern Ghats by Non-Destructive Method

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***Abstract***: The present study deals with the estimation of tree biomass by non destructive method in dry deciduous forest (DDF) of Seshachalam hill ranges in 18 (1-ha) study sites. The biomass of each species was estimated by calculating its volume by means of its specific equation and species specific gravity. The range of above ground biomass was (AGB) 70.36 t ha$^{-1}$ to 156.64 t ha$^{-1}$ and below ground biomass (BGB) was in the range of 34.45 t ha$^{-1}$ to 74.39 t ha$^{-1}$. AGB showed a significant positive relationship with Basal area; while non-significant positive relation with tree density and tree diversity was recorded. But the top ten rank species AGB values has showed significant positive relationship with tree density. Below ground biomass (BGB) was derived from above ground biomass (AGB) and the total tree biomass was estimated.

***Keywords***: Above ground biomass, Belowground biomass, Dry deciduous forest, Tree volume equation, Wood specific gravity

Forests play a significant role in the global carbon cycle as they store large quantities of carbon in vegetation and soil (Padmakumar et al 2018). Among terrestrial ecosystems, forests are one of the major pools that have the ability to fix atmospheric carbon in to their phytobiomass. Among them, tropical forests not only have the highest potential and also show diverse range in their capacity to sequester large amounts of carbon (Metz et al 2001). Thus, forests and climate change are strongly interlinked (Mohanta et al 2020). The aboveground biomass (AGB) of a forest ecosystem is one of the fundamental parameters that can describe its functioning and carbon storage potential (Brown et al 1997). Among Indian forests, total above ground and below ground biomass has been estimated as 6865.1 Mt (79%) and 1818.7 Mt (21%) respectively (ISFR 2019).

Trees play major roles in carbon storage and forest ecosystem functioning (Pragasan 2020). Assessing the above ground biomass (AGB) of dominant tree species in different forest tree species functional types is of great importance (Behera et al 2017). Tree inventories are still an efficient way for assessing the forest carbon stock (Rao and Rao 2015). The forest ecosystem captures and retains large volume of carbon over long periods. The young trees have the potential to sequester large amount of carbon, where as an old mature trees acts as a reservoir holding large volume of carbon even it is not experiencing net growth, thus a young forest holds less carbon but it is sequestering additional carbon overtime (Baishya et al 2009). In a forest ecosystem tree biomass varies with forest type (Li et al 2008), species composition (Behera et al 2017), girth class (Mohanta et al 2020; Mani and Parthasarathy 2007), stand age (Paragasan 2020), site conditions (Chave et al 2005), edaphic factors (Clark and Clark 2000) and altitude (Swapna and Jayakumar 2020). Studies on biomass of forest vegetation are essential for determining storage of the carbon in the dominant tree component which will help in computing the carbon cycling at regional as well as global level (Behera et al 2017).

## MATERIAL AND METHODS

**Study area:** The present study was carried out in the forests that lie in the foot hills of Seshachalam Biosphere Reserve which include low hill ranges such as Thurupukondalu, Palakondalu and Veligondalu (Fig. 1) which occur in between (14°04'24.3'' N 78° 57' 44.2'' E and 13°48'25.1'' N 79° 25.1' 13.9'' E of southern Eastern Ghats. These hill ranges mainly comprise of dry deciduous forests with an elevation range of (160-851m). *Pterocarpus santalinus* (Red sanders) was found to be the most dominant tree followed by *Anogeissus latifoliia* and *Chloroxylon swietenia*. The climate is hot and dry with a maximum mean temperature rising to 45.3°C and the minimum mean temperature of 16°C. The mean annual normal rainfall is 677 mm. The study sites comprise of shallow red ferruginous loam soil derived from Shales, Quartzites and Sandstone primary rocks. Soils are weak acidic in nature in the range of 5.8 to 6.5, soil organic carbon (SOC) was in the range of 0.34 % to 1.59% and soil bulk

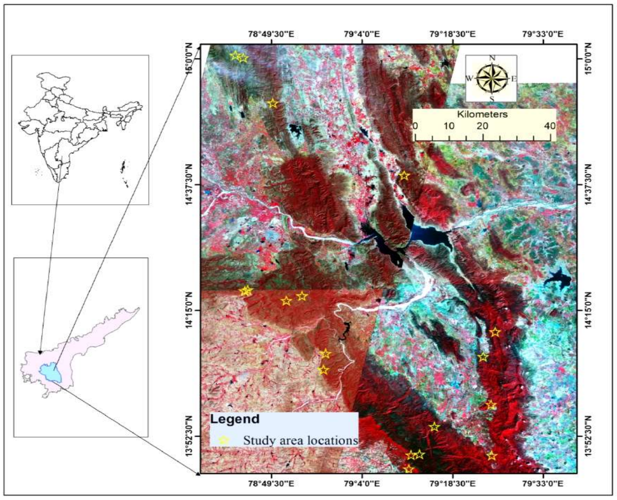


**Fig. 1.** Location of the 18 (1-ha) forest study sites in Seshachalam hill ranges

density in the range of 1.13 g/cm$^3$ to 1.67 g/cm$^3$ (Ramana and Reddy 2019).

**Field methodology:** A total of ten belt transects of (10 x 100 m) size were randomly laid at each of the 18 dry deciduous forest study sites of Seshachalam hill ranges totaling to 18 (1 ha) study area with the help of Global Positioning System (Model Garmin GPS map 78s). A marked distance of at least 50-200m was kept in laying the ten transects to cover the variations in the forest due to altitude, slope, topography and aspect in the field. Non-destructive methods (indirect method) are often preferred over destructive methods (direct method) for the estimation of AGB owing to their increasing reliability in the estimation (Brahma et al 2021). In each transect, all trees that have attained ≥30 cm gbh were enumerated and AGB was calculated by using the species specific volume equation for each of the tree individual (≥30 cm gbh) in one hectare and all these individual AGB values were added to a get a cumulative value at 1-ha level. The volume allometric equations comprise of variables such as tree diameter, tree height and wood specific gravity (Mohanta et al 2020). Above Ground Biomass (t ha$^{-1}$) = Volume of tree × Wood specific gravity (g cm$^{-3}$) (FSI 1996, Rao and Rao 2015, Salunkhe et al 2016). The relationship between estimated above ground biomass and forest structural variables such as tree density, tree diversity, basal area was carried out by Pearson correlation method. Below ground biomass (BGB) of a tree is related to its above ground biomass (AGB) and is estimated based on the Regression equation provided by (Cairns et al 1997). BGB = exp [-1.059+0.884*ln (AGB) +0.284].

## RESULTS AND DISCUSSION

The tree inventory in 18 (1-ha) dry deciduous forest study sites of Seshachalam hill ranges has yielded a total of 6483 tree individuals (≥30cm gbh) belonging to 110 tree species and 43 families. The range of tree density was 193-471 tree individuals ha$^{-1}$ and tree diversity was in the range of 19-47 ha$^{-1}$. (Ramana and Reddy 2020). The estimated mean above ground biomass (AGB) was 109.17 ± 23.35 t ha$^{-1}$ and it varied from 70.36 t/ha to 156.64 t ha$^{-1}$ for trees ≥30cm gbh across the study sites. Across the study sites at 1-ha scale the BGB was in the range of 34.45 t ha$^{-1}$ to 74.39 t ha$^{-1}$ with a mean of 53.35 t ha$^{-1}$. AGB was found to have positive relationship with tree density (r = 0.4) and tree diversity (r = 0.42) although the relation was non-significant. While, AGB showed a significant positive relationship with Basal area (r = 0.89).

The top ten species have accounted for 90.1% of the total AGB (Table 1) which include *Pterocarpus santalinus*, *Gardenia resinifera, Gardenia latifolia, Anogeissus latifolia, Hardwickia binata, Chloroxylon swietenia,* and *Pterocarpus marsupium* etc. While among them *Pterocarpus santalinus* is ranked as the top most species with high biomass stock contributing 62.2% of total AGB with a range of 46.8% to 79.4% at 1-ha scale and remarkably, *Pterocarpus santalinus* was the top most species in all the 18 study sites. When the top ten rank tree species with respect to AGB was considered, their respective tree density (r = 0.71; P<0.05) and basal area (r = 0.75; P<0.05; Fig. 2) values have shown a significant positive relationship with the logarithmic AGB values. The GBH class wise distribution of AGB revealed a reverse J shape curve with 70.9% of total AGB was contributed by lower gbh class (30-50 cm). The medium gbh class (51-70 cm) has featured 16.7% of total AGB and higher

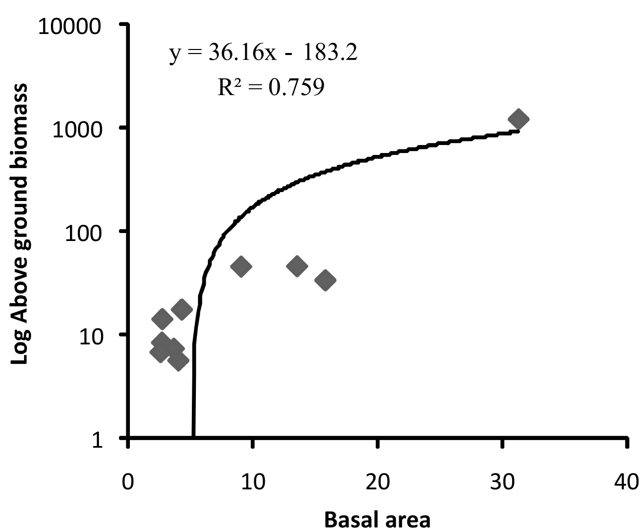


**Fig. 2.** Relationship between basal area and above ground biomass for top ten AGB dominant tree species

**Table 1.** Top ten tree species above ground biomass (AGB) values in 18 study sites

| Name of the tree species | Tree density | Basal area | Above ground biomass |
|---|---|---|---|
| *Pterocarpus santalinus* | 1666 | 31.23 | 1221.79 (62.17%) |
| *Gardenia resinifera* | 86 | 0.86 | 235.41 (11.98%) |
| *Gardenia latifolia* | 32 | 0.35 | 90.95 (4.62%) |
| *Anogeissus latifolia* | 863 | 13.52 | 46.74 (2.37%) |
| *Hardwickia binata* | 305 | 9.05 | 46.35 (2.35%) |
| *Gardenia gummifera* | 14 | 0.14 | 38.26 (1.94%) |
| *Chloroxylon swietenia* | 834 | 15.78 | 34.19 (1.73%) |
| *Pterocarpus marsupium* | 53 | 1.29 | 27.58 (1.40%) |
| *Terminalia chebula* | 169 | 4.31 | 17.87 (0.90%) |
| *Dalbergia panicualta* | 90 | 2.21 | 16.42 (0.83%) |

gbh class (>70 cm) has included just 12.3% of the total AGB. The major tree species in the higher gbh category include *Hardwickia binata, Shorea tumbuggaia, Ficus benghalensis, Terminalia alata, Madhuca indica* and *Boswellia serrata.*

Dry deciduous forests of the present study, like other tropical forest types of Eastern Ghats have showed variation in carbon stock stored in above ground biomass (C.V = 21.38% ) and below ground biomass (C.V = 20.67%). The range of the AGB values (70.36 to 156 t/ha is found to be occur within the range of AGB values that occur in dry deciduous forests of Eastern Ghats 64.81 t $ha^{-1}$ - 624.94 t $ha^{-1}$ (Gandhi and Sundarapandian 2017), Nallamalais in Southern Eastern Ghats 0.74 t $ha^{-1}$ - 205.95 t $ha^{-1}$ (Rao and Rao 2015), Bhoramdeo Wildlife Sanctuary, Chhattishgarh 101.43 t $ha^{-1}$ - 192.36 t $ha^{-1}$ (Jhariya et al 2014). But is lower than the results reported in tropical moist deciduous forest of Simplipal Biosphere Reserve 379.09 t $ha^{-1}$ - 440.62 t $ha^{-1}$ (Mohanta et al 2020), Mahendragiri hills in Eastern Ghats 102.4 t $ha^{-1}$ - 202.85 t $ha^{-1}$ (Swapna and Jayakumar 2020) and tropical moist in Brazil and dry forest in Venezuela, 144 t $ha^{-1}$ - 513 t $ha^{-1}$ (Brown et al 1989). While the results are found to be higher than the values reported from tropical deciduous forest, Madhya Pradesh 3.99 t $ha^{-1}$ - 53.90 t $ha^{-1}$ (Salunkhe et al 2014), tropical dry forest, Brazil 19.27 t $ha^{-1}$ (Junior et al 2016) and Chinnar Wildlife Sanctuary, Kerala 50.86 t/ha (Padmakumar et al 2018). Estimation of tree biomass involves mainly forest structure variables like tree density, tree height and basal area calculated from tree girth (Chave et al 2005). Hence the variation in tree biomass can be related to these variables as they vary in accordance to habitat, succession stage, tree species composition, elevation etc (Mohanta et al 2020) and the present study, the average tree GBH was 45.5cm and tree height ranged between 5m and 12m.

The correlation results indicated a positive relationship with tree diversity as also observed in dry deciduous forests of Northern Eastern Ghats (Swapna and Jayakumar 2020) indicating that increase in tree diversity may lead to increase in tree biomass to certain extent by formation of two layered forest structure leading to better partitioning of resources and light penetration (Wang et al 2010). Similarly non-significant and positive relationship with tree density and AGB can be assumed due to the presence of large number of lower girth trees in comparison to large girthed trees. The positive relationship may arise through positive feedback mechanisms like facilitation or complementary use and more species rich plant communities have a higher chance of the presence of highly productive species those may dominate the forest community (Tilman et al 1996). The results in these dry forests support the inference that the selection effect is dependent on complementarily as few dominant species such as *Pterocarpus santalinus*, *Anogeissus latifolia*, *Hardwickia binata* have contributed higher AGB and tree density. While tree species such as *Syzygium alternifolium, Dalbergia paniculata, Gardenia resinifera, Gardenia latifolia* occurred in few specific areas like water sources and middle mountain elevation sites that have led to higher species richness as well as biomass enhancement.

A strong and significant positive correlation of AGB with basal area from each of across the study sites was recorded. This kind of significant relation between AGB and basal are among different study sites having varied tree density and at species level also was observed in dry forests of Western Ghats (Padmakumar et al 2018). It suggests that among the forest structure variables like, tree density, species richness and basal area, the later variable (basal area) can be ideally used as an indicator for biomass as it combines the effect of both the number and size of trees. The results of the present study are in line with the reports provided from dry forests of Western Ghats (Padmakumar et al 2018) that low girthed young trees have shared substantially higher (70.96%) proportion of the total AGB and negated with the results showed in moist deciduous forests of Northern Eastern Ghats (Swapna and Jayakumar 2020), and Chitteri reserve forests of the Eastern Ghats (Pragasan 2014) where larger trees have majorly contributed to higher biomass values than the small girth trees. Although site wise relationship between tree density and AGB did not yield significant results but species wise relationship between tree density and respective AGB values have yielded significant relationship as also reported in dry forests of Western Ghats (Padmakumar et al 2018). Estimation of AGB studies tries to identify the predominant carbon storing species which arise due to their high wood specific gravity and greater girth values. It was suggested that IVI, AGB, and basal area are the important factors to be taken to identify the potential carbon storing species (Pattnayak et al 2020). The results suggests that Red sanders can be considered as key species with high carbon potential as it has not only high wood specific gravity, well distributed across the forest study sites in all the considered gbh class and has high ecological amplitude across the varied disturbance levels (Ankalaiah et al 2017). Thus, it can be explained due to the high dominance of few tree species and tree density being positively skewed towards the lower girth class. has influenced the AGB distribution across the study. The results indicate that the regenerating type of the present forest study sites have the potential for future carbon sequestration and especially the dominant tree species like Red sanders will play a major role in future also in these dry deciduous forests.

## CONCLUSIONS

AGB has showed a significant positive relationship with basal area and hence it can be used for biomass estimation as it combines the effect of both tree abundance and girth size in dry forests. While, the top ten rank species in regard to AGB has significant positive relationship with tree density indicating the importance of dominant tree species in the contribution to total forest biomass. *Pterocarpus santalinus* was the top dominant tree species with high biomass stock contributing 62.2% of total above ground biomass, while the top ten tree species have accounted for 90.1% of the total above ground biomass. The reverse J-shape curve of AGB distribution with 70.09% of total AGB being contributed by lower gbh class (30-50cm) indicate that these forests are of regenerating type of forests. Among the 18 study sites, lower gbh class have shared AGB in the range of 43.9%-92.4% indicating that these younger stands may take few decades to reach the equilibrium in the carbon storage ability. The estimated data is important in framing forest management plans and strategies aimed at enhancing carbon sequestration potential of dry deciduous forests.

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# Fungal Decomposition of Tree Leaf Litters in Tropical and Sub-Tropical Forests of Mizoram, Northeast India: A Laboratory Microcosm Experiment

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***Abstract***: The decomposing ability of two fungal groups was studied on two dominant tree leaf litters from tropical and sub-tropical forests of Mizoram in relation to total fungi under laboratory condition. Litters were sterilized and inoculated with 5 major fungi isolated from forest litter in relation to a control on 4 litter types from two forests. Out of ten fungi isolated, eight belongs to ascomycota and two zygomycota. Decomposing ability of the major filamentous fungi was evaluated at early (35 day, 5weeks) and later stage (70 days, 10 weeks) of litter inoculation. Percent mass lost were significantly higher at early stage (10-51% after 5 weeks) than late stage (16-85% after10 weeks) of decomposition. Corresponding values of lignin mass lost were 73-90 and 76-96, respectively at two stages. Inoculation of total and major fungi in two forest litters induced mass loss differently with a greater mass loss occurred in tropical forest litter. This study indicates considerable role of 5 major fungi in litter decomposition which will have profound effect on soil fertility management in tropical forests.

***Keywords***: Decomposing ability, Major fungi, Ascomycota, Lignin, Zycomycota

Litter decomposition is one of the major processes of cycling of carbon (C) and nutrients in the natural forest ecosystems (Krishna and Mohan 2017). In forest, the process of litter decomposition is strongly influenced by the abiotic variables, litter chemistry and microorganisms (Tripathi and Singh 1992 a & b, Tripathi et al 2006, Pandey et al 2007, Song et al 2010, Lalnunzira and Tripathi 2018, Cid et al 2019). Decomposition has been reported to be considerably higher in the tropical ecosystems than temperate or sub-tropical ecosystems (Tripathi and Singh 1992 a & b, Tripathi et al 2005, 2006, Pandey et al 2007). Further, the breakdown of organic compounds such as lignin, cellulose and holocellulose is strongly affected by the microorganism (Song et al 2010, Rahman et al 2013, Wapongnungsang et al 2017). In addition, studies suggested that organic matter decomposition in forest soil is strongly affected by certain groups of fungi which are responsible for decomposing lignocellulose matrix of the leaf litter that is not decomposed by other organism and facilitates the recycling of nutrients in the forests (Osono and Takeda 2002, Hauchhum and Tripathi 2017, Singh and Tripathi 2020).

Plant tissue consists of about two-third of organic material such as lignin and celluloses which are degraded by fungal mycelia (Pandey et al 2007, Song et al 2010, Valencia and Chambergo 2013), and thus the role of fungi in organic matter decomposition, nutrient release and plant growth (De Melo et al 2018). Studies on the abilities of pure culture of fungal inoculations in litter decomposition have been carried out under laboratory conditions with proper management implications (Osono and Takeda 1999, Song et al 2010, Do Nascimento Barbosa et al 2016, De Melo et al 2018). However, studies on tropical forests are highly limited. This study was conducted under laboratory conditions understand the decomposing abilities of total and major filamentous fungi on litter decomposition in tropical and sub-tropical forests of Northeast India.

In temperate and sub-tropical ecosystems, studies reported that fungi belonged to Taxa (Ascomycota) have the ability to decompose lignin during litter decomposition (Osono and Takeda 1999, Song et al 2010). Litter decomposing abilities of fungi reported from various ecosystems have been reported as tool for the nutrient management in the forest ecosystem under oligotrophic conditions (Osono and Takeda 2002, Song et al 2010, Valencia and Chambergo 2013). Most of the tropical forest soils are highly weathered leached and often exhibit nutrient limitations (Tripathi and Singh 1994, Tripathi et al 2008). Therefore, such studies will be useful in regulating soil nutrients in these forests by microbial management of litter decomposition.

Ascomycota and Zycomycota are two important fungal groups reported to be responsible for litter decomposition (Štursová et al 2020, Osono and Takeda 2002, Voříšková and Baldrian 2013, Jiang et al 2014). In this study, we isolated ten major filamentous fungi (5 from each forest)from the forest litter based on frequency of their occurrences, and inoculated them separately on two dominant species of tropical (i.e. *Melocanna baccifera* and *Tectona grandis*) and sub-tropical (i.e. *Quercus floribunda* and *Drypetes indica*) litters in relation to total fungi. This study aims to understand decomposing ability of major fungi and total fungi in litter of two forest settings. We hypothesize that fungi with higher frequency of occurrence in the organic layer of the forest has greater ability to decompose litter and the decomposing ability of fungi changes with litter types from two forest settings. The study has strong implications for nutrient management in tropical and sub-tropical forests.

## MATERIAL AND METHODS

**Study sites:** Two forest sites were selected in Aizawl district of Mizoram (Fig. 1) with varying altitudes. Tropical moist deciduous forest was situated in Sairang at 23°49.2`N lat and 92°39.5`E long at an elevation of 110 m amsl. Sub-tropical semi-evergreen forest was located at Hmuifang at 23°27.2`N lat and 92°45.2`E long at 1455 m amsl**.** Tropical forest at Sairang was dominated by naturally occurring species of bamboo (*Melocanna baccifera,* MB) and Teak (*Tectona grandis,* TG) planted 20-30 years ago. In sub-tropical forest at Hmuifang, naturally occurring Oak (*Quercus floribunda,* QF) and (*Drypetes indica,* DI) were the most dominant tree species (>80 yrs old).

**Isolation and identification of fungi:** Fermented litter collected from the forest floor were cut into small pieces (approx. 3 mm) and5 pieces were placed on PDA (Potato Dextrose Agar) containing Rose4Bengal (~ 0.8%) in Petridish, and a total 200 pieces were placed in 40 petri plates from each site. The petridish containing media were incubated in the dark at 25°C which was monitored regularly (every day) for the growth of occurring fungi (total fungi). Samplings of five major fungi from each site having highest frequencies of occurrence were sub-cultured onto other petridish containing PDA media for pure culture and identification. All 5 pure cultures of fungi were transfers to one petridish (PDA media) and mark as major fungi (Fig. 2). Spores of pure cultures of fungi were identified to genus and species level with the help of a microscopic (10x and 40x magnification, Olympus CX41, Japan) on the basis of culture characteristics. Identification was based on the published descriptions as described by Gilman (1957), Ellis (1976), Nelso et al (1983), Barnett and Hunter (1972).

**Experiment design and Procedure:** Modified experiment based on Song et al (2010), freshly collected leaf litter of the dominant tree species were disked (2.0 cm in diameter) with a metal borer. Disks were dried (36 h at 60°C) and weighed to obtain the original weight of the leaf disks. Leaf disks were pressed in moistened paper towels between the base and lid of a petridish, then autoclaved at 120°C for 20 min. The sterilized leaf disks were inoculated with the 5 major fungal colonies and other sterilized disk were inoculated to total fungal colonies which were placed on petridishes and uninoculated dishes served as control. A total of 100 leaf disks (25 disks from each tree species including control) were incubated at 26°C and 90% humidity in the dark (Osono and Takeda 2002).

**Estimation of litter mass loss rates:** After incubation, leaf litter was removed carefully and cleaned with a small brush and sterilized with water and oven dried at 60°C for 36 h and weighed. The original mass was estimated with the same method before inoculation. Mass loss of leaf litter at early stage and late stages were considered from the initial dry weight. Decomposing ability of the two groups of major filamentous fungi was evaluated as litter weight lost (%). Samples of decomposing material were harvested at the end

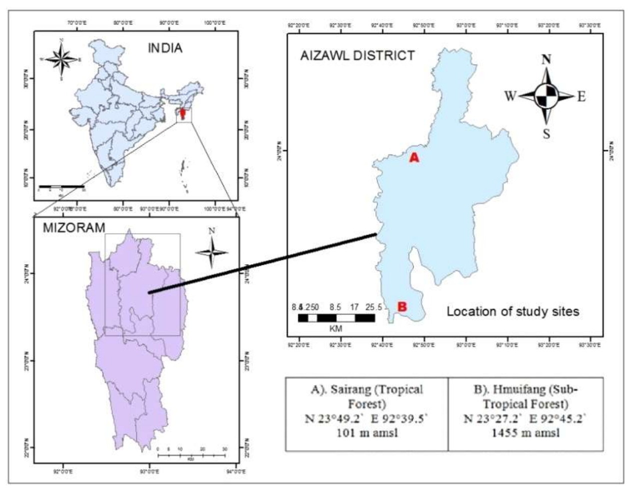


**Fig. 1**. Geographical map of Mizoram showing two forest sites


**Fig. 2.** Inoculation of litter in media (Potato Dextrose Agar) showing different stages of decomposition

of the 5th week (day 35) and 10th week (day 70). Weeks 1–5 marked as early stage and weeks 6-10 week marked as late stage of litter decomposition.

The mean relative decomposition rate (RDR) was calculated by using the following formula:

RDR (mg $g^{-1}$ $d^{-1}$) = *ln* ($W_1$– $W_0$)/($t_1$– $t_0$), where $W_0$ = weight of litter present at time $t_0$; $W_1$ = weight of litter present at time $t_1$, and $t_1$ – $t_0$ = sampling interval (days). The equation gives the relative weight loss rate; the negative sign of the value, however, was ignored.

**Analysis of lignin and nutrients**: Oven dried leaf samples were ground in the laboratory for analysis of lignin using Fibrotron Automatic Fibre Analysis, Model: FRB 6, Version 1, Tulin Equipments, Chennai, India. Nutrient concentrations in initial litter were analyst using CHNS Analyser and Thermo Scientific ICP spectrometer (iCAP 6000 series), Manchester Metropolitan University (UK). Data were compared and analyzed statistically using one-way ANOVA (Analysis of Variance) followed with Least Significant Difference (LSD) in MS Excel 7, and compared between leaf species from both sites. Pearson correlation was performed to determine relation of litters within initial dried mass and mass remaining at the end of each stage (i.e. early and later stage) inoculated with major fungi and total fungi and the mass remaining between two stages. Lignin mass loss rates in initial, early and later stages were individually performed for each litter type.

## RESULTS AND DISCUSSION

**Initial litter chemical composition and isolation of major fungal groups from two forests:** Chemical composition of different leaf litter categories showed wide variations, for example, variations in lignin concentrations were 15–23%. Per cent lignin concentration was higher in QF, whereas, per cent C concentration and C:N ratio were higher in TG. MB had minimum lignin, C and C:N ratio, whereas, concentrations of N, P, Mg and Mn were higher compared to other leaf litter categories. K content was higher in TG and lower in DI while Na content (~ 0.01%) did not vary with different species litters (Table 1). The major fungal groups from two sites belonged to Ascomycota (*Phoma* spp., *Cladosporium* spp., *Aspergillus niger, Myrothecium verrucaria, Tricoderma viride, Periconia macrospinosa, Penicilium* spp. and *Chaetomium* spp.) and Zygomycota (*Rhizopus* spp. and *Mucos* spp.) (Table 2). After 2 weeks of fungal inoculation, 2-3 (dominant) fungal colonies began to occupy the whole petridish (Fig. 2), reflecting strong competition within fungal colonies for resource utilization during the course of litter decomposition.

**Litter dry matter and nutrient dynamics during decomposition:** After 5 weeks, maximum mass loss (51.8%) occurred in TG litter inoculated with total fungi followed by 45.8% in MB inoculated with major fungi. Whereas, minimum mass loss (9.8%) occurred in QF inoculated with major fungi. In litter (e.g. MB and DI) higher mass loss (45.8 and 22.6%) rates were observed in plates inoculated with major group of fungi compared to total fungi (42.8% and 17.3%) (Fig. 3). Significantly strong to moderate positive correlations were observed between initial and final weight remaining in MB (r = 0.88), QF (r = 0.79) and TG (r =

**Table 2**. Major filamentous fungi isolated from two forest sites

| Sites | Fungal species | Taxa |
|---|---|---|
| Tropical forest | *Phoma* spp. | Ascomycota |
| | *Cladosporium* spp. | Ascomycota |
| | *Aspergillus niger* | Ascomycota |
| | *Myrothecium verrucaria* | Ascomycota |
| | *Tricoderma viride* | Ascomycota |
| Sub-tropical forest | | |
| | *Rhizopus* spp. | zygomycota |
| | *Mucos* spp. | zygomycota |
| | *Periconia macrospinosa* | Ascomycota |
| | *Penicilium* spp. | Ascomycota |
| | *Chaetomium* spp. | Ascomycota |

**Table 1**. Initial chemical composition of four litter types used for fungal inoculations to assess the decomposing ability of two groups of fungi

| Litter type | ADL (%) | C (%) | N (%) | CN ratio | P (%) | Na (%) | Mg (%) | K (%) | Mn (%) |
|---|---|---|---|---|---|---|---|---|---|
| MB | 15.2 ± 0.34 | 37.04 ± 0.02 | 2.22 ± 0.02 | 16.69 | 0.09 ± 0.003 | 0.01 ± 0.003 | 0.19 ± 0.006 | 0.36 ± 0.004 | 0.04 ± 0.001 |
| TG | 21.6 ± 0.32 | 47.64 ± 0.03 | 1.04 ± 0.04 | 45.68 | 0.05 ± 0.001 | 0.01 ± 0.00 | 0.13 ± 0.003 | 0.45 ± 0.007 | 0.01 ± 0.00 |
| DI | 22 ± 0.38 | 42.85 ± 0.09 | 0.99 ± 0.09 | 43.46 | 0.02 ± 0.00 | 0.01 ± 0.00 | 0.06 ± 0.001 | 0.09 ± 0.004 | 0.03 ± 0.002 |
| QF | 22.4 ± 0.29 | 47.54 ± 0.15 | 1.14 ± 0.02 | 41.63 | 0.02 ± 0.003 | 0.01 ± 0.00 | 0.10 ±0.002 | 0.14 ± 0.004 | 0.06 ± 0.005 |
| LSD (p=0.05) | 1.1 | 0.31 | 0.05 | NA | 0.006 | 0.01 | 0.015 | 0.03 | 0.008 |

# (mean±1SE, n=3, NA = Not applicable)

65) litters inoculated with major fungi ($p < 0.05$). In case of total fungal inoculation significant ($p<0.05$) positive correlation was observed between initial and final weight remaining of MB ($r = 0.5$), QF ($r = 0.72$), TG ($r = 0.77$) and DI ($r = 0.81$). Mass remaining in MB litter inoculated with major and total fungi was weakly positively correlated ($r = 0.24$), whereas, moderately positively correlated with TG ($r = 0.67$, $p <0.05$) and QF ($r = 0.69$, $p <0.05$) litters and strongly negatively correlated ($r = - 0.72$) with DI.

After 10 weeks, significantly higher mass loss rates 85.2% and 79.1% occurred in TG and QF inoculated with total fungi compared to other leaf litter. Whereas, minimum mass loss (15.8%) was recorded in QF inoculated with major fungi. Maximum mass loss (51.8%) occurred in MB and minimum in QF (15.8%) with major fungal inoculation. In case of total fungi inoculation, greater mass loss 85.2% and 79.1% occurred in TG and QF, whereas, minimum mass loss (32.2%) was observed in DI (Fig. 3).

In general, per cent litter mass loss ranged from 10-46 in early stage and 16-52 in later stage in MF inoculation (Fig. 4). The per cent mass loss (6-12%) varied between the two stages of decomposition. Among the litters, maximum mass loss was observed in MB followed by TG and minimum in QF during the two stages of decomposition. TG showed maximum mass loss (11 %) between two stages followed by 9 % in DI and minimum (5 %) in MB and QF inoculated with

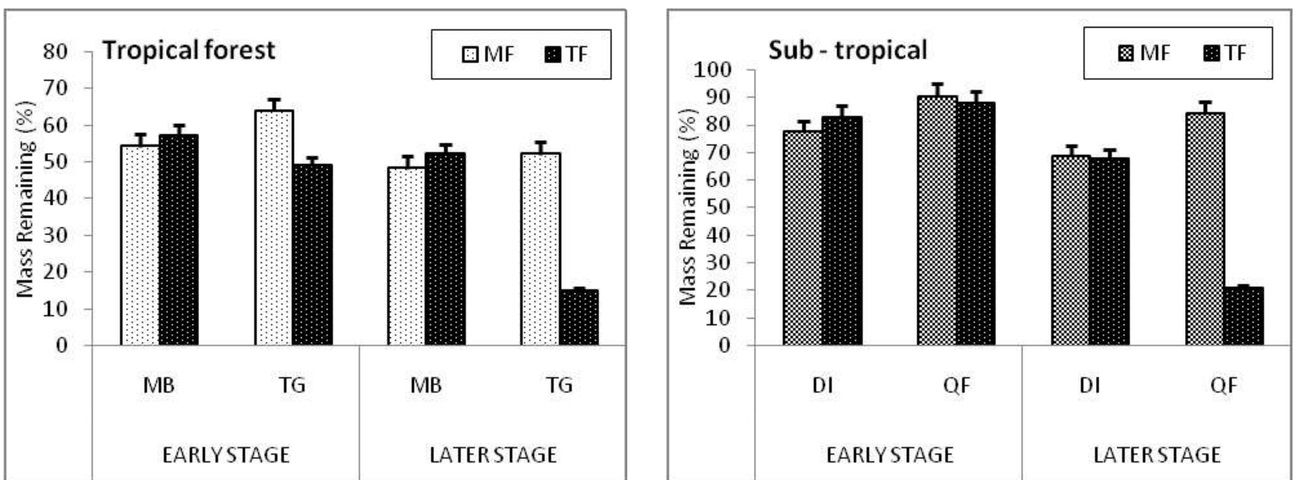


**Fig. 3.** Mass remaining (%) of litter (MB and TG ) in tropical forest and sub tropical forest (DI and QF) after 5 weeks (early stages) and 10 weeks (later stages). Mean ± 1SE, n=5; MF= Major fungi; TF= Total fungi

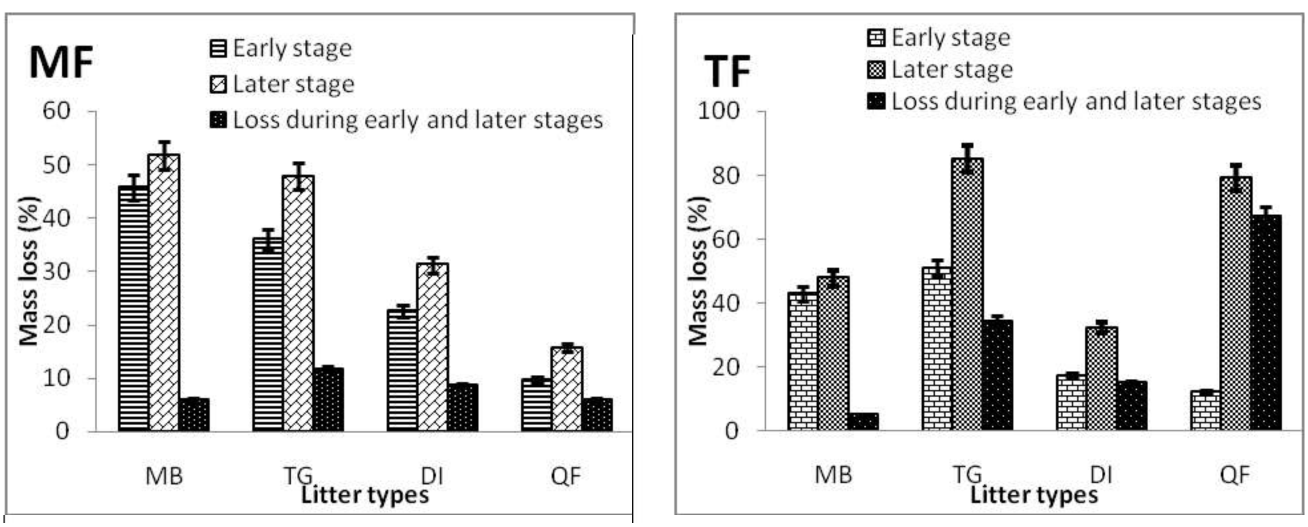


**Fig. 4**. Per cent litter mass loss in various leaf litter types inoculated with major fungi (MF) and total fungi (TF) at different time period (i.e. early stage, later stage and during early and later stage)

MF (Fig. 4). In TF inoculation, per cent litter mass loss ranged from 12-51 in early stage, 32-85 in later stage and 5-67 per cent between two stages of decomposition (Fig. 4). In early stage, maximum mass loss was observed in TG (51%) followed by MB (43%) and minimum in QF (12%). At later stage, maximum per cent litter mass loss was observed in TG (85%) followed by QF (79%) and minimum in DI (32%). About 5-67% of litter mass loss occurred during early and late stage in all litter inoculated with TF. Maximum mass loss occurred in QF (67%) followed by TG (34%) and minimum in MB (5%) (Fig. 4).

Overall RDR (mg $g^{-1}$ $d^{-1}$) ranged from 0.03-0.1 (Fig. 5). In general, higher RDR values were observed during early stage (i.e. 0.06-0.1) of decomposition in different litters compared to late stage (i.e. 0.03-0.09) except MB. Among the two fungal inoculations, litters inoculated with MF showed higher RDR (i.e. 0.06-0.1) compared to TF (i.e. 0.03-0.9). Among various litter types, highest RDR value (0.1) was observed in QF litter inoculated with MF and lowest RDR value (0.03) was recorded in QF litter inoculated with TF (Fig. 5).

**The effect of tree species litters, duration and fungal inoculations on mass loss rates:** Mass remaining of four litter types (i.e. MB, TG, DI & QF) after two stages (i.e. 5 weeks & 10 weeks) of decomposition and two inoculations (major and total fungi) showed significant differences (Table 3). More specifically, in MB litter significant difference in the mass loss was observed between two stages of decomposition (F= 55, P<0.01) and fungal inoculations (F=4.6, P<0.05), whereas, interaction was not significant (Table 3). In TG litter, mass loss was significantly different with two stage of decomposition (F=229, P<0.01), fungal inoculations (F=310, P<0.01) and their interactions (F=96, P<0.01) were also significant. Similarly, effects of two stages

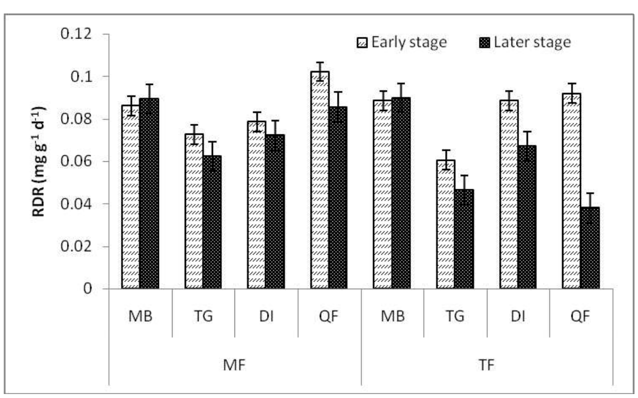


**Fig. 5.** RDR (mg $g^{-1}$ $d^{-1}$) value in various litter types, inoculated with major fungi (MF) and total fungi (TF) at different time period (i.e. early stage, later stage and during early and later stage)

**Table 3**. Lignin remaining after 5 weeks (early stage), 10 weeks (later stage) and loss during 5-10 weeks of different leaf discs from two sites inoculation with major fungi and total fungi

| Leaf Species | Incubation type | After 10 weeks (%) | Upto 5 weeks (%) |
|---|---|---|---|
| MB | Major fungi | 10.42 | 10.21 |
| | Total fungi | 12.53 | 11.88 |
| TG | Major fungi | 13.13 | 14.65 |
| | Total fungi | 4.14 | 10.96 |
| DI | Major fungi | 17.87 | 17.64 |
| | Total fungi | 14.37 | 19.85 |
| QF | Major fungi | 24.25 | 22.01 |
| | Total fungi | 4.85 | 27.41 |
| LSD (0.05) | - | 17.51 | 6.96 |

(F=167 & 915, P<0.01), fungal inoculations (F=20 & 552, P<0.01) and their interactions (F=37 & 1077, P<0.01) on mass loss rates were significant in DI and QF (Table 3).

**Initial litter quality and fungal isolation:** In the present study, initial litter quality varied in different litters. Wide variations have been noted in the litter quality of various litters in different tropical and sub-tropical forest ecosystems (Tripathi and Singh 1992a, Pandey et al 2007). Per cent lignin concentration in the presently studied litter (15-23%) was comparable to lignin content in oak leaf litter sub-tropical forests in northeast India (Pandey et al 2007). However, the values were lower to recent findings of Razak and Anwar (2020) in mahogany leaf (41.17%) in sub-tropical region of Indonesia and Trevisan and Rezende (2020) in elephant grass (25%) of humid and sub-tropical region of Brazil. The variations in initial quality of litter in different tropical species litter may be related to the intrinsic quality of species leaf, prevailing climate conditions and soil parameters of the region.

In the present study, 10 major fungi isolated out of total fungi from organic layer of two forests belonged to Ascomycota and Zygomycota. Voříšková and Baldrian (2013) reported that majority of the sequences of fungi from senescent oak leaves belonged to Ascomycota. Štursová et al (2020) also reported that dominance of Ascomycota in initial period of decomposition in montane forest of Czech Republic. Further, in another report from cool temperate deciduous forest of Japan, about 60 belonged to Ascomycota out of total 79 isolated fungi (Osono and Takeda 2002). *Penicillium* sp. and *Aspergillus* sp. were the dominant species belonging to Ascomycota have been found to potentially affect litter decomposition in mixed pine and broad leaf forest of China (Jiang et al 2014). The presence of these two species in the present study is in conformity with other studies reported elsewhere (Štursová et al 2020, Osono and Takeda 2002, Voříšková and Baldrian 2013, Jiang et al 2014).

**Mass loss and substrate utilization pattern:** In the present study at the end of 5 weeks, significantly higher mass loss in TG and MB inoculated with total fungi compared to other species litter like DI & QF is related to the initial litter chemistry particularly lower initial lignin content and the dominance of major fungi belonging to Ascomycota. Initial lignin content and dominance of group of fungi belonging to Ascomycota have been reported to significantly affect mass loss in different ecosystems over the world (Song et al 2010, Wapongnungsang et al 2017). However, at the end of 10 weeks, significantly higher mass loss rates in TG and QF inoculated with total fungi compared to other leaf litter were the result of the presence of certain fungal species with lower frequency of occurrences and higher ability to decompose litter from the total fungal group. Further studies would be required to identify such species from tropical regions.

Overall, significantly greater mass loss in the early stage than the late stage in both (MF & TF) inoculations (Fig. 3) indicated availability of more resources in the initial litter that fuel the fungal growth and as a result the causes higher decomposition (Song et al 2010, Wapongnungsang et al 2017, Osono and Takeda 2002). According to the substrate utilization fungi can be divided into three functional groups, for example, sugar fungi, cellulose decomposers and lignocellulose decomposers which are responsible for utilizing/degrading soluble sugars, carbohydrates with slight lignin and lignocellulose complexes (lignin and carbohydrate in various proportions), respectively (Song et al 2010, Osono and Takeda 2002). The litter material had abundant soluble sugars in the early phase of decomposition which are preferentially utilized by fungi causing significant mass loss in early stage (Tripathi and Singh 1992b). Further, results suggest that the group of 5 major fungi and the total fungi from the two forests are diverse in terms of their substrate utilization pattern and therefore, they degrade sugars, carbohydrates and celluloses in the first phase of decomposition causing greater loss followed by the degradation of lignocellulose in the second stage of decomposition exhibiting slow decomposition.

The two forest sites vary with respect to their climate and vegetation, and thus they differ in the quality of their litter which attracted variety of fungal organisms based on their feeding habits. The group of major fungi belonged to Ascomycota and Zycomycota observed more ability to decompose the selected leaf litter in both early and later stages (Osono and Taketa 2006). However, the group of total fungi in TG observed higher rate of decomposition compared to other litters. In some plates (e.g. DI), mass loss rates in both major and total fungi were lower compared to other litters. Group of major fungi (e.g. MB) showed higher decomposition in leaf litter as result of changes in the litter substrate of this species (Fig. 3), particularly lowest lignin. Litter with low initial lignin content decomposes faster than the litter having high lignin as lignin is recalcitrant to decomposition (Tripathi and Singh 1992a, Pandey et al 2006). *Aspergillus* and *Penicillium* have been reported as lignocellulose decomposers which cause high mass loss in different litter types (Song et al 2010). Osono and Takeda (2002) reported that Ascomycota and Zygomycota do not show bleasing and caused low weight losses of litter mass. Fungi belongs to Basidiomycota have more ability to decompose lignin and carbohydrate in different stages of litter decomposition (Osono and Takeda 2002, Trevisan and Rezende 2020).

During the study, litter mass remaining at the end (after 10 weeks) ranged from 14-85%. The group of fungal Ascomycota isolated from tropical forest on MB and TG showed maximum decomposition ability compared to mix group of fungal Ascomycota and Zygomycota from sub-tropical forest on DI and QF (Fig. 3). Some Ascomycetes species showed decomposition ability of cellulose in *Abies* and *Betula* (Osono and Takeda 2002). Fungal species such as *Penicillium* have shown decomposition of carbohydrate in some leaf litter (Song et al 2010, Osono and Takeda 2002, Lindeberg 1946, Saito 1960, Hering 1967). On other hand, Osono and Takeda (2002) have reported Basidiomycetes, Ascomycetes and Zygomycetes caused very less or negligible amount of mass loss in *Abies* and *Betula* leaf litter. *Trichoderma* species have less ability to decompose litter and regarded as parasitic fungi (Osono and Takeda 2002 & 2003). Greater lignin degradation in major fungal inoculation than in total fungi reflects major role of dominant fungi over the total fungi on the organic layer. The uses of lignin in leaf litter by fungal diversity depend on the availability of soluble matters such as the amount of nutrients (particularly N) in the litter that influences the ability of the fungi to decompose litter on the forest floor.

## CONCLUSIONS

Two most abundant fungi found on the forest litters are responsible for causing considerable mass loss in the litter because of the strong competition for resource utilization between the two major fungal groups (i.e. Ascomycota and Zygomycota). Few fungal species out compete the initial dominant fungal species during the course of decomposition, for example, after 10 weeks of inoculation requires proper identification and their ability to degrade lignocellulose. This study indicates considerable role of major fungi in litter decomposition which will have profound effect on soil fertility management in tropical and subtropical forests.

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# Diversity and Composition of Herbaceous Component in Behali Reserve Forest of Biswanath District, Assam, India

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***Abstract***: The present paper was aimed to study the flora and composition of the herbaceous species in Behali Reserve Forest (Assam, India). For this purpose, in 2019-2020, we established nine study sites with various number of 1 × 1 m study plots per each. In total, 77 plant taxa belonging to 71 genera and 33 families were reccorded. The families Acanthaceae (7 species), Asteraceae (6 species), Poaceae (6 species), Lamiaceae (5 species) and Asparagaceae (5 species) were richest in terms of number of species. The most abundant species were *Cynodon dactylon* (235 individuals), *Piper sylvaticum* (145 individuals), *Ageratum conyzoides* (103 individuals), and *Amischotolype hookeri* (101 individuals). The same species plus *Rhaphidophora glauca* were considered as dominant in herbaceous plant communities studied in the Behali Reserve Forest. The protected area is recognized as one of the richest herbaceous flora among other Protected Areas in the Northeast India. At the same time, some "white gaps" remained in the Behali Reserve Forest. Only the establishing of dense and relatively even net of the study sites along this Protected Area will allow to obtain the complete knowledge about herbaceous flora of the Behali Reserve Forest, although the main proportion of the flora and vegetation structure will be changed insignificantly.

***Keywords***: Eastern Himalaya, Flora structure, Semi-evergreen forest, Species richness, Ground flora

Forests are complex ecosystem composed of trees that buffer throughout the globe supporting numerous life forms and creating a special environment. In turn, it affects the diversity of animals and plants that can exist in these ecosystems (Wanjohi et al 2017). FAO (2010) defines forest as land with trees that covers more than 10% of the total area over 0.5 hectares. They are home to 80% of the world's terrestrial biodiversity including 60 million indigenous people (FAO 2018). The herbaceous vegetation represents less than 1% of the biomass of the forest, yet contains about 90% of the plant species and contributes up to 20% of the foliar litter (Gilliam 2007). Though being the site of intense competitive interactions, the herb layer directs the development of forests (Gilliam 2007). The biodiversity loss occurs worldwide and at an ever increasing rate seen so far (Pimm et al 2014, Le Roux et al 2019). This especially concerns the forest ecosystems near the areas with the high human population density (Coritico and Amoroso 2020). The high land use can negatively influence the ecosystem through extinction of native elements and introduction of invasive species (Gilliam 2007). The species richness is higher in the herbaceous layer than in any other forest strata, however threat level assessments are often provided for the arboreal species (Ungricht et al 2005, Callmander et al 2007, Linnik and Khapugin 2020) by neglecting the herbs (Inceer and Kalmuk 2019, Wagensommer et al 2020). This is ironic because herbaceous species have higher natural extinction rates than plant species in other strata. Even, Levin and Wilson (1976) found that extinction rates of herbs are three times more than of hardwood tree species and approximately five times more than of gymnosperms. Biological diversity is a central topic of ecological theory and is the subject of many recent discussions (Loreau 2010). Currently, researchers have developed a large number of parameters for the measurement of biodiversity as an indicator of the status of ecological systems, with practical applicability for conservation, management and environment monitoring (Kanieski et al 2017). Yet the basic knowledge on the flora and structure needs to be addressed first.

Some studies has been conducted on determining the structure and composition of the forests as well as private gardens in Assam (Borah and Garkoti 2011, Dutta and Devi 2013 a,b, Sarkar and Devi 2014, Sarma and Borah 2014, Borah et al 2016, Deka and Sharma 2016, Saikia and Khan 2016, Bora and Bhattacharyya 2017, Borogayary et al 2017, Barua et al 2018). However, by considering the diversity of elements in this wide range of forests, the data seem to be negligible. One of the only left pristine forest patches in the entire Biswanath district of Assam is the Behali Reserve Forest (BRF) known in the foothills of Eastern Himalaya. So far no data on the vegetation cover structure and plant diversity is known for this Protected Area. However, the recently intensified floristic studies have been carried out during last five years, which resulted in the discovery of two new species *Chlorophytum assamicum* (Asparagaceae) (Borah et al 2019a), *Aristolochia assamica* (Aristolochiaceae) (Borah et al 2019b), and a new variety *Peliosanthes macrophylla* var. *assamensis* (Asparagaceae) (Borah et al 2020a). As well as one more species, *Tupistra stoliczkana* (Asparagaceae) (Borah et al 2020b) has been rediscovered after a century. In present paper we aimed to study the diversity, composition and structure of herbaceous angiosperms in the Behali Reserve Forest of the Assam state.

## MATERIAL AND METHODS

The study was conducted in BRF, a semi-evergreen forest (Champion and Seth 1968), presented in the foothills of the Himalayas. It is located in the Biswanath administrative district of Assam state, northeastern India between 26°52′20.08″N and 26°57′33.17″N, and 93°11′30.58″E and 93°23′21.09″E. BRF spans over an area of 14.16 $km^2$ area bordering River Buroi in the east, River Borgang and Singlijan Reserve Forest in the west, Papum Reserve Forest in the north, and tea plantations and human habitations in the south (Sarma et al 2009). It experiences a tropical monsoon, with 1800 mm mean annual precipitation and 24.8°C mean annual temperature (Sarma et al 2009, Upadhaya 2016-2017). The elevation ranges between 90 m a.s.l. and 110 m asl, comprising mostly plains, with some undulating hillocks on the north (Sarma et al 2009, Upadhaya 2016-2017). Numerous annual and perennial streams, wetlands, swamps, mixed grasslands are also present within the reserve that provides shelter to different forms of life. Agriculture is present in almost all sides of the reserve and degradation has severely hampered its boundaries. Shifting cultivation is seen in the North boundaries, whereas settled agriculture is predominant in the south and east.

**Sampling and study design:** We studied the herbaceous cover of semi-evergreen forests in BRF. We were not sampling the total species diversity because of the large coverage of the Protected Area. The study was based from randomly sampled nine plots namely falling in three zones, east (Naharjan-Thandapani, Sialmari and Serelia Bongaon), central (Hatidipu, Hatimara and Rangagorha beat) and west (Dikal, Radhasu and Siklibandha Tiniali) parts. The nine study sites have been studied from October 2019 to January 2020. They covered three zones (east, central and west) of the forest. The study plots spanned almost all the sides and sub-vegetation types based on the forest homogeneity (dense forests, grasslands, open forests and wetlands). The location characteristics of the study sites are presented in Table 1. At the selected plots, a total of 96 quadrats of 1 × 1 m were placed randomly by representing 32 quadrats per each zone in October 2019-January 2020. All the plant individuals were tagged, measured and collected giving a specific collection number that was used throughout the field studies. The collected samples were later processed following the methods of Jain and Rao (1977). It was then identified using Hooker (1872-1897), Kanjilal et al (1934-1940) and consulting the regional herbaria (ASSAM) and submitted in HAU (Herbarium of Rajiv Gandhi University, Arunachal Pradesh).

**Data analysis:** Abundance, density, frequency occurrence, species richness were calculated according to Magurran (1988). For each species, Importance Value Index (IVI) was calculated, expressed as the sum of relative density, relative abundance, and relative frequency of species in and among plots (Curtis 1959, Bhadra and Pattanayak 2016). On the basis of these parameters, the flora of the Behali Reserve Forest was characterized. All calculations were performed using PAST (Hammer et al 2001) and Microsoft Excel.

## RESULTS AND DISCUSSION

**List of herbaceous plants in the Behali Reserve forest:** The forest of BRF was found to be very rich and diverse in terms of herbaceous plant species. The analysis of selected nine study plots indicated instance of a total 77 species belonging to 71 genera representing 33 families. A list of taxonomic diversity in terms of family and their occurrence in BRF are provided in Table 2.

The following plant families were characterized by the highest number of species: Acanthaceae (7 species), Asteraceae and Poaceae (6 species each), Lamiaceae and Asparagaceae (5 species each), Zingiberaceae, Commelinaceae, Orchidaceae, Fabaceae, Rubiaceae and Polygonaceae (3 species each), Araceae, Lythraceae, Cyperaceae, Urticaceae, Vitaceae, Onagraceae, Marantaceae and Pontederiaceae (2 species each). The rest 14 families contain one species each. The genera *Clerodendrum*, *Ludwigia*, *Persicaria* and *Pontederia* included two species each, while the rest genera contained one species only.

**Herbaceous flora characteristics:** For the studied flora, we distinguished four species group in terms of the species abundance (Fig. 2). The abundant species were represented by four species, *Cynodon dactylon* (235 individuals), *Piper sylvaticum* (145 individuals), *Ageratum conyzoides* (103 individuals), and *Amischotolype hookeri* (101 individuals). The group of common species included twelve taxa (in total, 611 individuals), including *Axonopus compressus* (92 individuals), *Rhaphidophora glauca* (73 individuals), *Acmella paniculata* (63 individuals), *Eleusine indica* (62 individuals), *Tupistra stoliczana* (53 individuals), *Digitaria* sp. (50 individuals), *Bonnaya ruellioides* (48 individuals), *Oplismenus burmannii* (45 individuals), *Dracaena petiolata* (37 individuals), *Cuphea carthagenensis* (34 individuals) and *Kyllinga* sp. (29 individuals). The group of rare species consisted of 36 species (in total of 317 individuals). Examples include some narrow endemic species such as *Strobilanthes paniculiformis*, *Chlorophytum assamicum*, etc. And the group very rare species consisted of 20 taxa. Of them, eight species were represented by two individuals each such as *Aristolochia assamica*, *Begonia silletensis, Corymborkis veratrifolia*, and twelve species by one individual each such as *Lepidagathis incurva, Peliosanthes macrophylla var. assamensis, Pontederia vaginalis*.

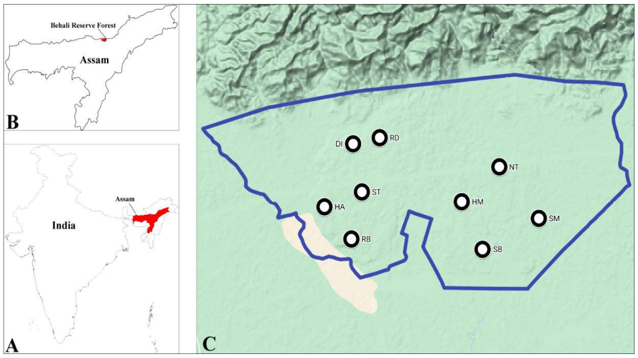


**Fig. 1.** Location of the Behali Reserve Forest (Assam, India) in India (A, Assam state is marked dark), Assam state (B, Behali Reserve Forest is marked dark) and study sites (C, black line indicates the border of the protected area)

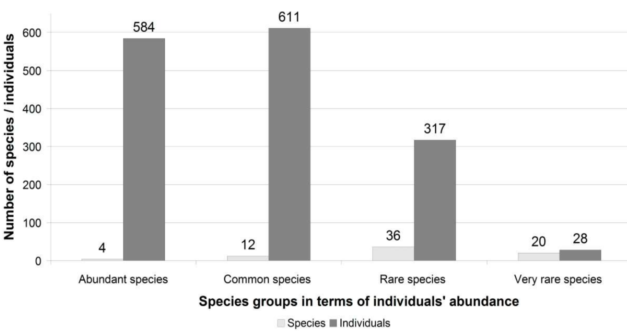


**Fig. 2.** Number of species and individuals for each of herbaceous plant group in terms of individuals' abundance in the Behali Reserve Forest (Assam, India)

The dominance of species has been estimated on the basis of Importance Value Index. In the study area, the most dominant species were *Piper sylvaticum* Roxb. (Piperaceae) IVI=52.48), *Amischotolype hookeri* (Hassk.) H. Hara (Commelinaceae) (IVI=30.03), *Cynodon dactylon* (L.) Pers. (Poaceae) (IVI=29.99), *Ageratum conyzoides* L. (Asteraceae) (IVI=20.04), *Rhaphidophora glauca* (Wall.) Schott (Araceae) (IVI=18.17).

Using data about species richness and some flora characteristics on the nine study sites, we created heat maps of spatial distribution of species richness, individuals' density and dry weight biomass in the Behali Reserve Forest (Fig. 3). We may see that the species richness and density of

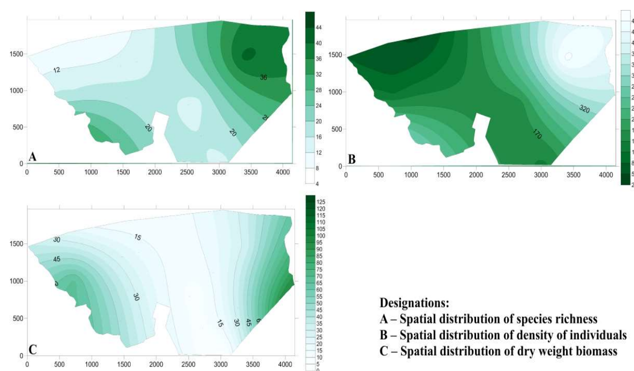


**Fig. 3.** Spatial distribution of species richness, individuals' density and dry weight biomass along the Behali Reserve Forest (Assam, India)

**Table 1.** Characteristics of the study sites in the Behali Reserve Forest (Assam, India)

| Zone | Name | Abbreviation | Coordinates; altitude |
|---|---|---|---|
| East | Serelia Bongaon | SB | 26°52′56.5″N 93°20′00.7″E; 103 m a.s.l. |
| West | Dikal | DI | 26°55′37.0″N 93°16′04.5″E; 98 m a.s.l. |
| Central | Hatidipu | HA | 26°54′01.1″N 93°15′12.2″E; 101 m a.s.l. |
| Central | Hatimara | HM | 26°54′08.9″N 93°19′22.6″E; 97 m a.s.l. |
| West | Radhasu | RD | 26°55′46.5″N 93°16′53.1″E; 95 m a.s.l. |
| Central | Rangagorha Beat | RB | 26°53′12.2″N 93°16′01.6″E; 92 m a.s.l. |
| East | Naharjan-Thandapani | NT | 26°55′02.1″N 93°20′31.3″E; 101 m a.s.l. |
| East | Sialmari | SM | 26°53′43.4″N 93°21′43.3″E; 104 m a.s.l. |
| West | Siklibandha Tiniali | ST | 26°54′23.9″N 93°16′20.7″E; 99 m a.s.l. |

Based on the individuals' density in the sampled plots, species were grouped into following five categories: a) abundant species (species with ≥ 100 individuals); b) common species (species with 25 to 99 individuals); c) rare species (species with 3 to 24 individuals); d) very rare species (species with < 3 individuals).

**Table 2.** Total number of individuals, density, frequency and Importance Value Index (IVI) of the studied herbaceous vegetation in Behali Reserve Forest (India)

| Species | Family | Total number of individuals | Density* | Frequency (%) | IVI |
|---|---|---|---|---|---|
| *Achyrospermum wallichianum* (Benth.) Benth. ex Hook.f. | Lamiaceae | 6 | 0.06 | 0.01 | 0.79 |
| *Acmella paniculata* (Wall. ex DC.) R.K.Jansen | Asteraceae | 63 | 0.66 | 0.06 | 8.68 |
| *Ageratum conyzoides* L. | Asteraceae | 103 | 1.07 | 0.14 | 20.04 |
| *Ajuga* spp. | Lamiaceae | 3 | 0.03 | 0.01 | 0.58 |
| *Alocasia fornicata* (Kunth) Schott | Araceae | 21 | 0.22 | 0.07 | 4.91 |
| *Alpinia nigra* (Gaertn.) Burtt | Zingiberaceae | 7 | 0.07 | 0.03 | 1.71 |
| *Amischotolype hookeri* (Hassk.) H.Hara | Commelinaceae | 101 | 1.05 | 0.24 | 30.03 |
| *Aristolochia assamica* D.Borah & T.V.Do | Aristolochiaceae | 2 | 0.02 | 0.01 | 0.52 |
| *Axonopus compressus* (Sw.) P.Beauv. | Poaceae | 92 | 0.96 | 0.07 | 12.60 |
| *Bambusa* sp. 1 | Poaceae | 7 | 0.07 | 0.02 | 1.28 |
| *Begonia silletensis* (A.DC.) C.B.Clarke | Begoniaceae | 2 | 0.02 | 0.01 | 0.52 |
| *Boeica filiformis* C.B.Clarke | Gesneriaceae | 6 | 0.06 | 0.01 | 0.79 |
| *Bonnaya ruellioides* (Colsm.) Spreng. | Linderniaceae | 48 | 0.50 | 0.02 | 4.40 |
| *Calamus* sp. 1 | Arecaceae | 1 | 0.01 | 0.01 | 0.45 |
| *Calanthe sylvatica* (Thouars) Lindl. | Orchidaceae | 7 | 0.07 | 0.04 | 2.13 |
| *Camonea umbellata* (L.) A.R.Simões & Staples | Convolvulaceae | 7 | 0.07 | 0.02 | 1.28 |
| *Centella asiatica* (L.) Urb. | Apiaceae | 11 | 0.11 | 0.02 | 1.59 |
| *Chlorophytum assamicum* D.Borah & A.P.Das | Asparagaceae | 8 | 0.08 | 0.03 | 1.79 |
| *Chromolaena odorata* (L.) R.M.King & H.Rob. | Asteraceae | 18 | 0.19 | 0.02 | 2.12 |
| *Clerodendrum infortunatum* L. | Lamiaceae | 12 | 0.13 | 0.04 | 2.57 |
| *Clerodendrum* sp. | Lamiaceae | 1 | 0.01 | 0.01 | 0.45 |
| *Corymborkis veratrifolia* (Reinw.) Blume | Orchidaceae | 2 | 0.02 | 0.01 | 0.52 |
| *Crinum amoenum* Ker Gawl. ex Roxb. | Amaryllidaceae | 4 | 0.04 | 0.02 | 1.06 |
| *Cuphea carthagenensis* (Jacq.) J.F.Macbr. | Lythraceae | 34 | 0.35 | 0.04 | 4.53 |
| *Curcuma* sp. | Zingiberaceae | 1 | 0.01 | 0.01 | 0.45 |
| *Cynodon dactylon* (L.) Pers. | Poaceae | 235 | 2.45 | 0.08 | 29.99 |
| *Cyperus* sp. | Cyperaceae | 7 | 0.07 | 0.06 | 2.97 |
| *Desmodium* sp. | Fabaceae | 3 | 0.03 | 0.01 | 0.58 |
| *Digitaria* sp. | Poaceae | 50 | 0.52 | 0.07 | 8.05 |
| *Dracaena petiolata* Hook.f. | Asparagaceae | 37 | 0.39 | 0.04 | 4.80 |
| *Elatostema* sp. | Urticaceae | 4 | 0.04 | 0.02 | 1.06 |
| *Eleusine indica* (L.) Gaertn. | Poaceae | 62 | 0.65 | 0.05 | 7.80 |
| *Exallage ulmifolia* (Wall.) Bremek. | Rubiaceae | 14 | 0.15 | 0.01 | 1.35 |
| *Floscopa scandens* Lour. | Commelinaceae | 10 | 0.10 | 0.02 | 1.51 |
| *Globba multiflora* Wall. ex Baker | Zingiberaceae | 6 | 0.06 | 0.02 | 1.21 |
| *Gomphostemma niveum* Hook.f. | Lamiaceae | 2 | 0.02 | 0.01 | 0.52 |
| *Grangea maderaspatana* (L.) Poir | Asteraceae | 9 | 0.09 | 0.01 | 1.00 |
| *Hygrophila polysperma* (Roxb.) T.Anderson | Acanthaceae | 15 | 0.16 | 0.01 | 1.42 |
| *Kyllinga* sp. | Cyperaceae | 29 | 0.30 | 0.07 | 5.77 |
| *Laportea interrupta* (L.) Chew | Urticaceae | 1 | 0.01 | 0.01 | 0.45 |
| *Lasiobema scandens* (L.) de Wit | Fabaceae | 4 | 0.04 | 0.01 | 0.65 |

**Cont...**

**Table 2.** Total number of individuals, density, frequency and Importance Value Index (IVI) of the studied herbaceous vegetation in Behali Reserve Forest (India)

| Species | Family | Total number of individuals | Density* | Frequency (%) | IVI |
|---|---|---|---|---|---|
| *Leea* spp. | Vitaceae | 16 | 0.17 | 0.03 | 2.45 |
| *Lepidagathis incurva* Buch.-Ham. ex D.Don | Acanthaceae | 1 | 0.01 | 0.01 | 0.45 |
| *Ludwigia perennis* L. | Onagraceae | 9 | 0.09 | 0.02 | 1.44 |
| *Ludwigia* sp. | Onagraceae | 12 | 0.13 | 0.03 | 2.12 |
| *Mikania micrantha* Kunth | Asteraceae | 9 | 0.09 | 0.04 | 2.30 |
| *Mimosa pudica* L. | Fabaceae | 3 | 0.03 | 0.01 | 0.58 |
| *Murdannia japonica* (Thunb.) Faden | Commelinaceae | 2 | 0.02 | 0.01 | 0.52 |
| *Oldenlandia corymbosa* L. | Rubiaceae | 2 | 0.02 | 0.01 | 0.52 |
| *Ophiopogon micranthus* Hook.f. | Asparagaceae | 13 | 0.14 | 0.05 | 3.12 |
| *Oplismenus burmanni* (Retz.) P.Beauv. | Poaceae | 45 | 0.47 | 0.03 | 4.84 |
| *Paederia foetida* L. | Rubiaceae | 1 | 0.01 | 0.01 | 0.45 |
| *Peliosanthes neilgherriensis* Wight | Asparagaceae | 1 | 0.01 | 0.01 | 0.45 |
| *Persicaria hydropiper* (L.) Delarbre | Polygonaceae | 17 | 0.18 | 0.02 | 2.05 |
| *Persicaria praetermissa* (Hook.f.) H.Hara | Polygonaceae | 4 | 0.04 | 0.02 | 1.06 |
| *Phlogacanthus curviflorus* (Nees) Nees | Acanthaceae | 23 | 0.24 | 0.06 | 4.60 |
| *Phrynium pubinerve* Blume | Marantaceae | 7 | 0.07 | 0.05 | 2.55 |
| *Piper sylvaticum* Roxb. | Piperaceae | 145 | 1.51 | 0.34 | 52.48 |
| *Polygonum* sp. | Polygonaceae | 3 | 0.03 | 0.01 | 0.58 |
| *Pontederia crassipes* Mart. | Pontederiaceae | 1 | 0.01 | 0.01 | 0.45 |
| *Pontederia vaginalis* Burm.f. | Pontederiaceae | 1 | 0.01 | 0.01 | 0.45 |
| *Potentilla indica* (Andrews) Th.Wolf | Rosaceae | 11 | 0.11 | 0.01 | 1.14 |
| *Pseuderanthemum latifolium* (Vahl) B.Hansen | Acanthaceae | 1 | 0.01 | 0.01 | 0.45 |
| *Rhaphidophora glauca* (Wall.) Schott | Araceae | 73 | 0.76 | 0.17 | 18.17 |
| *Rotala rotundifolia* (Buch.-Ham. ex Roxb.) Koehne | Lythraceae | 25 | 0.26 | 0.01 | 2.12 |
| *Rungia* sp. | Acanthaceae | 4 | 0.04 | 0.01 | 0.65 |
| *Scoparia dulcis* L. | Plantaginaceae | 5 | 0.05 | 0.01 | 0.72 |
| *Stephania japonica* (Thunb.) Miers | Menispermaceae | 3 | 0.03 | 0.02 | 0.98 |
| *Strobilanthes paniculiformis* J.R.I.Wood | Acanthaceae | 20 | 0.21 | 0.04 | 3.28 |
| *Synedrella nodiflora* (L.) Gaertn. | Asteraceae | 9 | 0.09 | 0.01 | 1.00 |
| *Tacca integrifolia* Ker Gawl. | Dioscoreaceae | 2 | 0.02 | 0.02 | 0.90 |
| *Thunbergia grandiflora* (Roxb. ex Rottler) Roxb. | Acanthaceae | 2 | 0.02 | 0.01 | 0.52 |
| *Tropidia curculigoides* Lindl. | Orchidaceae | 4 | 0.04 | 0.02 | 1.06 |
| *Tupistra stoliczana* Kurz | Asparagaceae | 53 | 0.55 | 0.09 | 9.81 |
| *Urena lobata* L. | Malvaceae | 1 | 0.01 | 0.01 | 0.45 |
| *Vitis* sp. | Vitaceae | 1 | 0.01 | 0.01 | 0.45 |

**Note:** * – individual per 1 $m^2$

individuals are unevenly distributed along the area of Behali Reserve Forest. So, these characteristics are higher in south-western and north-eastern parts of the Protected Area, while there is a lack of species and their individuals through the line from northwestern to southeastern parts of the Behali Reserve Forest. A little similarly, the central part of the Behali Reserve Forest was characterized by low values of dry weight biomass. This parameter increases towards western and eastern parts of the Protected Area.

The taxonomic richness (77 species) of herbaceous plants recorded in the study area was higher than reported in Perumudiyoor Sacred groves, Kerala, India (43 species)

(Parappurath and Paul 2016), Hojai Reserve Forest and Kumorkata Reserve Forest of Nagaon district, Assam, India (66 species) (Dutta and Devi 2014), Antaibao in Shanxi Province, China (44 species) (Wang et al 2012). However, our results were lower than it was reported from some other areas in the Northeast India, namely Barnadi Wildlife Sanctuary, Assam (Deori and Talukdar 2015), Hollongapar Gibbon Wildlife Sanctuary, Assam (Sarkar and Devi 2014), Eastern Himalayan forest, Arunachal Pradesh (Saikia et al 2017), Sariska Tiger Project, Rajasthan (Yadav and Gupta 2007). In our opinion, this is explained by the various knowledge levels about local floras. At the same time, we undoubtedly state a need to increase in coverage of Behali Reserve Forest by study plots to fill “white gaps” in terms of investigated area, namely northern, westernmost and easternmost parts of the Protected Area. Obviously, the best output could be obtained under even and dense distribution of study sites in the research area (Khapugin 2019). Therefore, the herbaceous plant diversity is presumably higher than it was found during the previous study period.

Data we obtained for the most dominant plant species in the Behali Reserve Forest are consistent with data from other areas in India. So, Saikia and Khan (2014) listed *Piper sylvaticum* as a dominant herb in home gardens of Jorhat and Golaghat districts (Upper Assam, Northeast India). *Amischotolype hookeri* was also recognized as dominating herb species in Abhoypur Reserve Forest of Assam (Barua and Hazarika 2020) and in Hollongapar Gibbon Wildlife Sanctuary of Assam (Sarkar 2015). *Ageratum conyzoides* has been considered to be abundant and dominant species in moderately disturbed stands of Namdapha National Park, Arunachal Pradesh (Nath et al 2005), in tropical mixed and broad leaved evergreen forests of Arunachal Pradesh (Saikia et al 2017) and in secondary forests of Jorhat of Assam (Das and Duarah 2013). Finally, Singh and Singh (2012) stated that *Rhaphidophora glauca* dominated in plant communities of the Dihang Dibang Biosphere Reserve of Arunachal Pradesh. The data about families characterized by higher abundance, corroborate with Wang et al (2012) which conducted the similar study in Antaibao, Shanxi Province, China, as well as with Parappurath and Paul (2016) investigating the sacred grooves of Perumudiyoor, Muthathala Gramapanchayath, Pattambi, Kerala. We believe that the further investigations of this area in terms of the plant diversity spatial distribution could change insignificantly this picture, while the general amount of the most abundant plant families will be the same.

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# Species Diversity, Population Structure and Regeneration of Tree Species in Kuldiha Wildlife Sanctuary, Odisha, India

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***Abstract*:** Phyto-sociological analysis of vegetation in Kuldiha Wildlife Sanctuary revealed presence of 43 numbers of tree species (37 genera and 23 families), 12 numbers of shrub species (12 genera and 11 families) and 17 numbers of climbers (14 genera and 10 families). *Shorea robusta, Syzygium cumini, Terminalia tomentosa, Schleichera oleosa* and *Madhuca indica* were the predominant tree species having important value index 48.70, 19.13, 17.50, 15.84 and 14.46 respectively. The forest stand density was 488 trees $ha^{-1}$ with basal area 36.17 $m^2$ $ha^{-1}$. The Shannon-Weiner index of diversity for trees, shrubs and climbers was 4.81, 1.41 and 1.15, respectively. The Simpson Index of dominance, Margalef index for species richness and Pielou index for species evenness were also calculated. Many species were found rare and clumped pattern of dispersion was predominant among trees. The population structure of tree species showed reverse J-shaped pattern. Sapling and seedling density was 350 numbers $ha^{-1}$ and 50750 numbers $ha^{-1}$ respectively. Overall regeneration status of the forests was fair.The study will be helpful in understanding changes in the plant community and there by developing location specific strategies for conservation of valuable rare plants of the sanctuary as well as sustainable utilization of biodiversity in future.

***Keywords*:** Population structure, Tree diversity, Important value Index, Floristic composition

Tropical forest ecosystems are very special areas in the earth surface occupying only 7% of area, harbours about two third of global terrestrial biodiversity and provides innumerable services to mankind having local, regional and global significance (Gardner et al 2009). During last few decades irreparable changes in the structure and functionality of this complex ecosystem has been observed in addition to loss of 0.8-2.0% area per year. Currently losing about 14-40 thousand species per year as a result of destruction of tropical forest habitat (Narayan and Anshuali 2015a).The interaction of continued deforestation, woodland fire, alien species invasion, faulty land utilization pattern and climate change hastening the process of species extinctions (Bradshaw et al 2009). Wilderness and virginity of many tropical forests have been lost due to severe human intrusion, demanding conservation oriented management interventions for maintaining sustainability (Kumar et al 2006). Major threat is from forest land conversion for different land use types which drastically alters nutrient cycling, net ecosystem production and ultimately the diversity of species (Srivastava et al 2020). Till today species composition of many tropical forest ecosystems are not fully unraveled and so also the interaction among themselves. Every day new species are being added and many listed under threat category. Species composition, diversity, dominance, distribution pattern, physiognomy are some of the principal attributes of plant community and any alteration natural or human induced, may significantly modify the entire community stratification as well as its functionality (Suchiang et al 2020). It is well established that a balanced ecosystem is diverse in species composition, where almost all species are directly or indirectly interdependent on each other making the system sustainable. A proper knowledge of age groups viz. seedlings, saplings, juvenile and tree density in a crop helps to understand population structure and sustainability of any forest community. It also depicts the regeneration status of forest crop. Competitive interactions between tree species of same or different families, biotic and abiotic disturbances immensely affect recruitment patterns. Regeneration is fundamental for the maintenance of natural populations. Initial biodiversity survey reports are an important tool in field of scientific conservation as they document the biodiversity temporally and spatially. Plants particularly trees constitute a major structural and functional unit of tropical forest ecosystem. They provide recourses and habitat for many other species and plays substantial role in shaping community characteristics. Hence phyto-sociological survey of tree species in a forest ecosystem is vital for assessing the structural and functional stability, sustainable supply of tangible services.

Kuldiha Wildlife Sanctuary comes under Deccan Peninsula in Chhotnagpur plateau of Garjat Hills. The Sanctuary comprises of Kuldiha, Devgiri and Tenda Reserved Forests and other Protected Forests. Kuldiha sanctuary is a typical representative of mixture of coastal peninsular Sal forest and moist mixed deciduous forest. It comes in the Biogeographic region of Deccan peninsular zone having variety of flora and fauna with a substantial genetic and ecological importance. Vegetation structure, species composition and regeneration status of tropical dry deciduous forests of eastern India have been inventoried persistently by many workers to unravel impact of human intrusion and species loss (Narayan and Anshuali 2015a, Gupta and Misha 2019, Mastan et al 2020). The quantitative assessments of plant diversity in tropical moist and dry deciduous forests of Odisha have been done by many workers (Panda et al 2013, Sahoo et al 2017, Paul 2017, Dash et al 2020 and Nayak and Sahoo 2020). Rout et al (2018) enlisted floristic composition in tropical moist deciduous forest of Kuldiha WLS and documented the plant species. Saravanan et al (2019) enumerated plant diversity in the sanctuary but information regarding distribution pattern, rarity, regeneration status of individual tree species, immigrant species are lacking. Proper balance in seedling, sapling, juvenile and adult class that commonly found in a tropical forest was not observed. In the present investigation an attempt has been made to elucidate the forest structure and floristic composition with special emphasis on distribution pattern, recruitment status of rare trees and alien plant invasion to Kuldiha Wildlife Sanctuary.

## MATERIAL AND METHODS

The study was carried out in Kuldiha Wild Life Sanctuary (KWLS) which situated between 21º20'31" to 21º29'08"N latitude and 86º25'23" to 86º 44' 50"E longitude in Balasore district of Odisha (Fig. 1). KWLS spreads across an area of 272.75 km$^2$ covering the Nato hills and the Sukhupata hills join to the Similipal biosphere reserve (SBR). The landscape is hilly with moderate to steep slopes whose altitude ranges from 80 m to 682 m. Climate is sub-tropical hot, with temperature ranging from 42$^0$C to 8$^0$C, relative humidity 88-62% and rainfall 1460 mm/annum. Red lateritic soils form a major part of edaphic mass. Seasonal rivers, Tangna, Kamala, and Usatalnala flows across the sanctuary. Vegetation comprises of northern tropical semi-ever green forests, peninsular (coastal) Sal forests, moist mixed deciduous forests and mixed dry deciduous forests. KWLS abode many large carnivores (tiger, leopards etc.), herbivores (elephant, sambar, bison, Indian gaur and giant squirrels), birds (hornbills, hill myna and peafowl etc.) and reptiles (king kobra, monitor lizard etc.).

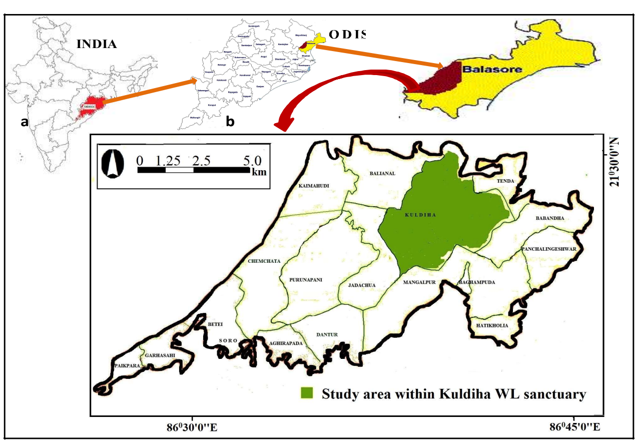



**Fig. 1.** Location of Kuldiha wild life sanctuary and study area

Phyto-sociological study for assessing tree diversity, population structure and regeneration status was carried out during April 2017 to March 2018 in Kuldiha section and its adjoining areas of the sanctuary spreading over 40 ha. The section was selected purposefully because it harbours all four kinds of forests seen in the sanctuary. The study area covered Kuldiha-1, Kuldiha-2, Jadachua beats which possesses about half of the area under very dense forest and more than one third of moderately dense forest cover of the sanctuary (Increasing Forest Cover of Odisha 2015-17, PCCF, Forest Department Govt. of Odisha). Random sample plot method was adapted to analyse the floristic composition and other related numeric attributes. A total of fifty sample plots of 20m ×10 m (equalling to 1.0 hectare) were delineated randomly in the study area. Every species and individuals found in each sample plot were identified and reckoned. Circumference of tree and sapling trunk was measured at breast height (1.37 m from ground) over bark. Individuals having girth ≥30 cm were considered as adult and ≥10 cm to < 30 cm as saplings. Seedling class consisted of individuals having girth < 10 cm but height ≥30 cm. Both trees and saplings were sampled (counting of number of individuals and measuring GBH) in the entire 1.0ha area. Seedlings were sampled (number of seedlings) by laying 2 m ×2m sub-sample plots within each main plot accounting an area of 400m$^2$ (Fig. 2). Distribution pattern of each species was analysed by computing variance to mean ratio. Dispersion was considered random, if ratio is 1.0, uniform if <1.0 and clumped if >1.0. Quantitative parameters such as frequency, density, abundance, basal area (BA) were determined following Mishra (1968) and Curtis and Cottam (1956). Importance Value Index (IVI) for each species was calculated as the sum of relative values of three quantitative characters i.e. dominance, density and frequency following Muller-Dombois and Ellwnberg (1974). Family importance value index (FIV) was calculated by summing relative dominance, relative density and relative frequency of represented families (Mori et al 1983). Shannon's diversity index

($H' = -\Sigma_{i=1}^{n}(ni/N)\log(ni/N)$ where ni =IVI of species "i" and N = ∑ IVI for the community) was calculated following Magurran (1988) and evenness index (($E = H'/\ln S$) where H'=diversity index and S=number of species) by Pielou (1975). The dominance was calculated by Simpson's index $Cd = \sum\left(\frac{ni}{N}\right)^2$ where ni = ∑individuals of a particular tree species and N= ∑ individuals of all tree species (Simpson 1949). Population structure of each species was analysed by comparing species, individual and basal area in four girth classes *viz.* sapling (≥10 to <30 cm), juvenile (≥30 to <60 cm), young (≥60 to <90 cm) and elder (≥90 to <180 cm). Regeneration status of each species was assessed by comparing number of seedlings, saplings and adults in the population. The regeneration was rated (i) 'good', if seedlings > saplings > adults; (ii) 'fair', if seedlings > saplings ≤ adults; (iii) 'poor', if a species survives only in sapling stage, but no seedlings (though saplings may be <, > or = adults); (iv) 'none', if it is absent both in sapling and seedlings stages, but found only in adults and (v) 'new', if a species has no adults, but only saplings and/or seedlings (Shankar 2001). Each plant species was identified with the help of published literature and floras of the region.

## RESULTS AND DISCUSSION

**Floristic composition:** Phyto-sociological investigation ravelled presence of 488 numbers of stems per hectare representing 43 species, 37 genera and 23 families. The family Combretaceae was dominant with five species followed by Anacardiaceae, Euphorbiaceae, Ebenaceae and Rubiaceae (Table 4). Within family Combretaceae, the genus Terminalia was represented by four species and in family Ebenaceae, three species were in genus Diospyros. Among the total documented families, 56.5% were having singletons species and 21.7% were by doubletons, whereas, 21.8% had more than two species. Based on IVI value *Shorea robusta, Diospyros melanoxylon* and *Syzygium cumin* were observed abundant (Table 2). Shrub density was 3000 individuals per hectare belong to 12 species and 11 families. Apocynaceae was the dominant family with two species. 17 species of climbers belonging to 14 genera and 10 families were identified with a density of 228 individuals per hectare. Climbers belonging to family Dioscoreaceae, Fabaceae and Vitaceae were the dominant one having three genera each. Among the recorded genera Dioscorea was dominant one having three species viz. *Dioscorea oppositifolia, D. pentaphylla* and *D. wallichii.*

**Species abundance and rarity:** Analysis of frequency distribution (Raunkiaer's frequency class) of tree species in each sample plot indicated that many species are rare. Among the observed species, 82% have low frequency distribution in the forest community (Fig. 3). *Shorea robusta, Syzygium cumini* and *Terminalia tomentosa* were the most frequent ones where as *Pterocarpus marsupium, Strychnos potatorum, Grewia tiliaefolia* were among the least occurrent

**Table 1.** Key diversity attributes of the sampling sites in Kuldiha WL sanctuary, Odisha

| Variables | | Tree | Shrub | Climber |
|---|---|---|---|---|
| Number of individuals $ha^{-1}$ | | 488 | 3000 | 228 |
| Number of species $ha^{-1}$ | | 43 | 12 | 17 |
| Number of genera $ha^{-1}$ | | 37 | 12 | 14 |
| Number of family $ha^{-1}$ | | 24 | 11 | 10 |
| Basal area ($m^2$ $ha^{-1}$) | | 36.17 | 4.39 | - |
| Dispersion | a) Uniform | 6 | 1 | 7 |
| | b) Random | 15 | 0 | 3 |
| | c) Clumped | 22 | 11 | 7 |
| Shannon index of diversity (H') | | 4.81 | 1.41 | 1.15 |
| Species richness (Margalef index) | | 7.97 | 2.55 | 3.55 |
| Species evenness (Pielou Index) | | 1.28 | 0.57 | 0.41 |
| Species dominance [Simpson index (Cd)] | | 0.21 | 0.02 | 0.01 |

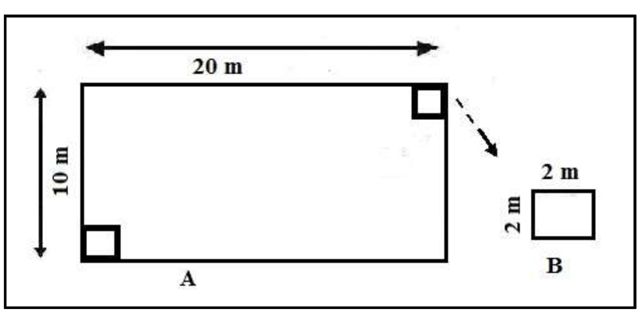



**Fig. 2.** Sample plot design (A) for trees and shrubs, (B) for seedlings

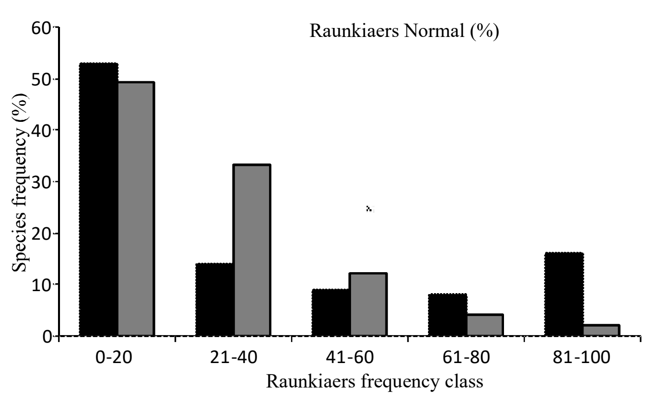



**Fig. 3.** Frequency distribution of observed tree species in Kuldiha WL Sanctuary

**Table 2.** Composition, distribution pattern and regeneration status of tree species (≥ 10 cm girth) in KLWS

| Species name | Family | A | RF | RDe | RBA | IVI | DP | SD |
|---|---|---|---|---|---|---|---|---|
| *Shorea robusta* Roxb. | Dipterocarpaceae | 7.0 | 6.94 | 18.44 | 23.31 | 48.70 | $4.40^{C}$ | $5000^{F}$ |
| *Syzygium cumini* L. | Myrtaceae | 3.8 | 5.56 | 7.68 | 5.89 | 19.13 | $4.48^{C}$ | $2000^{G}$ |
| *Terminalia tomentosa* Roxb. | Combretaceae | 1.8 | 2.78 | 5.64 | 9.09 | 17.50 | $1.10^{C}$ | $1750^{F}$ |
| *Schleichera oleosa* (Lour) Oken | Sapindaceae | 2.4 | 3.47 | 6.15 | 6.22 | 15.84 | $3.78^{C}$ | $1000^{F}$ |
| *Madhuca indica* J.F.Gmel. | Sapotaceae | 2.0 | 5.56 | 4.10 | 4.81 | 14.46 | $3.83^{C}$ | $3000^{F}$ |
| *Adina cordifolia* (Roxb.) Brandis | Rubiaceae | 2.8 | 4.17 | 5.64 | 4.63 | 14.43 | $3.40^{C}$ | $750^{F}$ |
| *Tectona grandis* L.f. | Lamiaceae | 3.0 | 4.17 | 4.61 | 5.24 | 14.02 | $3.67^{C}$ | $2750^{F}$ |
| *Mangifera indica* L. | Anacardiaceae | 1.7 | 2.08 | 2.56 | 5.50 | 10.15 | $1.89^{C}$ | $2750^{F}$ |
| *Terminalia arjuna* (Roxb.) Wight and Arn. | Combretaceae | 2.5 | 3.47 | 2.56 | 2.12 | 8.16 | $3.22^{C}$ | $500^{G}$ |
| *Phyllanthus emblica* L. | Phyllanthaceae | 1.0 | 2.78 | 3.59 | 1.79 | 8.15 | $1.60^{C}$ | $0^{N}$ |
| *Buchnania lanzan* Spreng. | Anacardiaceae | 2.0 | 2.78 | 3.07 | 2.05 | 7.90 | $1.56^{C}$ | $500^{G}$ |
| *Bursera serrata* Wall. ex Colebr. | Burseraceae | 2.0 | 3.47 | 2.56 | 1.41 | 7.44 | $2.33^{C}$ | $1000^{G}$ |
| *Lannea coromandelica* (Houtt.) Merr. | Anacardiaceae | 3.0 | 2.78 | 2.56 | 1.74 | 7.08 | $1.89^{C}$ | $1000^{G}$ |
| *Diospyros malabarica* (Desr.) Kostel. | Ebenaceae | 1.0 | 2.08 | 2.56 | 2.30 | 6.95 | $1.44^{C}$ | $250^{F}$ |
| *Terminalia chebula* Retz. | Combretaceae | 1.3 | 2.08 | 2.05 | 2.75 | 6.88 | $1.22^{C}$ | $1000^{F}$ |
| *Semecarpus anacardium* L. f. | Anacardiaceae | 2.0 | 2.78 | 2.05 | 1.45 | 6.28 | $2.33^{C}$ | $0^{N}$ |
| *Soymida febrifuga* (Roxb.) Juss. | Meliaceae | 3.0 | 2.08 | 2.05 | 2.08 | 6.21 | $2.11^{C}$ | $0^{P}$ |
| *Diospyros melanoxylon* Roxb. | Ebenaceae | 3.0 | 1.39 | 2.05 | 1.97 | 5.41 | $0.67^{U}$ | $1000^{F}$ |
| *Saraca indica* (Roxb.) Willd. | Caesalpiniaceae | 2.0 | 2.78 | 1.02 | 1.11 | 4.92 | $2.00^{R}$ | $0^{P}$ |
| *Azadiratchta indica* A. Juss. | Meliaceae | 1.0 | 2.78 | 1.02 | 0.69 | 4.49 | $2.00^{C}$ | $1500^{F}$ |
| *Bridelia retusa* (L.) A. Juss. | Euphorbiaceae | 1.0 | 2.08 | 1.54 | 0.75 | 4.37 | $1.52^{C}$ | $1250^{F}$ |
| *Lagerstroemia parviflora* Roxb. | Lythraceae | 1.0 | 2.08 | 1.54 | 0.70 | 4.32 | $1.52^{C}$ | $0^{P}$ |
| *Cassia fistula* L. | Papilionaceae | 1.0 | 2.08 | 1.54 | 0.67 | 4.29 | $1.52^{C}$ | $500^{F}$ |
| *Tamarindus indica* L. | Caesalpiniaceae | 1.0 | 1.39 | 1.54 | 1.29 | 4.22 | $0.78^{U}$ | $1000^{F}$ |
| *Strychnos nux-vomica* L. | Loganiaceae | 2.0 | 1.39 | 1.02 | 1.09 | 3.51 | $0.89^{U}$ | $1000^{F}$ |
| *Casearia graveolens* Dalzell. | Samydaceae | 1.0 | 1.39 | 1.02 | 0.64 | 3.05 | $0.89^{U}$ | $0^{F}$ |
| *Pterocarpus marsupium* Roxb. | Fabaceae | 1.0 | 1.39 | 0.51 | 1.05 | 2.95 | $1.00^{R}$ | $0^{N}$ |
| *Diospyros sylvatica* Roxb. | Ebenaceae | 4.0 | 1.39 | 1.02 | 0.53 | 2.94 | $0.89^{U}$ | $1000^{G}$ |
| *Sterculia villosa* Roxb. | Sterculiaceae | 1.0 | 1.39 | 1.02 | 0.48 | 2.89 | $0.89^{U}$ | $1000^{F}$ |
| *Limonia acidissima* L. | Rutaceae | 1.0 | 1.39 | 0.51 | 0.82 | 2.73 | $1.00^{R}$ | $500^{G}$ |
| *Careya arborea* Roxb. | Lecythidaceae | 2.0 | 1.39 | 0.51 | 0.78 | 2.68 | $1.00^{R}$ | $250^{F}$ |
| *Chloroxylon swietenia* (Roxb.) Dc. | Rutaceae | 2.0 | 1.39 | 0.51 | 0.56 | 2.46 | $1.00^{R}$ | $0^{N}$ |
| *Grewia tiliaefolia* Vahl. | Tiliaceae | 1.0 | 1.39 | 0.51 | 0.56 | 2.46 | $1.00^{R}$ | $0^{N}$ |
| *Gmelina arborea* Roxb. ex Sm. | Verbenaceae | 1.8 | 1.39 | 0.51 | 0.50 | 2.40 | $1.00^{R}$ | $0^{P}$ |
| *Mallotus philippinensis* (Lam.) Mull.Arg. | Euphorbiaceae | 1.0 | 1.39 | 0.51 | 0.49 | 2.39 | $1.00^{R}$ | $3000^{G}$ |
| *Mitragyna parvifolia* (Roxb.) Korth. | Rubiaceae | 1.0 | 1.39 | 0.51 | 0.49 | 2.39 | $1.00^{R}$ | $0^{G}$ |
| *Oroxylum indicum* (L.) Kurz. | Bignoniaceae | 1.0 | 1.39 | 0.51 | 0.49 | 2.39 | $1.00^{R}$ | $0^{N}$ |
| *Terminalia bellerica* (Gaertn.) Roxb. | Combretaceae | 1.0 | 1.39 | 0.51 | 0.48 | 2.38 | $1.00^{R}$ | $3750^{G}$ |
| *Cleistanthus collinus* (Roxb) Benth. | Euphorbiaceae | 1.0 | 1.39 | 0.51 | 0.43 | 2.33 | $1.00^{R}$ | $500^{G}$ |
| *Millettia pinnata* (L.) Pierre | Fabaceae | 1.0 | 1.39 | 0.51 | 0.37 | 2.27 | $1.00^{R}$ | $750^{F}$ |
| *Aegle marmelos* L. | Rutaceae | 1.0 | 1.39 | 0.51 | 0.25 | 2.16 | $1.00^{R}$ | $250^{G}$ |
| *Miliusa velutina* (Dunal.) Hook.f. andThomson | Anonaceae | 1.0 | 1.39 | 0.51 | 0.22 | 2.12 | $1.00^{R}$ | $0^{N}$ |
| *Strychnos potatorum* L. | Loganiaceae | 1.0 | 1.39 | 0.51 | 0.22 | 2.12 | $1.00^{R}$ | $0^{N}$ |
| Total | | 78.10 | 100 | 100 | 100 | 300 | 72.8 | 40500 |

A- Abundance, RF- Relative Frequency, RDe- Relative Density, De- Density ($m^2$/ha),DP- Distribution Pattern, SD- Seedling Density (Individual/ha), Superscripts for distribution pattern denotes: U-Uniform, R-Random, N-Near random and C-Clumped. Superscripts for seedling density denotes:G-Good, F-Fair, P-Poor,N-No regeneration and I-Immigrant species in the forest. The analysis of dispersal pattern of dominant species in KWLS revealed that, forest community is highly heterogeneous with respect to composition and one species *Shorea robusta* is the dominant species (Fig. 4).

**Stand density and basal area:** The forest crop in KLWS consisted of 488 individuals per hectare of which Dipterocarpaceae reckoned 18.44%, followed by Combretaceae (11.78%) and Anacardiaceae (10.25%). Rest of the family have relative density less than ten (Table 4). *Shorea robusta* (Dipterocarpaceae) was the most abundant species having relative density 18.44% followed by *Syzygium cumini* (7.68%), *Schleichera oleosa* (6.15%), *Adina cordifolia* (5.64%) and *Terminalia tomentosa* (5.64%). The mean basal area of rest was 36.17 $m^2$ $ha^{-1}$ (Table 3). The members of the family Dipterocarpaceae constituted 23.31% of the total basal area, followed by Combretaceae, Anacardiaceae with 15.55% and 10.74%, respectively (Table 4). *Shorea robusta* and *Terminalia tomentosa* were dominant and co-dominant having 23.31% and 9.09% of the total basal area (Table 2, Fig. 6).

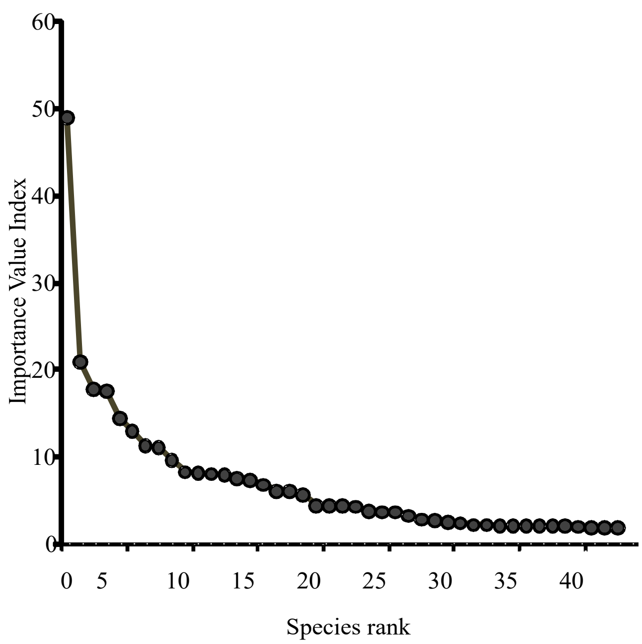



**Fig. 4.** Dispersal pattern of dominant tree species in KWLS

**Species richness, diversity and species accumulation curve:** The species richness of 43 species was observed in 1.0 ha of the Kuldiha WL sanctuary. Shannon- Wiener diversity (H'), Species richness, (Margalef index) and evenness index (Pielou Index) values were 4.81, 7.97 and 1.28 respectively. The ratio of the number of individuals to the number species (N/S) in the studied plots was 11.35. The species accumulation curve was initially steep as the area of sample plots increased up to 0.72 ha but then the rise with increasing number of sampling plots was much slower. However the species area curve did not reach an asymptote (Fig. 5).

**Distribution pattern (variance to mean ratio):** Dispersion pattern of tree species was predominantly clump pattern (51.16%). Fifteen species were randomly dispersed and only six species have shown uniform pattern (Table 1). *Diospyros melanoxylon, Tamarindus indica, Strychnos nuxvomica, Casearia graveolens, Diospyros sylvatica,* and *Sterculia villosa* have sown uniform distribution (Table 2). Random dispersion was observed in all rare species in the forest, whereas dissemination of abundant species was in a clumping manner. *Shorea robusta, Syzygium cumini, Schleichera oleosa* have showed variance to ratio more than 2.0.

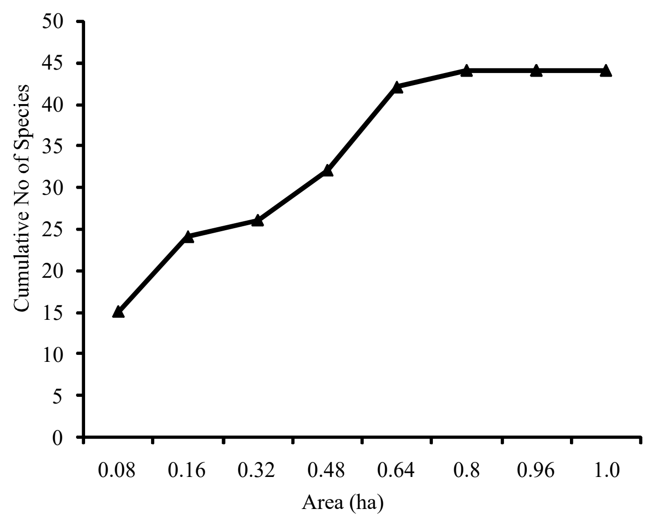



**Fig. 5.** Tree species accumulation curve based on cumulative sample area

**Table 3.** Species richness, density and basal area for different girth classes of trees in KWLS

| Girth class (cm) | Species richness | Density (Ind. $ha^{-1}$) | Per cent contribution to density | Basal area ($m^2$ $ha^{-1}$) | Per cent contribution to Basal area |
|---|---|---|---|---|---|
| 10-30 (Sapling) | 33 | 350 | 41.77 | 4.28 | 10.58 |
| 31-60 (Juvenile) | 40 | 407 | 48.57 | 33.43 | 82.65 |
| 61-90 (Young) | 16 | 73 | 8.71 | 2.29 | 5.66 |
| 91-180 (Elder) | 3 | 8 | 0.95 | 0.45 | 1.11 |

**Table 4.** Family composition and FIV of tree species in Kuldiha WL Sanctuary, Odisha

| Family | A | SPP | Rdi | Rde | RBA | FIV |
|---|---|---|---|---|---|---|
| Anonaceae | 1.00 | 1(1) | 2.33 | 0.51 | 0.22 | 3.06 |
| Sterculiaceae | 1.00 | 1(1) | 2.33 | 1.02 | 0.48 | 3.83 |
| Bignoniaceae | 1.00 | 1(1) | 2.33 | 0.51 | 0.49 | 3.33 |
| Tiliaceae | 1.00 | 1(1) | 2.33 | 0.51 | 0.56 | 3.39 |
| Salicaceae | 1.00 | 1(1) | 2.33 | 1.02 | 0.64 | 3.99 |
| Lythraceae | 2.00 | 1(1) | 2.33 | 1.54 | 0.70 | 4.56 |
| Lecythidaceae | 1.00 | 1(1) | 2.33 | 0.51 | 0.78 | 3.62 |
| Loganiaceae | 3.00 | 2(1) | 4.65 | 1.54 | 1.32 | 7.50 |
| Burseraceae | 2.00 | 1(1) | 2.33 | 2.56 | 1.41 | 6.30 |
| Fabaceae | 2.00 | 2(2) | 4.65 | 1.02 | 1.42 | 7.09 |
| Rutaceae | 3.00 | 2(2) | 4.65 | 1.54 | 1.63 | 7.82 |
| Euphorbiaceae | 4.00 | 3(3) | 6.98 | 2.56 | 1.67 | 11.21 |
| Phyllanthaceae | 1.80 | 1(1) | 2.33 | 3.59 | 1.79 | 7.70 |
| Caesalpiniaceae | 3.00 | 2(2) | 4.65 | 3.07 | 1.96 | 9.69 |
| Meliaceae | 4.00 | 2(2) | 4.65 | 3.18 | 2.76 | 10.59 |
| Ebenaceae | 8.00 | 3(1) | 6.98 | 5.64 | 4.80 | 17.41 |
| Sapotaceae | 2.00 | 1(1) | 2.33 | 4.10 | 4.81 | 11.23 |
| Rubiaceae | 3.80 | 3(3) | 6.98 | 6.15 | 5.11 | 18.24 |
| Verbenaceae | 4.00 | 2(2) | 4.65 | 5.12 | 5.74 | 15.51 |
| Myrtaceae | 3.80 | 1(1) | 2.33 | 7.68 | 5.89 | 15.90 |
| Sapindaceae | 2.40 | 1(1) | 2.33 | 6.15 | 6.22 | 14.70 |
| Anacardiaceae | 8.70 | 4 (4) | 9.30 | 10.25 | 10.74 | 30.29 |
| Combretaceae | 7.60 | 5(2) | 11.63 | 11.78 | 15.55 | 38.96 |
| Dipterocarpaceae | 7.00 | 1(1) | 2.33 | 18.44 | 23.31 | 44.08 |
| Total | 78.10 | 43(37) | 100 | 100 | 100 | 300 |

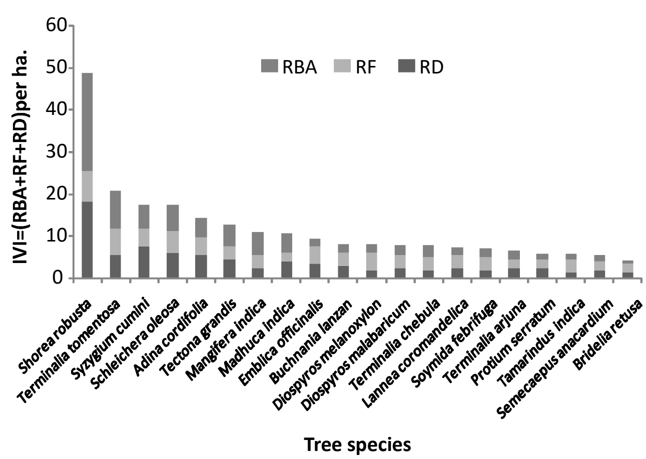
A-Abundance, Spp- Species number, $RD_i$ –Relative diversity, $RD_e$- Relative density, RBA-Relative basal area, FVI-Family importance value, Values in parenthesis are number of genera

**Table 5.** Density of Immigrant tree species and sapling Kuldiha WL Sanctuary, Odisha

| Immigrant tree species (having seedling class only) | | Sapling density of dominant trees | |
|---|---|---|---|
| Name | Seedlings $ha^{-1}$ | Name | Saplings $ha^{-1}$ |
| *Bauhinia purpurea* | 2500 | *Shorea robusta* | 50.0 |
| *Cordia obliqua* | 1250 | *Syzygium cumini* | 30.0 |
| *Ficus recemosa* | 2000 | *Terminalia tomentosa* | 5.0 |
| *Ficus microcarpa* | 500 | *Schleichera oleosa* | 5.0 |
| *Butea monosperma* | 1750 | *Madhuca indica* | 5.0 |
| *Artocrpus heterophyllus* | 250 | *Adina cordifolia* | 7.5 |
| *Trewia nudiflora* | 250 | *Tectona grandis* | 7.5 |
| *Dillenia pentagyna* | 1750 | *Mangifera indica* | 2.5 |
| Other tree species | 40500 | Rest of species | 237.5 |
| Total | 50750 | Total | 350 |

**Structure of plant community:** The grouping of species and individuals in size classes showed increase in sapling class to juvenile class and sharp decline from juvenile through elder class (Fig. 7a, c). A total of 40 species (out of 43 species in tree category) contributed 48.57% to stand density and the rest 51.43% by only three species. The contribution of different girth classes to total basal area of forest showed a progressive increase from sapling to juvenile and sharp decline from juvenile through young class (Fig. 7b). This signifies that, juvenile class shares a greater percentage of basal area (82.65% of stand basal area). Only three species *Shorea robusta, Terminalia tomentosa* and *Mangifera indica* represented elder girth class and accounted 0.95% to the stand density and 1.11% to the stand basal area (Table 3).

**Importance value:** The important value index (IVI) was maximum in *S. robusta* (48.70) followed by *S. cumin* (19.13), *T. tomentosa* (17.5), S. *oleosa* (15.84), *M. indica* (14.46), *A. cordifolia* (14.43), *T. grandis* (14.02) and *M. indica* (10.05). The remaining thirty-fivespecies were found having IVI of < 10.0 (Table 2, Fig. 6). Only ten species contributed to 56.85% of the overall IVI of which *S. robusta* alone contributed to 16.23% (Table 2).The maximum family importance value (FIV) was recorded in Dipterocarpaceae (44.08) followed by Combretaceae (38.96), Anacardiaceae (30.29), Rubiaceae (18.24), Ebenaceae (17.41), and Myrtaceae (15.90) and Verbenaceae (15.51). Ten families reckoned for 72.51% of the overall FIV (Table 4). Twelve families (Anonaceae, Bignoniaceae, Tiliaceae, Lecythidaceae, Sterculiaceae, Salicaceae, Lythraceae, Burseraceae, Fabaceae, Loganiaceae, Phyllanthaceae, Rutaceae and Caesalpiniaceae) had FIV values less than 10.0 (Table 4).




All values for the most abundant 20 species have been converted to values per hectare. IVI (Important value index) = RD (Relative density) + RF (Relative frequency) + RBA (Relative basal area)

**Fig. 6.** Detailed tree composition in Kuldiha sanctuary

**Regeneration:** Regeneration status of trees in Kuldiha forest was satisfactory. Among 43 tree species 11 species have good regeneration and 24 have fair regeneration. Dominant species *S. robusta* was found regenerating fairly (seedlings 5000 ha$^{-1}$, saplings 50 ha$^{-1}$) in the forest while as major associate *Syzygium cumini* in a good manner (seedlings 2000 ha$^{-1}$, saplings 30 ha$^{-1}$). A disproportionately lower sapling density in comparison to sapling and adult was observed in almost each studied sites (Fig. 7). Among all tree species, density of seedlings and saplings were found high in *S. robusta*, *Mallotus philippinensis, Terminalia bellerica, Madhuca indica, Mangifera indica, Tectona grandis* and *Syzygium cumini.* No regeneration was observed in eight species *Pterocarpus marsupium, Emblica officinalis, Strychnos potatorum, Miliusa velutina, Oroxylum indicum, Grewia tiliaefolia, Chloroxylon swietenia, Semecaepus anacardium.* Seedlings of eight number of immigrant tree species (having no adult and sapling class) were recorded among which density of *Bauhinia purpurea, Ficus recemosa* was maximum (Table 5).

**Floristic composition:** The predominant forest type of Kuldiha WL sanctuary is northern tropical semi-ever green, moist mixed deciduous, mixed dry deciduous forests and peninsular sal forests. The observed tree density of 43 species ha$^{-1}$ indicates to moderate level of diversity in KWLS. The observed diversity in KWLS is comparable with other phyto-sociological assessments in tropical forests of Odisha and other regions of India (Sunil et al 2016, Kumar et al 2006). In a similar type of floristic assessment, 91 species of trees were recorded from 4.0 ha sample area in tropical dry deciduous forest of Boudh division and 177 species of trees from 60.0 ha sample area in tropical forests of Nayagarh forest division situated in Eastern Ghats of Odisha (Sahu et al 2007, Sahoo et al 2017). Rout et al (2018) recorded 38 numbers of tree species from 0.4 ha sample area at Kuldiha WL sanctuary. Saravanan et al (2019) reported presence of 35 species in periphery zone, 63 in buffer zone and 105 in core zone of the sanctuary including seedlings and saplings class. The observation of 43 species per hectare in this study was due to location of study site in between core and peripheral zone, size of sampling unit, consideration of individuals having GBH ≥30 cm in tree class count and season of study. High species richness in the sanctuary is because of site quality and ecotonal location; that is

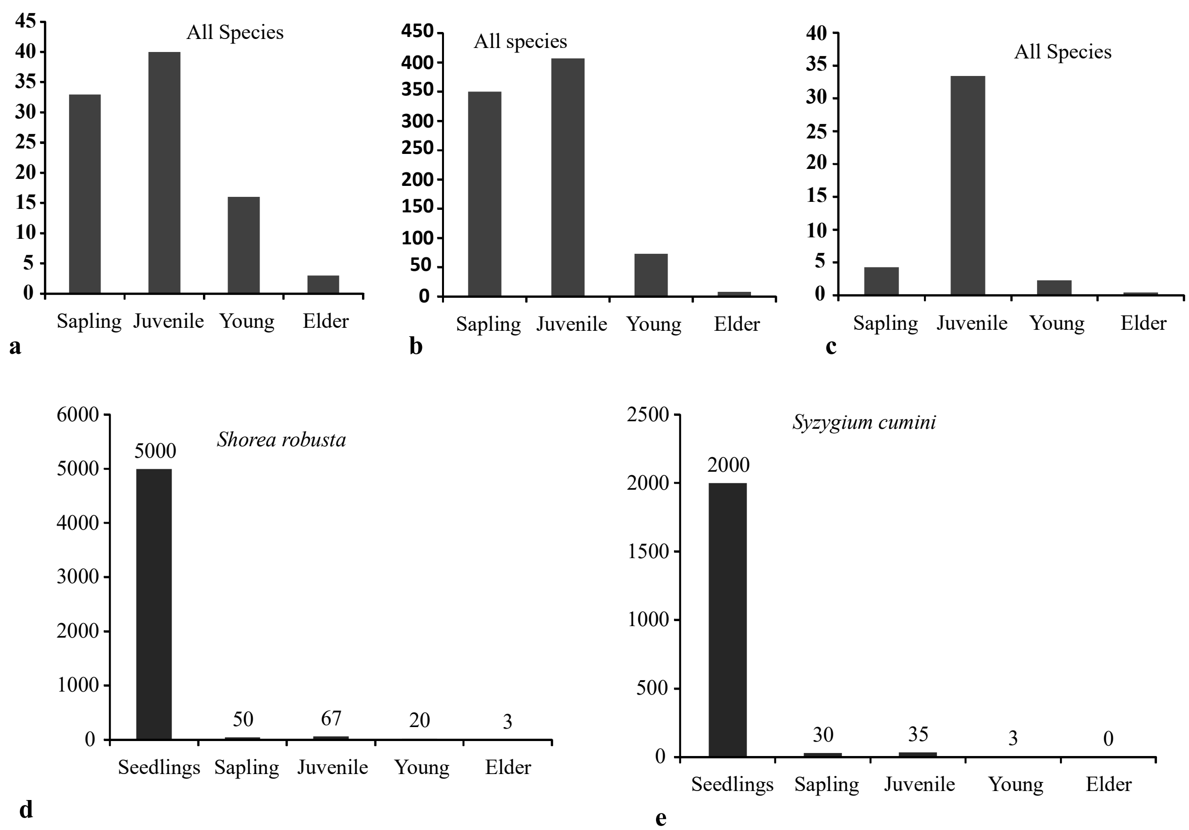



**Fig. 7.** Population structure of all species based on (a) No. of Species, (b) Density per ha. (c) Basal area (m$^2$ ha$^{-1}$), (d-e) two dominant species. Girth Class is seedling ≤ 10cm, Sapling ≥10 − <30 cm, Juvenile ≥30 − <60cm, Young ≥60 − <90 cm and Elder ≥90 − < 180cm

conjunction with the Similipal biosphere reserve through the Sakhuapada and Nato Hill ranges where the tree density range is between 650-970 individuals $ha^{-1}$ (Mishra et al 2008).

Forests in KWLS were dominated by Dipterocarpaceae, Combretaceae, Anacardiaceae, and Rubiaceae. The dominance of family Dipterocarpaceae and Combretaceae in terms of FIV was attributed to higher abundance and basal area of the constituent species. But dominance of family Anacardiaceae and Rubiaceae was because of higher species richness, density and basal area. The species rank curve gives a clear insight into species diversity (Fig. 4). Relative abundance of species in a plant community indicates towards the distribution of commonness and rarity of individuals (Sugihara 1980). Frequency class analysis of data revealed that many species are having low frequency and abundance indicating their rarity. Variability in climatic and pedogenic resources, competition, poor dispersability, heavy biotic pressure is among few factors responsible for the rarity of species. When number of individuals of each species are same in all parts of community its distribution pattern is considered homogeneous which was not found in present study. Random distributions of 18 species confirms to the heterogeneity of forest.

Quantified index IVI (RD+RF+RBA) in ecological studies signifies ecological importance of a species in a community. It helps ecologist in prioritizing species for conservation whereby species having low IVI value need immediate attention compared to the ones with high IVI (Kacholi 2014). The high IVI exhibited by *Shorea robusta* (48.70) was largely due to higher relative frequency, density and basal area compared to other species which is very close to the observations by Saravanan et al (2019) in peripheral zone in the same sanctuary (IVI-55.647) and at par with reporting of Sahu et al (2007) for tropical forest in Odisha (IVI-43.88). Shannon Index of diversity ($H'$) for trees calculated for the present set of observation (4.81) was close to earlier observations (3.11 in buffer to 4.332 in core region) of Saravanan et al (2019). It is also comparable to the reported range 3.76-3.96 for tropical forests of the Eastern Ghats, Andhra Pradesh and 0.83-4.1 for Indian sub-continent (Sahoo et al 2017). It was also comparable with reporting of Reddy et al (2007) for tropical semi-evergreen, moist deciduous and dry deciduous forests of Similipal biosphere reserve which is adjoining to KWLS.

**Forest structure:** Kuldiha is rich in flora diversity owing to its location. The observation of stand density 488 stems $ha^{-1}$ under present study is due to location of study site in between buffer and peripheral zone and counting of individual plants having GBH≥ 30 cm as tree. It is within the range reported for other tropical forests of Indian Sub-continent; 486.76 stems $ha^{-1}$ in Nayagarh Forest Division of Odisha (Sahoo and Panda 2015), 443 stems $ha^{-1}$ in Malayagiri hills of Odisha (Sahu et al 2012) and 352 stems $ha^{-1}$ in North-Eastern Ghats (Panda et al 2013). This observation was in conformity with the earlier reporting of 256-512 trees per ha by Rangaswamy et al (2007). Population structure based on number and density of species showed that sapling and juvenile class were prevalent revealing almost reverse J-shaped pattern (Fig. 7). The stem cross sectional area of stand acts as a key attribute for judging the site quality as well as floristic composition of forests (Ramanujam and Kadamban 2001). Reddy et al (2007) reported basal area 52.82 $m^2$ $ha^{-1}$ for tropical semi-evergreen, 42.08 $m^2$ $ha^{-1}$ for tropical moist deciduous and 26.30 $m^2$ $ha^{-1}$ for tropical dry deciduous forest of adjoining Similipal Biosphere Reserve (SBR). The lower tree basal area in Kuldiha than SBR in spite of more or less similar ecosystem might be due to juvenility of forest (absent of higher girth classes), poor site quality and degradation.

**Dispersion pattern:** Spatial dispersion of trees and their assemblages in a forest illustrates spacing of individuals corresponding to each other. Many autogenic and habitat factors such as seed dispersal ability, patchiness of habitat, density dependant mortality factors during recruitment stage, coppicing ability, microhabitat characteristics and degree of disturbances determines distribution pattern of tree species in a forest (Datta and Rawat 2008). Clumped distribution pattern was observed in 22 species (51.16%) and this type distribution is common in tropical forests (Reddy and Ugle 2008). Clumping of these tree species may be due to poor seed dispersal, coppicing ability of stumps after deforestation and patchiness of microhabitat (Narayan and Anshumali 2015b) Uniform distribution was observed in 6 tree species (13.95%) owing to their small densities in the population. However facilitating such type of distribution in tropical forests allows preservation of high level of biodiversity (Narayan and Anshumali 2015b).

**Regeneration:** The overall regeneration status of plant communities in Kuldiha was satisfactory as indicated from population structure of all species (Fig. 7a). Among the ten dominant tree species only *Syzygium cumini* showed good regeneration indicating proper distribution of age classes seedlings > saplings> adults. The rest of nine species including *S. robusta* have fair regeneration due to less number of saplings per ha than adult one. Out of 43 tree species, no seedlings were recorded in eight tree species. The disproportionate in seedling, sapling and adult population indicates disturbance in ecological processes which might be climatic, biotic or anthropogenic. Poor sapling density than adult in most of dominant and co-dominant tree species might be due to heavy biotic pressure and degradation of site quality. In a similar type of Sal dominated forest of Odisha in Eastern Ghats, poor natural regeneration was attributed to recurrent ground fire, over grazing by livestock, climatic and edaphic factors (Panda et al 2013). Kuldiha forest is receiving heavy pressure from fringe villagers for many tangible non-timber forest products, illegal quarrying, and other commercial activities.

## CONCLUSION

Species diversity in tree species has a significant role in contributing the extent of biodiversity in an ecosystem. In a plant ecosystem, study of vegetation structure throws light on the age classes including regeneration pattern of the forest which determines future species composition and stocking. Quantitative assessment of tree diversity in Kuldiha WL sanctuary revealed that sapling class is disproportionately lower compared to seedlings and adult class. Many species are rare and needs immediate attention for conservation. Overall the natural regeneration is fair except for few species like *Pterocarpus marsupium, Strychnos potatorum, Miliusa velutina, Oroxylum indicum, Semecaepus anacardium* where no seedling class was found. Observation of eight immigrants in seedling class is an indication of progress of natural plant succession and positive sign for enhancing plant diversity. The present study highlighted the status of plant community which will serve as a primary input towards monitoring and sustaining phyto-diversity in the sanctuary. It will further assist in structuring management strategies for conservation of rare plant species in the sanctuary as well as similar forest types of the region.

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# Standardization of Fast, Efficient and Improved Genomic DNA Extraction Protocol for *Melia dubia* Cav. using RAPD and ISSR Marker Assay

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***Abstract***: *Melia dubia* Cav. is an important fast growing multipurpose tree species and recently, it is recognized as prominent species for pulp and paper. Due to its demand, farmers have started planting this species. In order to improve the growth and yield, tree improvement work has already initiated in the country. In the present study, DNA extraction techniques have been standardized by modifying available protocol using RAPD and ISSR marker assay for assessment of genetic diversity in *M. dubia*. The standardized protocol yielded 301-961 ng/µl of quality genomic DNA with 1.77 to 1.90 absorbance ratio 260/280 nm. The quality of isolated DNA was also assessed by 0.8% agarose gel electrophoresis which evidenced a single compact band without shearing. Further, it was successfully tested for PCR amplifications using RAPD and ISSR markers assay. The isolated DNA from this method can be used in approximately 3000 PCR reactions for molecular studies in *M. dubia*.

***Keywords***: *Melia dubia*, Multipurpose tree species, DNA isolation, RAPD and ISSR markers

*Melia dubia* Cav. (Malabar Neem) is a very fast growing deciduous tree species belonging to Meliaceae family. It is commonly known as “Maharukh, Burma Neem and Maha Neem”. It is native to Indian sub-continent and introduced in many counties of the globe (Anand et al 2012). The species has a wide range of adaptability and successfully grown on all types of soils. The wood of the species is a fantastic raw material for paper and plywood industries and also used for furniture, construction, match boxes, packing cases, pencils, splints, agricultural implements and fuel wood (Parthiban et al 2009). Various parts of the plant such as leaves, fruit, roots and bark are also used in Ayurvedic medicine (Kirtikar and Basu 1999, Kumar et al 2013). Thus, due to diverse uses, this versatile tree species is recognized as good multipurpose tree species. The selection of species on commercial scale is very essential for fulfilling the increasing gap between demand and supply of wood raw materials. After selection of desired species, large scale production of superior quality planting material of the species is essential for large scale block plantations and agroforestry plantations. Due to huge demand of *M. dubia* wood for pulp and plywood, a tree improvement programme on *M. dubia* was initiated at Navsari Agricultural University, Navsari, Gujarat, India during 2013 (Chauhan et al 2019). The tree genetic improvement programme of any tree species is always started with germplasm collection and genetic studies (Kumar et al 2013). Genetic diversity assessment was carried out using molecular marker technique such as RAPD (Random Amplification of Polymorphic DNA; Johar et al 2017) and ISSR (Inter Simple Sequence Repeat; Rawat et al 2018) for further improvement of the species. Marker assisted selection (MAS) became more reliable than morphological selection because it is not affected by environmental factors. However, the first and most crucial step for molecular studies is DNA isolation which should be pure and intact for its quantity and quality. Several problems in DNA isolation were reported in *M. dubia* which may be due to large variation in chemical composition in the cell such as polysaccharides, polyphenolics, bio-actives. *etc*. (Johar and Rachana 2015, Rawat et al 2016, Sharma et al 2018 and Phukan et al 2018). Hence, in the present study, an assay was made to develop a fast and efficient genomic DNA isolation protocol for *M. dubia* in order to workout genetic diversity among selected genotypes.

## MATERIAL AND METHODS

**Plant material:** The young fresh green leaf samples of 12 genotypes of *M. dubia* were collected from the progeny trial established at NAU campus, Navsari, Gujarat and carried to the laboratory in thermocol box with ice packs and stored in deep freezer (-20ºC) in Forest Biotechnology Laboratory, College of Forestry, NAU, Navsari for the study.

**DNA isolation:** Five different DNA isolation protocols, namely CTAB (Doyle and Doyle 1990), Johar and Rachana (2015), Rawat et al (2016), Sharma et al (2018) and Phukan et al (2018), were employed for extraction of genomic DNA from fresh green leaves of *M. dubia* for RAPD and ISSR molecular marker analysis. However, none of the methods was able to extract optimum quantity and quality of DNA required from *M. dubia* leaves. Hence, some modifications were accomplished in each method and finally slight modifications were carried out in the methods given by Rawat et al (2016) and Phukan et al (2018). The new protocol was developed which improved the quantity and quality of genomic DNA and found to be suitable for RAPD and ISSR molecular studies in *M. dubia*. The standardized protocol is presented in Table 1 and optimized CTAB extraction buffer for DNA isolation is presented in Table 2.

**DNA Quantification:** The yield of extracted DNA was checked using spectrophotometer (Thermo Scientific NanoDrop) through measuring spectral wavelength absorbance ratio 260/280. The quantification of isolated DNA was assessed to find out purity of DNA. The 260/280 ratio for pure double stranded DNA is 1.80. If the ratio is greater than 1.80 suggest RNA contamination, whereas less than 1.80 suggests protein contamination in the sample (Table 3). Further, the purity, size and integrity of DNA was also studied through electrophoresis on 0.8 % (w/v) agarose gel prepared using 1X TBE buffer containing ethidium bromide (0.5 µg/ml) at 80 volt for 45 minutes and quality was judged by viewing the image of single compact DNA band (Fig. 1 and 2). The genomic DNA of 12 genotypes of *M. dubia* was visualized on 0.8 % agarose gel under the gel documentation system (BioRad Universal Hood II Gel Doc System) and depicted in Figure 2.

**RAPD and ISSR PCR Amplifications:** The isolated DNA was subjected to PCR amplification using RAPD and ISSR molecular markers in the present study. The RAPD primer OPL-12 (5' GGGCGGTACT 3', Operon Technology Incorporated Company, USA) and the ISSR primer UBC-874 (5' CCCTCCCTCCCTCCCT 3', University of British Columbia, Canada) were employed to check the PCR amplifications of 12 genotypes of *M. dubia*. The PCR amplification was carried out in 25 µl reaction volume mixture (Table 4) as described by Williams et al (1990) and Zietkiewicz and his associates (1994) with some modifications in a PCR thermocycler (Applied Biosystems, Veriti 96-Well Thermal Cycler, Thermo Fisher Scientific).

The PCR amplification was programmed as under: initial cycle of denaturation at 95°C for 4 minutes, followed by 35 cycles of 95ºC for 1 minute, 40°C for 1.20 minute, 72ºC for 2 minutes and final extension cycle of 72ºC for 10 minutes and hold at 4°C for ∞ for RAPD assay. Similarly, initial cycle of denaturation at 95°C for 4 minutes, followed by 35 cycles of 95ºC for half minute, 50°C for 1 minute, 72ºC for 1.20 minute and final extension cycle of 72ºC for 10 minutes and hold at 4°C for ∞ for ISSR assay. The electrophoresis was done in 1.8 % agarose gel under 1X TBE electrophoresis buffer at 80 volt for 105 minutes. After running, the amplification products were visualized and photographed under the gel documentation system (BioRad Universal Hood II Gel Doc System) and stored in computer (Fig. 3 and 4).

## RESULTS AND DISCUSSION

In the present study, five different DNA isolation methods Doyle and Doyle (1990), Johar and Rachana (2015), Rawat et al (2016), Sharma et al (2018) and Phukan et al (2018) were employed to extract DNA in optimal quantity and quality for RAPD and ISSR studies in *M. dubia*. CTAB method (Doyle and Doyle 1990) yielded medium DNA concentration (351-462 ng/µl) with high 260/280 nm value (2.11-2.14) and gel documented photograph also revealed dark brown coloured shearing bands which indicate high RNA contamination as well as secondary metabolites (Fig. 1). The shearing of bands was noticed in CTAB method, which was also reported by Rawat et al (2016) and this may be due to RNA contaminations as well as organic substances. The protocol of Johar and Rachana (2015) revealed a very dark brown colour contaminated bands with shearing which indicate high amount of RNA and secondary metabolites with gelatinous materials. Further, low (1.65-1.71) absorbance ratio 260/280 nm and quantity (165-221 ng/µl) was also recorded, which may be due to high amount of secondary metabolites such as carbohydrates, phenols *etc.* and this method was time consuming. Whereas, the protocol suggested by Rawat et al (2016) showed that DNA concentration was 121-226 ng/µl and absorbance ratio 260/280 nm was 1.92-1.95 with slight shearing of bands. The result indicates good bands; however, DNA quantity was low and the treatment of DNase and Proteinase was effective. In the case of protocol developed by Sharma et al (2018), there was a low (122-186 ng/µl) amount DNA concentration with high (2.01-2.07) absorbance ratio of 260/280 nm, which show some shearing bands on the agrose gel. This indicates the contamination of RNA and other substances. Whereas Phukan et al (2018), extracted *M. composita* DNA without liquid nitrogen and this method yielded very low (55-81 ng/µl) amount of genomic DNA as well as low (1.93-1.96) absorbance ratio 260/280 with slight shearing bands on the 0.8 % agrose gel, this result may be due to lack of fine powder of leaf materials used without liquid nitrogen. Thus, all these protocols did not provided desired quantity and quality of isolated genomic DNA for molecular studies. However, methodology

**Table 1.** Steps for total genomic DNA isolation from fresh green leaf samples of *M. dubia*

| Steps | Description |
|---|---|
| 1 | Collected fresh leaves (300 mg) of *Melia dubia* and washed in millipore water, air-dried and removed mid-ribs from each leaf. |
| 2 | Ground the leaf samples in autoclaved pre-chilled mortars and pestles using liquid nitrogen (-196º C) to make very fine powder. |
| 3 | Immediately, fine powder of leaves was transferred to 2.0 ml autoclaved microcentrifuge tubes and added 1 ml pre-warmed extraction buffer (Table 2). |
| 4 | Suspension inverted and mixed gently and incubated at 65ºC for 60 minutes with intermittent shaking at the interval of every 15 minutes. |
| 5 | This emulsion centrifuged at 10000 RPM for 5 minutes at room temperature and transferred the supernatants to another 2 ml autoclaved microcentrifuge tube. |
| 6 | Equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed vigorously by inverting 15-20 times to form an emulsion and centrifuged at 12000 RPM for 10 minutes at room temperature. |
| 7 | Carefully, the upper aqueous phase pipetted out and transferred into a fresh autoclaved 2 ml microcentrifuge tube. |
| 8 | Added 5 µl of RNase (5 mg/ml) and 5 µl of Proteinase K (5 mg/ml) to the microcentrifuge tube and incubated at 65ºC for 10 minutes. |
| 9 | Again, equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed vigorously by inverting 15-20 times to form an emulsion and centrifuged at 12000 RPM for 10 minutes at room temperature. |
| 10 | Carefully, the upper aqueous phase pipetted out and transferred into a fresh autoclaved 1.5 ml microcentrifuge tube and added 1/4$^{th}$ volume of 5M NaCl with an equal volume of chilled isopropanol (-20°C) for about 3 hours to precipitate DNA at the bottom of the microcentrifuge tube. |
| 11 | This precipitated mixture centrifuged at 12000 RPM for 10 minutes at 4°C temperature to get DNA pellet at the bottom of 1.5 ml microcentrifuge tube. |
| 12 | Later, the supernatant was discarded from the microcentrifuge tube and washed the DNA pellet with 70% ethanol for 2 times by centrifugation at 7000 RPM for 5 minutes at 4°C temperature. |
| 13 | DNA pellet was air dried for 30-40 minutes and then dissolved in 100 µl of TE buffer and stored at -20°C for further use. |

standardized in present study resulted in very clear band without shearing of bands on the 0.8 % agrose gel (Fig. 1 and 2) as well as it provided very good concentration of genomic DNA which ranged from 301 to 961 ng/µl (Table 3). The good absorbance ratio 260/280 nm varied from 1.77 to 1.90, which indicates very low amount of RNA and protein contaminations. When RAPD and ISSR analysis was performed using the isolated DNA, various monomorphic and polymorphic PCR bands were observed in *M. dubia* samples (Fig. 3 and 4).

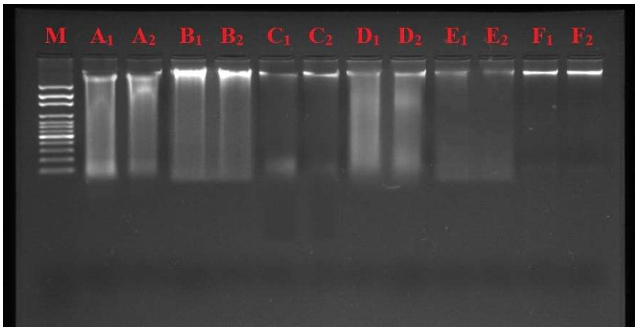
*M. dubia* is a source of medicinal substances and natural or bioactive products including large amounts of secondary metabolites such as polysaccharides, polyphenols and gelatinous materials which impede the isolation of DNA in optimal quantity and quality for molecular assay. In higher plants, especially in medicinal plants, secondary compounds such as polysaccharides (Sarwat et al 2006, Sahu et al 2012, Rawat et al 2016), polyphenols (Katterman and Shattuck 1983, Sahu et al 2012, Sharma et al 2018) and other organic substances (Khanuja et al 1999, Rawat et al 2016, Thakur et al 2017) present in the leaves get accumulated and it may create problems while DNA isolation and purification. Further, phytochemical composition and its quantity in any

**Table 2.** Composition of CTAB extraction buffer for DNA isolation

| Stock solutions | Concentration | Amount per 10 ml |
|---|---|---|
| CTAB | 3 % | 300 mg |
| 5M NaCl | 1.5 M | 3.0 ml |
| 1M TrisHCl (pH 8.0) | 100 mM | 1.0 ml |
| 0.5 M EDTA (pH 8.0) | 50 mM | 1.0 ml |
| PVP | 2 % | 200 mg |
| β-mercaptoethanol | 0.1 % | 10 µl |
| Distilled water | - | Remaining |
| Total | - | 10.0 ml |

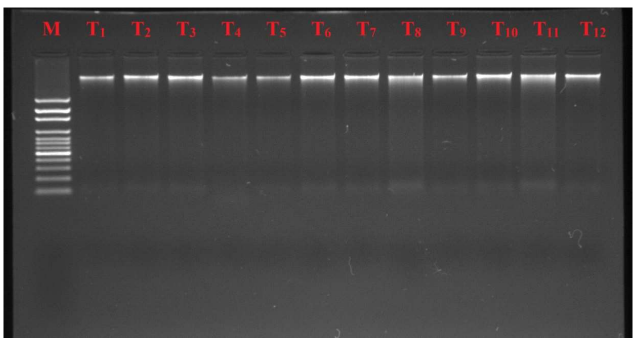
**Table 3.** DNA yield (nanogram) of *M. dubia* used for molecular study

| Genotypes | Nucleic acid concentration (ng) | 260/280 ratio |
|---|---|---|
| $T_1$ | 301 | 1.88 |
| $T_2$ | 402 | 1.85 |
| $T_3$ | 533 | 1.90 |
| $T_4$ | 829 | 1.87 |
| $T_5$ | 719 | 1.90 |
| $T_6$ | 930 | 1.77 |
| $T_7$ | 581 | 1.89 |
| $T_8$ | 565 | 1.90 |
| $T_9$ | 526 | 1.90 |
| $T_{10}$ | 916 | 1.89 |
| $T_{11}$ | 961 | 1.86 |
| $T_{12}$ | 893 | 1.87 |



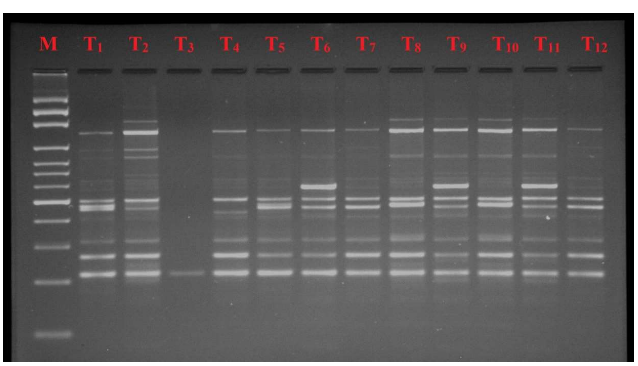

**Note:** M= DNA marker (100-3000 bp); $A_1$&$A_2$= Isolated DNA using CTAB method; $B_1$&$B_2$= Isolated DNA using Johar and Rachana method; $C_1$&$C_2$= Isolated DNA using Rawat et al method; $D_1$&$D_2$= Isolated DNA using Sharma et al method; $E_1$&$E_2$= Isolated DNA using Phukan et al method; $F_1$ & $F_2$= Isolated DNA using standardized protocol in the present study

**Fig. 1.** Isolated genomic DNA from fresh leaves of *M. dubia* under 0.8 % agarose gel using different standardized protocols




**Note:** M= DNA marker (100-3000 bp); $T_1$-$T_{12}$ = Isolated DNA of 12 genotypes of *M. dubia* using standardized protocol in the present study

**Fig. 2.** DNA quality of 12 genotypes of *M. dubia* used for RAPD and ISSR finger printings under 0.8 % agarose gel




**Note:** M= DNA marker (100-3000 bp); $T_1$-$T_{12}$ = RAPD-PCR products of 12 genotypes of *M. dubia*

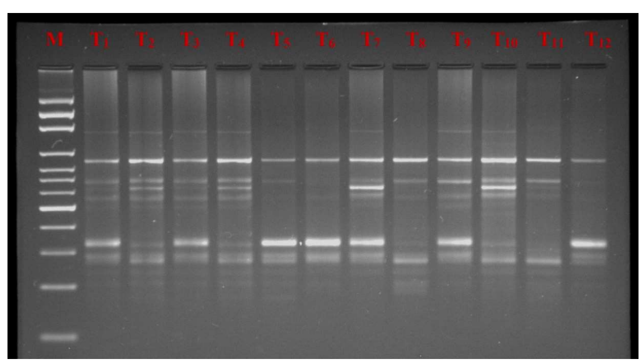
**Fig. 3.** RAPD-PCR amplification marker profile of 12 genotypes of *M. dubia* on 1.8 % agarose gel using OPL-12 primer

plant species varies from species to species as well as between individuals within a species (Kirtikar and Basu 1999, Sahu et al 2012). It has also been reported that even closely related plant species require different protocol (Rawat et al 2016). Therefore, it is quite necessary to develop a high precision DNA isolation protocol that suits each and every plant species as well as between individuals within a species. Such efforts have been attempted in *Melia* species for DNA isolation protocols by several scientists (Johar and Rachana 2015, Rawat et al 2016, Sharma et al 2018, Phukan et al 2018), however, they are unable to get a high pure DNA.

Moreover, polysaccharide contaminations are very problematic due to inhibition the activity of molecular enzymes such as polymerases, ligases and restriction endonucleases (Khanuja et al 1999, Sahu et al 2012, Rawat et al 2016). This creates a gelatinous DNA pellet due to polysaccharide contaminations and therefore, it can be removed to optimize the concentration of CTAB and NaCl in extraction buffer. Polyphenol contaminations inhibit the activity of restriction enzymes and interact with proteins and nucleic acids because it is a strong oxidizing agent (Katterman and Shattuck 1983, Sahu et al 2012, Sharma et al 2018). The oxidized form of polyphenols covalent coupling binds to nucleic acid giving a brown colour of pellet and

**Table 4.** PCR reaction mixture for RAPD and ISSR assay

| Components | Final concentration | Single PCR tube |
|---|---|---|
| *Taq* Buffer (10X) | 2X | 02.5 µl |
| 1.5 mM $MgCl_2$ | 6 mM | 01.5 µl |
| dNTPs mix (25 mM each) | 10 mM (2.5 mM each) | 01.0 µl |
| *Taq* DNA polymerase (5U $\mu l^{-1}$) | 5 Units | 00.5 µl |
| Primer (50 ng $\mu l^{-1}$) | 50 ng | 01.0 µl |
| DNA template (50 ng $\mu l^{-1}$) | 50 ng | 01.0 µl |
| *Taq* Nuclease free water | 7.0 µl | 17.5 µl |
| Final volume | | 25.0 µl |




**Note:** M= DNA marker (100-3000 bp); $T_1$-$T_{12}$ = ISSR-PCR products of 12 genotypes of *M. dubia*

**Fig. 4.** ISSR-PCR amplification marker profile of 12 genotypes of *M. dubia* on 1.8 % agarose gel using UBC-874 primer

polyphenols are exhausted from vacuoles to react quickly with cytoplasmic enzymes during homogenization process. The higher concentrations of CTAB, polyvinylpyrrolidone (PVP) and the addition of β-Mercaptoethanol (antioxidants) help to prevent interaction of phenolic compounds with DNA during DNA extraction (Rawat et al 2016). The use of liquid nitrogen also facilitates to make very fine powder which helped in creating non-oxidative environment during homogenization. Further, Rawat et al (2016) also suggested that incorporation of 5M NaCl along with chilled isoporpanol precipitate the DNA pellet without browning. The RNA and protein contaminations can be easily removed by giving additional treatments of RNase and Proteinase K (Rawat et al 2016, Phukan et al 2018). The washing of DNA pellet with 70% ethanol facilitated the removal of co-precipitate substances. Thus, this highly precise protocol was standardized to improve the quantity and quality of genomic DNA which is found to be suitable for RAPD and ISSR molecular analysis in *M. dubia*. The isolated DNA from this protocol can be used in approximately 3000 PCR reactions for molecular studies in short span time period.

## CONCLUSION

The study pointed out that modification done in the earlier DNA extraction protocols resulted in a fast, efficient and improved genomic DNA extraction with high quality and quantity pure DNA from fresh green leaves of *M. dubia.* The extracted DNA can be used in approximately 3000 PCR reactions for RAPD and ISSR molecular studies in *M. dubia* genetic resources assessment.

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# Phytosociology of Invasive Alien Plant Species and Anthropogenic Disturbances in the Community Forests of Haryana, India

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***Abstract***: Different types of anthropogenic disturbances along with the diversity of invasive plant species were studied in the selected community forests. During the study, a total of 14 invasive plant species (1 tree, 2 shrubs, and 11 herbs) belonging to 11 families were found and the selected community forests were seen to be under severe stress by disturbances like overgrazing, indiscriminate & uncontrolled tree felling, and habitat fragmentation which further increase the susceptibility to invasion. So, disciplinary actions should be taken in the direction to conserve these ecosystems and the biodiversity they contain.

***Keywords***: Anthropogenic disturbances, Biodiversity, Conservation, Habitat fragmentation, Species invasion

According to India State Forest Report (2019), Haryana comprises only 0.22% of the total forest cover of the country i.e., 1602 sq. km only. As Haryana is mainly an agricultural state, more than half of the geographical area is under agriculture and only 3.62% of the geographical area is covered by forests. The community forests of villages comprise a significant portion of this forest cover. The study of community forestry can prove to be a very promising approach to joining forest conservation with community empowerment by rural development and reduction in poverty. The Village community forests of Haryana are known by name of *Banis* and play a vital role in the lives of the village people including forest upkeep by the community as well as by the interaction of forest; agriculture and livestock maintain the forest condition and alleviate the poverty also. It has been estimated that community forests account for nearly 14% of all forests in developing countries and come under Community Forest Management (White and Martin 2002). In Asia, Africa, Latin America, and North America, forest land of around 370 million ha is thought to be conserved under community forestry.

The significance of the community forests can be predicted by the fact that in this developing world millions of poor village people are dependent on these ecosystems for their livelihood. Along with this, community forests also deliver several benefits such as biodiversity conservation, storage of carbon in soil and biomass as well as climate regulation. But these community forests are under stress due to various anthropogenic disturbances and alien plant species invasion. Due to hasty extraction of resources including over-grazing by herbivores; uncontrolled tree-felling and habitat fragmentation leads to species loss and degradation of community forests. Disturbances are considered a significant component of natural ecosystems because they affect community structure and functioning in an ecosystem. Human induced land-use or cover changes cause degradation of soil that adversely affect the productivity and wellbeing of ecosystems. To satisfy needs of rapidly growing population, land resources were exploited unsustainably (Ellis 2010) leading to depletion and continuous disturbances in forests, grasslands and wetlands (Bahn et al 2006) resulting in change of 70% land of world (FAO 2015, IPCC 2018). Thus, disturbances should be considered explicitly during the conservation efforts to preserve the functioning and biodiversity of ecosystems. Other than this, disturbances are also known to promote the invasion of weeds and non-native plant species in ecological communities. (Hobbs 1989, Rejmanek 1989). Therefore, pose an important problem for management and conservation practices.

Invasive species are recognized as one of the most stressful environmental issues (Enserink 1999, Mack et al 2000a), and man acts as the major vector in their dispersal all over the world (Hodkinson and Thompson 1997, Palumbi 2001). Invasive alien species are declared as the second largest threat for biodiversity loss in the world by CBD (Convention on Biological Diversity). The degree and quick spread of invasive alien species is homogenizing the flora and fauna throughout the world (Mooney and Hobbs 2000). Thus, important steps should be taken in direction of plant species invasion to identify and thus to remove or halt their growth to maintain the natural state of the different forest ecosystems. Therefore, to know the conservation status and sustainable management of community forests, proper documentation should be done in these ecosystems. The present study documents the phytosociology of invasive plant species in the selected community forests of three districts of Southwest Haryana. As well as it also aims to describe various benefits that local people derive from the community forests and in turn the anthropogenic pressure created by them.

## MATERIAL AND METHODS

**Study site:** The present study was performed in the community forests of Dhangar village (Fatehabad), Daya village (Hisar), and Bhera village (Bhiwani) of Southwest Haryana (Fig. 1) in the arid regions, characterized by local steppe climate. The rainfall is little throughout the year. The average rainfall and temperature of the three districts are shown in the climographs given in Figure 2. The area covered by the three community forests is 35 ha, 50 ha, and 22 ha *viz.* Dhangar village, Daya village and Bhera village, respectively.

**Vegetation sampling:** For analysis of vegetation composition and diversity, the quadrat method was used following Curtis and McIntosh (1950). A total of 15 quadrats of 20×20m were placed randomly with sub-quadrats of 5×5m and 1×1m respectively were placed with large quadrats in each of the community forests. The quantitative analysis of the vegetation was done following Misra (1968). The parameters calculated during the study are density, frequency, basal area (B.A.), Important Value Index (IVI), and diversity indices such as Shannon Weiner index (H') for species diversity, Simpson index (Cd) for the concentration of dominance, Pielou index (E) for species evenness and Margalef index (d) for species richness following Shannon and Weiner (1963), Simpson (1949), Pielou (1966) and Margalef (1958).

**Statistical analysis:** To understand the relationship among the studied parameters, Pearson correlation was analysed using R Studio and a heatmap was prepared.

## RESULTS AND DISCUSSION

Community forests form a part of Tropical Dry forests which used to cover nearly 50% of the Tropical forests of the world (Murphy and Lugo 1986, Janzen 1988b). Rural people depend on the community forests for their subsistence as the biodiversity of these ecosystems offers wonderful socio-economic as well as environmental benefits to them. Hence, they perform an integral role in the lives of rural people. During phyto-sociological analysis of the selected community forests, a total of 14 invasive plant species (1 tree, 2 shrubs, and 11 herbs) belonging to 11 families were recorded (Table 1). This can be compared with the results of Das and Duara (2013), as they found a total of 18 invasive plant species of 10 families during their study from the roadside areas of Jorhat, Assam. Other than this, Sakachep and Rai (2021) recorded 36 genera and 44 species of invasive alien plants belonging to 23 families, in the Hailakandi district (Barak valley) of North-East (NE) India. The invasive plant species encountered during the study were found to be predominantly of American origin and herbaceous life form (Table 1). A variety could also be seen in their composition as only three invasive plant species were found to be common among the three community forests, *viz. Argemone Mexicana, Croton bonpandlianum, and Sonchus oleraceous.*

Out of 11, four plant species belong to the family Asteraceae whilst the rest of the plant species belong to various other families i.e., Amaranthaceae, Asclipiadaceae, Convolvulaceae, Euphorbiaceae, Malvaceae, Mimosaceae, Oxalidaceae, Papaveraceae, Poaceae, and Verbenaceae.

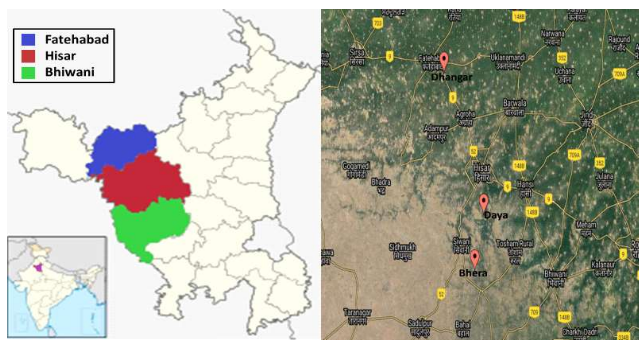


**Fig. 1.** Map showing the location of three districts in Haryana and Google imagery of the selected community forests

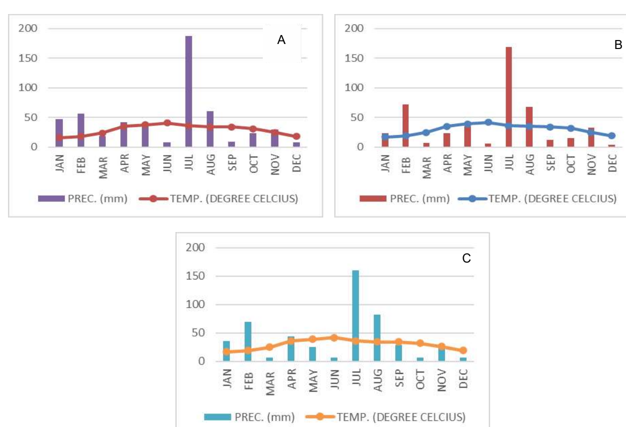


**Fig. 2.** Climographs of the three districts under study showing monthly average temperature and rainfall of the year 2019; Fatehabad (A), Hisar (B), and Bhiwani (C)

Asteraceae was also reported as the most specious family by Kosaka et al 2010, Reshi et al (2017) and Sakachep and Rai (2021) during their investigation of invasive plant species from their study site, Arunachal Himalayas, Madhya Pradesh and Hailakandi district- Barak valley (NE India), respectively. The floristic composition and diversity of invasive plant species varied in the selected community forests (Dhangar, Daya, and Bhera). The maximum diversity of invasive plant species was found in Bhera village community forest, Bhiwani (13), followed by Daya village community forest, Hisar (11), and Dhangar village community forest, Fatehabad (5). While the value of density of invasive plant species was found highest in Dhangar (20482.5 ind. $ha^{-1}$), followed by Bhera (6295 ind. $ha^{-1}$) and Daya (4170 ind. $ha^{-1}$) village community forests (Table 2-4).

The maximum value of IVI was obtained by *Prosopis juliflora* (105.366) followed by *Croton bonpandlianum* (0.84526) and others in Dhangar village community forest, (Table 2). While in Daya village community forest, the maximum IVI was observed for *Parthenium hysterophorus* (118.516) followed by *Calotropis procera* (41.264) and others (Table 3). In the case of Bhera village community forest, the maximum value of IVI was obtained by *Lantana camara* (85.390), followed by *Parthenium hysterophorus* (51.146), *Croton bonpandlianum* (33.899), and others (Table 4). Thus, *Lantana camara* was found to be the dominant invasive plant species in Bhera village community forest, Bhiwani. While in Dhangar village community forest, Fatehabad, and Daya village community forest, Hisar, the dominant plant species were *Prosopis juliflora* and *Parthenium hysterophorus* respectively. These three plant species i.e., *Lantana camara, Parthenium hysterophorus*, and *Prosopis juliflora* are also reported to be highly invasive by Aravindhan and Rajendran (2014) during their study in Boluvampatti forest Range, India.

Other than this, the diversity and richness of invasive plant species was seen to be highest in Bhera followed by

**Table 1.** Summary of Invasive alien plant species encountered during the study

| Name of the plant | Family | Habit | Nativity |
|---|---|---|---|
| *Aerva jawanica* | Amaranthaceae | Herb | Tropical America |
| *Ageratum conyzoides* | Asteraceae | Herb | Tropical America |
| *Argemone mexicana* | Papavaraceae | Herb | Tropical and Central America |
| *Calotropis procera* | Asclipiadaceae | Shrub | Tropical Africa |
| *Croton bonpandlianum* | Euphorbiaceae | Herb | Temperate South America |
| *Evolvulus nummularis* | Convolvulaceae | Herb | Tropical America |
| *Lantana camara* | Verbenaceae | Shrub | Tropical America |
| *Malvastrum coromandelianum* | Malvaceae | Herb | Tropical America |
| *Oxalis corniculata* | Oxalidaceae | Herb | Europe |
| *Parthenium hysterophorus* | Asteraceae | Herb | Tropical north America |
| *Prosopis juliflora* | Mimosaceae | Tree | Mexico |
| *Saccharum spontaneum* | Poaceae | Herb | Tropical west Asia |
| *Sonchus oleraceous* | Asteraceae | Herb | Mediterranean |
| *Xanthium strumarium* | Asteraceae | Herb | Tropical America |

**Table 2.** Phytosociological characteristics of Invasive alien plant species found in Dhangar village community forest, Fatehabad

| Name of the plant | D | B.A. | IVI | Cd | H' | E | d |
|---|---|---|---|---|---|---|---|
| *Argemone Mexicana* | 32.5 | 0.00159 | 3.146 | 0.00011 | 0.04780 | 0.02970 | 1.55948 |
| *Croton bonplandianum* | 6630 | 0.84526 | 83.771 | 0.07797 | 0.35622 | 0.22133 | 0.50741 |
| *Oxalis corniculata* | 6532.5 | 0.01132 | 43.991 | 0.02150 | 0.28151 | 0.17491 | 0.50837 |
| *Prosopis juliflora* | 667.5 | 2.46465 | 105.36 | 0.12335 | 0.36749 | 0.22833 | 0.71591 |
| *Sonchus oleraceous* | 6620 | 0.06753 | 63.723 | 0.04511 | 0.32907 | 0.20446 | 0.50751 |
| Total | 20482.5 | 3.39035 | 300 | 0.26806 | 1.38211 | 0.85875 | 3.79868 |

D= Density (individuals/hectare); B.A.= Basal Area ($m^2$/hectare); IVI= Important value index; H'= Shannon Wiener Index; Cd= Simpson Index, E= Pielou index and d= Margalef index

Daya and Dhangar village community forest as depicted from their values of Shannon-Weiner index and Margalef index (Table 2-4). While the dominance and evenness of invasive plant species was found maximum in Dhangar village community forest followed by Daya and Bhera village community forest as portrayed by values of their Simpson index and Pielou index. After the assessment of all phytosociological parameters, Pearson correlation was analysed using Rstudio and a heatmap was prepared (Fig. 3) to understand the relationship among them. From this, it is clear that the D is positively correlated to Cd, E and B.A. while negatively correlated to H' and d. Other than this, Cd is positively correlated with D and E while negatively correlated with H', d and B.A. During positive correlation, if the value of one parameter increases the other parameter also increases but during negative correlation with increase in value of one parameter, the value of other parameter decreases.

During the survey, it was found that the rural people derive a variety of benefits from these community forests or *Banis* (Fig. 4, g-i) such as fuelwood by cutting trees & shrubs as well as water, fodder & shelter for their animals. The community forests are also rich in medicinally important

**Table 3.** Phytosociological characteristics of invasive alien plant species found in Daya village community forest, Hisar

| Name of the plant | D | B.A. | IVI | Cd | H' | E | d |
|---|---|---|---|---|---|---|---|
| *Aerva javanica* | 40 | 0.0069 | 4.529 | 0.00022 | 0.06331 | 0.02640 | 3.606 |
| *Argemone Mexicana* | 147.5 | 0.0298 | 18.232 | 0.00369 | 0.17020 | 0.07098 | 2.452 |
| *Calotropis procera* | 175 | 0.1125 | 41.264 | 0.01891 | 0.27286 | 0.11379 | 2.353 |
| *Croton bonplandinus* | 472.5 | 0.0351 | 31.568 | 0.01107 | 0.23693 | 0.09880 | 1.907 |
| *Evolvulus nummularius* | 300 | 0.0011 | 16.492 | 0.00302 | 0.15947 | 0.06650 | 2.088 |
| *Malvastrum coromandelianum* | 42.5 | 0.0013 | 5.809 | 0.00037 | 0.07637 | 0.03185 | 3.529 |
| *Oxalis corniculata* | 912.5 | 0.0075 | 30.111 | 0.01007 | 0.23074 | 0.09622 | 1.694 |
| *Parthenium hysterophorus* | 1942.5 | 0.2858 | 118.516 | 0.15606 | 0.36689 | 0.15300 | 1.502 |
| *Prosopis juliflora* | 57.5 | 0.0069 | 16.312 | 0.00295 | 0.15833 | 0.06603 | 3.189 |
| *Saccharum spontaneum* | 25 | 0.0441 | 11.166 | 0.00138 | 0.12249 | 0.05108 | 4.342 |
| *Sonchus oleraceus* | 55 | 0.0007 | 5.996 | 0.00039 | 0.07820 | 0.03261 | 3.235 |
| Total | 4170 | 0.5317 | 300 | 0.20819 | 1.93583 | 0.80730 | 29.903 |

D= Density (individuals/hectare); B.A.= Basal Area ($m^2$/hectare); IVI= Important value index; H'= Shannon Wiener Index; Cd= Simpson Index, E= Pielou index and d= Margalef index

**Table 4.** Phytosociological characteristics of invasive alien plant species found in Bhera village community forest, Bhiwani

| Name of the plant | D | B.A. | IVI | Cd | H' | E | d |
|---|---|---|---|---|---|---|---|
| *Aerva javanica* | 225 | 0.05558 | 11.733 | 0.00152 | 0.12677 | 0.04942 | 2.666 |
| *Ageratum conyzoids* | 715 | 0.07193 | 16.142 | 0.00289 | 0.15724 | 0.06130 | 2.121 |
| *Argemone Mexicana* | 27.5 | 0.00738 | 2.372 | 0.00006 | 0.03827 | 0.01492 | 5.004 |
| *Calotropis procera* | 95 | 0.06107 | 18.847 | 0.00394 | 0.17386 | 0.06778 | 3.298 |
| *Croton bonplandianum* | 930 | 0.17314 | 33.899 | 0.01276 | 0.24637 | 0.09605 | 2.027 |
| *Lantana camara* | 272.5 | 4.51135 | 85.390 | 0.08101 | 0.35765 | 0.13944 | 2.557 |
| *Malvastrum coromandelianum* | 327.5 | 0.01986 | 10.973 | 0.00133 | 0.12101 | 0.04718 | 2.461 |
| *Oxalis corniculata* | 1652.5 | 0.00789 | 31.831 | 0.01125 | 0.23802 | 0.09279 | 1.847 |
| *Parthenium hysterophorus* | 1537.5 | 0.53534 | 51.146 | 0.02906 | 0.30160 | 0.11758 | 1.868 |
| *Prosopis juliflora* | 7.5 | 0.34394 | 7.424 | 0.00061 | 0.09154 | 0.03568 | 10.922 |
| *Saccharum spontaneum* | 207.5 | 0.27527 | 14.960 | 0.00248 | 0.14952 | 0.05829 | 2.715 |
| *Sonchus oleraceous* | 7.5 | 0.00137 | 1.959 | 0.00004 | 0.03285 | 0.01281 | 10.922 |
| *Xanthium strumarium* | 290 | 0.20415 | 13.318 | 0.00197 | 0.13827 | 0.05390 | 2.524 |
| Total | 6295 | 6.26827 | 300 | 0.14898 | 2.17304 | 0.84720 | 50.940 |

D= Density (individuals/hectare); B.A.= Basal Area ($m^2$/hectare); IVI= Important value index; H'= Shannon Wiener Index; Cd= Simpson Index, E= Pielou index and d= Margalef index plants. Thus, the local people make ayurvedic medicines using them. Sometimes, people also harm these ecosystems by using the area as dumping ground and also perform soil mining (Fig. 4, f) which leads to the degradation of the land. Inclusive of this, some other benefits are also obtained from the plants growing in the Community forests such as the fruits of *Capparis decidua* are used to make the pickle, fruits of *Cucumis callosus* are used in making vegetable curries while those of *Salvadora oleoides*, *Ziziphus nummularis*, *Coccinia grandis,* and *Physalis minima* are eaten raw by people. In this way, these community forest ecosystems play a very significant role in the lives of rural people. But, due to the rapid increase in population rendering urbanization, need for agricultural land as well as industrialization, the community forest ecosystems are declining and getting lost with time. Thus, phyto-sociological studies of community forests to understand the cause of their degradation and the pertaining anthropogenic pressure may help in the direction of conservation of these forest ecosystems and the biodiversity they comprise. The present study was performed by keeping this in mind and it was seen that the three community forests are under acute anthropogenic pressure as well as invaded by many alien plant species.

The selected study sites are found to be under high pressure due to grazing by herbivores. Excessive grazing causes a decrease in species richness; a profound change in soil properties, and the formation of bare land (Podwojewski et al 2002). But along with this grazing may also contribute to alien plant species invasion. As the grazers may import the propagules of invasive alien plant species to the native land and may also offer microsites for invasion. This leads to a change in vegetation structure along with soil disturbance and other conditions, just like that it ultimately establish the alien species invading the ecosystem. Similarly, by continued tree felling performed by rural people in the community forests to fulfil the fuelwood requirements, canopy openings are created which cause regeneration problems due to exposed conditions (rapid soil drying out and nutrient loss through run-off). These canopy openings allow the growth of invasive weeds and other herbaceous plants which usually hinder the regeneration of native plants and impede their recovery (Hawthorne 1994, Madoffe et al 2006).

Other than this, due to the high growth of the human population and urbanization fragmentation of forests occurred leading to the clearing of forest land for human settlements and agriculture. It is still being continued in the community forests such as land encroachment in the name of building schools or temples and by the owners of adjacent agriculture fields (Fig. 4, d-e). It leads to a decrease in the land area of these ecosystems which may also cause

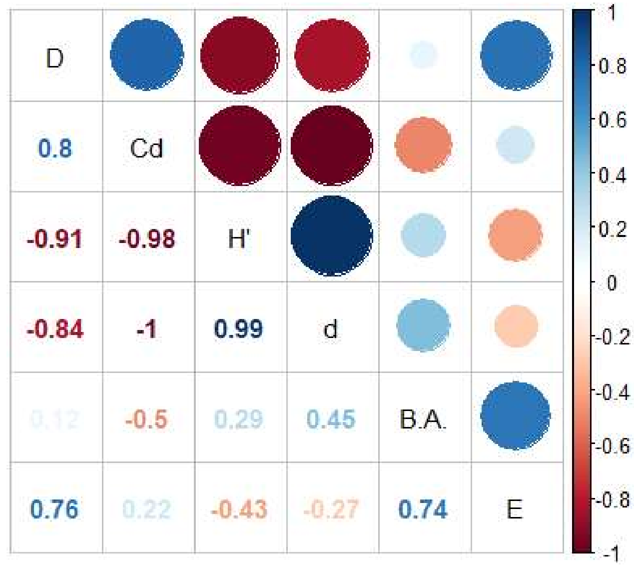


**Fig. 3.** Heat map showing the correlation of different parameters calculated during the study

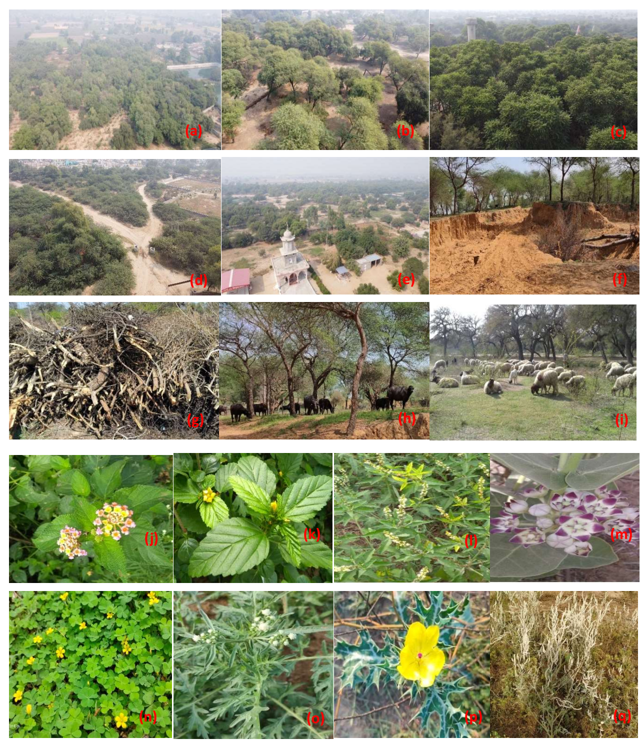

**Fig. 4.** Pictures showing the status, various activities, and some of the invasive plant species documented during the present study. **(a)** Dhangar village site, **(b)** Daya village site **(c)** Bhera village site **(d)** Habitat fragmentation at Bhera **(e)** Land encroachment at Dhangar **(f)** Soil mining at Bhera **(g)** Fuelwood collected by cutting trees and shrubs at Daya **(h)** & **(i)** Cattle grazing **(j)** *Lantana camara* **(k)***Malvastrum coromandelianum* **(l)** *Croton bonpandlianum* **(m)** *Calotropis procera* **(n)** *Oxalis corniculata* **(o)***Parthenium hysterophorus* **(p)** *Argemone mexicana* **(q)** *Aerva javanica*.

species loss. The species loss is apparent in the case of fragmentation, as the smaller patches cannot sustain as much species diversity in comparison to a large forest patch (Game and Peterken 1984, Dzwonko and Loster 1989). In this way, anthropogenic disturbances encourage the process of alien plant species invasion (Elton 1958, Lodge 1993, Burke and Grime 1996) and may cause species loss. Despite the mechanism which facilitates invasion in case of disturbance is rarely examined due to high availability of resources, decrease in competition, and increase in propagule pressure (Hobbs and Huenneke 1992, D'Antonio 1993, Davis and Thompson 2000).

Invasive species have been seen to be linked to human-induced environmental change for a long period, having adverse effects on ecosystem services as well as human well-being (Pejchar and Mooney 2009). Species invasion may cause local declines (Islam 2001) and also result in the extinction of native species (Pimm 1986) therefore variation in the species richness of a forest fragment (Carey et al 1996). Because, once they are established over an area, some of them can replace the native flora, disturb the nutrient cycles, and cause variations in the process of plant succession. Thus, it would not be wrong to say that right now we are fighting on two fronts; one is to maintain the natural flora of the community forests which is native to them and the second is to halt the growth of invasive alien species growing in them. Hence, we suggest that proper management activities should be performed in the given community forests to control the growth of these invasive alien plant species as well as to restore the natural ecology of the area.

## CONCLUSION

The community forests are not only getting degraded in their state but also becoming less diverse and species-poor due to anthropogenic pressure leading to change in community organization. The invasive plant species create a homogeneous, mono-specific understorey that not only disturbs the environment but may also threaten the indigenous flora, as many of the plant species are getting vulnerable. Hence grave actions are required for the conservation of these community forests in Haryana state so that the vanishing of these species-rich communities can be ceased. Hence, it is imperative to put a check on anthropogenic activities and create an effective database to manage and control the invasive plant species, and also progress the information regarding their diversity, habitat, life form, and usage for future studies.

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# Comparative Assessment on Riparian Soil Characteristics at Three Lateral Buffer Zones in Riparian Forest of Dikhu River, Nagaland

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***Abstract***: The present study aimed to estimate the heterogeneity of soil physicochemical properties in riparian zones at the three lateral buffer of riparian forest of the Dikhu river, Nagaland. Soil physicochemical properties of three lateral buffers i.e.,10~100 m (zone –III, the intact forest, which is undisturbed by human activities), 100~300 m (zone-II, the cultivate land) and >300 m (zone-I, the human habited land) from river edge along one side of the riparian zones were measured. The soil pH, soil moisture, organic carbon, organic matter and available nitrogen were higher in the intact forest as compared to the other two zones i.e., cultivated area and the human habited area . Seasonal variations, distance from river edge and nature of different land use system were the main responsible factors affecting the riparian soil physicochemical properties in this study.

***Keywords***: Riparian soil, River edge, Riparian buffer, Nutrients, Dikhu river

Riparian forests are vegetated areas that locate immediately adjacent of streams, lakes, swamps, or other surface waters and differ from the uplands because of periodical flooding, stagnant water, high soil moisture condition and the unique assemblage of living organisms found there (Mongkolsawat 2008). The effects of disturbance on riparian soils are variable. Riparian zones are unique and dynamic systems. Major types of disturbance that impact these systems include management activities such as livestock grazing, timber harvest, recreational use, and the creation of physical structures like dams and roads, or natural perturbation such as fire, beavers, wind throw, and the action of water. In particular, impacts of livestock grazing on riparian soils include soil compaction, breakdown of undercut stream banks, and increased loss of sediment due to excessive removal of stabilizing vegetation. Timber harvest increases soil erosion and alters soil microclimates by increasing soil temperatures. Standing water that is present during all or part of the year produces unique water and soil conditions in riparian zones, which results in plant communities that are distinct from upland habitats (Michelson and Veto 2000). Riparian zones possess high levels of soil moisture due to both the presence of water in the stream and the movement of groundwater into the rooting zone of riparian vegetation (Bilby 1988). Fluctuations in groundwater levels that influence the nature and extent of plant production and microbial activity are responsible for much of the variation in riparian zone soil (Michelson and Veto 2000). Accelerated decomposition rates result in rapid nutrient release to riparian soils (Edmonds 1980). One of the major difference of riparian soils compared to upland is that they tend to be wetter and are subjected to fluctuation water tables that may reach the soil surface (USDA-NRCS 2005). In general, because of their position within the landscape, riparian soils are recipients of sediments and other materials from the watershed and are also important regulators and transformers of energy and materials between terrestrial and aquatic ecosystems (Caiman and Decamps 1997, Hill and Cardiac 2002). The changing land use practices, deforestation in the catchment and river banks, agricultural expansion, increasing population and pollution are some of the major threats to the riparian ecosystem (Motet and Savant 2013). The spatial heterogeneity of riparian soil properties is mainly affected by parent material, topography, vegetation, climate, biological conditions and human activities such as land use changes and agriculture (Liu et al 2013). Analysing the relationship between soil organic carbon (SOC) and N, P, pH values and bulk density in riparian zone, is important to understand the condition of soil fertility of riparian zone, the composition of soil organic matter and the condition of soil quality as well as the supply ability of nutrients (Quaint et al 2017). The present study was conducted to evaluate the variation in soil physico-chemical properties of riparian soil at the three lateral buffer zones of the riparian forest of Dikhu river, Nagaland.

## MATERIAL AND METHODS

**Experimental area and sampling site:** The study site lies between 26$^{0}$15′11″ to 26$^{0}$5′18″ latitude and 94$^{0}$31′43″ to

$94^0$31′49″ longitude with an altitude ranging from 600-665 metres above mean sea level. Soil samples were collected from river edge along one side of the riparian zones on three different lateral buffer zones of the riparian forest of Dikhu river in Moalenden village of Mokokchung, Nagaland and represented as human habited land (zone-I), cultivated land (zone-II) and intact forest (zone-III) area between September, 2018 and April, 2019. Distances of the lateral buffer were: 10~100 m (zone –III), 100~300 m (zone-II) and >300 m (zone-I) away from the river edge. In each zone, soil was collected at 0-10cm, 10-20cm and 20-30cm layers depth. The collection was done once in three seasons *viz*., autumn (September), winter (November) and summer (April). After collection, the soils were packed in labelled polythene bags. Soil pH of 1:2.5 (v/v) soil: water suspension was measured by using a pH glass electrode, soil temperature was estimated using a digital soil thermometer, soil moisture content by gravimetric method, soil bulk density and particle density by core method, soil texture by using Pipette method (Piper 1950) and soil temperature by using soil thermometer. Soil organic carbon (SOC) by the $K_2Cr_2O_7$ wet oxidation method (Walkley and Black's 1934), available nitrogen ($N_{av}$) using $KMnO_4$ oxidation method following Kjeldahl (1883), Available phosphorous was determined using sulphuric acid digestion followed by colorimetric determination (Bray and Kurtz 1945) while Potassium concentration was analyzed after the sample has been digested by sulphuric acid following Jackson (1973).

## RESULTS AND DISCUSSION

**Soil temperature:** There was significant seasonal change in soil temperature. Soil temperature was maximum rising to 29ºC in the upper layer during autumn and low during winter (20ºC). During autumn the maximum temperature was at zone I (26ºC-29ºC) and the minimum temperature at zone III (23º-26ºC) (Table 1). During winter, the maximum temperature was in zone I (23ºC-26ºC) and the minimum temperature was recorded from zone III (20ºC-23ºC). During spring the maximum temperature was recorded from the zone I (25ºC-28ºC) and the minimum temperature from zone III (21ºC-24ºC). The soil temperature decreases with increase in depth. This was similar to the work done by Leishangthem and Singh (2021) which also shows a decreasing soil temperature with increase in soil depth. Soil temperature of intact forest was less because the soil is under the shed of trees and plants and also the intact forest has more moisture content compared to human habited area and the cultivated area. Scull (2007) showed that the vegetation cover was negatively correlated with soil temperature.

**Soil pH:** Soil pH was third in the lower layer (20-30 cm) in all the seasons. During autumn pH lies within the range 5.61 to 6.93, in winter it lies between 5.02 and 6.11 and in spring, it lies between the ranges 5.13 to 5.96. Quaint et al (2017) stated that soil pH range from 4.76 to 7.79 in riparian areas of inflowing streams of Taihu lake and concluded that soil pH decreases with soil depth in riparian soil. Norton et al (2011) also reported soil pH from 4.99 to 6.13 in the riparian soils.

**Bulk density:** Bulk density was highest during spring and relatively low in autumn in all the 3 zones (Table 1). It ranges from 0.8-1.11 g $cm^{-3}$ in autumn, 0.97-1.31 g $cm^{-3}$ in winter and 1.17-1.59 g $cm^{-3}$ during spring. Similar observations was made by Qianet al (2017) in the riparian soils where he found the bulk density between 0.76 to 1.53 g $cm^{-3}$. The bulk density was found to be higher in the human habited area as compared to the other two zones i.e., cultivated area and intact forest. This could be due to the presence of high sand percentage since total pore space in sand is less than that of silt and clay soils (Muhamad et al 2011).

**Particle density:** Particle density was maximum during spring (1.76–2.02 g $cm^{-3}$) followed by autumn (1.67-2.08 g $cm^{-3}$) and winter (1.58-1.94 g $cm^{-3}$). The mean particle density was observed highest at zone III while the lowest was observed in zones I (Table 1). The soil particle density increases as the soil depth increases in all the three zones because of the concurrent decrease in organic matter (Schiavo et al 2012).

**Soil porosity:** The soil porosity was found maximum during autumn (37-52%) followed by winter (35-44%) and the lowest was observed during spring (25-35%). On average, soil porosity was higher at zone II in the upper layer at all seasons (Table 1) and decreases with an increase in soil depth. Subsurface layers have reduced organic matter, aggregation and root penetration compared to surface layers and therefore contains less pore space which was in conformity with Muhammad et al (2011).

**Soil texture:** The content of sand ranges from 26-46%, silt from 19-47% and clay from 25-44%. The percentage of silt was observed highest at zone III during autumn season (Table 1). This may be due to the fact that the riparian areas contain higher concentrations of minerals. The soil texture in intact riparian forest shows the dominance of silt and clay soils which is ideal for plant growth. A similar observation was made by Arshid (2012) in intact forest of riparian areas of Himalayas. In case of zone I, the percentage of sand was higher due to various anthropogenic activities, which have influenced higher soil compaction.

**Soil moisture:** The moisture content was highest at the top layer (0-10cm) in the three zones at all seasons (Table 1). Soil moisture ranges maximum during autumn (9.65-52.35%)

**Table 1.** Seasonal variation in various physico-chemical properties of riparian soil at three different lateral buffer zones from Dikhu riparian forest

| Parameters | Zone/Layer | Autumn | | | Winter | | | Spring | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0-10 cm | 10-20 cm | 20-30 cm | 0-10 cm | 10-20 cm | 20-30 cm | 0-10 cm | 10-20 cm | 20-30 cm |
| Soil temperature (°C) | Zone I | 29 | 28 | 26 | 26 | 25 | 23 | 28 | 26 | 25 |
| | Zone II | 27 | 26 | 25 | 25 | 23 | 22 | 27 | 24 | 23 |
| | Zone III | 26 | 24 | 23 | 23 | 22 | 20 | 24 | 23 | 21 |
| Soil pH | Zone I | 5.99 | 5.98 | 5.96 | 5.23 | 5.21 | 5.18 | 5.22 | 5.19 | 5.13 |
| | Zone II | 5.7 | 5.68 | 5.61 | 5.18 | 5.12 | 5.02 | 5.62 | 5.38 | 5.23 |
| | Zone III | 6.93 | 6.91 | 5.72 | 6.11 | 6.08 | 6.02 | 5.96 | 5.95 | 5.92 |
| Bulk density ($g/cm^3$) | Zone I | 0.92 | 0.99 | 1.11 | 1.12 | 1.25 | 1.31 | 1.29 | 1.36 | 1.59 |
| | Zone II | 0.8 | 0.86 | 1 | 0.97 | 1.08 | 1.11 | 1.17 | 1.21 | 1.29 |
| | Zone III | 0.88 | 0.93 | 1.1 | 1.03 | 1.16 | 1.19 | 1.25 | 1.28 | 1.39 |
| Particle density ($g/cm^3$) | Zone I | 1.58 | 1.67 | 1.76 | 1.67 | 1.82 | 1.84 | 1.87 | 1.94 | 2.02 |
| | Zone II | 1.67 | 1.76 | 1.94 | 1.67 | 1.82 | 1.83 | 1.76 | 1.82 | 1.87 |
| | Zone III | 1.76 | 1.82 | 1.94 | 2 | 2.02 | 2.08 | 1.87 | 1.94 | 2.02 |
| Porosity (%) | Zone I | 42 | 41 | 37 | 38 | 36 | 35 | 31 | 30 | 25 |
| | Zone II | 52 | 51 | 48 | 42 | 40 | 39 | 34 | 33 | 31 |
| | Zone III | 50 | 49 | 43 | 44 | 38 | 37 | 35 | 34 | 31 |
| Soil moisture (%) | Zone I | 13.68 | 13.33 | 9.65 | 28.24 | 26.33 | 21.12 | 21.33 | 19.97 | 19.62 |
| | Zone II | 37.97 | 34.08 | 28.53 | 25.06 | 21.33 | 20.37 | 23.94 | 22.97 | 21.98 |
| | Zone III | 52.35 | 48.76 | 44.92 | 30.21 | 25.03 | 23.92 | 31.82 | 27.32 | 26.52 |
| Sand (%) | Zone I | 40 | 30 | 26 | 36 | 37 | 26 | 39 | 30 | 31 |
| | Zone II | 41 | 30 | 26 | 37 | 30 | 33 | 40 | 28 | 34 |
| | Zone III | 34 | 37 | 28 | 46 | 32 | 31 | 35 | 37 | 32 |
| Silt (%) | Zone I | 35 | 36 | 42 | 32 | 34 | 42 | 32 | 37 | 37 |
| | Zone II | 32 | 26 | 44 | 26 | 32 | 38 | 32 | 30 | 35 |
| | Zone III | 35 | 30 | 47 | 19 | 33 | 40 | 32 | 32 | 36 |
| Clay (%) | Zone I | 25 | 34 | 32 | 32 | 29 | 32 | 29 | 33 | 32 |
| | Zone II | 27 | 44 | 30 | 37 | 38 | 29 | 28 | 42 | 31 |
| | Zone III | 31 | 33 | 25 | 35 | 35 | 29 | 33 | 31 | 33 |
| Organic Carbon (%) | Zone I | 3.87 | 3.72 | 3.62 | 3.52 | 3.47 | 3.41 | 3.77 | 3.67 | 3.46 |
| | Zone II | 3.93 | 3.83 | 3.72 | 3.62 | 3.52 | 3.47 | 3.87 | 3.72 | 3.62 |
| | Zone III | 4.03 | 3.93 | 3.827 | 3.724 | 3.57 | 3.58 | 4.13 | 4.08 | 3.98 |
| Organic matter (%) | Zone I | 2.25 | 2.16 | 2.1 | 2.04 | 2.01 | 1.98 | 2.19 | 2.13 | 2.01 |
| | Zone II | 2.28 | 2.22 | 2.16 | 2.1 | 2.04 | 2.01 | 2.25 | 2.16 | 2.1 |
| | Zone III | 2.34 | 2.28 | 2.22 | 2.16 | 2.07 | 2.04 | 2.4 | 2.37 | 2.31 |
| Available nitrogen (kg/ha) | Zone I | 150.52 | 125.44 | 100.35 | 163.07 | 137.98 | 112.89 | 200.7 | 188.16 | 163.07 |
| | Zone II | 163.01 | 125.44 | 112.9 | 150.52 | 112.89 | 100.35 | 188.16 | 163.07 | 150.52 |
| | Zone III | 175.01 | 137.99 | 100.35 | 225.79 | 213.24 | 200.7 | 250.88 | 238.33 | 200.7 |
| Potassium (kg/ha) | Zone I | 173.97 | 86.58 | 150.08 | 134.23 | 102.42 | 62.72 | 301 | 229.54 | 94.47 |
| | Zone II | 324.86 | 205.69 | 102.42 | 324.86 | 277.2 | 150.08 | 380.24 | 388.08 | 293.1 |
| | Zone III | 261.69 | 388.64 | 269.36 | 380.24 | 245.39 | 86.24 | 412.16 | 332.64 | 237.49 |

followed by spring (19.62-31.82%) and winter (20.37-30.21%). The highest soil moisture was recorded during autumn at the top soil layer of zone III. Soil moisture showed direct relation with precipitation and vary with seasons. In the present study, the more exposed area in human habited area might have contributed for lower value of soil moisture than in riparian forest. Similar observation was made by Zamias et al (2010) where he found that soil moisture were higher in riparian forest compared to surrounding uplands. Moreover, riparian zones possess high levels of soil moisture due to both the presence of water in the river and the movement of groundwater into the rooting zone of riparian vegetation (Bilgy and Robert 1988).

**Organic matter:** The organic matter in autumn ranges from 2.1-2.34%, winter from 2.01-2.16% and spring from 2.01–2.4%. As observed in the study, soil organic matter decreases with depth which was in conformity with Barzanji et al (2011). Cultivated soils generally have low organic matter content compared to native land, since cultivation increases aeration of soil, which in turn enhances decomposition of soil organic matter. In addition, most of the organic matter produced in the cultivated lands is removed during the harvest period. Previous studies also reported that conversion of forest to cultivated land significantly decrease soil organic matter content (Jaiyeoba 2003).

**Organic carbon:** Organic carbon ranges from 3.62-4.03% during autumn, 3.41- 3.72% in winter and 3.46-4.13 during spring which was similar with the findings of Diane et al (2017).It was observed that the organic carbon decreases with increasing soil depth (Table 1). In general, the amount of soil organic carbon is greater in forest than in arable land used for cropping soils (Vesterdal et al 2002). Maximum organic carbon was recorded in intact forest of riparian areas as compared to cultivated land and human habited areas because in intact forest there are higher amount of litter and root decomposition.

**Available nitrogen:** Available nitrogen in autumn ranges from 2.1-2.34 kg $ha^{-1}$, winter from 2.01-2.16 kg $ha^{-1}$ and spring from 2.01-2.4 kg $ha^{-1}$. The maximum available nitrogen content was recorded at zone III in all the seasons (Table 1) which could be due to moist condition of the zone that may have enhanced the activity of nitrogen fixing bacteria. High amount of nitrogen from the adjacent farmland leach or flow along the surface of the stream water which enter into the intact forest and eventually accumulate in riparian soils and vegetation.

**Potassium:** Potassium content ranged from 261.68 kg $ha^{-1}$ to 388.64 kg $ha^{-1}$ at zone-III, 102.42 kg $ha^{-1}$ to 324.85 kg $ha^{-1}$ at zone-II and 86.57 to 173.93 kg $ha^{-1}$ at zone-I (Table 1). It was observed that potassium content in the surface soil was higher than that of the sub-surface soils which was in conformity with Barzani et al (2011). The more amount of potassium in intact forest is due to leaching of inorganic fertilizers from the agricultural fields during rainy seasons that got accumulated in the riparian soil. Moreover, higher percentage of organic carbon and clay can elevate the content of potassium and other nutrient elements in the forest soil (Semy and Singh 2021).

## CONCLUSION

The nutrients were comparatively higher in the intact forest of riparian areas as compared to the other two zones (cultivated area and the human habited area). The parameter such as soil pH, soil moisture, organic carbon, organic matter and available Nitrogen were higher in the intact forest. Available nitrogen, soil organic carbon, soil organic matter and soil moisture was higher at the surface soil (0-10 cm) than the deeper layers (10-20 cm and 20-30 cm). Soil analysis showed that the soil of riparian area was nearly acidic; Nitrogen content decreased with depth and was found highest in intact riparian forest; Potassium content was higher in dry season and soil moisture was directly related to precipitation i.e. higher in rainy season and lower in dry season. As observed in the study, the capacity of soil to be productive depends not only on the plants nutrient stores but also on the physical characteristics of the soils such as bulk density and porosity. Significant variations among the soil nutrients and physical parameters such as soil porosity, bulk density, soil moisture and soil temperature were associated to different landuse pattern in the riparian zone showing sensitivity to changes to landuse. The results from this study indicates that there is a need to assess and protect the riparian zones, and proper land use planning and adoption of sustainable farming systems within the zone should be inculcated in order to prevent further deterioration of the riparian soils along the stretch of Dikhu river.

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# Changes in Soil Microbial Population Following Decomposition of Different Litters Under Shifting Cultivation in Mizoram

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***Abstract***: Shifting cultivation is a traditional agricultural practice being carried out on steep slopes of hills by the tribal population of Mizoram. The present study aims to determine the changes in soil microbial population due to decomposition of different leaf litters {i.e., *Tephrosia candida* (Roxb.) DC, *Oryza sativa* L. and mixture of both} in two shifting fallows (FL-3 years and FL-8 years old) and under laboratory microcosm. Litter components were enclosed in nylon net bags and placed over the soil surface for decomposition in July 2019 in Tanhril village of Mizoram, and the same litter bags were placed over soil in laboratory microcosm. Four litter bags were retrieved at monthly interval for eight months, and soil beneath the litter bags were collected to determine microbial population (i.e., Bacteria, Fungi and Actinomycetes) using serial dilution method. Results of the present study indicate that soil microbial population increased initially for a period of five months of litter decomposition, and thereby a decrease was noticed. *T. candida* litter decomposed faster than other two litter types and subsequently showed higher soil microbial counts. Bacterial population was considerably higher than the populations of fungi and actinomycetes. Soil microbial diversity was significantly higher in longer fallow land than the shorter. It is concluded that *T. candida* leaf litter of decomposes faster in longer fallow land due to higher microbial activity which plays an important role in soil fertility and subsequently sustainable agricultural practices in Northeast hill region of Mizoram.

***Keywords***: Shifting Cultivation, Decomposition, Microbial population, *Tephrosia candida*, *Oryza sativa*

Northeast India (NEI) is one of the richest reservoirs of biodiversity with large number of flora and fauna showing high endemism (Singh et al 2015, Tripathi et al 2016). Shifting cultivation, a primary agricultural system in NEI, is also known as slash and burn or *jhum* cultivation. Mizoram is one of the seven sister states of Northeast India with undulating areas dominated by tribal populations, which are involved in shifting cultivation for their livelihood through centuries. Traditional practices of shifting cultivation involved slashing a piece of forest land, waiting it to dry and burn *in situ* followed by cropping for few years depending on the level of soil fertility (Grogan et al 2012). When crop productivity decreases due to decrease in soil fertility, farmers move to other forested land for cultivation and abandon this land to resume soil fertility through natural regeneration. Earlier shifting cultivation was capable of regenerating soil fertility through land abandonment (20-30 years) but now a days due to increasing population, this has led to reduce the length of fallow period (<5 years years), which resulted in decreased soil fertility and crop productivity (Grogan et al 2012). Trends of decreasing soil fertility and crop productivity has badly hampered the livelihood options of farming communities (Tripathi et al 2017) and thereby there is a need to reverse this trend through improving soil fertility and crop productivity under short fallow (Wapongnungsang et al 2021). In this study, an effort has been made to energies this agricultural system through litter amendments and assessing the role of amended litters on soil microbial population. Organic matter decomposition has been widely reported to regulate soil fertility and rate of nutrients availability through soil microorganisms like bacteria, fungi and actinomycetes (Tripathi 2009, Manpoong et al 2020). Soil microorganisms are essential for maintaining soil fertility and plant growth by playing important role in nutrient cycling (Nannipieri et al 2003). Soil microbes are involved in decomposition of organic matter which affects soil carbon stabilization that regulates soil fertility and nutrient availability (Ramesh 2019).

Soil consists of large number of microbial population but the proportions of bacteria, fungi and actinomycetes in soil vary dramatically. For example, soil contains large number of bacteria, one tenth of actinomycetes and small number of fungi which become dominant in undisturbed soil (Hoorman 2010). Litter inputs through litterfall and root mortality are the main source of nutrients to the soil through microbial transformation which considerably affect soil fertility and crop productivity (Tripathi 2010). *Tephrosia candida* (Roxb.) DC is forming dense vegetation cover, and adding atmospheric nitrogen through fixation and litter input through high biomass production (Wapongnungsang et al 2017). *Oryza sativa* L is the major crop plant under shifting cultivation in NEI region and can add 15.4% to 38.4% of total organic carbon in the soil (Pangga et al 2000). *O. sativa* litter affects C and N cycle in the soil and makes chemical speciation and biological efficiency of soil nutrients. Therefore, keeping these facts in mind, this study aims to determine the changes in soil microbial population during the course of decomposition of leaf litters of *T. candida* and *O. sativa* alone and in combination (*T. candida.* + *O. sativa*).

**(A)** **(B)**

**Fig. 1.** A- 8 years fallow jhum field, B- 3 years fallow jhum field

**Fig. 2.** Pot experiment

## MATERIAL AND METHODS

**Site description:** The study was conducted in Tanhril, Mizoram on two fallow lands of 3 years (23°39´55´´N, 92°31´48´´-92°33´24´´E) and 8 years (23°43´55´´-23°44´48´´N, 92°38´43´´-92°40´4´´E). Tanhril is located about 15 km away from the capital city Aizawl. The average temperature and humidity during the experiment varies from 26°C to 11°C and 82% to 90% respectively. The total rainfall of the year was 1418.1 mm. The ages of the fallow lands were identified by interviewing the land owner. The soil of the study sites was slightly strongly acidic (pH=4-5) and belongs to the order inceptisol and falls under red soil group having light to medium texture and slope of land varied between ~35° and 40° (Hauchhum and Tripathi, 2017).

**Field experiment:** *T. candida* and *O. sativa* leaf litters were collected from the field and air dried. Ten grams of two leaf litters were enclosed in nylon bags (20cm x 20cm, 2mm mesh) alone (*T. candida*, *O. sativa*) and in combinations (*T. candida* +*O. sativa*). A total of 50 bags were prepared for each litter types and placed randomly over the soil in two fallows (3 years fallow and 8 years fallow; Fig. 1) in July 2019.

**Pot experiment:** Additionally, soils were collected from the study sites and brought to the laboratory for setting microcosm experiment. One fifty pots were set for three different litter types (i.e. *T. candida* and *O. sativa* alone and in combination of *T. candida* + *O. sativa*) for 3 years and 8 years fallow separately (Fig. 2). Each pot was filled with 3 kg of soil and 10g of leaf litters were added on the top of the soil. Pot experiment was conducted under control condition.

**Collection of samples from field and pot experiments:** Every month for field and pot experiment, surface soil (10 cm) was collected in four replicates for each litter types and sieved through a 2-mm mesh screen after removing the visible plant debris. Soil samples were retrieved monthly for eight months. A total eight recoveries were made for each litter type for each experimental setup. Collected soils were stored at 4°C for microbial study. Microbial population count was estimated by serial dilution method within one month.

**Sterilization of glass wares:** All the glass wares (Petri plate, test tubes, slides conical flask, inoculum needle, measuring jar, and beaker) were kept in cleaning solution for 12 hrs. Then, they were washed in soap water and tap water. Finally, they were cleaned with distilled water and dried. The dried glass wares were sterilized at 120°C for 15 minutes in autoclave. All the chemical media were sterilized using the autoclave.

**Serial dilution method:** Sterilized test tubes were taken and the first test tube was taken with 10 ml of 0.9% NaCl solution, and remaining test tubes were with 9 ml of saline solution. 1 gm of soil sample was dissolved in the first test tube and its dilution factor is $10^{-1}$. Then, 1 ml of sample was transferred from first tube ($10^{-1}$) to second test tube and its dilution factor is $10^{-2}$, similarly samples were transferred to remaining test tubes, and dilution factor obtained as $10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$, $10^{-7}$, $10^{-8}$, $10^{-9}$, and $10^{-10}$ (Martin 1950). From these diluted samples, 0.1 ml of sample was taken and transferred to a separate Nutrient agar media plate, Potato Dextrose Agar (PDA) plate and Starch Casein Agar (SCA) plate and spread with L-spreader carefully which is then incubated in BOD at 37°C, 24°C and 28°C for 24 hours, 48 hours and 72 hours for bacteria, fungi and actinomycetes, respectively.

**Microbial population count:** Plates were observed after incubation and developed colonies were counted under colony counter. Colonies were over populated in low diluted plate and were very less in high diluted plates. So, we selected $10^{-4}$ dilution for our study. The population of microbes was examined and expressed as cfu $g^{-1}$.

Colony Forming Unit (cfu $g^{-1}$) = (Number of colonies x Dilution factor)/ (Volume of culture plate)

## RESULTS AND DISCUSSION

Microorganisms are known to play an important role in decomposing organic matter, cycling nutrients, maintaining structural and functional properties of soil and making nutrients available to the plants through microbial succession during the course of decomposition (Paul and Clark, 1989). In this study, microbial population (i.e., total number of bacteria, fungi and actinomycetes) in soil increases initially after the application of different leaf litters in field and pot experiments in the laboratory which decreases later (Fig. 3). Microbial populations are shown in the plates (Fig. 4). Bacterial and fungal populations ranged from $34.2x10^6$ - $35.4x10^6$ cfu $g^{-1}$ and $15.4x10^6$ - $26.7x10^6$ cfu $g^{-1}$, respectively in *O. sativa* and *T. candida* in 8 years fallow field, and from $32.5x10^6$- $29.1x10^6$ cfu $g^{-1}$and $11.1x10^6$ - $23.7x10^6$ cfu $g^{-1}$, respectively in *O. sativa* and *T. candida* in 3 years fallow field. Whereas, actinomycetes population ranged between $12x10^5$ and $15x10^5$ cfu/g in *T. candida* and *O. sativa*, respectively in 8 years fallow field and $4x10^5$ and $17x10^5$ cfu $g^{-1}$in *O. sativa* and *T. candida,* respectively in 3 years fallow field and pot experiments. Generally, higher soil microbial populations were recorded beneath the *T. candida* leaf litter and lower under *O. sativa* leaf litter. In response to combined effect of both litters (*O. sativa* + *T. candida*), soil microbial populations were in the mid of the range obtained for two litters except in case of *O. sativa* litter in 8 years old fallow. Interestingly, 8 years fallow exhibit higher microbial population compared to 3 years fallow, which may be related to higher litter input in the former than later. Higher litter input in longer fallow promotes greater soil organic matter, which consequently supports large number of microbes through feeding relations (Lalnunzira and Tripathi 2018, Manpoong et al 2020). Holtkamp et al (2011) showed an increase in soil microbial community following land abandonment. Bacterial and fungal population ranged from $27.8x10^6$-$31.7x10^6$ cfu $g^{-1}$ and $13.9x10^6$-$25.9x10^6$ cfu $g^{-1}$ in *O. sativa* and *T. candida*, respectively. However, actinomycetes population ranged from $12x10^5$-$15x10^5$ cfu $g^{-1}$ in *O. sativa* and *T. candida,* respectively in 8 years fallow soil. The rate of increase in microbial population was quite less in pot experiment as compared to field experiment. A possible explanation of higher abundance of microbial group in fallow fields soil than fallow pots soil is the larger segment of intractable soil organic matter in the carbon pool of fallow field soil (Kramer and Gleixner 2006, 2008, Manpoong et al 2020). Bacterial population in field experiment gradually increases as the decomposition proceeds and reaches maximum nearly on fifth month whereas fungal population was quite less initially but it increased noticeably during fifth to sixth month of decomposition. Actinomycetes count was quite less during our entire study. We sought to examine how soil microbial communities respond to litter addition and subsequent litter decomposition in field and pot soil of different fallow period. In the present study, there was a clear succession of microbial community in order of: Bacteria > Fungi > Actinomycetes. This pattern was remarkably comparable across all fallow land and pot soil.

The bacterial community show a larger increase in response to addition of *T. candida* litter which may be related to low C/N ratio of the litter that allow bacteria to rapidly utilize labile fractions of litter including the transport of soil inorganic nitrogen to stimulate the production of bacterial community (Tripathi and Singh 1992, Pandey et al 2007). Other microbial groups followed the pattern similar to that of bacterial population in response to different types litter addition. Studying the mineralization of nitrogen in *Sesbania sp.* leaves, Mafongoya et al (1998) showed that rapid decomposition may be due low protein binding polyphenols which can be associated to fast decomposition of *T. candida* as both belongs to the same family, Fabaceae. The readily mineralizable liable C and N released from *T. candida* may have caused a significant surge in microbial community during decomposition (Singh et al 2007). *T. candida* is a tree legume which helps in soil fertility management and gives higher biomass dry matter that can add large quantity of C and N to the soil. *T. candida* biomass is a composed of high-quality litter having low C/N ratio that decomposes faster and thus can be used for short term enrichment of soil fertility (Wapongnungsang et al 2017). Present study showed that *T. candida* decomposing soil contain more microbial population which may be due to its nitrogen fixing ability and high-quality litters but *O. sativa* being low quality litters with high C/N ration showed less microbial population. This study also revealed that microbial population was higher for *T. candida* treatment than *O. sativa* and *T. candida* + *O. sativa* treatment.

## CONCLUSION

The increase in soil microbial community is strongly influenced by organic matter inputs of different litter types (high-quality litter versus low-quality litter) because of varying decomposition rate in fallow lands following *jhum* cultivation. The presence of higher organic matter and soil microflora speeded up the process of litter decomposition rate in the long fallow compared to short fallow. High-quality (*T. candida*) litter decomposes faster than low-quality (*O. sativa*) litter as a result of faster nutrient release in former than later because of higher microbial count on soft litter. Further *T. candida* litters can be recommended for application in *jhum*

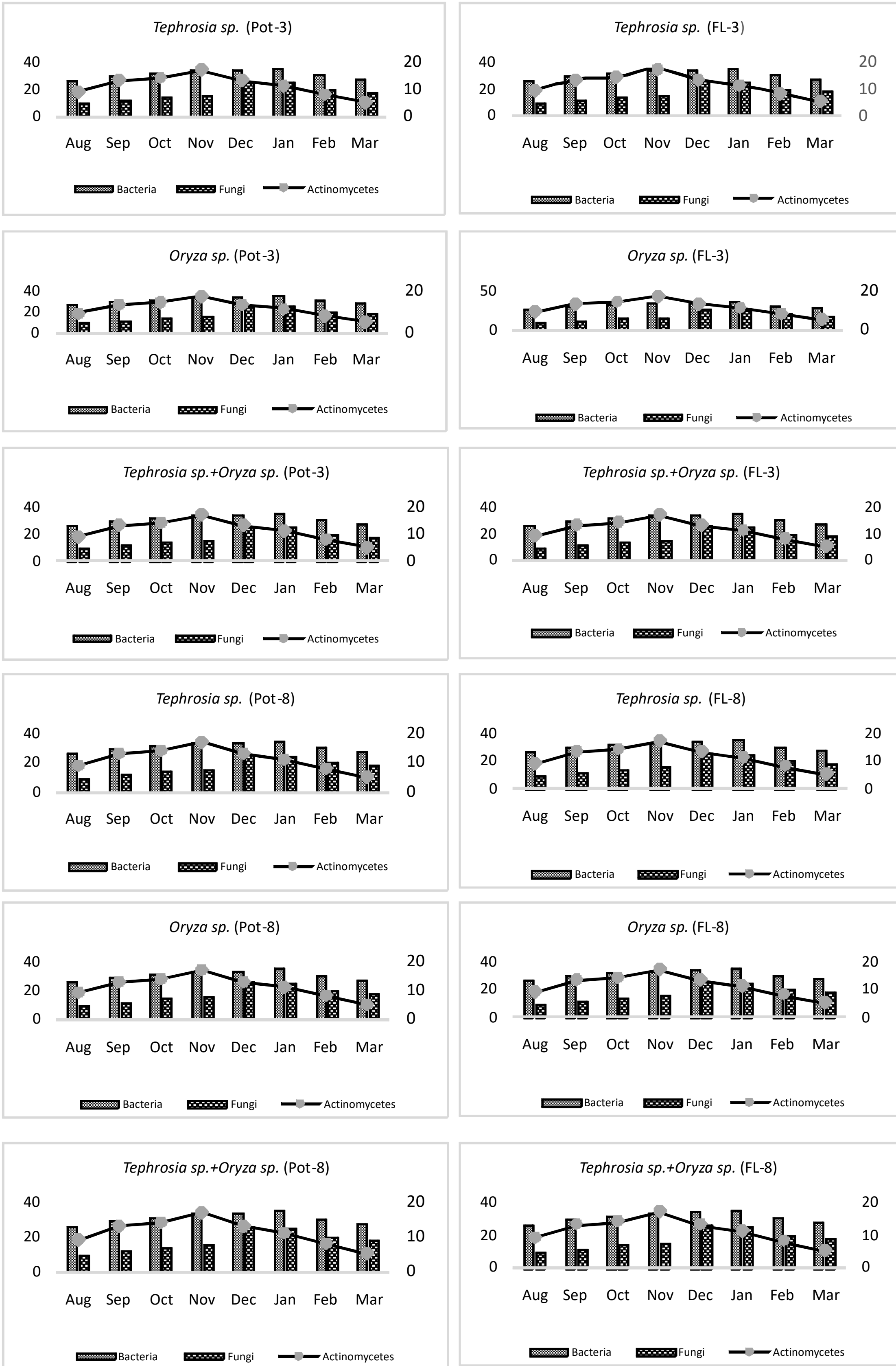


**Fig. 3.** Microbial population in field and pot experiment. Bacteria and Fungi expressed in $10^6$ and actinomycetes in $10^5$ Pot-3= 3 years fallow soil of pot, Pot-8= 8 years fallow soil of pot, FL-3= 3 years fallow land, FL-8= 8 years fallow land

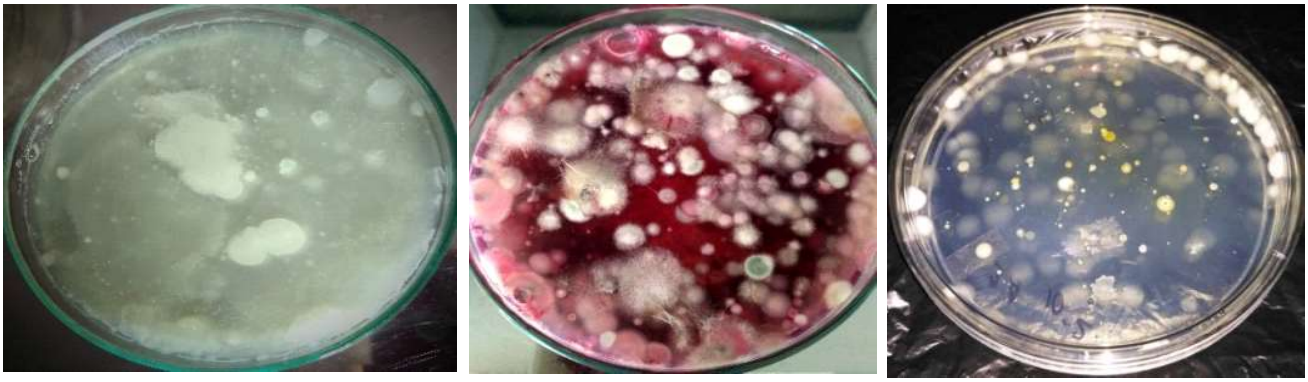

**Fig. 4.** Microbial population plate images. A representative of bacterial population, B representative of fungal population and C representative of actinomycetes population

field for the improvement of soil microflora and fertility status that ultimately enhanced crop productivity. Therefore, *T. candida* leaf litter can be considered as good quality litter resources to be applied in *Jhum* field of shorter fallow to meet the crop demand for growing population by maintaining the soil fertility.

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# Population Dynamics and Land use Pattern in City of Bhubaneswar, India: A Case Study

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***Abstract:*** The present study has been carried out in Bhubaneswar which has been declared as a smart city in the recent past by the Indian Government. The objective of the study is to understand the spatio-temporal dynamics of population growth and land use pattern in the perspectives of its future growth and development. The population has grown from 16,000 at the time of its inception to more than 10 lakhs presently with an annual growth of 9.148 percent. The population density has gone up from 4444 in 2001 to 7292 persons /sq.km in 2016 with an annual increase of 110/sq. km. The fast growth of population has led to expansion of the geographical area of the city from 28.7 sq.km to 148 sq.km. Of 13 different land uses recognized, residential area has increased from 6.20 to 23.04% of the total area. With increase in residential and commercial areas, agriculture and forest land areas are shrinking at a rate of 0.717% and 0.449% per annum respectively. Water bodies in the city are declining in area and getting polluted. The number of slums has increased from 50 in1981 to 436 in 2016 with an average increase of 10.82 slums per year. Promotion of vertical growth of the city, rejuvenation of water bodies, availability of open space, development of sewage network and drainage systems were realized in order to make the city green, resident friendly and sustainable.

***Keywords***: Land use, Population, Water bodies, Slum, Environment, Open space

Globalization and industrialization has brought rapid urbanization and fast growth of cities that are considered as the engines of economic growth (Kumar and Chithra 2012). The continuous and unabated migration from rural to urban areas over the years has made cities densely populated and unsustainable. Over the years, the global urban population is expected to rise and the number of people in Indian towns and cities will reach at 814 million by 2050. Realizing the need for transformation of the urban life Government of India has launched the smart city mission in June 2015 for comprehensive development of physical, institutional, social and economic infrastructure and the quality of life of people in 100 selected cities in the country (Kulshrestha 2016). In the first phase, Bhubaneswar stands number one in the smart city pilot project and attracts the attention of policy makers, planners and researchers to make it a model city for others. Bhubaneswar, the Capital of Odisha with hundreds of temples is widely known as the “Temple City” of India. The capital city came into existence in 1948 with a total area of 510 ha has increased tremendously in recent times because of rapid growth of infrastructure. The capital city at the time of its design was planned to accommodate a population of 20,000. But its population stands at more than 10 lakhs presently. The original master plan of 1946 that was based on the neighborhood concept of living is being replaced by Comprehensive Development Plan to address diverse issues in order to turn Bhubaneswar into a modern city (BDA 2015).Though some study has been conducted by Geographers and Botanists on the land use modeling (Mishra 2012) and flora of Bhubaneswar (Choudhury 1980), there is no comprehensive study reflecting the ground realities that could throw light for its future planning and development. Under this background the present study was conceptualized. The objectives of the present study were to understand the spatio-temporal dynamics of Bhubaneswar city in terms of population and land use, analyse the pattern and nature of land use and establish correlation, if any between population growth and land use.

## MATERIAL AND METHODS

**Study site:** Bhubaneswar is located in the Khurda district of Odisha, India between 20°12'N to20°25'N latitude and 85°44'E to 85°55'E longitude on the Western fringe of the coastal plain across the main axis of the Eastern Ghats. The city stands at the western side of the “Mahanadi Delta” on the bank of river Kuakhai, a distrbutory of Mahanadi River, 30 km South-West of Cuttack city. The river Daya flows along the South-eastern part of the city. Topographically Bhubaneswar forms an undulating hilly terrain. The average height of the town is about 43.5 m above mean sea level. Broadly the city area is divided into North-Western Upland Zone consisting of various denudated hills and the South-Eastern zone consisting of flood plain of three rivers, Kuakhai, Bhargavi and Daya. Geologically, Bhubaneswar comes under

Gondwana landmass, one of the Oldest and stable landmasses in the world. The soil in Bhubaneswar is mostly laterite in the north and western parts and alluvial in the eastern and southern parts. Bhubaneswar enjoys a salubrious and moderately equable humid tropical climate and receives about 120 cm rainfall annually.

**Methodology**

The present study was carried out on Bhubaneswar city (confined to Bhubaneswar Municipality Corporation (BMC) area) having an area of 148 Sq.km. The GPS location of the study area is presented in Figure 1. The study is based on multi source data base, analysis of primary and secondary data. The primary data includes information collected from personal physical survey of localities and wards and study of satellite images. The secondary data involves analysis of published research reports, maps, census reports, Government documents, cadastral sheets, etc. The available satellite data has been visually interpreted and integrated with intensive field checks and land use maps available from BDA, BMC and revenue departments. For urban sprawl mapping and for temporal land use and land cover delineation, the following maps/ data base sets were used in the present study.

1930: Survey of India maps in 1:63360 (in inch = 1 mile) (From SOI)

1968: Directorate of Town Planning maps (From ORSAC)

1974: Aerial photographs of 1974 (Scale 1:25,000) (From ORSAC)

2000: IRS-ID PAN and LISS-III of Feb. 16, 2000(From OSAC)

2009: world view, CDP map of Directorate of Town Planning and BDA (From ORSAC)

2011: Cartosat-II of March, 2011(From ORSAC)

Toposheets of BBSR and its surrounding area (From BDA)

**Statistical analysis:** Simple regression was used to understand the population growth and pattern of land use over time. Linear correlation was established between population growth and land use to find out relation, if any using IRRISTAT software.

## RESULTS AND DISCUSSION

Bhubaneswar, the capital city is about 70 years old and has undergone tremendous changes in terms of population and infrastructure over time. The urban sprawl which was confined to the central part of the city has expanded on north and south direction taking a dumbbell shape (Map 2)

**Population dynamics:** The population was only 8170 in the year 1921. In 2011 the number touched at 8.81 lakhs. In 2016, the population crossed 9.711 lakhs with growth rate of 9.148 % per year, with $R^2$ as 0.779. This fast growth of population is possibly linked to expansion of infrastructure such as housing complexes, educational institutions, public

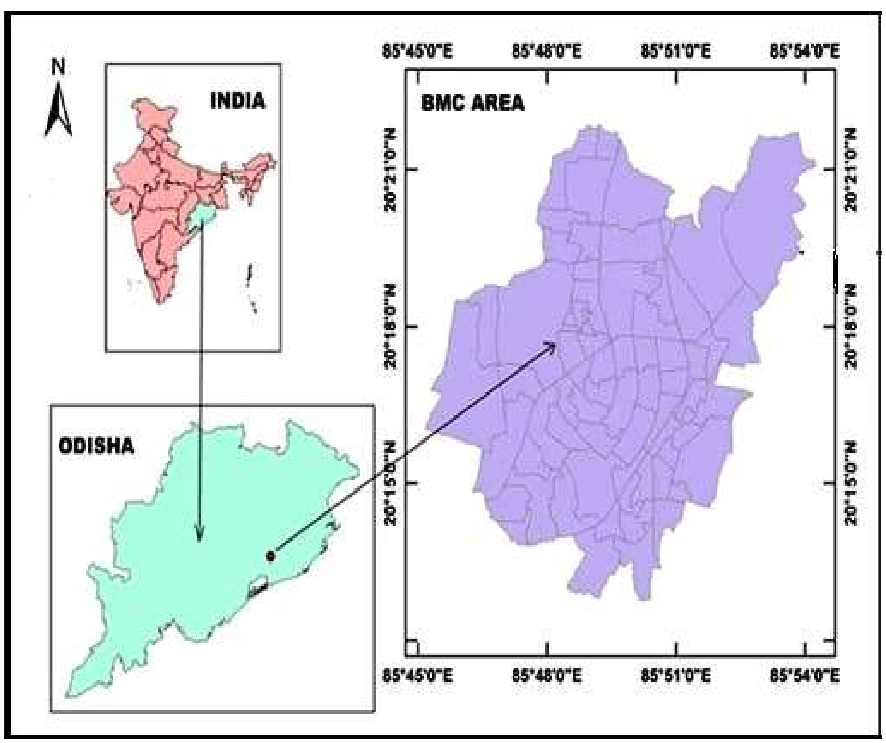



**Fig. 1.** GPS location of the study (Bhubaneswar city) area

sector units, small manufacturing industries, IT hubs etc. Looking in to the decadal growth of population, growth was minimum between 1931-41 and the highest during 1961-71 (176.07%). Though there was increase in absolute number, the decadal growth in population decreased after 1971 (Fig. 2) reaching at 36.1% between 2001- 11. This could be due to the reason that just after formation of the capital more people migrated from surrounding villages and neighboring districts for construction work and other activities that made the city crowded. The cost of living, the possible other factor subsequently reduction in the flow of population was due to rise in cost of land besides cost of living. Further development of satellite towns towards capital periphery with basic facilities such as school and health services with good communication facilities made people to stay out of the city. As per the statistical calculations, the rate of decrease in decadal population is by 24.45 % per year ($R^2$ = 0.807)**.** The increase in population density showed that there is an annual increase of 110 persons per sq. km. as noted from the linear logistic model ($R^2$= 0.923) (Fig. 3.)

**Land use pattern**: Bhubaneswar started its master plan since 1968 and the city had only a geographical area of 9,621 ha. During this period residential zone covered the maximum area (22.93%) followed by green belt zone (19.54%). After 1968, the city expanded at a fast rate and new zones were created and presently a total of 13 land use types have been recognized (Table 1). In between 1970 and 2017, two Comprehensive Development Plans (CDPs) were developed and the CDP under 2016 is under finalization (IIT 2016) Each CDP has tried to make Bhubaneswar more resident friendly. The city is expanding at an average rate of 2.032 sq. km per year (Fig. 9). The positive correlation between population growth and land area change (Fig. 10) reflected that the increase in population could be the cause behind expansion of Bhubaneswar city area.

**Residential land use:** Till 1968, the residential areas were mostly confined to Units planned in the form of self-contained neighborhoods, with each unit having its own school, shopping centers and play areas. Till 1974 the residential areas covered about 4.30 Sq.km representing 6.20% ha of the total area (Table 1)**.** With involvement of public and private sectors, the residential areas grew and reached at 17.59% of the total area in 2000. This increase in residential area reached 31.61 sq. km. in 2010, representing 21.85% of the total area and 32.29% in 2016 (Fig. 6). Even agricultural lands and low lying areas have been converted into residential colonies making the city unhygienic.

**Commercial land use:** With growth of population and residential complexes, the commercial areas came up accordingly. There has been fast growth of commercial areas in Bhubaneswar city from 0.131 sq. Km. in 1974 to 2.474 sq. Km in 2010 (Table 1). The increase in land value in the central areas has led to transform residential areas into commercial areas (Ravinder and Kaushik 2014)

**Agricultural land use:** With growth of population followed by increase in residential areas, agricultural land areas are

**Table 1.** Changes in land use pattern over the years from 1974-2016 in Bhubaneswar city*

| Land use type | 1974 | | 1990 | | 2000 | | 2010 | | 2016 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Area (Sq km) | Area (%) | Area (Sq km) | Area (%) | Area (Sq km) | Area (%) | Area (Sq km) | Area (%) | Area (Sq km) | Area (%) |
| Residential | 4.30 | 6.2 | 13.41 | 10.74 | 23.97 | 17.59 | 31.61 | 21.85 | 32.29 | 23.04 |
| Commercial | 0.131 | 0.19 | 0.957 | 0.77 | 1.472 | 1.08 | 2.474 | 1.71 | ---- | ---- |
| Industrial | 0.159 | 0.23 | 1.928 | 1.55 | 3.14 | 2.31 | 3.47 | 2.4 | 3.69 | 2.48 |
| Traffic and transportation | 0.596 | 0.86 | 1.219 | 0.98 | 1.635 | 1.2 | 12.73 | 8.8 | ---- | ---- |
| Public /Semi Public, Institutional | 1.422 | 2.05 | 3.893 | 3.13 | 5.82 | 4.27 | 10.79 | 7.46 | 12.2 | 8.2 |
| Utility services | 0.131 | 0.19 | 0.335 | 0.27 | 0.517 | 0.38 | 0.781 | 0.54 | ---- | ---- |
| Recreational | 0.242 | 0.35 | 1.094 | 0.88 | 1.690 | 1.24 | 2.691 | 1.86 | ---- | ---- |
| Vacant land | 2.86 | 4.13 | 10.68 | 8.59 | 18.04 | 13.24 | 32.99 | 22.8 | ---- | ---- |
| Agricultural land | 25.33 | 36.5 | 42.70 | 34.33 | 32.24 | 23.66 | 18.01 | 12.45 | 16.07 | 10.8 |
| Vegetation / Forest land | 17.21 | 24.8 | 19.07 | 15.33 | 19.57 | 14.36 | 16.78 | 11.6 | 16.60 | 11.2 |
| Waste land | 14.28 | 20.58 | 23.38 | 18.8 | 19.45 | 14.27 | 6.279 | 4.34 | 5.23 | 3.52 |
| Water bodies/ Wet land | 2.22 | 3.2 | 4.366 | 3.51 | 3.70 | 2.72 | 3.646 | 2.52 | 3.60 | 2.42 |
| Others | 0.499 | 0.72 | 1.39 | 1.12 | 5.01 | 3.68 | 2.416 | 1.67 | ----- | ---- |
| Total | 69.4 | 100 | 124.4 | 100 | 136.3 | 100 | 144.7 | 100 | 148.8 | |

* = Data from BMC and BDA reports

shrinking at a fast rate. Agricultural land use was 36.5% of the city area during 1974 and it has declined to 10.81% in 2016 with an average of 0.717% Sq. Km per year (Fig. 6).

**Forest land use:** At the time of development of Bhubaneswar city, there was dense forest of broad leaved trees beyond the core area (Dash and Satapathy 2020) towards the north and the west. The forests were full with wild animals However with expansion of road networks, development of the area under forest/vegetation cover declined from 24.8% in 1974 to 11.2% in 2016 (Fig. 5). As a whole it is observed that vegetation cover decreased at a rate of 0.343% sq. Km per year (Fig. 5).

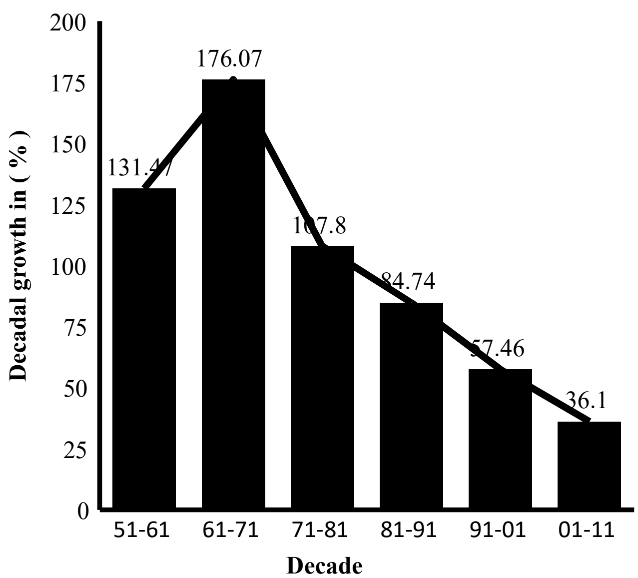



**Fig. 2.** Decadal growth in population of Bhubaneswar city

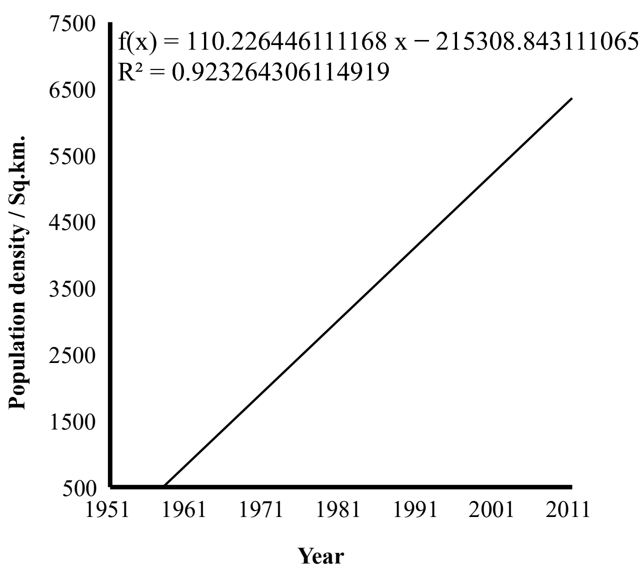



**Fig. 3.** Increase in population density (per Sq km) Bhubaneswar city over the years

**Industrial use:** Along with increase in commercial activities, land use under industrial sector has gone up over the years from 0. 159 sq. Km. in 1974 to 3.69 sq. in 2016 (Table 1).

**Public and semi-public institutional areas**: Various institutions those came up during 1960-70 and upto 1974, the Institutional areas covered only 2.5% of the total geographical areas of the city (Table 1). However with the development of centrally funded institutions and State Government funded/private institutes institutional areas have increased reaching up to 8.20% of the total city area.

**Recreational land use:** Recreation is very important for

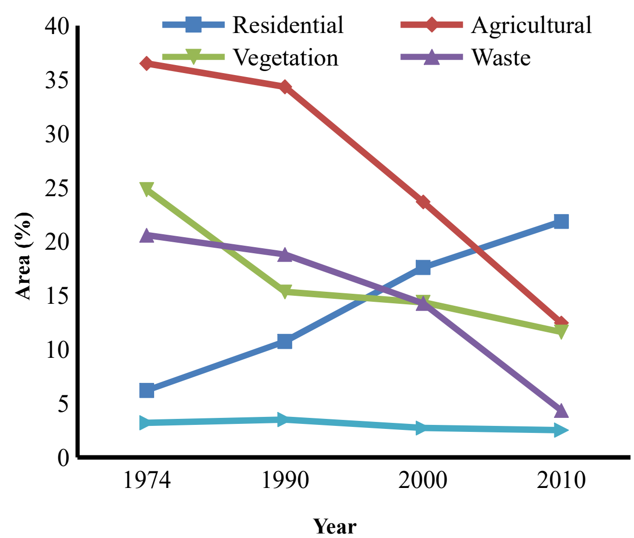



**Fig. 4**. Change in land use area for residential, agriculture, vegetation, waste land and water bodies over the years in Bhubaneswar city

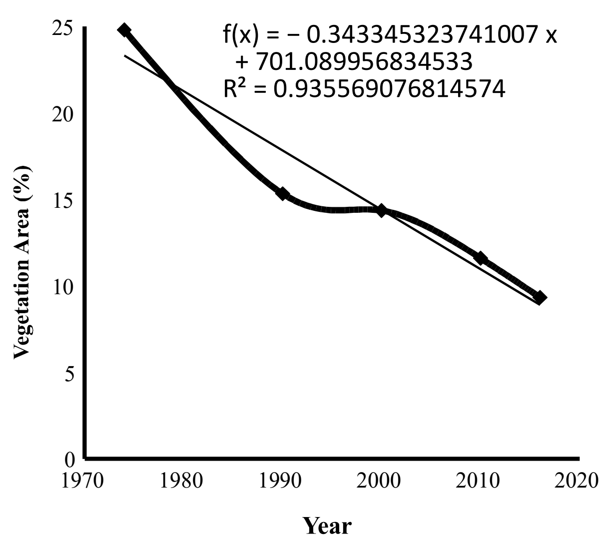



**Fig. 5**. Change in vegetational cover (%) over the years in Bhubaneswar city

quality of life. Over the years, a number of parks were developed in different parts of the city and the area covered for this land use has increased from 0.242 sq. km. (0.35%) in 1970's to 2.691 sq. km. (1.86%) in 2010.

**Land use for Traffic and Transportation:** The lands used for traffic and transportation have increased ten times from 0.86% to 8.8% in last 40 years.

**Water bodies:** In the city, a total of 128 small and big water bodies ranging in area from 01 to 100 acres existed. These ponds and water bodies acted to store rain water besides recharging ground water and thus could influence the microclimate of the city. However water bodies and low land areas in the city are declining over the years (Table 1). Presently, there are 67 ponds in renovation stage. Sludge and weeds have proliferated in the ponds making them unsuitable for use. There are 12 heritage ponds such as Bindusagar pond, Devipadata, Ramakunda, GangaJamula, GodipokhariPaidadhara Pond, Bhaskareswar, Meghaswar, Nageswar Temple Pond, Sukhameswar, Baniswar Pond and Kotitirtha which are very much polluted because of flow of untreated sewage and growth of weeds and aquatic plants such as *Hydrilla* and *Eichhornia*. All most all of these drains are filled with garbage and algal blooms, wild plants and aquatic weeds restricting the flow of water in rainy season

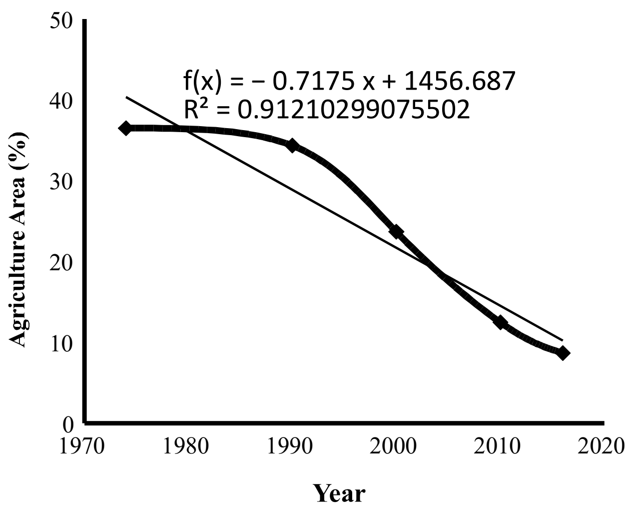



**Fig. 6.** Change in agricultural area (%) over the years in Bhubaneswar city

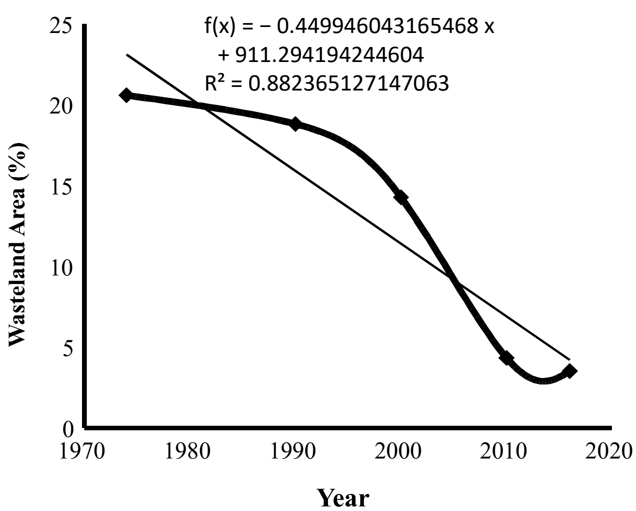



**Fig. 7.** Change in waste land (%) Bhubaneswar over the years (1974-2020)

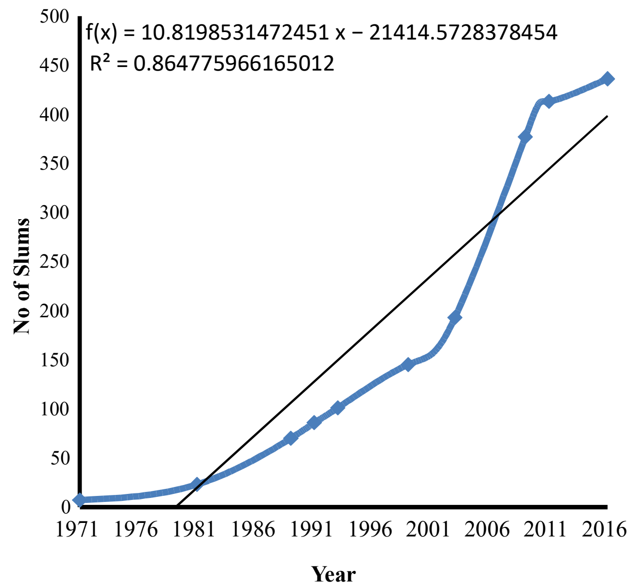



**Fig. 8**. Increase in number of slums over the years in Bhubaneswar city

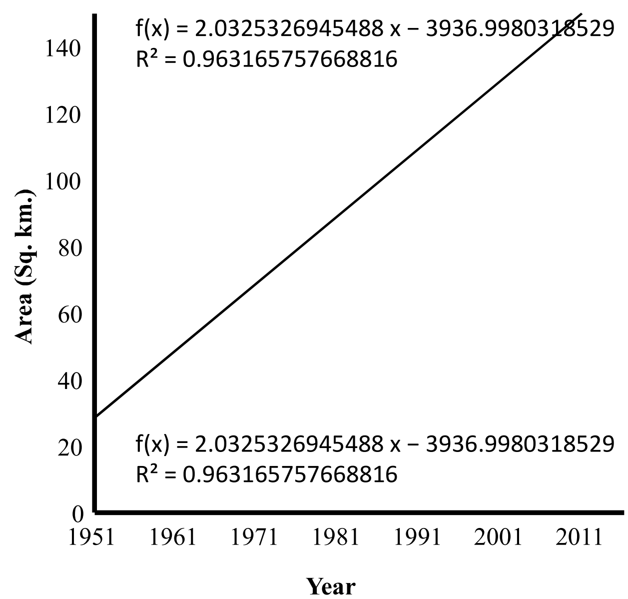
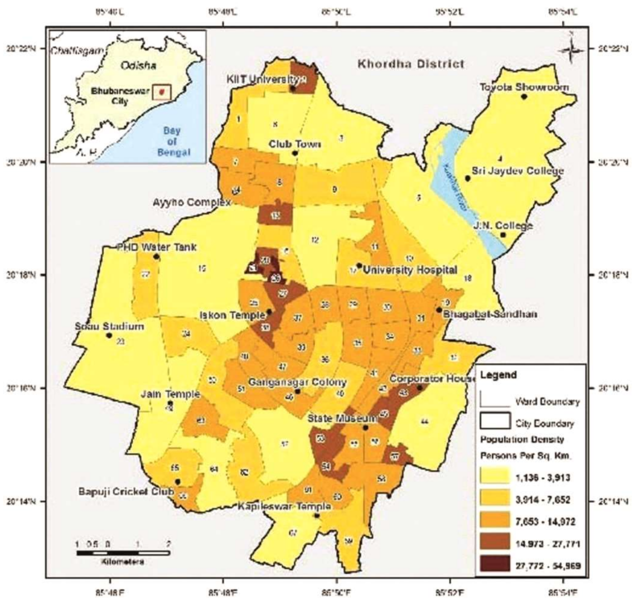



**Fig. 9.** Increase in geographical area in Bhubaneswar city over the years

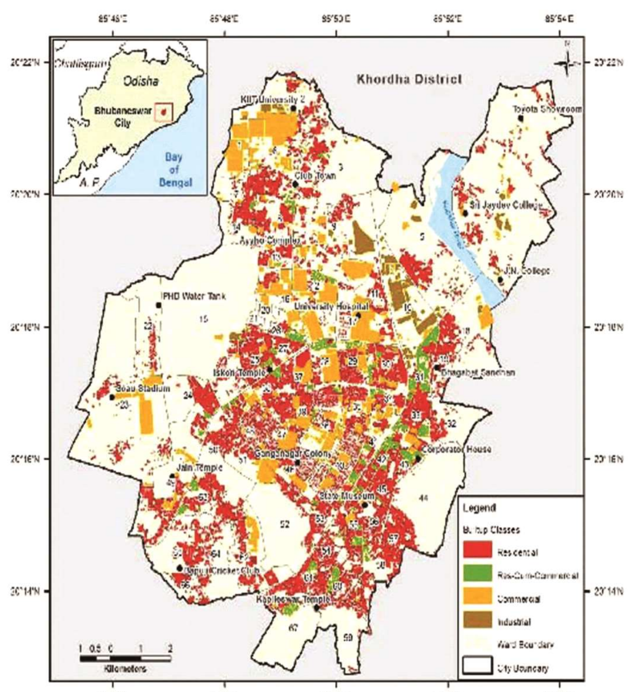
leading to flooding of roads and residential areas.

**Vacant and waste land:** Vacant lands are about 5% the total land area in the city. There is a decline in the waste land over the years. Presently these areas have often become the sites for growth of slums. There is a reduction of waste land at a rate of 0.449% sq. Km per year (Fig. 7).




**Source:** ORSAC

Map - 1 Ward wise population density in Bhubaneswar city




**Source:** ORSAC

Map -2 Distribution of residential, commercial and industrial areas in Bhubaneswar city

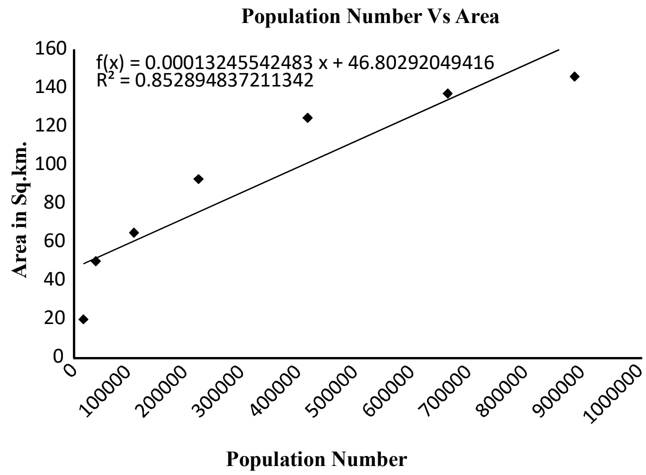



**Fig. 10**. Correlation between population number and area (in Sq. Km.) in Bhubaneswar

**Land use by slum dwellers:** In Bhubaneswar, there were less than 50 slums in 1981 and increased 436 in 2016 with an average increase of 10.82 slums per year (Fig. 8). The 62% of the slums are in encroached (public /private) lands and 15% are in stitiban lands. Only 23% of total slums are in rehabilitated colonies. With unorganized growth of slums, besides unhygienic environment and outside defecation, extending service at a later stage becomes difficult as planning fails as per the standard. (Jai Prakash and Najmmuddin 2015).

## CONCLUSION

The continued urbanization pattern will increase land and resource consumption over time besides putting stress on the environment. As availability of land is limited, it is essential that utilization of available land in the city is judicious and in the best interest of the community through the implementation of developmental plan(s).Instead of horizontal growth of the city, vertical growth need to be encouraged to reduce land area utilization and pressure on natural resources such as water, electricity etc. In order to reduce traffic congestion, there is need for construction of a circular road at the periphery of the capital city besides many service roads inside the city. There is an urgent need to rejuvenate the ponds/lakes/water bodies in the city by desilting and cleaning along with building bunds and fencing to prevent encroachment

Open space provides opportunity for healthy living. However, with the development of the city in terms of construction of roads, buildings, and commercial complexes etc. the environment has deteriorated. Besides avoiding mixed land use, rehabilitation of slum dwellers needs to done to avoid unhygienic environment. In order to save residents from heat waves and to get cool weather in the city, there is an urgent need to plant trees particularly broad leafed perennial wind resistant species on waste lands, degraded forests and along the road sides. As such besides judicious utilization of available land, there is a need to extend modern facilities and technology to its people in terms of better mobility, waste management and citizen centric services. The tradition and heritage of the city is not to be compromised as Bhubaneswar has become a city of both tradition and modernity.

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# Biological Activity of Soils of Low-Mountain Reliefs in Adygeya after Forest Felling

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***Abstract:*** Studies of the suite of metrics have been carried out which characterize the ecological condition of forest soils of low-mountain reliefs of the North-Western Caucasus Mountains. The goal of carried-out research was to evaluate the biological activity of soils that have different genesis at a different level of anthropogenic impact. Objects of research were Greyic Phaeozem of natural and disturbed ecosystems of the Adygeya Republic. For this, plots were investigated after forests clear felling that have different ages from 10 to 40 years old. Among used metrics, the following were present: the content of humus and active carbon, the activity of enzymes (catalase, invertase, dehydrogenase, urease, phosphatase), plenty of nitrogen-fixing bacteria of the Azotobacter genus. Their contingency with hydrothermal conditions was evaluated, also with bulk density, penetration resistance, medium reaction, hydrolytic acidity, the sum of absorbed bases, and other soil parameters. Features of biological activity of soils of low-mountain reliefs in Adygeya depending on terms of recovery after forest felling have been detected. The application of complex researches with the use of methods of biodiagnostics has shown, generally, the increased stability of Greyic Phaeozem of low-mountain reliefs in comparison with soils of areas of medium-altitude Mountains in Adygeya (Cambisol and Rendzik). The primary factor of degradation, which lowers the biological activity of soils, is erosion.

***Keywords*:** Forest soils, Adygeya Republic, Biodiagnostics, Anthropogenic impact

Soils are an important component part of terrestrial ecosystems that determines their producing capacity. In submountain and mountain areas of the South of the European Part of Russia, soils undergo the essential impact because of their intensive use. Zonal soils of mountain territories of the Western Caucasus Mountains are Phaeozem, Cambisols, and Rendzik (Valkov et al 2008). The anthropogenic impact, especially forest felling, leads to the degradation of natural ecological systems and soil covering. The ecological condition of postforest soils of the Western Caucasus is much different from natural undisturbed soils (Soldatov et al 2020a,b, Kazeev et al 2021). Biological properties of soils and bioindication are widely used in the diagnostics of the ecological condition of the environment (Thiele-Bruhn et al 2020, Zhadobin et al 2020). At the diagnostics of the fertility and quality of soils, methods of soil enzymology have shown good results (Sinsabaugh et al 2008, Luo et al 2017, Sudina et al 2021). These methods have shown themselves well in southern Russia at the evaluation of the ecological condition of soils after the agrogenic impact (Gorobtsova et al 2017, Azarenko et al 2020, Kazeev et al 2020 a,c) and fires (Kazeev et al 2019, 2020b, Odabashyan et al 2019). The purpose of the presented work was the evaluation of the biological activity of soils of low-mountain reliefs of the Western Caucasus Mountains after forest felling, depending on their genesis and the age of felling.

The researched territory is situated near Dakhovskaya Cossack village, Maikop Territory, Adygeya Republic (Fig. 1). On plot №1, at the elevation of 540 meters above sea level, the reference area is presented with oak and hornbeam forest with poorly expressed young growth. The plot is divided into 2 zones: reference area (forest) and clear felling area. Afterward, in 2019 in the felling area, the repeated felling of the tree undergrowth with the age of 10 years old was carried out. As a result, the felling territory was differentiated into 2 areas: regenerating clear felling area and repeated clear felling area. At the area of the repeated clearcut, the flora is represented by the meadow mixed cereals grass family. The soil of this plot is Greyic Phaeozem Vertic. The plot №2 is situated on slopes of the Una-Koz range and the Gud mountain, near the confluence of the Dakh river and Belaya river. On this territory, several postforest areas at different stages of the progressive succession were investigated 10-40 after forest felling. Three plots of broad-leaved forests were researched at the elevation of 500-675 meters above sea level (Table 1).

The reference oak and hornbeam forest with the bonitet 4 and the composition 3 oak 4 hornbeam 1 ash tree has the age of 250-300 years old. The soil here is Greyic Phaeozem Eutric. Insomuch as these soils are formed on limestones, in their genesis and properties, they are similar to Rendzic Leptosols that is abundant on carbonate rocks of the North-Western Caucasus Mountains (Valkov et al 2008). In some cases, these soils can border on each other and create soil combinations (Valkov et al 2007, Kazeev et al 2021). On this plot, 4 areas more have been examined that formed after forest felling and that are in different stages of recovery. These areas differ from each other according to their age (10-40 years old) and based on carrying out reafforestation work**s**. On the area №868, the red oak (*Quercus rubra*) was planted out; by now, it is practically overgrown with other kinds of trees (predominantly, hornbeam and asp).The third plot is situated on the graded terrace at the elevation of approximately 500 meters above sea level near the place where the Syuk River mouths into the Belaya River. The reference area is an oak, maple, and ash forest. The soil of

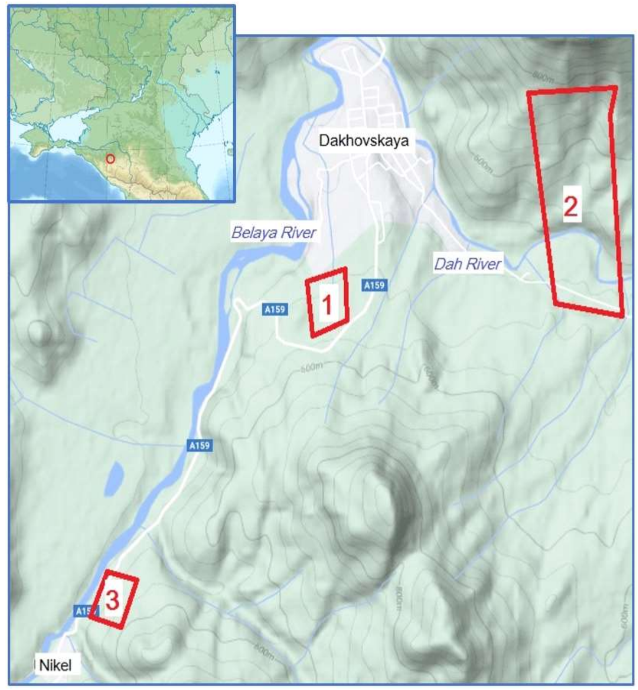


**Fig. 1.** Location of the study area with plot numbers

**Table 1.** Specification of the study plots

| No | Location and relief | Vegetation | Age after felling | Soil |
|---|---|---|---|---|
| Plot No 1 | | | | |
| 858 | Leveled terrace on the lower part of the slope to the Belaya River. Height above sea level - 541 m | Oak and hornbeam forest. Age 120-130 years | Control area of the forest | Greyic Phaeozem Vertic on pebble deposits |
| 858 | Leveled terrace on the lower part of the slope to the Belaya River. Height above sea level - 542 m | wild fruit garden with grassy canopy | 10-12 | Greyic Phaeozem Vertic on pebble deposits |
| 858 | A levelled terrace on the lower part of the slope towards the Belaya River. Height above sea level - 542 m | High grass meadow vegetation | 12 and again in 2019 | Greyic Phaeozem Vertic on pebble deposits |
| Plot No 2 | | | | |
| 865 | The eastern slope 8-10º of the Una-Koz Range (middle part). Height above sea level - 675 m | Oak-hornbeam-ash forest. Age 250-300 years | Control area of the forest | Greyic Phaeozem Eutric on the weathering products of limestone |
| 866 | The eastern slope 6º of the Una-Koz Range (middle part). Height above sea level - 672 m | Hornbeam forest | 40 | Greyic Phaeozem Eutric on the weathering products of limestone |
| 867 | The southern slope 12-15º of the Una-Koz Range (lower part). Height above sea level - 558 m | Hornbeam forest | 25 | Greyic Phaeozem Eutric on the weathering products of limestone |
| 868 | The middle lower part of the southern 12-15º slope of the Una-Koz Range. Height above sea level - 552 m | Hornbeam forest | 22 | Greyic Phaeozem Eutric on the weathering products of limestone |
| 869 | The lower part of the northern slope of Mount Hood 15-20º. Height above sea level - 508 m | Mixed deciduous forest | 12 | Greyic Phaeozem Eutric on the weathering products of limestone |
| Plot No 3 | | | | |
| 871 | A terrace on the lower part of the 3-5º slope to the Belaya River. Height above sea level - 502 m | Oak-maple-ash forest | Control area of the forest | Greyic Phaeozem Pachic on pebble deposits |
| 872 | Terrace on the lower part of the slope 5-7º.Height above sea level - 505 m | Abandoned planting of hazel and young ash, maple, hornbeam and elm undergrowth | 12 | Greyic Phaeozem Sceletic on pebble deposits |

this plot is Greyic Phaeozem Pachic. In the clear felling area nearby, the soil has degraded as a result of the development of erosion processes that have increased abruptly after forest felling. As a result, the soil in this territory is Greyic Phaeozem Sceletic.

## MATERIAL AND METHODS

Fieldwork and analytical laboratory researches have been carried out from 2014 to 2020. On every investigated plot, sections and heeling-ins were laid down, 3 soil samples from the upper soil layer (0-10 cm) and one sample from every one of the underlying beds have been selected. Temperature, humidity, the density of soil texture, the content of humus and active carbon, the number of microorganisms, the intensity of carbon dioxide emission by the soil, enzymic activity, and other metrics have been investigated (Kazeev et al 2016). The soil temperature was detected layer-by-layer, with a HANNA CHECTEMP electronic thermometer. The temperature dynamics within the period of 2018-2020 were investigated using Thermochron DS1921 temperature sensors at a depth of 10 cm with a measurement periodicity of 6 hours. The soil humidity has been detected afield using a Fieldscout TDR 100 humidimeter with the tenfold replication. The density of texture of soil was detected by the volume-weight method using steel rings with the volume of 135 $cm^3$ with the three-fold replication. The soil enzymic activity was judged based on the activity of different enzyme classes: oxidoreductase (catalase, dehydrogenase) and hydrolyzing enzyme – (fructofuranosidase (invertase), phosphatase, and urease). The activity of catalase, invertase, urease, phosphatase, and dehydrogenase was detected based on reaction products at the substrate decomposition (hydric dioxide, saccharose, urea, sodium phenolphthalein-phosphate, and triphenyl tetrazolium chloride). The humus content was detected in the way of bichromatic oxidation in the acid environment at the temperature of 150 degrees with the spectrophotometric end. The plenty of nitrogen-fixing bacteria of the *Azotobacter* genus was detected using the Ashby medium by the method of encrustation balls. The total bacterial count has been detected using the luminescence microscopy method. All measurements have been carried out with a 3-6-fold replication.

For detecting differences at the level of biogenesity and biological activity of different soils, the integrated index of the biological condition (Integrated Index of the Biological Condition) of the soils was determined. This metric is used to evaluate the complex of biological parameters expressed in different units and makes it possible to align random fluctuations characteristic for the most biological parameters (Kazeev et al 2015, 2016). For the calculation of the integrated index of the biological condition, the highest value of each metric is taken as 100%, and the value of the same metric in other samples is expressed relative to it in percentage terms:

$$Б_1 = (Б_x / Б_{max}) \times 100\%,$$

where $Б_1$ is a relative point of the metric, $Б_x$ is an actual value of the metric, $Б_{max}$ is the highest value of the metric.

After that, the average score point of investigated metrics of the integrated index of the biological condition of the soil is calculated – similarly to the calculation of the relative point of the metric. The statistical processing of findings of the investigation has been carried out using Statistica 10.0 and MS Excel software.

## RESULTS AND DISCUSSION

The Greyic Phaeozem territory of felling was characterized by the essential change of hydrothermal conditions. This is connected with the thinning-out of the forest crop that results in the supply of the bigger rainfall amount to this territory. Also due to the shadowing, the soil heating happens, and as a result of this, the lowering of the air humidity is noted. in turn, this leads to the increasing of the water evaporation. The stored moisture storage on deforested territories is higher than under the forest cover; this is also connected with the plenty of rainfall that gets onto the soil surface. In comparison with the forest, 20-23% more atmospheric precipitates are getting onto the felling territory. On the felling territories, the depth of penetration of biologically active temperatures is increasing; in particular, this is expressed on clean felling territories. The temperature increased by 25-27%. Microclimate changes in connection with forest crop destruction persist within 4–6 years after the tree felling (Gerwing 2002). On felling plots, the increased temperature and humidity were recorded, in comparison with reference values under the forest cover. This is connected with the lower foliage cover of the flora in plots with the strong violation. Minimal temperature and humidity values were recorded on reference forest plots. In general, the temperature conditions of Greyic Phaeozem are favorable. In the summer months, the soil temperature at a depth of 10 cm achieves the value of 20°C. On Figure 2, the temperature dynamics in the reference plot №1 are presented. There was no frost penetration in the soil even in the winter months. Greyic Phaeozem Vertic of the reference plot №1 is characterized with the high content of the organic substance in the upper level – 7,3 %, neutral medium reaction – pH = 7,3, heavy clay loam granulometric composition, medium biological activity.

Within the first several years after forest felling, no significant excess of the soil texture density in comparison with the reference area was recorded. In 2014, an increase of the metric value by 10-15% was recorded. The higher temperature values on the felling plot were connected with the openness of the plot that is not protected with the forest crop shadow. At the end of 10 years after the forest felling, the physical parameters of the soil have practically recovered and achieved reference values. The flora species composition on the felling plot is represented by the tree and shrub vegetation (the height of trees is 8-10 m) with the crop formed with meadow mixed cereals grass vegetation with the height of 50-100 cm and 100 percent foliage cover. In 2019, the understory of young regeneration was cut over on the part of the overgrown cutover area and left unremoved. Many properties of Greyic Phaeozem Vertic on different cutover plots were significantly different. The soil medium reaction varied not so much on different plots. The excess of the soil pH in the cutover area was less than 5%. The dynamics of enzyme ferments activity are also different within the first years after the deforestation. The catalase activity changes with the same regularities as for the invertase activity. For both enzymes, similar nature of the dynamics was detected. The high grade of violation results in the significant increase of values practically without the trend to the recovery for the catalase activity, the invertase activity approaches to reference values. The dehydrogenase activity varies within significantly lower limits than the activity of catalase and invertase. However, within the overall period of observation of the cutover area, the dynamics of lowering the activity concerning the reference area were noticed. Values of the integrated index of the biological condition calculated based on 10 metrics in soils of the plot No. 1 situated on Greyic Phaeozem Vertic differ much less in comparison with meadow and carbonate soils that can degrade significantly at the forest felling (Soldatov et al 2020a,b, Kazeev et al 2021). The highest discrepancy was noticed for the soil of the reference forest and the overgrown cutout area (Fig. 3). The values of the integrated index of the biological condition in the reference area are higher by 23% than on the cutout area. However, after the repeated felling of the understory of young regeneration in the same cutout area, values of the integrated index of the biological condition practically do not differ with the reference area (only –2%). This is connected with the increase of insolation on the glabrate plot of the cutout area, increase of the temperature, intense development of the meadow mixed cereals grass vegetation. Besides, the content of calcium carbonates increases as well as pH, texture density, and soil humidity. Different biological metrics on this plot behaved in different ways. The content of humus and active carbon on both plots of the cutout area

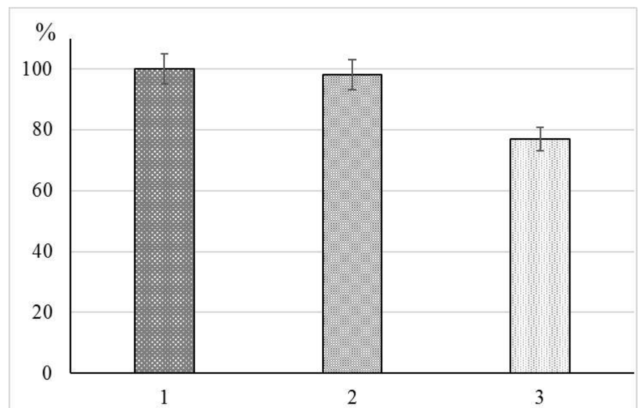


**Fig. 3.** Integral index of biological activity of Greyic Phaeozem Vertic at plot 1, 2019: 1) control forest, 2) felling, 3) re felling

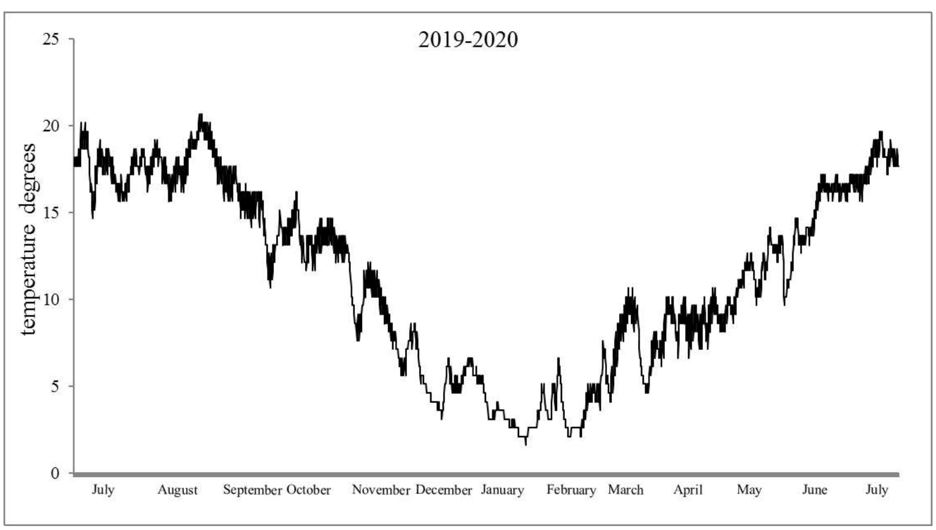


**Fig. 2.** Soil temperature at 10 cm depth, control forest near felling plot No.1

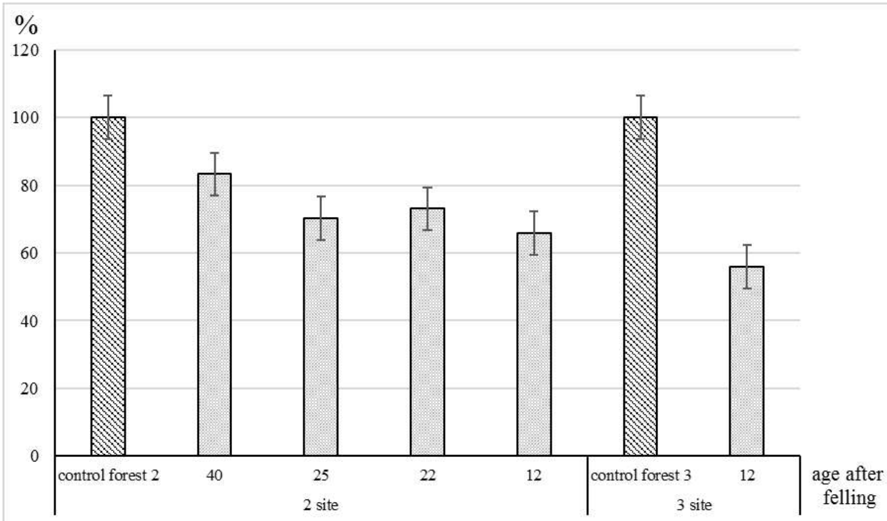


**Fig. 4.** Integral index of biological activity of lowland post-forest soils at plots 2 and 3, 2020. (column captions correspond to the age of felling)

decreases by 4-23% and 11-28% in comparison with control values. Besides, the bacterial count is decreasing (by 19-10%), the urease activity is decreasing (by 10-75%), and the peroxidase activity is decreasing by 48-44% However, the catalase activity increased, by 13-41%. The behavior of other metrics is different depending on the type of vegetation on the felling plot that has been cleared repeatedly and on the felling plot that has overgrown.

In plot no. 2, the reference soil under the forest contains 9.7% of humus at the neutral reaction of the medium and a high degree of base saturation. Such favorable conditions in soils are also formed as a result of the affinity of carbonate soil-forming materials. That's why the humus content and biological properties in this soil are slightly decreased downwards the profile what is characteristic for meadow and carbonate soils of the examined region (Valkov et al 2007, Kazeev et al 2012). The reference soil is characterized by a high level of catalase, phosphatase, and invertase: their values decrease significantly in soils violated due to fellings as well as the humus content. The same regularity was also noticed in plot №3. The humus content decreases here from 9.0% in the reference soil up to 4.7% in the washed-off soil of the cutover area. Besides, in plot №3 the degree of the base saturation of the soil and the medium reaction decrease. This happens as a result of the water denudation of the soil surface layer in the felling area after the forest felling. The biological activity also decreases, especially the activity of the urease (by 2.5 times) and dehydrogenase (by 4 times). On soils of the plot №2, lowering of the enzyme strength was expressed to a lesser degree. Values of the integrated index of the biological condition in soils at plots №2 and 3 are uppermost in reference areas (Fig. 4). The forest felling has led to the lowering of values of the integrated metric of Greyic Phaeozem. At the plot №2, the gradual increase of values of the integrated index of the biological condition has been detected, in proportion to the increase of the age from the date of the forest felling. The lower difference of the integrated index of the biological condition is recorded for the soil that is 40 years old; the highest discrepancy has been detected for the soil of the territory that is 12 years old. At the plot №3, an almost twofold decrease of values of the integrated index of the biological condition in the soil in the felling area happened in comparison with the reference forest plot.

## CONCLUSION

The biological activity of soils of low-mountain reliefs of the North-Western Caucasus Mountains changes significantly after the forest clear felling. After increasing the age of felling, the biological activity of soils recovers expectedly. However, the values of the biological activity don't recover to the initial values even within 40 years of the progressive succession. The main degradation factor that lowers the biological activity is erosion that develops in slope territories after the forest felling.

## ACKNOWLEDGEMENTS

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# Yield Advantage Assessment of Sweet Corn Based Intercropping Systems Through Indices Under Irrigated Condition of Kashmir Valley

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***Abstract***: A field study to assess the yield advantage and economics of sweet corn-based intercropping systems under irrigated condition, was conducted on silty-clay loam soil at SKUAST-Kashmir, Wadura, Sopore during *Kharif* season of 2017. The experiment was in randomized block design with ten treatment combinations of row. The intercrops (bean or soybean) were grown in additive series with sweet corn as regular rows of 1:1 and paired rows of 2:1 and 2:2 including sole cropping of each crop. System productivity of intercropping system of sweet corn and intercrops in terms of corn equivalent yield was significantly higher with sweet corn + soybean (1:1). Yield advantage in terms of land equivalent ratio indicated that sweet corn + soybean (1:1) produced 55 % more yield compared to sole sweet corn followed by sweet corn + soybean (2:2). The maximum monetary advantage index, net returns and B:C ratio also obtained with sweet corn + soybean (1:1) system. In crux, amongst different cropping system, intercropping of sweet corn with soybean in regular rows of 1:1 was more biologically and economically viable intercropping system for irrigated silty- clay loam soil of Kashmir valley.

***Keywords***: Corn equivalent yield, Intercropping, Land equivalent ratio, Soybean and Sweet corn

Corn (*Zea mays* L.) is a versatile crop, globally grown under a wide range of agro-ecological situations of tropical, sub-tropical and temperate regions over an area of 193.7 million hectare (mha) with production of 1147.7 million metric tonnes (m Mt) and average productivity of 5.75 t ha$^{-1}$ (FAOSTAT 2020). In India as a third most important cereal crop grown over an area of 9.2 million hectare (mha) with grain production of 27.8 million metric tonnes (m Mt) and average productivity of 2.96 t ha$^{-1}$ during 2018-19 (DACNET 2020). In Jammu and Kashmir, it is the second most important cereal crop after rice and is cultivated over an area of 0.30 mha with 0.54 mt production during 2017-18 (DESJK 2019). Canopy structures and root systems of cereal crops are generally different from those of legume crops. Cereal crops form relatively higher canopy structures than legume crops and the roots of cereal crops grow to a greater depth than those of legume crops. This indicates that the component crops probably have differing spatial and temporal use of environmental resources such as radiation, water and nutrients (Willey 1990). Spatial arrangement of crops in mixture is an important management practice that can provide complete ground cover and hence improve solar radiation interception. Yield advantage occurs because growth resources such as light, water and nutrients are more efficiently utilized and converted into crop biomass by the intercrop over time and space as a result of differences in competitive ability for growth resources between the component crops, which exploit the variation of the mixed crops in characteristics such as rates of canopy development, final canopy size (width and height), photosynthetic adaptation of canopies to irradiance conditions and rooting depth. Component crop yield decreases in intercropping but system efficiency increases in terms of benefit cost ratio, net income and land equivalent ratio (Banik et al 2006) because intercropping enhances farm income by using land resources efficiently. Individual crops in intercropping show different competitive behaviours that can be assessed in terms of competitive ratio and relative crowding coefficient. The aim of this present study is to assess yield advantage and competition indices and economics influenced by intercropping of sweet corn with bean and soybean.

## MATERIAL AND METHODS

A field experiment was conducted at SKUAST-K, Wadura, Sopore, Kashmir; during *Kharif* season of 2017 that lies between 34˚17' N latitude and 74˚33' E longitude with an altitude of 1524 meters above mean sea level. The experimental site was well drained silty-clay loam in texture, non-saline (EC 0.37 dS m$^{-1}$) with pH 7.2, medium in organic carbon (0.68 %), available N (244.5 kg $ha^{-1}$), available P (19.28 kg $ha^{-1}$) and available K (163.6 kg $ha^{-1}$). The experiment consisted of ten treatments in randomized complete block design with three replications. The 'Mithas' sweet corn, 'French Yellow' bean and 'Shalimar Soybean-1' soybean were used as test crops. Sweet corn, bean and soybean were sown in the second week of May, 2017 and sweet corn was harvested on $10^{th}$, $13^{th}$ and $16^{th}$ September, 2017 and $11^{th}$ and $12^{th}$ October for the intercrops. Full dose of phosphorus (60 kg $ha^{-1}$) and potassium (30 kg $ha^{-1}$) and half dose of nitrogen (60 kg $ha^{-1}$) were applied as basal dose before the sowing of sweet corn seeds while the remaining half dose of nitrogen (60 kg $ha^{-1}$) was top dressed at respective critical stages i.e. first at 35 DAS and second at 65 DAS. In case of sole bean and soybean, 30:60:30 and 30:90:60 kg N, $P_2O_5$, $K_2O$ per hectare was applied as basal dose through urea, Diammonium phosphate (DAP) and Murate of Potash (MOP), respectively. Data on yield components were recorded from randomly selected five plants for all the three crops. The yields of different intercrops are converted into equivalent yield of anyone crop based on price of the produce. The crop equivalent yield (CEY) was calculated for each crop was added to give system productivity as follows:

CEY sweet corn = (Ymb) × (Pm/Pm)

CEY bean = (Ybm) × (Pb/Pm)

CEY soybean = (Ysm) × (Ps/Pm)

System productivity = CEY sweet corn + CEY bean

Or,

System productivity = CEY sweet corn + CEY soybean

Where,

CEY sweet corn: Crop equivalent yield of sweet corn

CEY bean: Crop equivalent yield bean

CEY soybean: Crop equivalent yield of soybean

Ymb: Yield of sweet corn in intercropping system

Ybm: Yield of bean in intercropping system

Ysm: Yield of soybean in intercropping system

Pm: Price of sweet corn

Pb: Price of bean

Ps: Price of soybean

**Table 1.** Green cob yield (t $ha^{-1}$) of sweet corn as influenced by cropping system

| Treatment | Green cob yield (Without husk) (t $ha^{-1}$) |
|---|---|
| Sole cropping system | |
| Sweet corn sole (75 cm) | 23.1 |
| Sweet corn paired sole (50/100 cm) | 22.7 |
| Intercropping system | |
| Sweet corn + Bean (1:1) | 22.6 |
| Sweet corn + Soybean (1:1) | 23.0 |
| Sweet corn paired + Bean (2:1) | 20.1 |
| Sweet corn paired + Soybean (2:1) | 20.1 |
| Sweet corn paired + Bean (2:2) | 18.2 |
| Sweet corn paired + Soybean (2:2) | 18.5 |
| CD (p=0.05) | 3.1 |

## RESULTS AND DISCUSSION

**Green cob yield of sweet corn:** There was significantly higher green cob yield was recorded with the sole system of sweet corn both in regular and paired rows. Among intercropping system, regular rows of sweet corn in association with soybean in 1:1 row proportion was significantly (23.0 t $ha^{-1}$) superior over sweet corn when intercropped with bean and soybean in 2:2 paired row ratios (Table 1). This might be attributed to limited disturbance of the habitat and interspecific competition in the sole cropping environment. Similar findings were also observed by Aynehband and Behrooz (2011) and Takim (2012).

**System productivity:** Marked variation in sweet corn equivalent yield (CEY) was observed with the cropping systems under present study (Table 2). System productivity was significantly higher with the intercropping of regular rows of sweet corn with soybean (1:1) which was at par with sweet corn when intercropped with bean in the same row ratio. Intercropping of paired rows sweet corn with bean or soybean (2:1 and 2:2) showed poorer CEY. The regular row arrangement might had provided sufficient inter-row space for raising intercrops of soybean and bean with 1:1 row proportion. The results are in the conformity with the findings of Liu et al (2006) and Solanki et al (2011).

**Land equivalent ratio (LER):** All the intercropping combinations recorded greater than unity land equivalent ratio (LER), indicating yield advantage over sole cropping, ranging from 11 to 55%. The highest LER was computed with sweet corn + soybean (1:1) followed by sweet corn + soybean (2:2). The least LER was computed with sweet corn + bean (2:1). These results agree with the results of Yilmaz et al (2008) and Solanki et al (2011).

**Aggressivity (A):** An aggressivity value of zero indicated that component crops are equally competitive. The greater the numerical value, the more is the difference in competitive abilities and higher will be the differences between actual and expected yields. In the present study, the positive values of aggressivity were in sweet corn which indicated that base crop was the dominant crop, whereas the associated intercrops dominated ones (Table 2). Similarly, Sawargaonkar et al (2008) reported that the maize-based intercropping systems were more remunerative than sole maize.

**Relative crowding coefficient (K):** The relative crowding coefficient plays a vital role in determining the competition effects and advantages of intercropping. The relative crowding coefficient were more than 1.00 indicated yield advantage to grow more sweet corn with bean and soybean in all the intercropping situations (Table 2). This might be due to better utilization of land with intercropping than sole crops. Intercropping of sweet corn with soybean under 1:1 row proportion recorded more yield advantage (353.38) which was comparable with the other intercropping treatments.

**Monetary advantage index (MAI):** The monetary advantage was based on land equivalent ratio indicated superior economic viability of sweet corn + soybean intercropping in 1:1 row proportion over other intercropping system.

**Economics:** The difference in cost of cultivation among treatments was because of the variation in seed rate and seed price of intercrops. Sweet corn in association with soybean in regular rows of 1:1 ratio made it more remunerative and economically more viable as it provided higher gross return (₹ 562099 ha$^{-1}$), net return (₹ 505874 ha$^{-1}$) and B:C ratio (9.00) compared to other intercropping treatments as represented in Table 3.

**Table 2.** System productivity, yield advantage indices, monetary advantage index and aggressivity as influenced by intercropping systems of sweet corn, bean and soybean

| Treatment | System productivity (CEY) (t ha$^{-1}$) | Land equivalent ratio (LER) | Relative crowding coefficient (K) | Monetary advantage index (MAI) (×10$^{4}$) | Aggressivity (A) |
|---|---|---|---|---|---|
| Sole cropping system | | | | | |
| Sweet corn sole (75 cm) | 23.1 | - | - | - | - |
| Sweet corn paired sole (50/100 cm) | 22.7 | - | - | - | - |
| Bean sole | 4.1 | - | - | - | - |
| Soybean sole | 3.2 | - | - | - | - |
| Intercropping system | | | | | |
| Sweet corn + Bean (1:1) | 24.3 | 1.39 | 37.10 | 13.39 | 1.15 |
| Sweet corn + Soybean (1:1) | 24.8 | 1.55 | 353.38 | 16.93 | 0.88 |
| Sweet corn paired + Bean (2:1) | 21.0 | 1.11 | 2.16 | 4.41 | 0.66 |
| Sweet corn paired + Soybean (2:1) | 21.2 | 1.21 | 3.72 | 8.15 | 0.34 |
| Sweet corn paired + Bean (2:2) | 19.7 | 1.16 | 2.29 | 6.13 | 0.87 |
| Sweet corn paired + Soybean (2:2) | 20.5 | 1.42 | 6.87 | 13.43 | 0.41 |
| CD (p=0.05) | 2.7 | - | - | - | - |

**Table 3.** Economics as influenced by cropping systems of sweet corn, bean and soybean

| Treatment | Total cost of cultivation (₹ ha$^{-1}$) | Gross return (₹ ha$^{-1}$) | Net return (₹ ha$^{-1}$) | B: C ratio |
|---|---|---|---|---|
| Sole cropping system | | | | |
| Sweet corn sole (75 cm) | 52475 | 523344 | 470869 | 8.97 |
| Sweet corn paired sole (50/100 cm) | 52475 | 516318 | 463843 | 8.84 |
| Bean sole | 34375 | 92582 | 58207 | 1.69 |
| Soybean sole | 34135 | 73239 | 39104 | 1.15 |
| Intercropping system | | | | |
| Sweet corn + Bean (1:1) | 57225 | 551109 | 493884 | 8.63 |
| Sweet corn + Soybean (1:1) | 56225 | 562099 | 505874 | 9.00 |
| Sweet corn paired + Bean (2:1) | 56025 | 476462 | 420437 | 7.50 |
| Sweet corn paired + Soybean (2:1) | 55025 | 481401 | 426376 | 7.75 |
| Sweet corn paired + Bean (2:2) | 57825 | 444912 | 387087 | 6.69 |
| Sweet corn paired + Soybean (2:2) | 55825 | 463995 | 408170 | 7.31 |

## CONCLUSION

Sweet corn has been proved an attractive crop has an immense economic potential under sufficient resource condition. The highest CEY and LER were with sweet corn (75 cm) + soybean in 1:1 row ratio. The aggressivity (A) was lower when sweet corn was intercropped with soybean compared to bean, thus showing less dominancy of sweet corn with soybean in all the intercropping systems. The relative crowding coefficient (K) were higher when sweet corn was intercropped with soybean, again showing better performance of soybean compared to bean as intercrop with sweet corn. The monetary advantage index (MAI), net return and B: C ratio was also higher with sweet corn (75 cm) + soybean (1:1) system. Therefore, we recommend the farmers of Kashmir valley to adopt sweet corn with soybean in regular rows of 1:1 ratio as it is biologically and economically sustainable intercropping system for irrigated silty clay loam soil.

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# Optimum Dose of Phosphorus and Potassium Fertilization for Enhancing Productivity and Profitability of Rice (*Oryza sativa* L.) in Irrigated Sub Tropics of Jammu

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***Abstract*:** This study was undertaken to assess the effects of different phosphorus and potassium fertilizer levels on yield and economics of rice (*Oryza sativa* L.). The experiment consisted of four levels of phosphorus (control, 20, 40 and 60 kg $P_2O_5$ $ha^{-1}$) as main factor and four levels of potassium (control, 10, 20, 30 kg $K_2O$ $ha^{-1}$) as sub factor in factorial randomized block design with three replication. 60 kg $P_2O_5$ $ha^{-1}$ and 30 kg $K_2O$ $ha^{-1}$ recorded significantly higher grain yield (4.31 and 4.33 t $ha^{-1}$, respectively) and straw yield (6.21 and 6.26 t $ha^{-1}$, respectively) as compared to control. Similarly 60 kg $P_2O_5$/ha and 30 kg $K_2O$ $ha^{-1}$ recorded numerically higher net returns (Rs. 53035 and 53564 $ha^{-1}$) with B: C ratio of 1.72 and 1.82, respectively. However, economic optimum dose of phosphorus and potassium can be 28 kg $P_2O_5$ $ha^{-1}$ and 18 kg $K_2O$ $ha^{-1}$.

***Keywords*:** Economic optimum dose, Net returns, Phosphorus, Potassium, Yield

Rice (*Oryza sativa* L.) is one of the most important cereal crops of the India as well as world and is grown on wide range of climatic zones. Globally rice is grown on an area of about 165.93 million hectares with production of about 480.76 million tonnes. In India, the crop occupies an area of about 43.90 million hectare with production of 107.80 million tonnes (Anonymous 2020a). Total area under rice in Jammu And Kashmir State is about 271.56 thousand hectare with production of about 5570 thousand quintals (Anonymous 2020b). Fine rice enjoys a special place both in domestic as well as international market. The productivity and quality of fine rice depends on the environmental conditions and the agronomic practices especially the proper and balanced application of fertilizers plays significant role in achieving higher productivity. Generally, in rice cultivation much attention is given only to nitrogen fertilization and very often phosphorus and potassium fertilizers applied at minimal level. This practice of imbalance and inadequate fertilizer application affects the soil and crop productivity. Therefore, judicious and proper use of fertilizers can markedly increase the yield and improve the quality of rice crop (Yoseftabar 2016).

Phosphorus plays a vital role in crop growth and development, plant metabolism, photosynthesis, glucose assimilation, peptide synthesis, respiration, cellular function, gene transfer, reproduction and transfer of energy. Likewise, potassium is not only a constituent of organic structure but also regulates enzymatic activities and translocation of photosynthates. It enhances the root development due to which vegetative growth and production is increased (Gupta et al 2019). Hence, combined application of phosphorus and potassium when applied in appropriate balanced quantities led to substantive amount of photosynthates which are utilized by plants and eventually increases the crop yield. Optimum dose of phosphorus and potassium fertilization plays a vital role in growth and development and grain formation which results in higher yield of rice plant. Excessive phosphorus and potassium fertilization affects the plant growth which makes the plant susceptible to insect, pest and diseases, which ultimately reduces yield whereas less than optimum rate affects both yield and quality of rice to remarkable extent (Regar and Singh 2014). Therefore, this study was planned to ascertain the economic optimum dose of phosphorus and potassium for attaining good yield levels of rice.

## MATERIAL AND METHODS

The field experiment was conducted during *kharif*, 2016 at research Farm of SKUAST-J, Chatha. The experimental site is located at $32^0 40'$ N latitude and $74^0 58'$ E longitude with an altitude of 332 meters above mean sea level in the Shiwalik foothills of North-Western Himalayas. The soil of the experimental site was sandy loam in texture, slightly alkaline in reaction (pH 8.10), low in organic carbon (4.4 g $kg^{-1}$) and available nitrogen (247.06 kg $ha^{-1}$), but medium in available phosphorus (14.28 kg $ha^{-1}$) and potassium (138.03 kg $ha^{-1}$).

The experiment was laid out in factorial randomised block design replicated thrice The experiment consisted of four levels of phosphorus as main factor viz. control, 20, 40 and 60 kg $P_2O_5$ $ha^{-1}$ and four levels of potassium as sub factor viz. control 10, 20 and 30 kg $K_2O$ $ha^{-1}$.

**Agronomic practices:** The rice crop was fertilized with 60 kg N/ha and phosphorus and potassium as per treatment. Full dose of phosphorus and potassium along with 1/3$^{rd}$ of nitrogen was applied manually as a basal dose at the time of puddling. The remaining 2/3$^{rd}$ of nitrogen was applied in two equal splits *i.e.* at mid-tillering stage and just before the panicle initiation stage.

**Grain yield and straw yield:** The rice crop harvested from the net plot area of each treatment was sun dried, threshed, cleaned and grain yield was recorded at a moisture level of 12 per cent. The straw yield was worked out by subtracting the grain yield from the biological yield.

**Optimum dose of phosphorus and potassium**: Optimum dose of phosphorus and potassium was worked out through quadratic model as given by Cochran and Cox (1957)

$$Y=a+bx-cx^2$$

Where,

Y= Predicted yield (t $ha^{-1}$),a, b and c= are the coefficients of the quadratic equation, x = the dose of the nutrient (phosphorus and potassium) applied in kg $ha^{-1}$.

## RESULTS AND DISCUSSION

**Growth**: Growth of rice in terms of plant height, number of tillers/m$^{2,}$ dry matter accumulation revealed that the growth characteristics of fine rice showed pronounced improvement at 60 DAT and at harvest with different levels of phosphorus and potassium (Table 1). Among phosphorus levels, significantly highest plant height, number of tillers $m^{-2}$, dry matter accumulation were recorded with 60 kg $P_2O_5$ $ha^{-1}$ which was statistically at par with 40 and 20 kg $P_2O_5$ $ha^{-1}$ respectively and might have happened due to better development of root system and nutrient absorption by the crop plants. These results were in conformity with the findings of Masood et al (2018). The significantly highest plant height, number of tillers $m^{-2}$ , dry matter accumulation were recorded with 30 kg $K_2O$ $ha^{-1}$ which was statistically at par with 20 and 10 kg $K_2O$ $ha^{-1}$ respectively. This might have happened probably due to highest level of potassium triggers the activation of enzyme which enhanced the photosynthetic efficiency leading to more number of leaves and higher dry matter production. A similar view of better performance was reported by Zayed et al (2019).

**Yield attributes:** Yield attributes of rice viz. number of panicles $m^{-2}$, number of grains/ panicle , number of filled grains/ panicle were significantly higher with different levels of phosphorous and potassium except for 1000-grain weight as compared to control (Table 2). Among phosphorus levels, significantly highest number of panicles $m^{-2}$ (239.51), number of grains /panicle (96.49), number of filled grains/ panicle (85.93) were recorded with application of 60 kg $P_2O_5$ $ha^{-1}$ which led to significant enhancement in grain yield of rice. These results were in conformity with the findings of Kumar and Malarvizhi (2017) and Ya-jie et al (2018). Among potassium levels, application of 30 kg $K_2O$ $ha^{-1}$ recorded significantly higher panicles $m^{-2}$ , number of grains/ panicle and number of filled grain/ panicle which was statistically at par with 20 and 10 kg $K_2O$ $ha^{-1}$. This could be because potassium helps in improving growth, promoting photosynthetic rate and increase in assimilates translocation

**Table 1**. Effect of different phosphorus and potassium levels on growth of fine rice

| Treatments | Plant height (cm) | | No. of tillers $m^{-2}$ | | Dry matter accumulation | |
|---|---|---|---|---|---|---|
| | 60 DAT | At harvest | 60 DAT | At harvest | 60 DAT | At harvest |
| Phosphorus levels (kg $ha^{-1}$) | | | | | | |
| $P_0$ | 88.42 | 95.43 | 244.36 | 244.83 | 283.75 | 807.50 |
| $P_{20}$ | 94.33 | 101.83 | 254.41 | 252.16 | 315.59 | 962.08 |
| $P_{40}$ | 96.42 | 104.77 | 256.50 | 252.86 | 321.58 | 973.00 |
| $P_{60}$ | 96.58 | 104.91 | 258.07 | 254.62 | 329.73 | 987.43 |
| CD (p=0.05) | 5.83 | 6.27 | 9.20 | 6.82 | 17.02 | 29.01 |
| Potassium levels (kg $ha^{-1}$) | | | | | | |
| $K_0$ | 88.50 | 94.41 | 245.11 | 245.30 | 296.03 | 833.83 |
| $K_{10}$ | 94.67 | 102.03 | 254.72 | 250.91 | 312.50 | 954.74 |
| $K_{20}$ | 95.67 | 104.95 | 256.20 | 253.39 | 318.43 | 966.53 |
| $K_{30}$ | 96.92 | 105.54 | 257.32 | 254.87 | 323.69 | 974.92 |
| CD (p=0.05) | 5.83 | 6.27 | 9.20 | 6.82 | 17.02 | 29.01 |

to grains contributed to improvement in yield attributes. Similar findings were reported by Mahajan et al (2019).

**Grain and straw yield:** Different levels of phosphorus and potassium resulted in significant impact on grain yield and straw of fine rice (Table 3). Among phosphorous, 60 kg $P_2O_5$ $ha^{-1}$ recorded significantly highest grain yield and straw yield, which was at par with 40 and 20 kg $P_2O_5$ $ha^{-1}$. This might be due to cumulative effect of increased translocation of photosynthates which further had supplemental effect on the grain and straw yield of rice crop. (Ya-jie et al 2018, Gupta et al 2019). However among potassium levels, 30 kg $K_2O$ $ha^{-1}$ recorded significantly higher grain and straw yield which was statistically at par with 20 and 10 kg $K_2O$ $ha^{-1}$ (Table 1). This would probably due to mobilization of nutrients, enhanced the enzymatic activities and translocation of photosynthates from source to sink (Nanda et al 2016, Gupta et al 2019,).

**Table 2**. Effect of different phosphorus and potassium levels on yield attributing characters

| Treatments | Number of panicles $m^{-2}$ | Number of grain $panicle^{-1}$ | Number of filled grain $panicle^{-1}$ | 1000-grain weight (g) |
|---|---|---|---|---|
| Phosphorus levels (kg $ha^{-1}$) | | | | |
| $P_0$ | 226.67 | 89.39 | 74.43 | 23.43 |
| $P_{20}$ | 236.14 | 94.98 | 83.59 | 23.77 |
| $P_{40}$ | 238.55 | 95.70 | 85.67 | 24.15 |
| $P_{60}$ | 239.51 | 96.49 | 85.93 | 24.18 |
| Potassium levels (kg $ha^{-1}$) | | | | |
| $K_0$ | 225.40 | 89.95 | 73.64 | 23.18 |
| $K_{10}$ | 236.12 | 94.28 | 84.04 | 23.93 |
| $K_{20}$ | 239.10 | 95.89 | 85.54 | 24.19 |
| $K_{30}$ | 240.24 | 96.44 | 86.39 | 24.22 |
| CD (p=0.05) | 9.92 | 3.97 | 4.12 | NS |

**Economics:** Among the phosphorous levels, the highest net returns of Rs. 53,210 $ha^{-1}$ was in 40 kg $P_2O_5$ $ha^{-1}$ which was closely followed by 60 kg $P_2O_5$ $ha^{-1}$ respectively (Table 3). However, highest B: C ratio of 1.81 was in 20 kg $P_2O_5$ $ha^{-1}$. The possible reason could be lowest price of phosphorus applied through DAP resulted in enhancement of B: C ratio. However, among potassium levels, the highest net returns (Rs. 54460 $ha^{-1}$) and B: C ratio (1.83) was obtained in 30 kg $K_2O$ $ha^{-1}$.

**Optimum dose:** The model variables that made significant contributuion to the regression and the respective coefficient response to phosphorous perfectly fitted with a quadratic equation (equation 2) with 99.92 per cent of the variance in the grain yield of rice presented in Table 4 ( Motaka et al 2016)

$$Y=a+bp-cp^2 \quad \text{--- (1)}$$

Where,Y= Predicted grain yield (t $ha^{-1}$),p = phosphorus applied (kg $ha^{-1}$),a,b.c= regression coefficients

**Quadratic equation for grain yield**

$$Y=3.44+0.042p-0.0005p^2 \quad \text{--- (2)}$$

By differentiating the equation 2 optimum phosphorous dose was worked out. The optimum phosphorous dose for rice appeared as 28 kg $P_2O_5$ $ha^{-1}$ (Fig. 1).

Grain yield of rice response to potassium perfectly fitted with a quadratic equation (equation 4) with 99.90 of the variance presented

**Table 4.** Grain yield phosphorous and potassium response function parameters and coefficients

| Parameter | Phosphorus | | Potassium | |
|---|---|---|---|---|
| | Coefficient | $R^2$ | Coefficient | $R^2$ |
| A | 3.44 | 0.9992 | 3.42 | 0.9992 |
| B | 0.042 | | 0.0839 | |
| C | -0.0005 | | -0.0018 | |

**Table 3.** Effect of different phosphorus and potassium levels on yield and economics of rice

| Treatments | Grain yield (t $ha^{-1}$) | Straw yield (t $ha^{-1}$) | Cost of cultivation (Rs $ha^{-1}$) | Gross returns (Rs $ha^{-1}$) | Net returns (Rs $ha^{-1}$) | B : C ratio |
|---|---|---|---|---|---|---|
| Phosphorus levels (kg $ha^{-1}$) | | | | | | |
| $P_0$ | 3.41 | 4.98 | 27826 | 66428 | 38602 | 1.38 |
| $P_{20}$ | 4.17 | 6.06 | 28773 | 81105 | 52332 | 1.81 |
| $P_{40}$ | 4.26 | 6.17 | 29722 | 82932 | 53210 | 1.79 |
| $P_{60}$ | 4.31 | 6.21 | 30670 | 83706 | 53035 | 1.72 |
| CD (p=0.05) | 0.41 | 0.54 | | | | |
| Potassium levels (kg $ha^{-1}$) | | | | | | |
| $K_0$ | 3.41 | 4.97 | 28848 | 66439 | 37591 | 1.30 |
| $K_{10}$ | 4.15 | 6.00 | 29115 | 80678 | 51563 | 1.77 |
| $K_{20}$ | 4.26 | 6.19 | 29381 | 82946 | 53564 | 1.82 |
| $K_{30}$ | 4.33 | 6.26 | 29648 | 84108 | 54460 | 1.83 |
| CD (p=0.05) | 0.41 | 0.54 | | | | |

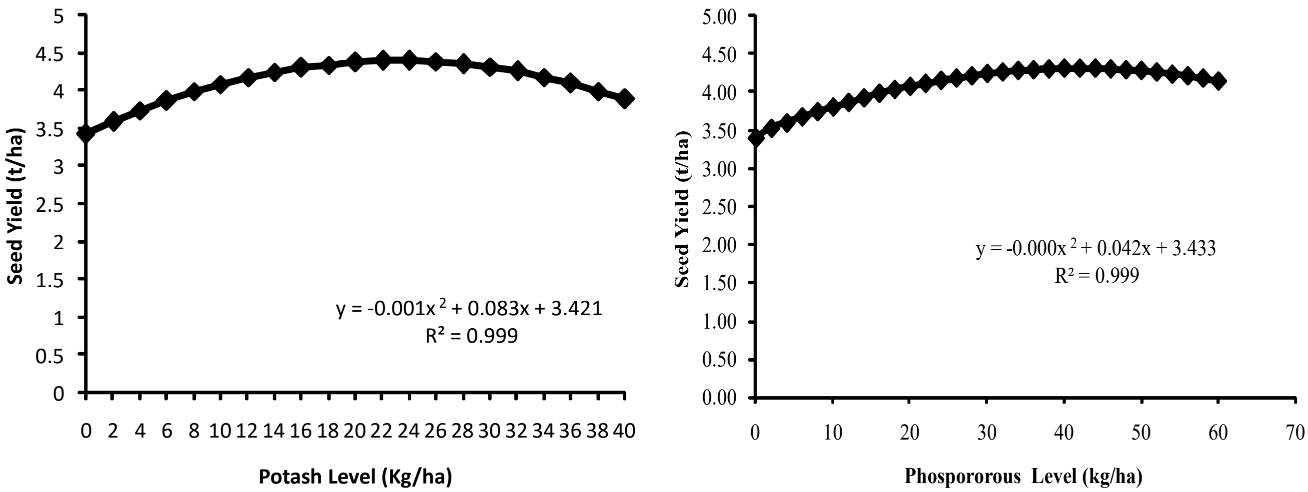


**Fig. 1.** Optimum dose of phosphorus and potassium for rice (*Oryza sativa* L.)

**Quadratic equation for grain yield**

$Y=3.422+0.0839k-0.018k^2$ --- (4)

By differentiating the equation 4 optimum potassium dose was worked out. The optimum potassium dose for rice appeared as 18 kg $K_2O$ $ha^{-1}$ (Fig. 1).

## CONCLUSION

Application of 60 kg $P_2O_5$ $ha^{-1}$ and 30 kg $K_2O$ $ha^{-1}$ were suitable dose of phosphorus and potassium for yield maximization irrespective of economic returns. Economically optimum level can be realized with 28 kg $P_2O_5$ $ha^{-1}$ and 18 kg $K_2O$ $ha^{-1}$ for yield optimization of fine rice in irrigated sub tropics of Jammu region. Regar, yield of rice in eastern plain zone of Utter Pradesh.

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# Assessment of Nutrient Management Practices on Productivity and Profitability of Fodder Maize+Ricebean Intercropping under Irrigated Condition

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***Abstract:*** Agronomic experiment entitled “Study on effect of nutrient management practices on fodder maize and ricebean intercropping in irrigated condition” was carried out during rainy season of 2019 at Research Farm of Agronomy Section, ICAR-NDRI, Karnal. The experiment results revealed that the higher growth attributes *viz*., plant height, leaf length, leaf width, leaf number, stem girth and leaf stem ratio were higher in sole maize. Whereas in ricebean, maximum plant height, leaf length and width were observed under intercropped condition by sowing maize and ricebean in 1:1 row ratio with 100% RDF + PGPR, while number of leaves, branches and nodules per plant were obtained greater in sole ricebean. Significantly higher green fodder yield (452.5 q $ha^{-1}$) were achieved in Maize + Ricebean (1:1) ratio with 100% RDF + PGPR application. Furthermore, the monetary returns of Maize+Ricebean (1:1) row ratio with 100% RDF+PGPR application accounted highest net returns (Rs. 41,969 $ha^{-1}$) and maximum B: C ratio (2.27) in comparison with rest of the treatments. The growing of maize and ricebean in 1:1 ratio with 100% RDF + PGPR realized higher production along with profitable returns to the farmers. The biometric parameters of each crop have high and positive correlation with their respective green fodder yield.

***Keywords***: Correlation, Growth, Intercropping, Maize, PGPR, Ricebean, Yield

Livestock sector is said to be a boon to rural economy by contributing 4.11% to our total GDP and 25.6% to agricultural GDP (Anonymous 2019). Scarcity of fodder is the major constraint in livestock sector all around the country. At present, India is facing net deficit of 35.6% of green fodder, 10.95% of dry fodder and 44% of concentrates (IGFRI Vision 2050). Along with fodder shortage area under grass land and pastures are in decreasing trend, nearly 50% of animals of our country directly depend on them for their foraging (Ghosh et al 2016). Most of the productive land is occupied by field crops and there is less scope to extent area under fodder crop the only option is left to increase the productivity per unit area basis with scientific crop rotation, cropping system/intercropping with fodder cereals. Legumes plays a significant role in enhancing the total productivity along with improve the quality of fodder by increasing its protein content and digestibility (Javamard et al 2009). Studies have reported that inclusion of legumes with fodder cereals helps to reduce pest and disease incidence by increasing predators and parasites population. Furthermore, intercropping with tall fodder crops such as maize, sorghum and pearl millet helps to prevent spread of pest and disease from one field to another field as it acts as barrier crops around the legume (Dwivedi et al 2015). Maize and ricebean is the important fodder crop grown by farmer mainly as a sole crop especially ricebean crop has been neglected and underutilized legume crop among the farming community. Hence, considerable efforts need to make for develop proper agronomic practices along with proper nutrient management to grow as intercrop with other crops which has an ability to supply nitrogen to the main crop by symbiotic nitrogen fixation through their root nodules. The rice bean has erected to semi erect growth habit and some varieties have twining characteristics which make most suitable to grow along with maize to get support from maize for climbing. Besides that, it also helps in solubilizing unavailable form of nutrients to make in available form, control weeds due to its smothering effect, increases microbial activity in soil and also supply the nutrients to succeeding crop by its legume effect (Ghosh et al 2007). Hence, a study was undertaken to assess the productivity and profitability of maize with ricebean intercropping under different nutrient management with their correlation studies.

## MATERIAL AND METHODS

**Site details:** Agronomic experiment was performed at Research Farm of Agronomy Section, ICAR-NDRI, Karnal during rainy season of 2019. Geographically, the experimental site situated at 29°45' N latitude, 76°58' E longitude and at an altitude of 245 m above mean sea level (MSL).

**Soil status:** The soil of experimental site was neutral in pH (7.24), clay loam in texture, medium in organic carbon (0.62%), low in available N (147.4 kg $ha^{-1}$) and medium in available P (24.5 kg $ha^{-1}$) and K (251.2 kg $ha^{-1}$).

**Treatments description:** The experiment was laid out in Randomized Block Design (RBD) with 14 treatments *viz*., $T_{1=}$ Maize sole + RDF; $T_{2=}$ Ricebean sole + RDF; $T_{3=}$ Maize + Ricebean (1:1) + RDF; $T_{4=}$ Maize + Ricebean (1:1) + 50% RDF; $T_{5=}$ Maize + Ricebean (1:1) + 50% RDF + PGPR; $T_{6=}$ Maize + Ricebean (1:1) + 75% RDF; $T_{7=}$ Maize + Ricebean (1:1) + 75% RDF + PGPR; $T_{8=}$ Maize + Ricebean (1:1) + 100% RDF + PGPR; $T_{9=}$ Maize + Ricebean (2:1) + RDF; $T_{10=}$ Maize + Ricebean (2:1) + 50% RDF; $T_{11=}$ Maize + Ricebean (2:1) + 50% RDF + PGPR; $T_{12=}$ Maize + Ricebean (2:1) + 75% RDF; $T_{13=}$Maize + Ricebean (2:1) + 75% RDF + PGPR and $T_{14=}$ Maize + Ricebean (2:1) + 100% RDF + PGPR and replicated in thrice. The recommended dose of fertilizer for maize i.e., 120:60:40 kg NPK/ha and ricebean i.e., 20:50:20 kg NPK/ha. For sole crops their respective recommended dose of fertilizer was applied whereas, in intercropping considered the demand of only main crop (maize) and fertilizer varied as per the treatments (100, 75 and 50% RDF). The fodder maize (Cultivar J-1006) and Ricebean (Sikkim local) were sown with seed rate of 45 and 35 kg/ha respectively during 1st week of August at spacing of 30 × 10 cm for sole crop of maize and ricebean. The intercropped maize geometry was modified and sown at spacing of 45 ×7.5 cm to introduce ricebean in additive series.

**Biometric data observations:** To measure growth parameters five plants were randomly selected from the net plot area and tagged to take observation from same tagged plant throughout the experiment period. Forage crops were harvested manually by separating maize and ricebean in order to determine extra additional yield obtained from each treatment at 60 days after sowing.

**Economic analysis:** The economics of each treatment were worked out on the basis of prevailing market price of inputs and out puts for production and estimated as cost of cultivation, gross return, net return and the benefit: cost ratio.

**Statistical analysis:** The data were analysed as described by Gomez and Gomez (1984) in MS EXCEL by using DMRT test with SPSS (ver. 20) software.

## RESULTS AND DISCUSSION

**Effect of Nutrient management practices on growth attributes:** Different nutrient management significantly influence all the growth parameters and attributing higher yield in different treatments, accordingly (Table 1).

**Plant height:** The result pertaining to plant height of both maize and ricebean showed significantly difference and highest plant height of maize was in sole maize + RDF whereas, lower value in maize + ricebean (1:1) + 50% RDF. However, the ricebean planted in intercropped condition recorded greater plant height i.e., maize + ricebean (1:1) + 100% RDF + PGPR and minimum under sole ricebean + RDF.The higher plant height of maize under sole crop is due to higher uptake of nutrients along with good light interception which increased meristematic activity and photosynthesis of plant which in turn increased inter-nodal length of sole maize. Similar observation was also noticed by Zaman and Malik (2000). In ricebean, higher plant height under intercropped condition may be due to support got from maize which helped ricebean to trail on maize and competition from maize for light increased the auxin content in ricebean meristem as shade promotes elongation of internodes. The findings of experiments were supported by Ayub et al (2004) and Bavec et al (2005).

**Leaf length and leaf width:** Leaf length and width of both maize and ricebean was significantly influenced by various nutrient management practices, maximum leaf length and width of maize was observed in sole maize + RDF and lowest value was recorded in maize + ricebean (1:1) + 50% RDF. Among various ricebean treatments maximum leaf length and width whereby sowing maize + ricebean in 1:1 ratio with RDF and PGPR and least in maize + ricebean (1:1) + 50% RDF. Highest leaf length and width in sole maize is due to no competition from ricebean along with more space and nutrient availability help the maize plant to enhance its crop canopy which in turn increased leaf length and width. Similar observation was recorded by Bhakar et al (2019). Leaf length and width of ricebean was higher under intercropped condition it may be due to increase in dose of nutrient i.e., RDF which increased cell division and further increased elongation of leaves. Similar observation was reported in maize + cowpea intercropping by Ginwal et al (2019).

**Number of leaves:** Number of leaves was not significantly influenced by different nutrient management practices in maize, but higher number of leaves noticed in sole maize + RDF. This might be due to increase in plant height with RDF. The same trend was noticed in maize and cowpea intercropping by Ibrahim et al (2006). However, sole ricebean + RDF showed significantly greater number of trifoliate leaves. This is due to increase in number of branches under sole crop which makes the plant to appear bushy with more leaves than trailing habit which was noticed under intercropped condition. The present experimental result is in agreement with the results of Ibrahim et al (2006) and Onuh et al (2011).

**Table 1.** Effect of nutrient management practices on growth attributes of fodder maize and ricebean under intercropping system

| Treatments | Plant height (cm) | | Leaf length (cm) | | Leaf width (cm) | | Number of leaves | | Stem girth (cm) | Leaf: Stem | Nodule number | Nodule weight (g) | Number of branches | Green fodder yield (t/ha) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | R | M | R | M | R | M | R | M | M | R | R | R | M | R |
| Maize + RDF | $265.08^{a}$ | | $88.61^{a}$ | | $7.16^{a}$ | | $11.50^{a}$ | | $2.02^{a}$ | $0.25^{a}$ | | | | $341.67^{a}$ | |
| Ricebean + RDF | | $125.83^{c}$ | | $12.49^{ab}$ | | $8.64^{ab}$ | | $30.44^{a}$ | | | $52.08^{a}$ | $0.539^{a}$ | $4.14^{a}$ | | $151.67^{ab}$ |
| M + R (1:1) + RDF | $251.65^{ab}$ | $218.65^{a}$ | $84.07^{abcd}$ | $12.50^{ab}$ | $6.25^{abcd}$ | $8.76^{ab}$ | $10.59^{abcd}$ | $25.00^{bcd}$ | $1.83^{bcd}$ | $0.22^{abcd}$ | $31.73^{de}$ | $0.407^{bc}$ | $3.45^{abc}$ | $310.67^{ab}$ | $130.17^{abcd}$ |
| M + R (1:1) + 50%RDF | $205.17^{d}$ | $187.65^{ab}$ | $71.83^{e}$ | $10.50^{c}$ | $5.37^{d}$ | $7.25^{c}$ | $9.33^{d}$ | $18.56^{e}$ | $1.73^{d}$ | $0.19^{cd}$ | $46.52^{ab}$ | $0.505^{ab}$ | $2.70^{c}$ | $264.00^{b}$ | $101.00^{bcd}$ |
| M + R (1:1) + 50%RDF + PGPR | $211.57^{cd}$ | $190.40^{ab}$ | $73.37^{de}$ | $10.58^{a}$ | $5.59^{cd}$ | $7.25^{c}$ | $9.50^{cd}$ | $19.22^{a}$ | $1.77^{a}$ | $0.19^{cd}$ | $49.97^{a}$ | $0.525^{ab}$ | $2.87^{bc}$ | $276.17^{b}$ | $100.50^{bcd}$ |
| M + R (1:1) + 75% RDF | $218.97^{bcd}$ | $202.17^{ab}$ | $77.83^{abcde}$ | $11.46^{bc}$ | $5.74^{cd}$ | $7.57^{bc}$ | $10.16^{abcd}$ | $22.48^{de}$ | $1.82^{bcd}$ | $0.20^{bcd}$ | $32.99^{de}$ | $0.423^{abc}$ | $3.29^{bc}$ | $301.67^{ab}$ | $120.00^{bcd}$ |
| M + R (1:1) + 75% RDF + PGPR | $222.93^{bcd}$ | $203.27^{ab}$ | $79.91^{abcde}$ | $11.50^{bc}$ | $5.92^{bcd}$ | $7.69^{bc}$ | $10.32^{abcd}$ | $23.17^{d}$ | $1.81^{bcd}$ | $0.21^{abcd}$ | $44.06^{abc}$ | $0.514^{ab}$ | $3.31^{bc}$ | $296.67^{ab}$ | $125.17^{bcd}$ |
| M + R (1:1) + 100% RDF + PGPR | $252.80^{ab}$ | $220.00^{a}$ | $85.32^{abc}$ | $13.41^{a}$ | $6.41^{abc}$ | $8.82^{ab}$ | $10.82^{abcd}$ | $25.06^{bcd}$ | $1.96^{abc}$ | $0.22^{abcd}$ | $34.71^{de}$ | $0.452^{abc}$ | $3.49^{ab}$ | $320.00^{ab}$ | $132.50^{abcd}$ |
| M + R (2:1) + RDF | $246.65^{abc}$ | $206.97^{ab}$ | $85.36^{abc}$ | $12.07^{abc}$ | $6.52^{abc}$ | $8.33^{abc}$ | $11.00^{abc}$ | $28.00^{abc}$ | $1.86^{abcd}$ | $0.24^{ab}$ | $29.34^{e}$ | $0.383^{c}$ | $3.56^{ab}$ | $320.83^{ab}$ | $82.50^{cd}$ |
| M + R (2:1) + 50%RDF | $216.00^{bcd}$ | $177.41^{b}$ | $74.49^{cde}$ | $11.16^{bc}$ | $5.63^{cd}$ | $7.48^{bc}$ | $9.81^{bcd}$ | $21.14^{de}$ | $1.74^{d}$ | $0.20^{bcd}$ | $44.72^{abc}$ | $0.513^{ab}$ | $3.00^{bc}$ | $270.00^{b}$ | $73.33^{cd}$ |
| M + R (2:1) + 50%RDF + PGPR | $216.47^{bcd}$ | $180.83^{b}$ | $75.67^{bcde}$ | $11.24^{bc}$ | $5.65^{cd}$ | $7.50^{bc}$ | $10.12^{abcd}$ | $21.33^{de}$ | $1.78^{cd}$ | $0.20^{bcd}$ | $47.94^{ab}$ | $0.519^{ab}$ | $3.16^{bc}$ | $278.33^{b}$ | $71.67^{d}$ |
| M + R (2:1) + 75% RDF | $227.20^{bcd}$ | $192.25^{ab}$ | $81.59^{abcde}$ | $11.66^{bc}$ | $5.99^{bcd}$ | $8.15^{abc}$ | $10.33^{abcd}$ | $24.16^{cd}$ | $1.85^{abcd}$ | $0.21^{abcd}$ | $37.31^{cde}$ | $0.481^{abc}$ | $3.33^{bc}$ | $311.50^{ab}$ | $74.83^{cd}$ |
| M + R (2:1) + 75% RDF + PGPR | $235.93^{abcd}$ | $194.67^{ab}$ | $83.83^{abcd}$ | $12.14^{abc}$ | $6.10^{bcd}$ | $8.32^{abc}$ | $10.49^{abcd}$ | $24.50^{cd}$ | $1.86^{abcd}$ | $0.21^{abcd}$ | $39.14^{bcd}$ | $0.486^{abc}$ | $3.37^{bc}$ | $312.00^{ab}$ | $74.67^{cd}$ |
| M + R (2:1) + 100% RDF + PGPR | $248.83^{ab}$ | $210.00^{ab}$ | $86.29^{ab}$ | $12.24^{abc}$ | $6.94^{ab}$ | $8.35^{abc}$ | $11.17^{ab}$ | $28.66^{ab}$ | $1.97^{ab}$ | $0.24^{ab}$ | $33.78^{de}$ | $0.402^{bc}$ | $3.59^{ab}$ | $319.17^{ab}$ | $85.83^{cd}$ |
| SEm± | 11.47 | 10.14 | 3.42 | 0.54 | 0.28 | 0.36 | 0.48 | 1.23 | 0.05 | 0.007 | 2.48 | 0.022 | 0.21 | 13.42 | 6.24 |
| CD (p=0.05) | 33.48 | 29.59 | 10.00 | 1.59 | 0.83 | 1.04 | NS | 3.60 | 0.16 | 0.02 | 7.25 | 0.064 | 0.63 | 39.19 | 18.22 |

*a,b,c,d,e,f superscripts indicate significance of mean at different treatments; M-Maize; R-Ricebean; RDF-Recommended dose of fertilizers; PGPR- Plant growth promoting rhizobacteria

**Leaf stem ratio:** The result pertaining to leaf stem ratio revealed that maize showed significantly greater value in sole maize + RDF in comparison with various intercropping treatments and lower value observed in maize + ricebean (1:1) + 50% RDF. Increased in leaf stem ratio in sole maize may be due to increase in the dose of fertilizer (RDF). Under intercropping situation due to competition for available resources among both the crops decreased the photosynthetic activity which in turn decreased the leaf stem ratio the results are close agreement with the findings of Bhakar et al (2019).

**Stem girth:** Stem girth showed significant difference among various treatments while thicker stem girth of maize was recognized in sole maize + RDF and lower value observed under intercropped condition by sowing maize and ricebean in 1:1 ratio with 50% RDF. This might be due to more uptake of nutrient along with good light interception helps to increase the activity of lateral meristem which in turn increase stem girth. The findings are contradictory to the findings of Ayub et al (2004).

**Nodules:** Significantly higher number of nodules and nodule weight were found in sole ricebean + RDF and lowest number observed in 2:1 ratio with RDF. Comparatively, a greater number of nodules and nodule weight per plant obtained in sole ricebean treatment due to increase dose of phosphorous, which increased the root length and root biomass which eventually increase nodule numbers. Almost identical observation recorded by Khan et al (2012); however the findings were contradictory to Agegnehu and Ghizam (2006).

**Number of branches:** Number of branches is complementary to number of leaves which ultimately reflects the biomass of plant. The ricebean branches differ significantly by nutrient management practices greater number of branches recorded in sole ricebean + RDF and lowest in maize + ricebean (1:1) + 50% RDF. More numbers of branches in sole ricebean might be due to more space and nutrients available which make the plant to appear bushy. But in intercropped condition, ricebean height was more as compared to number of branches in order to found those compete for light and nutrient with maize crop.

**Green fodder yield:** Green fodder yield was significantly influenced by different nutrient management practices (Table1). Highest green fodder yield has noticed in sole crops (maize and ricebean) treatments and lowest under maize + ricebean (1:1) + 50% RDF. But combined yield of maize and ricebean was significantly higher under intercropped condition by sowing maize + ricebean in 1:1 ratio with RDF + PGPR treatment (Fig. 1). Although yield of sole crop was superior to intercropped treatments but total green fodder yield per plot basis was lower in comparison with intercropping due to extra additional yield obtained from ricebean. Increasing trend in green fodder yield with increased RDF might be due to its higher uptake of nutrients which increase its photosynthetic activity and growth parameters which were directly correlated with green fodder yield. The results are in tune with Zaman and Malik (2000) and Kheroar and Patra (2013).

***Effect of nutrient management practices on economics of maize-ricebean production****:* Among the various treatment the maximum cost of cultivation was noticed in Maize + Ricebean (1:1) + 100% RDF + PGPR (₹ 33,081/ha)

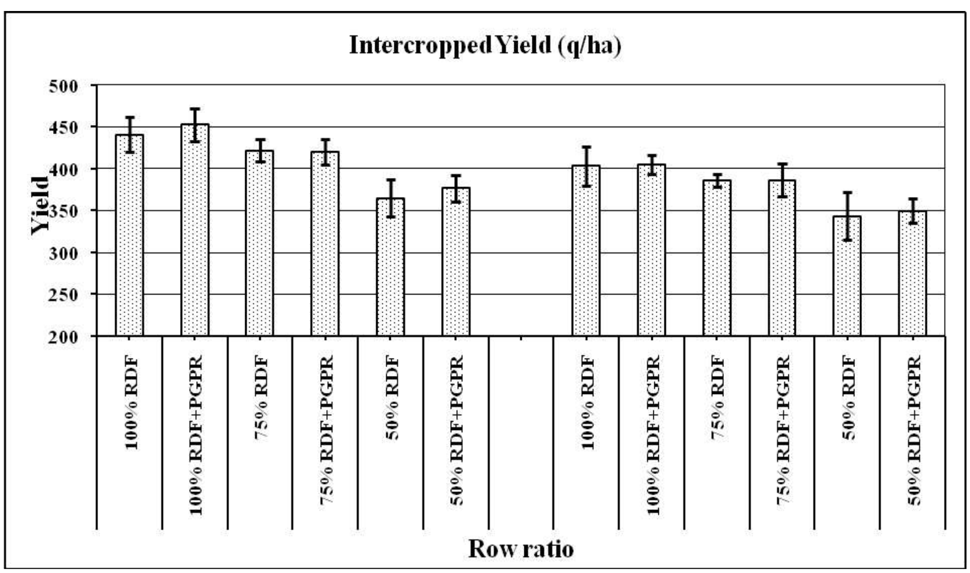


**Fig. 1.** Effect of nutrient management practices on total intercropped yield of fodder maize and ricebean under intercropping system

closely followed by Maize + Ricebean (1:1) + RDF this is due to extra seed cost of ricebean and labour (Table 4). However, lowest cost of cultivation recorded in sole ricebean treatment. But in terms of net returns highest net returns has been accounted in Maize + Ricebean (1:1) + 100% RDF + PGPR (₹ 41, 969/ha) and lowest with sole ricebean + RDF (₹

**Table 2.** Correlation studies of maize growth attributes under intercropping situation

| Pearson Correlations (n=13) | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Green fodder yield | Plant height | Leaf length | Leaf width | Number of leaves | Stem girth | Leaf stem ratio |
| Green fodder yield | 1 | | | | | | |
| Plant height | .920** | 1 | | | | | |
| Leaf length | .972** | .959** | 1 | | | | |
| Leaf width | .910** | .941** | .935** | 1 | | | |
| Number of leaves | .942** | .940** | .966** | .964** | 1 | | |
| Stem girth | .914** | .891** | .902** | .939** | .915** | 1 | |
| Leaf stem ratio | .881** | .916** | .915** | .973** | .962** | .860** | 1 |

**Correlation is significant at the 0.01 level (2-tailed)

**Table 3.** Correlation studies of ricebean growth attributes under intercropping situation

| Pearson Correlations (n=13) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Green fodder yield | Plant height | Leaf length | Leaf width | Number of leaves | Branches | Nodule number | Nodule weight |
| Green fodder yield | 1 | | | | | | | |
| Plant height | -.143 | 1 | | | | | | |
| Leaf length | .412 | .201 | 1 | | | | | |
| Leaf width | .375 | .136 | .953** | 1 | | | | |
| Number of leaves | .290 | -.142 | .774** | .828** | 1 | | | |
| Branches | .452 | -.268 | .787** | .812** | .955** | 1 | | |
| Nodule number | .073 | -.760** | -.512 | -.504 | -.366 | -.229 | 1 | |
| Nodule weight | .029 | -.690** | -.455 | -.470 | -.423 | -.261 | .949** | 1 |

**Correlation is significant at the 0.01 level (2-tailed)

**Table 4.** Effect of various nutrient management practices on economics of fodder maize and ricebean intercropping condition

| Treatments | Cost of cultivation (₹ $ha^{-1}$) | Gross return (₹ $ha^{-1}$) | Net return (₹ $ha^{-1}$) | B: C ratio |
|---|---|---|---|---|
| Maize + RDF | 26,108 | 54,672 | 27,365 | 2.09 |
| Ricebean + RDF | 23,077 | 27,306 | 4229 | 1.18 |
| M + R (1:1) + RDF | 33,019 | 73,148 | 40,129 | 2.21 |
| M + R (1:1) + 50%RDF | 29,943 | 60,420 | 30,477 | 2.02 |
| M + R (1:1) + 50%RDF + PGPR | 30,005 | 62,282 | 32,277 | 2.07 |
| M + R (1:1) + 75% RDF | 31,480 | 69,872 | 38,392 | 2.21 |
| M + R (1:1) + 75% RDF + PGPR | 31,542 | 70,008 | 38,466 | 2.22 |
| M + R (1:1) + 100% RDF + PGPR | 33,081 | 75,050 | 41,969 | 2.27 |
| M + R (2:1) + RDF | 30,384 | 66,178 | 35,794 | 2.17 |
| M + R (2:1) + 50%RDF | 27,308 | 56,394 | 29,086 | 2.06 |
| M + R (2:1) + 50%RDF + PGPR | 27,370 | 57,434 | 30,064 | 2.09 |
| M + R (2:1) + 75% RDF | 28,873 | 63,303 | 34,430 | 2.19 |
| M + R (2:1) + 75% RDF + PGPR | 28,935 | 63,366 | 34,431 | 2.19 |
| M + R (2:1) + 100% RDF + PGPR | 30,446 | 66,516 | 36,070 | 2.18 |

*RDF- Recommended dose of fertilizer; PGPR- Plant growth promoting rhizobacteria; M- Maize; R- Ricebean

4229/ha). In terms of B: C ratio maximum rupee obtained per rupee invested was noticed in the plot where Maize + Ricebean sown in 1:1 ratio with RDF + PGPR (2.27) and lowest in sole ricebean (1.18) treatment respectively. In comparison with sole crops intercropped treatment recorded higher biomass yield with only extra cost of ricebean seeds. This might be the possible reason for higher returns and B: C ratio under intercropped maize and ricebean. Similar findings related to high returns under intercropped situation were also described by Zaman and Malik (2000).

**Correlation studies :** The increasing trend of green fodder yield (GFY) of Maize fodder was highly correlated with biometric parameters i.e., plant height (0.920), leaf length (0.972), leaf width (0.910), number of leaves (0.942), stem girth (0.914) and leaf stem ratio (0.881) (Table 2). However green fodder yield of ricebean was positively correlated with most of the growth attributes (Table 3). These findings were also in line with the agreement of Dey et al (2004).

## CONCLUSION

The sole crop of both maize and ricebean with RDF was superior in terms of various growth attributes, even though some intercropped treatments which received 100% RDF with PGPR also performed similar growth as sole crops. However the total green fodder yield in intercropped treatments recorded significantly superior especially with combined application of 100% RDF + PGPR to Maize + Ricebean (1:1) intercropping and found to be more advantageous to the diary farmers.

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# Sustainable Production of Sesamum through Legume Intercropping: A Review

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***Abstract:*** Due to burgeoning human population and utilization of land for non-agricultural purpose, the demand for food crops is being increased day by day. To meet the growing food demand, we need to raise the production of all the food crops like cereals, pulses, oilseeds, etc. Under this situation, any strategy to boost agricultural production (especially oilseeds and legumes) is the expansion of an appropriate production-oriented multi-cropping system like intercropping system. Legumes intercropped with sesamum upsurges in productivity through better utilization of resources to stabilize the yield. To find out the advantages (yield, biological feasibility and economic) of any intercropping system, in general, the land equivalent ratio, area time equivalent ratio, relative crowding coefficient, aggressivity, competition ratio, net returns, benefit cost ratio, income equivalent ratio and monetary advantages are used. In this review article, the work on different sesamum based intercropping system carried out by researchers is discussed. Based on this review article, it is concluded that intercropping has beneficial effect on sesamum equivalent yield over sole sesamum, if desired short duration legumes be intercropped with it at an appropriate row ratio.

***Keywords***: Intercropping, Land equivalent ratio, Legumes, Profitability, Sesamum equivalent yield

Sesamum (*Sesamum indicum* L.) is one of the oldest oil crops grown for over 5000 years (Fuller 2003) and was cultivated/ and domesticated on the Indian subcontinent during Harappan and Anatolian eras (Bedigian 2003). It is an important oilseed crop of tropics and temperate zone of the world (Biabani and Pakniyat 2008) and widely grown in India, Myanmar, Sudan, Tanzania and China. It is a short-day plant and needs fairly hot conditions (25-27ºC) for its optimum growth and yield. Poor yield of sesamum was also due to low amount of daily sunshine (average 4-5 hours) in cloudy weather of rainy season; however, its productivity is higher under summer season compared with rainy season (Monpara and Gohil 2018). It is known as “The queen of the oilseed crops” due to excellent quality of its oil, flavor, taste and softness. It is considered to have both nutritional and medicinal values. Sesamum seeds are rich source of oil containing 46-52%, protein containing 18-20% (Kumar et al 2017b), calcium, phosphorus, potassium and vitamin E. Its high-quality oil has a high index of sesamin, sesamol and sesamolin antioxidants as well as monounsaturated and polyunsaturated fatty acids (Rangkadilok et al 2010). Sesamum oil does not get rancid early due to the presence of antioxidants which makes the sesamum oil highly preservable. Being having better oil quality and nutritional value, it plays a vital role in food system. Currently, sesamum is grown on around 23 states and union territory of India (Fig. 1) with annual production of 0.76 mt (Fig. 2).

Burgeoning human population is facing several problems in present day's agriculture like declining productivity, non-agricultural use of land, widening income gap between farmers and the rest of the workforce, etc. which will create pressure on land and food to have more agricultural intensification. The lower productivity of sesamum with respect to increasing population will threat an added pressure to achieve a targeted oilseed/ food production. However, the certainty in sesamum production system is irregular as it is highly sensitive to monsoon, biotic and abiotic stresses. Therefore, to make our food production system more vibrant, we need to cultivate legumes along with sesamum, which will not only stabilize sesamum production, but also increase foodgrain production through legumes and insurance against total sesamum failure for small and marginal farmers. Being short duration crop, sesamum has unique attributes that can fit almost any cropping system with a potential to get sustainable crop production through crop diversification (Oyeogbe et al 2015). Early maturing, relative thermo and photo-insensitivity and better canopy structure in pulses make them the ideal candidates for inclusion in multiple cropping systems. Legumes play a key role in atmospheric nitrogen fixation which contributes to valuable share of nitrogen for intercropped oilseeds and also total nutrient acquisition (Jiwan et al 2021). Besides it, the $N_2O$ losses from legumes grown field is also lower which indicates the healthy environment. Legumes also release some root exudates which aids in nutrient solubilization in rhizosphere, thus improves the soil fertility. The practice of intercropping among the multiple cropping has remained an exceptional asset of the tropical and subtropical areas and is getting popularity gradually amid small farmers (Kumar et al 2017a), even though it has been a common practice since long in developing countries (Wahla et al 2009). Oilseeds intercropped with short duration pulses/ legumes like green gram, black gram, moth bean, cowpea, clusterbean, etc. are popular intercropping systems followed in Indian regions. State wise total pulse's area and production in India are depicted in Figure 3 and 4.

**Current status of sesamum and total pulses:** The data on area, production and yield of sesamum in India (Anonymous 2019), Asia and World (Anonymous 2018) for the last three years depicted in Figure 5 showed the reduction in area of sesamum continuously for India and Asia since last three years whereas, it is constant for world. Sesamum production is also being declined in India, Asia and world. The productivity is, however being increased in India, but remained constant in Asia and world. Data on total pulse status presented in Figure 6 (Anonymous 2018); however, showed the continuous increment in total pulse's area and production in India and Asia since 2015, but remained constant in world.

**Intercropping systems:** Intercropping refers to the cultivation of two or more crops simultaneously on the same piece of land at same time which can provide yield benefit over sole cropping. It is a possibly advantageous system of crop production that may contribute yield gains over sole cropping. There are four major objectives of any intercropping systems. First objective of the intercropping system is to insure against total crop failure under aberrant weather conditions or pest epidemics; second objective is to enhance the total productivity per unit land area (Gao et al 2014a) in addition to stability in production (Mousavi and Eskandari 2011); third objective is to use resources like land, labour and inputs wisely/ judiciously (Nasri et al 2014) and fourth objective is to meet the domestic needs of the farmers.

**Additive and replacement series:** There are two design/ series for the sowing of crops under intercropping systems, *i.e.*, additive and replacement series (Fig. 7 and 8). Replacement series is sometimes known as substitutive designs, in which plant stand of main crop is less than 100%. Both the sown crops (may be more than two) are called as component crops. In additive series, however, pure stand of main crop is maintained (100%) and intercrop(s) is added by adjusting the planting pattern of main crop. The plant stand of intercrop is less than their pure stand.Intercropping always reduces the production potential of base crop/ component crop/ intercrop than their sole stand, but the overall performance of intercropping system (equivalent yield) is always higher than sole crop (Singh and Aulakh 2015, Kumar et al 2017a). Comparison between additive and replacement series, most of the findings (Kumar and Thakur 2006, Yogesh et al 2014, Hamzei and Seyedi, 2015, Zabih and Saeedipour 2015) confirmed the better performance of base crop under additive series due to have 100% plant population.

**Advantages:** The yield enhancement through the judicious use of resources including water, nutrients and solar energy over sole cropping is the major advantage of intercropping. It gives sustained yield advantages over sole cropping (Aulakh et al 2019) and this is especially achieved when legumes are intercropped with other crops which improves soil fertility (Wang et al 2014) due to higher $N_2$-fixation under legumes (Mawalia et al 2017). Due to the different nature of sole and intercrops, the light, water and nutrients could be more proficiently used which in turn poorer competitive ability. The variations in the competitive ability for growth characters between component crops were also reported by Amini et al (2013). Willey (1985) reported that intercropping has practical implications in ecology viz. diversity, crop interaction and other natural regulation mechanisms. Intercropping offers diversity at farm which leads to brings stability (Mousavi and Eskandari 2011); sustainability in crop production (Lithourgidis et al 2011) and productivity (Gao et al 2014a, Patel et al 2018); reduces the incidence of diseases (Zhang et al 2013, Gao et al 2014b), insects (Mitiku et al 2014, Uddin and Adewale 2014) and weed (Saudy and El-Metwally 2009, Aghaalikhani et al 2009) and improves the soil fertility (Bindhu et al 2014, Kumar et al 2017b, Patel et al 2017) and finally provides the economic benefit to farmers (Dwivedi et al 2015). Such benefits could be more important particularly when they are attained not by means of expensive inputs, but by the modest practice of growing crop together (Willey 1979). Spatial arrangement in intercropping is one of the important factors for higher yield. The major benefits of the intercropping systems are depicted in Figure 9.

Considering the above-mentioned roles of intercropping, attempts are, therefore, made in this paper to gather the findings of several research works carried out in India and abroad on sesamum + legumes intercropping systems.

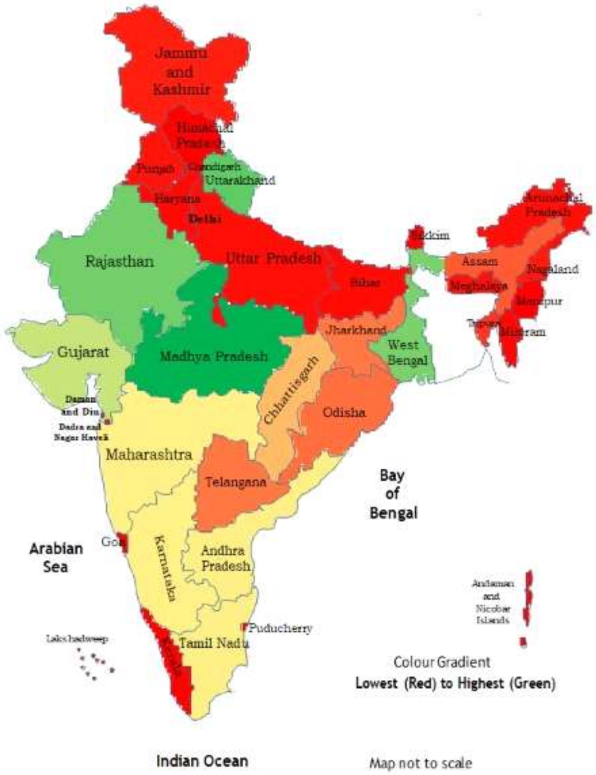



**Fig. 1.** State wise sesamum area in India during 2017-18 (Anonymous 2019)

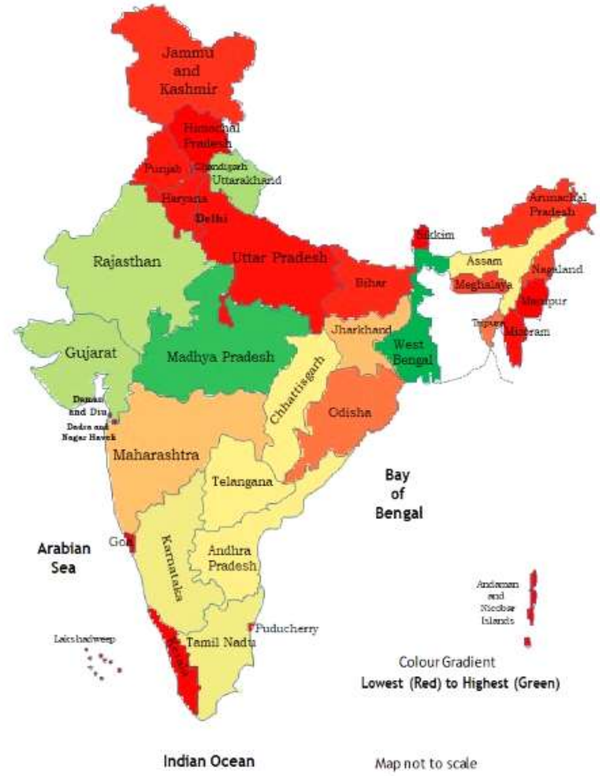



**Fig. 2.** State wise sesamum production in India during 2017-18 (Anonymous 2019)

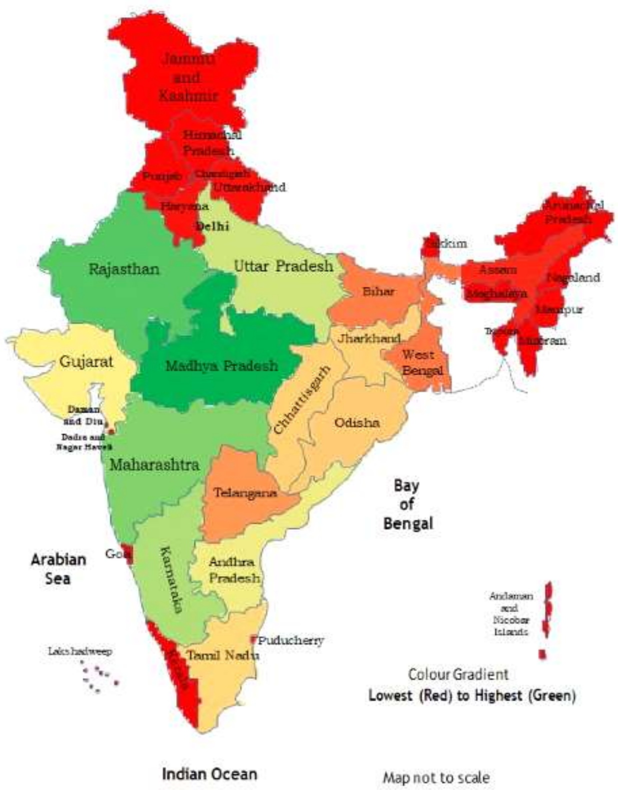



**Fig. 3.** State wise total pulses area in India during 2017-18 (Anonymous 2019)

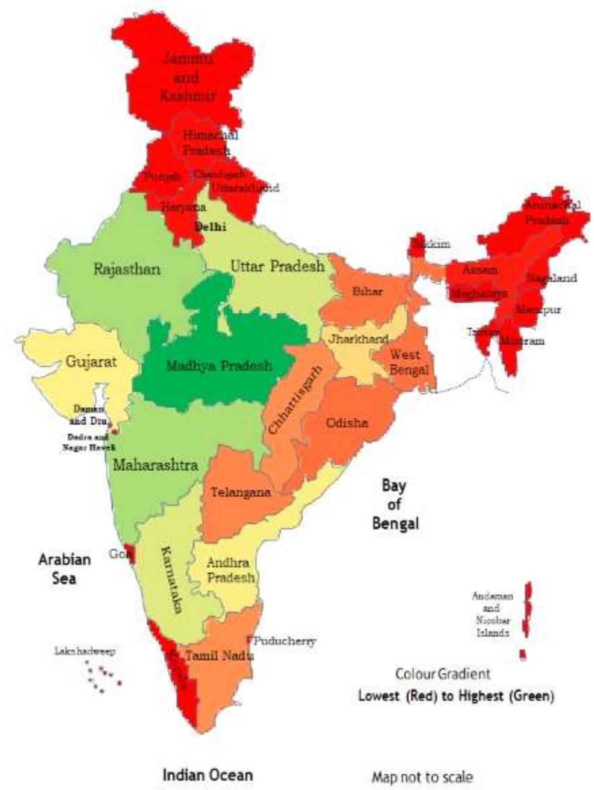



**Fig. 4.** State wise total pulses production in India during 2017-18 (Anonymous 2019)

## MATERIAL AND METHODS

**Economic assessment:** Economic assessment has paramount importance of any production system as it provides the information about cost involved, income and benefit cost ratio which is vital for farmers. The most commonly used economic parameters for intercropping system are income equivalent ratio (IER) and monetary advantages (MA).

**Income equivalent ratio:** IER is the relative land area required under sole crop to acquire the alike gross income as received from one ha of intercropping at the same management level. It is the conversion of LER in terms of economic returns. The value of IER>1 denotes advantages of intercropping system. The formula of IER given by Ghaffarzadeh (1997) is as follows:

$$IER = \frac{GI_{ab}}{GI_{aa}} + \frac{GI_{ba}}{GI_{bb}}$$

Where, GIaa: Gross income of base crop as sole crop; GIab: Gross income of base crop in intercropping treatment; GIba: Gross income of intercrop in intercropping treatment; Gibb: Gross income of intercrop as sole crop

**Monetary advantages (Rs ha$^{-1}$):** The formula of monetary advantage as suggested by Willey (1979) is as follows:

$$\text{Monetary Advantages (Rs. ha-1)} = \frac{\text{LER - 1}}{\text{LER}} \times \text{value of combined intercrop}$$

$$\text{Value of combined intercrop} = (Y_{ab} \times P_a) + (Y_{ba} \times P_b)$$

Where, LER: Land equivalent ratio; Yab: Yield of base crop in intercropping treatment (kg ha$^{-1}$); Yba: Yield of intercrop in intercropping treatment (kg ha$^{-1}$); Pa: Price of base crop; Pb: Price of intercrop

Higher MAI value indicates the more beneficial cropping system.

**Biological Assessment**

**Land equivalent ratio (LER):** LER is the relative land area required under sole crop to achieve the same yield as is obtained from one ha of intercropping at the same management level. The value of LER>1 denotes beneficial; LER<1 denotes harmful and LER=1 denotes neither benefit nor harmless of intercropping system. It is calculated by using the following formula as suggested by Mead and Willey (1980).

$$LER = \frac{Y_{ab}}{Y_{aa}} + \frac{Y_{ba}}{Y_{bb}}$$

Where, Yaa: Yield of base crop as sole crop (kg ha$^{-1}$); Yab: Yield of base crop in intercropping treatment (kg ha$^{-1}$); Yba: Yield of intercrop in intercropping treatment (kg ha$^{-1}$) and Ybb: Yield of intercrop as sole crop (kg ha$^{-1}$)

**Area time equivalent ratio (ATER):** ATER is the evaluation of crop yield per day basis. While considering the profitability of an intercropping system, LER takes into account only land area, whereas, ATER considers the time (duration of crop remained on field) along with land area. The value of ATER>1 shows beneficial; ATER<1 shows harm and ATER=1 shows neither benefit nor harm of any intercropping system. It is worked out by using the following equation (Hiebsch 1978).

$$ATER = \frac{(RY_a \times D_a) + (RY_b \times D_b)}{T}$$

$$RY_a = \frac{\text{Yield of sesamum in intercropping treatment}}{\text{Yield of sesamum as sole treatment}}$$

$$RY_b = \frac{\text{Yield of intercrop in intercropping treatment}}{\text{Yield of intercrop as sole treatment}}$$

Where, RYa: Relative yield of base crop (kg ha$^{-1}$); RYb: Relative yield of intercrop (kg ha$^{-1}$); Da: Duration of sesamum (Days); Db: Duration of intercrop (Days)

**Competitive Assessment**

**Relative crowding coefficient (RCC):** RCC is the measure of relative dominance of one species over the other in a mixture. It is denoted by K. The value of K>1, <1 and =1 shows yield advantage (more yield than expected); yield disadvantage (less yield than expected) and no differences in yield, respectively. It is calculated according to Agegnehu et al (2006) as follows.

$$K = K_{ab} \times K_{ba}$$

$$K_{ab} = \frac{Y_{ab} \times Z_{ba}}{(Y_{aa} - Y_{ab}) \times Z_{ab}}$$

$$K_{ba} = \frac{Y_{ba} \times Z_{ab}}{(Y_{bb} - Y_{ba}) \times Z_{ba}}$$

Where, Kab: Relative crowding coefficient of base crop in intercropping treatment; Kba: Relative crowding coefficient of intercrop in intercropping treatment; Zab: Proportion of sown spp. a (Base crop) in mixture with b (Intercrop); Zab: Proportion of sown spp. b (Intercrop) in mixture with a (Base crop)

**Aggressivity:** Aggressivity is calculated by using the following formula (Mc Gilchrist 1965). It is denoted by A. The value of A=0 shows both crops are equally competitive; A=

Positive shows dominant spp. and A= Negative shows weak spp.

$$A_{ab} = \frac{Y_{ab}}{Y_{aa} \times Z_{ab}} - \frac{Y_{ba}}{Y_{bb} \times Z_{ba}}$$

$$A_{ba} = \frac{Y_{ba}}{Y_{bb} \times Z_{ba}} - \frac{Y_{ab}}{Y_{aa} \times Z_{ab}}$$

Where, Aab: Aggressivity of base crop in intercropping treatment; Aba: Aggressivity of intercrop in intercropping treatment

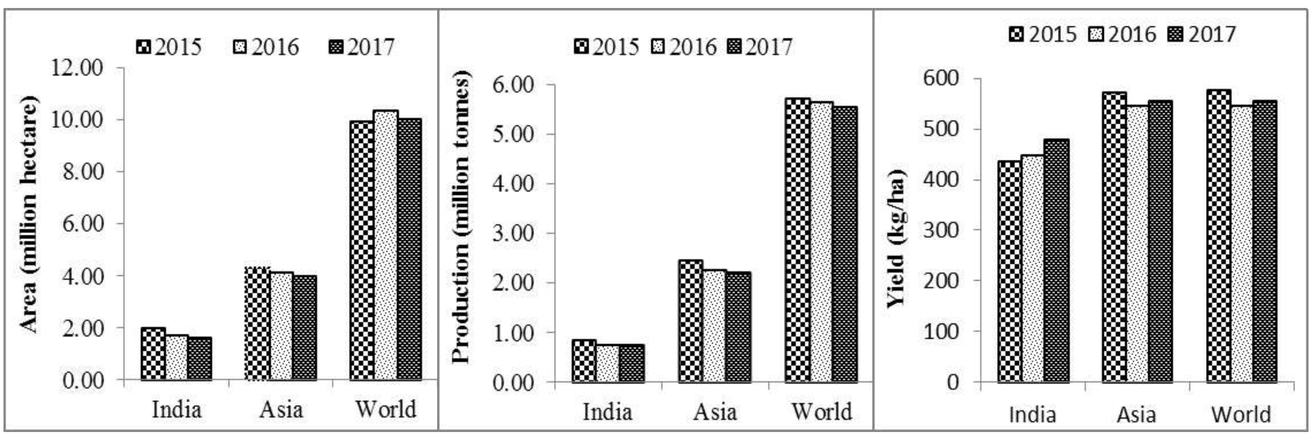
**Competition ratio (CR):** CR is the ratio of individual LER of the component crops. It involves the proportion of the crops sown in mixture. It provides more appropriate competitive ability for the plants and is also more beneficial index over RCC. It is calculated by using following equation (Willey and Rao 1980).

$$CR_a = \frac{LER_a}{LER_b} \times \frac{Z_{ba}}{Z_{ab}} \qquad CR_b = \frac{LER_b}{LER_a} \times \frac{Z_{ab}}{Z_{ba}}$$

Where, CRa: Competition ratio of base crop in intercropping treatment; CRb: Competition ratio of intercrop in intercropping treatment.

## RESULTS AND DISCUSSION

**Effect on growth:** Intercropping significantly increased the sesamum height (Kumar et al 2017a, Krishna and Reddy 2005, Puste et al 2014) over sole sesamum. The higher plant height of sesamum under intercropping treatments might be due to competition between plants for sunlight absorption as well as legumes fix N in soil which made available to sesamum (Kumar et al 2017a). However, Bhatti et al (2005) reported that intercropping reduced the sesamum height significantly and found the maximum and minimum values under sole and intercropped with cowpea (129.95 and




**Note:** Data depicted in Fig. 5 for India is for the years 2015-16, 2016-17 and 2017-18 instead of 2015, 2016 and 2017

**Fig. 5.** Status of sesamum in India, Asia and World

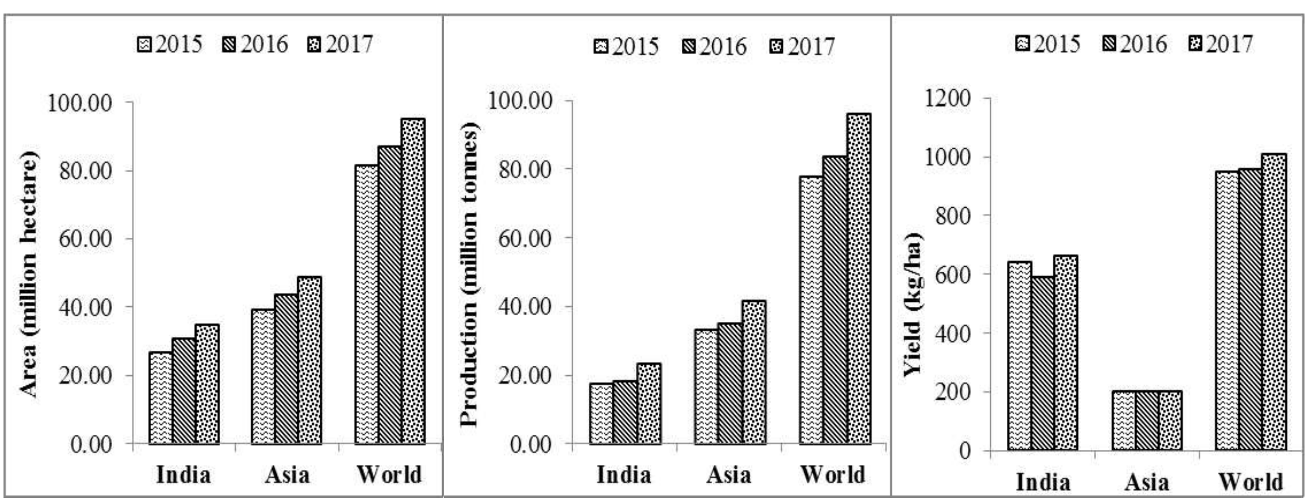



**Fig. 6.** Status of total pulses in India, Asia and World

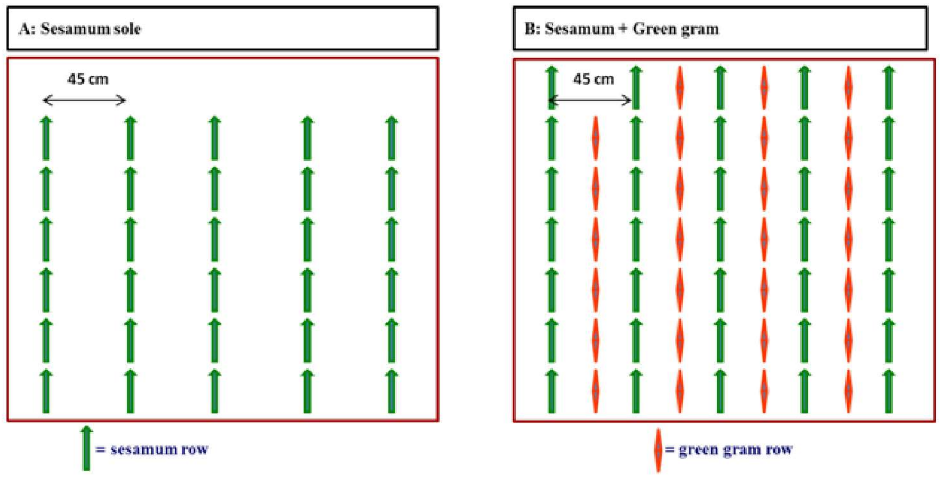



**Fig. 7.** Additive series

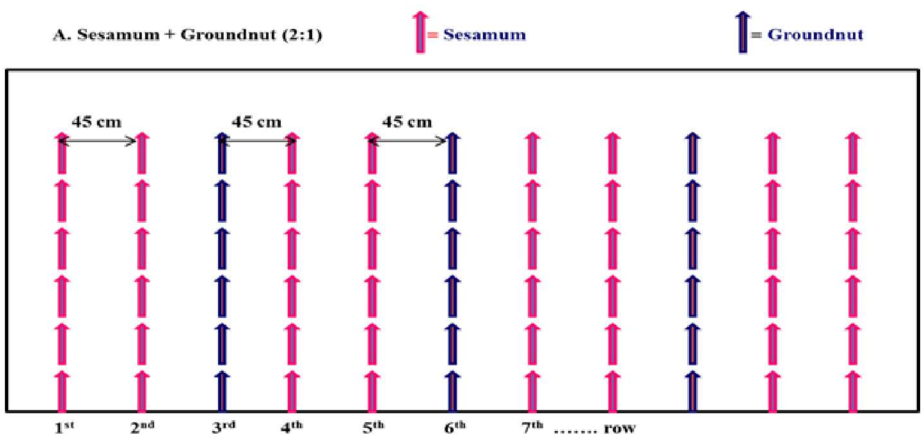



**Fig. 8.** Replacement series

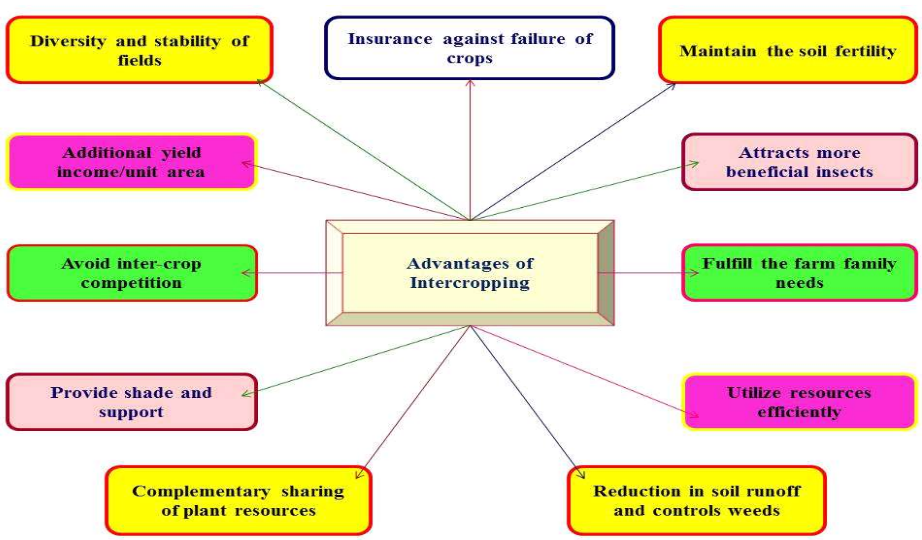



**Fig. 9.** Advantages of intercropping system

106.40 cm, respectively). Similar result was also reported by Sarma et al (2016). Bindhu et al (2014) reported that growth of both main crop as well as intercrop is reduced under intercropping treatment. Kumar and Thakur (2006) showed that sole sesamum recorded significantly higher number of branches per plant over intercropping treatments. Among intercropping systems, sesamum + black gram (1:1) gave the highest number of branches per plant of sesamum. Kumar et al (2017a) reported that intercropping significantly reduced the number of branches and dry matter congregation of sesamum (Fig. 10 and 11). Among the intercropping treatments (green gram and cowpea with sesamum at 2:1 and 3:2 row ratio), sesamum + green gram (Paired 3:2) recorded higher number of branches and dry matter accumulation per plant of sesamum.

**Effect on yield attributes and yield:** Intercropping significantly reduced the yield attributes and yield of sesamum over sole sesamum (Kumar et al 2017a). They (Kumar et al 2017a) found significantly greater number of capsules per plant, capsule length, number of seeds per capsule and test weight of sesamum with sesamum + green gram (3:2), when compared among intercropping treatments. Introducing the legumes into sesamum reduced the seed yield of sesamum up to a significant level (Hanumanthappa and Dalavai 2008, Kumar and Thakur 2006, Bhatt et al 2010) due to inter-crop competition. The intercropping of grain legumes statistically reduced the seed yield of sesamum due to adverse effects on its yield components; nonetheless, the sesamum equivalent yield (SEY) is higher under intercropping treatments compared with sole sesamum due to the additional harvest of intercrop (Bhatti et al 2005). Nurbakhsh et al (2013) reported the statistically equal grain yield of sesamum under sesamum + green gram (50:75%) and sole sesamum (100%). Bindhu et al (2014) also revealed that intercropping significantly reduced the test weight and seed yield of sesamum compared with sole sesamum. De et al (2002) reported that intercropping of sesamum + green gram (1:1) resulted into higher number of seeds per capsule, seed and stick yield compared with other intercropping treatments. Sesamum + groundnut (3:1) produced significantly higher weight of seeds per plant, seed as well as stalk yield (Mahale et al 2008). Highest sesamum yield (1.8 t/ha) was obtained from 100% sesamum + 20% chickpea intercropping system (Pouramir et al 2010). Significantly higher seed yield of sesamum was confirmed with sesamum + black gram (3:2) intercropping system (Yadav et al 2008). Sharma and Singh (2008) reported higher seed yields of sesamum when soybean and black gram were intercropped with it as compared to maize intercrop. The highest yield gain was obtained with sesamum + green gram (100:100%) compared with sesamum + green gram (100:50%) and sole sesamum (Krishna and Reddy 2005).

**Sesamum equivalent yield:** Intercropping increased total crop productivity (SEY) as compared to sole sesamum and significantly higher SEY was obtained from sesamum + green gram (1:1) (Sarkar and Chakraborty 2000). Sarkar et al (2003) reported that all the tested intercropping systems exhibited an increase in total productivity in terms of sesamum equivalent yield over sole sesamum. Sridhar et al (2002) reported that cultivation of sesamum + black gram (4:1) resulted into maximum mean SEY (872 kg/ha). Sharma and Singh (2008) reported higher SEY with sesamum + black gram (2:2) closely followed by sesamum + soybean (2:2) in comparison of sesamum + maize (2:2). Tripathi et al (2007) revealed that inclusion of clusterbean in sesamum at 3:1 row ratio exposed higher SEY followed by black gram in sesamum at 3:1 row ratio. The findings of some other researchers on yield of sesamum and intercrops; and SEY as influenced by intercropping are shown in Table 1.

**Fig. 10.** Sesamum + green gram (3:2) intercropping system

**Fig. 11.** Sole sesamum (Paired row sown at 30-60 cm)

**Competitive and biological feasibility:** Results from a field experiment conducted by Sarkar and Chakraborty (2000) revealed that intercropping of green gram with sesamum at 1:1 row ratio produced higher LER, ATER as well as product of crowding coefficient. The cultivation of sesamum cv. B-14 + green gram cv. B-105 (1:1) produced maximum ATER (1.25); though, higher competition ratio of sesamum (1.46) was obtained from sesamum cv. Rama + green gram cv. T-44 (2:2) (Dwary and Patra 2006). Kumar and Thakur (2006) reported that introduction of black gram to sesamum crop showed biologically sustainable intercropping system because of LER ranging from 1.09 to 1.17 over sole sesamum; however, intercropping of soybean to sesamum exhibited LER value lesser than one. They (Kumar and Thakur 2006) also reported the zero value of aggressivity index and highest relative value total (1.16) under sesamum + black gram (1:1). In comparison of sesamum + maize (2:2), sesamum + black gram (2:2) closely followed by sesamum + soybean (2:2) produced higher LER (Sharma and Singh 2008). Significantly higher LER as well as RCC was recorded with 50% sesamum + 50% bean as compared to 5% sesamum + 25% bean (Nurbakhsh et al 2013). Growing of sesamum + green gram (1:1) proved to be biologically advantageous intercropping system as it had higher value of the product of RCC (3.45) compared with sesamum + green gram (1:2), sesamum + black gram (1:1), sesamum + black gram (1:2) (Yadav et al 2013). Among the intercropping treatments (sesamum + green gram/ cowpea at 2:1 and 3:2 row ratio), maximum product of RCC (2.28), aggressivity (0.25) and competition ratio of sesamum (1.25) was recorded under sesamum + green gram (3:2) (Kumar et al 2017c). The competitive and biological feasibility of sesamum based intercropping system found by some other researchers are presented in Table 2.

**Economic assessment:** The maximum benefit in terms of net returns of Indian national rupee (INR) 39374/ha was obtained from sesamum + black gram (1:1) (Sarma et al 2016). Similarly, maximum net returns was received by intercropping of sesamum + black gram at 3:2 row ratio (INR 8997/ha) (Yadav et al 2008) and sesamum + black gram at 4:1 row ratio (INR 9068/ha) (Sridhar et al 2002). Sarkar and Chakraborty (2000) reported the maximum net returns under sesamum + green gram (1:1). Krishna et al (2004) obtained maximum net returns by sowing sesamum single row single skip with green gram (100%) in skip row. Higher net returns and B: C ratio was obtained from sesamum + clusterbean (3:1) followed by sesamum + black gram (3:1) (Tripathi et al 2007). Likewise, maximum B: C ratio (1.18) was also confirmed with sesamum + black gram (3:2) (Yadav et al 2008). Maximum net monetary returns as well as B: C ratio

**Table 1.** Effect of intercropping on yield of sesamum, intercrop and sesamum equivalent yield

| Treatment | Sesamum yield (kg ha$^{-1}$) | Intercrop yield (kg ha$^{-1}$) | SEY (kg ha$^{-1}$) | Increase in SEY over sole sesamum (%) | Reference |
|---|---|---|---|---|---|
| Sesamum sole | 667 | – | – | – | Sarkar and Kundu (2001) |
| Sesamum + groundnut (2:1) | 417 | 705 | 1245 | 86.66 | |
| Sesamum sole | 801 | – | – | – | Yadav et al (2013) |
| Sesamum + green gram (1:1) | 587 | 650 | 983 | 22.72 | |
| Sesamum sole | 456 | – | – | – | Kumar and Thakur (2006) |
| Sesamum + black gram (1:1) | 266 | 417 | 605 | 32.68 | |
| Sesamum sole | 1108 | – | – | – | Bhatt et al (2010) |
| Sesamum + cotton (3:1) | 921 | 716 | 1589 | 43.41 | |
| Sesamum sole | 853 | – | – | – | Mandal and Pramanick (2014) |
| Sesamum + green gram (2:2) | 571 | 407 | 1037 | 21.57 | |
| Sesamum sole | 540 | – | – | – | Bindhu et al (2014) |
| Sesamum + black gram (1:1) | 263 | 763 | 812 | 50.37 | |
| Sesamum sole | 1120 | – | – | – | Khan et al (2017) |
| Sesamum + groundnut (3:2) | 870 | 770 | 2330 | 108.04 | |
| Sesamum sole | 702 | – | – | – | Kumar et al (2017a) |
| Sesamum + green gram (Paired 3:2) | 530 | – | 944 | 34.47 | |
| Sesamum sole | 616 | – | – | – | Sarma et al (2016) |
| Sesamum + black gram (1:1) | 419 | 859 | 1385 | – | |

**Table 2.** Effect of intercropping on competitive ability, biological feasibility and economic assessment of sesamum

| Treatment | LER | ATER | Aggressivity index | B: C ratio | Reference |
|---|---|---|---|---|---|
| Sesamum + groundnut (2:1) | 1.35 | 1.30 | -1.26 | – | Sarkar and Kundu (2001) |
| Sesamum + groundnut (2:1) | 1.59 | – | 0.29 | – | Sarkar et al (2003) |
| Sesamum + black gram (1:1) | 1.17 | – | 0.00 | – | Kumar and Thakur (2006) |
| Sesamum + bean (1:1) | 1.59 | – | 0.42 | – | Nurbakhsh et al (2013) |
| Sesamum + green gram (1:1) | 1.29 | 1.12 | 0.25 | 2.97 | Yadav et al (2013) |
| Sesamum + green gram (2:2) | 1.21 | 1.16 | 0.11 | – | Mandal and Pramanick (2014) |
| Sesamum + green gram (3:2) | 1.18 | 1.13 | 0.25 | 3.01 | Kumar et al (2017c&d) |
| Sesamum + groundnut (3:2) | 1.64 | – | – | 2.15 | Khan et al (2017) |
| Sesamum + black gram (1:1) | 1.42 | – | – | 4.40 | Sarma et al (2016) |
| Sesamum + black gram (4:1) | 1.18 | – | – | 2.20 | Sridhar et al (2002) |

was attained from sesamum + black gram (2:2) closely followed by sesamum + soybean (2:2) (Sharma and Singh 2008). Introducing of grain legumes in sesamum produced higher net returns over sole sesamum owing to higher SEY in intercropping treatments. The cultivation of sesamum + black gram (1:1) gave 53.66% (Kumar and Thakur 2006), sesamum + groundnut (1:1) gave 79.64% (Sarkar and Kundu 2001), sesamum + green gram (1:1) gave 36.80% (Yadav et al 2013), sesamum + cotton (3:1) gave 58.57% (Bhatt et al 2010), sesamum + green gram gave 18.93% (Bhatti et al 2005) and sesamum + green gram (3:2) gave 52.42% (Kumar et al 2017d) higher net returns over sole sesamum. Sarkar and Kundu (2001) received the higher IER and monetary advantages from groundnut intercropped with at 2:1 row ratio compared with rest of the intercrops (green gram, black gram and sunflower) at different row ratios (2:1 and 3:2). Intercropping of cotton with sesamum at 3:1 row ratio proved superiority in terms of returns per rupee invested (4.44) and IER (1.37) over rest of the intercropping treatments (Bhatt et al 2010). Kumar et al (2017d) reported that sesamum + green gram (3:2) showed higher IER (1.18) and monetary advantages (INR 10515/ha) in comparison of rest of the intercropping treatments. Sesamum + green gram (2:2) proved superiority in terms of monetary advantages and relative value total (RVT) over rest of the treatments (Mandal and Pramanick 2014). Intercropping of sesamum with mashbean in pattern of 100 cm spaced 4-row strips has proved to be most economically feasible as compared to sole planting (Bhatti et al 2013). The superior B: C ratio of sesamum based intercropping system as obtained by some researchers is presented in Table 2.

Since, intercropping enhances the total crop productivity and resource-use efficiency; henceforth, it could be a viable approach for sustainable intensification of short duration legume intercrops in sesamum, particularly in regions where measured benefits have been greatest (Rusinamhodzi et al 2012) and farmers have marginal and small land holding. Based on the present review article, we found that most of the successful/ beneficial intercropping system had short duration grain legumes as intercrops with sesamum in additive series; therefore, region/ location-oriented research on sesamum based intercropping systems should be undertaken and developed for farmers.

## CONCLUSIONS

This review article shows the short duration of grain legumes like green gram, black gram, cowpea, etc. could fit well as intercrops in sesamum. Such intercropping system provide the higher production by utilizing land as well as other resources like light, water, nutrients, etc. effectively. Overall, it is concluded that beneficial effect of short duration grain legume intercrops aids in boosting the farm productivity which not only enables the farmers to have higher net returns, but also ensure the food security.

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# Effect of Organic Nutrients on Growth and Yield of Broccoli *Brassica oleracea* L. var. italica Plenck

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***Abstract***: The experiment was conducted during the winter season 2016-1017 under desert condition in south of Iraq, it included nine factorial layout with three genotypes of Broccoli Cavolo, Paraiso and Monaco as main treatments and three concentrations of liquid seaweed extract Algaton (0,1.5 and 3 ml.$L^{-1}$) . Three spays of Algaton was done at two weeks. Paraiso hybrid plants showed a significant increase in vegetative growth indicators including plant height, stem diameter, leaf area and both fresh and dry weights, in addition to yield indicators that include curd circumference, flower stalks, fresh weight and total yield and also in the qualitative characteristics of curd total including chlorophyll, vitamin C, total soluble carbohydrates, percentage of nitrogen, potassium, phosphorus, sulfur and protein. Spraying with Algaton spray at 3 ml.$L^{-1}$ caused a significant increase in vegetative growth and yield indicators. The interaction between the two factors was significant in some of the characteristics under study. Paraiso hybrid s sprayed with Algaton at 3 ml.$L^{-1}$ significantly exceeded in leaf area, fresh and dry weight of leaves, curd fresh weight, productivity and percentage of potassium.

***Keywords***: Broccoli, Genotypes and spraying with Algaton

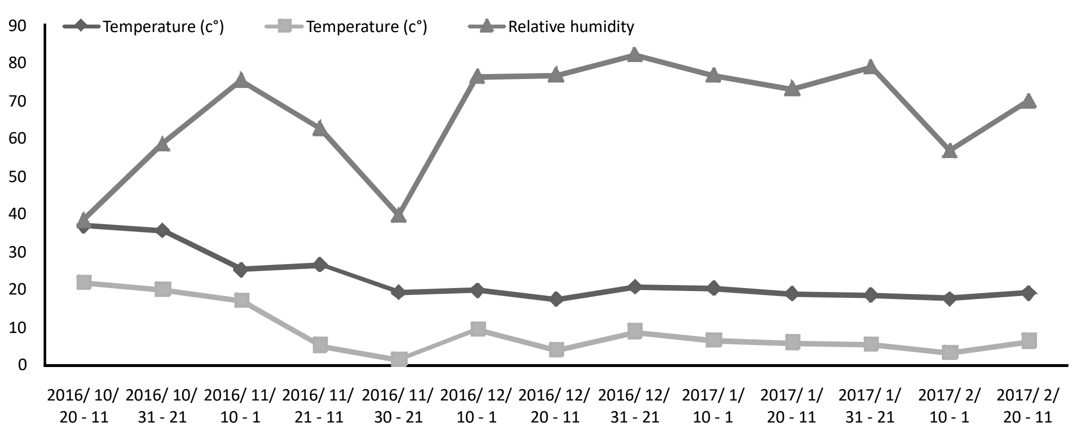
Broccoli (*Brassica oleracea* var.*italica*)is one of the winter vegetables crops that belongs to the Brassicaceae family. The planted area of broccoli with cauliflower in Iraq reached 989 hectares with a production of 7187 tons and productivity of 7.2669 ton. $ha^{-1}$ (FAO 2019). Foliar feeding is one of the preferred methods of fertilization because the plants take high benefit of it, as nutrients are added in low concentrations by spraying them on the plant, whether in traditional, organic or biological agriculture in order to nourish, accelerate the growth and improve production of the plant (Al-Gebory and Al-Khafagy 2011). Foliar fertilization provide necessary nutrients by spraying the total vegetative and absorption through the stomatal openings spread on the top and bottom surfaces of the leaf, but is not a substitute for ground fertilization, but rather due to quick response because the nutrient solutions contain amino acids and organic compounds (Sadiq et al 2002). The spraying with seaweed extracts, including Algaton, which is considered as organic primary products, is useful Al-Samaraee and Hassan (2012) concluded that spraying *Tagetes erecta* L. plant with seaweed extract Algaton at a 4 ml.$L^{-1}$ in two and three sprays significantly improved plant height, number of main branches, number and diameter of flowers. The three times spaying gave the highest values in the vegetative and floral growth characteristics. Kareem and Al-Ajil (2012) indicated that spraying cauliflower *Brassica oleracea* var. botrytis with seaweed extract Algaton in three concentrations: 0, 1.5 and 3 ml.$L^{-1}$ 15 days after transplanting and re-spraying every 15 days led to the significant superiority of the plants sprayed with the extract in the vegetative growth characteristics.Spray at of 1.5 ml.$L^{-1}$ exceeded in curd speed of ripening, while at 2 ml.$L^{-1}$ exceeded in weight characteristics and curd tenacity. Ali et al (2017) found that local garlic plants that were sprayed with foliar fertilizer Microm at the concentrations 0.5, 1 and 1.5 g.$L^{-1}$ were superior to control. Al-Zubaidi (2018) indicated that spraying eggplant plants with nutrient solutions Basfoliar Kelp and Fylloton at concentrations had a significant effect on growth. Fylloton at 3 g.$L^{-1}$ were distinguished by recording the highest average of plant height, leaf area, fruits number and weight, yield of the plant and the dry weight, also Abboud et al. (2020) indicated that spraying watermelon plants with Huzone nutrient had a significant effect on all growth and yield characteristics. Due to the importance of the broccoli crop the experiment was conducted on spraying with Algaton, which is a liquid seaweed extract on the growth and yield of broccoli in the desert region of southern Iraq.

## MATERIAL AND METHODS

The field experiment was conducted in winter season 2016-2017at Basra Agriculture Directorate, Khor Al-Zubair on a mixed sandy soil with a pH of 7.73, an electrical conductivity of 6.78 Des $m^{-1}$ and organic matter of 0.74%. The maximum, minimum temperatures and relative humidity of the experiment area during the planting season were recorded using the data of the Al-Barjisiah Agricultural Research Station (Fig. 1). The experiment included two factors in split plot design with the three varieties asmain treatment (Cavolo, Monaco, and Paraiso) and spraying with Algaton (1.5 and 3 ml.$L^{-1}$) as sub treatment. Three sprays were done at two weeks after a month of planting seedlings and the untreated control was also kept. The data was analyzed using Genstat, V.10.3 (2011) .Cavolo variety, seeds produced by the Italian company Hortus, hybrid variety Monaco with a germination rate of 96% and a purity of 99.9%, and hybrid variety Paraiso with a germination rate of 85% and a purity of 99% produced by the Australian company Syngenta and imported by Debana Modern Agriculture Company Ltd.was used in the experiment. The experiment included 27 experimental units with an area of 10 x 2 mfor each unit, with a distance of 50 cm between row and 50 cm between plants. The seeds were planted on September 1,2016 and the seedlings were transferred to the field at the age of 45 daysAll recommended agronomic practices were followed(Al-Khalidy 2018).Seaweed extract Algaton, produced by the Spanish company Valencia, and contains total nitrogen (N) 6% w/w, phosphoric anhydride ($P_2O_5$) soluble in water 3% w/w, potassium oxides ($K_2O$) soluble in water 10% w/w, molybdenum (Mo) soluble in water 0.3% w/w. In addition, Algaton contains auxin, cytokinin, gibberellin, amino acids, sugars and carbohydrates. Data was collected from plants selected randomly at the end of the season and included plant height (cm), stem diameter (cm), number of leaves, leaf area (m²), fresh and dry weight of leaves (g), curd fresh weight (kg), dry weight (g), curd circumference (cm), number of curd flower stalks and the total productivity (ton $ha^{-1}$), also total chlorophyll, vitamin C (mg $100g^{-1}$ fresh weight), total soluble carbohydrates (mg $g^{-1}$ dry weight), percentage of nitrogen and potassium, phosphorous, sulfur and protein in the curds.

## RESULTS AND DISCUSSION

The Paraiso variety was significantly superior in plant height, stem diameter, leaf area, and both fresh and dry weight of leaves, followed by Monaco hybrid then Cavolo. There was no significant difference between the genotypes in in number of leaves (Table 1). Plants sprayed with Algaton extract at 3 ml.$L^{-1}$were superior with a significant difference to those sprayed at 1.5ml.$L^{-1}$ in all characteristic with the exception of the number of leaves. Paraiso hybrid variety plants sprayed with Algaton extract at 3 ml.$L^{-1}$ were significantly superior in leaf area and both fresh and dry leaf weights (2.625 m², 549.67 g, and 41.59 g) compared to Cavolo. There was a significant difference between the genotypes in yield represented by the curd circumference, number of flower stalks of the curd, curd fresh weight and total productivity. Paraiso hybrid exceeded in the aforementioned characteristics, followed by the Monaco hybrid and Cavolo (Table 2). Plants sprayed with Algaton extract at 3 ml.$L^{-1}$ was significantly superior in all yield contributing parameters to those sprayed at 1.5 ml.$L^{-1}$with the exception of the dry weight of the curd as there was no significant difference between the genotypes, as well as the spraying with Algaton concentration. The interaction between the treatments had a significant effect on the fresh weight of the curd and total productivity as Paraiso hybrid variety sprayed with Algaton at 3 ml.$L^{-1}$ gave the higher yield. Paraiso hybrid variety was significantly superiorin total chlorophyllof curd, vitamin C, total soluble carbohydrates and the percentage of protein, followed by Monaco plants and then Cavolo (Table 3). The significant increase in the aforementioned characteristics was observed as spraying with Algaton concentration increased.




**Source:** Meteorology and meteorology authority at Al-Barjisia station for agricultural sciences

**Fig. 1.** Maximum, minimum temperatures and relative humidity during the experiment 2016-2017

**Table 1.** Effect of spraying with seaweed extract Algaton on growth indicators of broccoli genotypes

| Genotypes | Spraying with Algaton conncentration (ml.L$^{-1}$) | Plant Hight (cm) | Stem diameter (cm) | Number of leaves | Leaf area (m²) | Fresh weight of the leaves (g) | Dry weight of the leaves (g) |
|---|---|---|---|---|---|---|---|
| Cavolo | 0 | 53.33 b | 2.15 b | 19.00 | 1.215 b | 345.00 b | 20.14 b |
| | 1.5 | 59.00 | 2.42 | 21.33 | 1.513 | 352.67 | 23.87 |
| | 3 | 62.00 | 2.76 | 22.33 | 2.052 | 355.33 | 28.14 |
| Paraiso | 0 | 59.67 | 2.35 | 21.33 | 1.626 | 446.33 | 30.34 |
| | 1.5 | 71.33 | 2.72 | 23.33 | 2.564 | 539.33 | 37.71 |
| | 3 | 75.00 | 2.86 | 24.00 | 2.625 a | 549.67 a | 41.59 a |
| Monaco | 0 | 60.00 | 2.31 | 21.00 | 1.571 | 357.67 | 23.73 |
| | 1.5 | 64.67 | 2.52 | 22.00 | 2.135 | 423.33 | 26.37 |
| | 3 | 67.33 | 2.82 | 21.67 | 2.474 | 440.33 | 31.44 |
| LSD (p= 0.05) | | NS | NS | 1.51 | 0.003 | 5.03 | 0.05 |
| Averagegenotypes | Cavolo | 58.11 c | 2.44 c | 20.89 | 1.593 c | 351.00 c | 24.05 c |
| | Paraiso | 68.67 a | 2.64 a | 22.89 | 2.272 a | 511.78 a | 36.55 a |
| | Monaco | 64.00 b | 2.55 b | 21.56 | 2.060 b | 407.11 b | 27.18 b |
| LSD (p= 0.05) | | 1.10 | 0.04 | NS | 0.002 | 4.20 | 0.01 |
| Average concentration | 0 | 57.67 c | 2.27 c | 20.44 b | 1.471 c | 383.00 c | 24.74 c |
| | 1.5 | 65.00 b | 2.55 b | 22.22 a | 2.070 b | 438.44 b | 29.32 b |
| | 3 | 68.11 a | 2.81 a | 22.67 a | 2.383 a | 448.44 a | 33.72 a |
| LSD (p= 0.05) | | 2.04 | 0.04 | 0.94 | 0.002 | 2.75 | 0.03 |

**Table 2.** Effect of the spray with seaweed extract Algaton on the yield indicators of broccoli

| Genotypes | Spraying with Algaton concentration (ml.L$^{-1}$) | Crud circumference (cm) | Number of crud flower stalks | Crud fresh weight (kg) | Crud dry weight (g) | Total productivity (ton ha$^{-1}$) |
|---|---|---|---|---|---|---|
| Cavolo | 0 | 45.67 | 16.67 | 0.354 b | 62.25 | 6.24 b |
| | 1.5 | 53.33 | 20.00 | 0.428 | 64.25 | 7.53 |
| | 3 | 61.67 | 22.00 | 0.468 | 67.69 | 8.23 |
| Paraiso | 0 | 58.00 | 33.00 | 1.221 | 87.41 | 21.49 |
| | 1.5 | 76.67 | 35.33 | 1.321 | 90.27 | 23.26 |
| | 3 | 84.67 | 38.33 | 2.253 a | 93.70 | 39.65 a |
| Monaco | 0 | 59.00 | 20.67 | 0.744 | 76.21 | 13.09 |
| | 1.5 | 67.33 | 27.33 | 0.847 | 78.76 | 14.90 |
| | 3 | 73.00 | 35.33 | 0.997 | 81.51 | 17.55 |
| LSD (p=0.05) | | NS | 3.27 | 0.006 | 6.46 | 0.11 |
| Average genotypes | Cavolo | 53.56 c | 19.56 c | 0.417 c | 64.73 | 7.33 c |
| | Paraiso | 73.11 a | 35.56 a | 1.598 a | 90.46 | 28.13 a |
| | Monaco | 66.44 b | 27.78 b | 0.863 b | 78.83 | 15.18 b |
| LSD (p=0.05) | | 2.91 | 2.11 | 0.003 | 6.57 | 0.05 |
| Average concentration | 0 | 54.22 c | 23.44 c | 0.773 c | 75.29 c | 13.61 c |
| | 1.5 | 65.78 b | 27.56 b | 0.865 b | 77.76 b | 15.23 b |
| | 3 | 73.11 a | 31.89 a | 1.239 a | 80.97 a | 21.81 a |
| LSD (p=0.05) | | 2.75 | 2.07 | 0.004 | 1.06 | 0.07 |

**Table 3.** Effect of spraying with seaweed extract Algaton on the cruds quality broccoli

| Genotypes | Spraying with Algaton concentration ( $ml.L^{-1}$ ) | Total chlorophyll (mg $100g^{-1}$ fresh weight) | Vitamin C (mg $100g^{-1}$ fresh weight) | Total carbohydrates (mg $g^{-1}$ dry weight) | Protein (%) |
|---|---|---|---|---|---|
| Cavolo | 0 | 38.09 | 40.73 | 24.01 | 15.08 |
| | 1.5 | 47.46 | 50.79 | 25.77 | 17.48 |
| | 3 | 54.36 | 64.14 | 30.20 | 22.84 |
| Paraiso | 0 | 43.88 | 49.18 | 26.98 | 18.18 |
| | 1.5 | 59.52 | 73.45 | 34.84 | 19.68 |
| | 3 | 61.67 | 79.65 | 36.11 | 24.96 |
| Monaco | 0 | 43.01 | 40.67 | 25.02 | 16.24 |
| | 1.5 | 47.63 | 55.08 | 29.15 | 18.25 |
| | 3 | 56.59 | 68.28 | 32.04 | 23.61 |
| LSD (p=0.05) | | NS | NS | NS | NS |
| Average genotypes | Cavolo | 46.64 c | 51.89 c | 26.66 c | 18.46 c |
| | Paraiso | 55.02 a | 67.43 a | 32.64 a | 20.94 a |
| | Monaco | 49.08 b | 54.68 b | 28.74 b | 19.37 b |
| LSD (p=0.05) | | 1.77 | 1.38 | 0.80 | 0.35 |
| Average concentration | 0 | 41.66 c | 43.53 c | 25.34 c | 16.50 c |
| | 1.5 | 51.54 b | 59.77 b | 29.92 b | 18.47 b |
| | 3 | 57.54 a | 70.69 a | 32.78 a | 23.80 a |
| LSD (p=0.05) | | 1.89 | 3.32 | 0.96 | 0.37 |

**Table 4.** Effect of spraying with seaweed extract Algaton on the percentage of nutrients in the cruds of broccoli genotypes

| Genotypes | Spraying with Algaton concentration ($ml.L^{-1}$) | Nitrogen (%) | Phosphorous (%) | Potassium (%) | Sulfur (%) |
|---|---|---|---|---|---|
| Cavolo | 0 | 2.412 | 0.132 | 2.525 b | 0.225 |
| | 1.5 | 2.797 | 0.165 | 3.634 | 0.288 |
| | 3 | 3.654 | 0.214 | 4.185 | 0.434 |
| Paraiso | 0 | 2.909 | 0.160 | 3.056 | 0.268 |
| | 1.5 | 3.149 | 0.185 | 3.928 | 0.324 |
| | 3 | 3.984 | 0.237 | 4.356 a | 0.468 |
| Monaco | 0 | 2.598 | 0.142 | 2.827 | 0.246 |
| | 1.5 | 2.921 | 0.173 | 3.745 | 0.300 |
| | 3 | 3.777 | 0.225 | 4.274 | 0.446 |
| LSD (p=0.05) | | 0.092 | 0.005 | 0.005 | 0.006 |
| Average genotypes | Cavolo | 2.954 c | 0.171 c | 3.448 c | 0.315 c |
| | Paraiso | 3.348 a | 0.194 a | 3.780 a | 0.353 a |
| | Monaco | 3.098 b | 0.180 b | 3.615 b | 0.331 b |
| LSD (p=0.05) | | 0.060 | 0.002 | 0.003 | 0.005 |
| Average concentration | 0 | 2.640 c | 0.145 c | 2.802 c | 0.246 c |
| | 1.5 | 2.956 b | 0.174 b | 3.769 b | 0.304 b |
| | 3 | 3.805 a | 0.225 a | 4.271 a | 0.449 a |
| LSD (p=0.05) | | 0.058 | 0.004 | 0.003 | 0.004 |

The interaction between genotypes and spraying with Algaton had no significant effect on the qualitative characteristics of the curd. The study showed the superiority of the Paraiso hybrid variety plants followed by Monaco and Cavolo plants with significant difference in the percentage of nutrients in the curds represented by the percentage of nitrogen, phosphorus, potassium and sulfur. The significant increase in the percentage was observed as spraying with Algaton concentration increased. Paraiso plants sprayed with Algaton extract at 3 ml.$L^{-1}$ resulted in higher percentage of potassium.

The genotypes difference in growth and yield indicators may be attributed to the nature of the genotypes and their suitability to the environmental conditions of the region and hereditary factors in the genotypes (Fig. 1). The superiority spray with Algaton in the vegetative growth indicators might be due to the availability of the necessary nutrients in them and their effects on the process of photosynthesis and respiration, or that the foliar spray may lead to an increase in the biological processes responsible for cell division and elongation, which helps in obtaining good vegetative growth and the reflection of that on yield indicators and its qualitative and chemical characteristics (Saud 2013 on cucumber and Al-Mafraji, 2014 on tomato). In addition spraying seaweed extracts on the leaves may protect the plant from stress such as cold and drought, and this explains the superiority of plants sprayed with seaweed extract compared to control (O'Dell 2003 and Patil et al 2008 on onions). The increase in the total chlorophyll concentration might be attributed to the increase in the nitrogen component (Table 4) and the increase in the fresh and dry weights of the leaves might be due to the increase in the number of leaves and the leaf area (Table 1).

## CONCLUSION

The variety Paraiso with three sprays of organic nutrient Algaton at a concentration of 3 ml.$L^{-1}$ in Southern Iraq can be recommended for high productivity and good quality.

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# Economics of *Kharif* and *Rabi* Pea Cultivation in Kullu District of Himachal Pradesh

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***Abstract***: A study was conducted to look into the costs and returns involved in its cultivation during two the seasons. Two development blocks of district Kullu were selected purposively, one *Kharif* (Naggar) and other *Rabi* (Kullu) pea dominant block. Primary data were gathered by survey method. Average area under pea cultivation was 0.4 ha on Group I (Rabi pea growers) and 0.53 ha on Group II (*Kharif* pea growers) farms. The average yield was observed to be 91.48q ha$^{-1}$ on Group I farms, which was higher than the Group II farms i.e.38.17 q ha$^{-1}$. The total variable cost of pea per hectare on Group I farms was Rs. 85,393 and Rs. 59,277 on Group II farms. The total value of output was Rs. 2,28,700 and Rs. 1,71,765 per hectare on Group I and Group II farms, respectively. The net returns over variable cost were Rs. 1,43,306 and Rs. 1,12,487, respectively for Group I and Group II pea growers, respectively. Efficient extension services imparting proper knowledge of improved pea cultivation technology and availability of quality inputs to farmers on time should be ensured so that the production of pea, especially *Kharif* pea is enhanced to the level of potential yield and the farmers can earn more income.

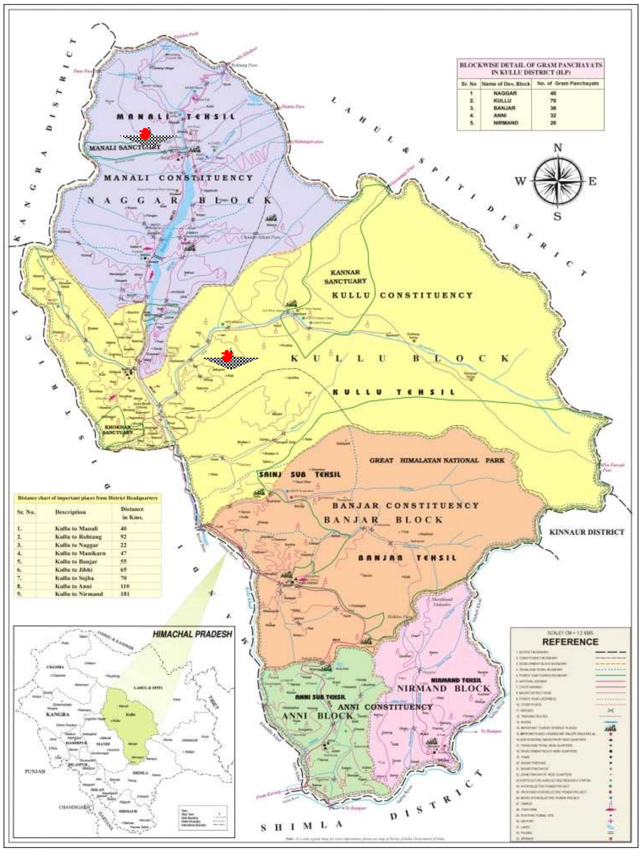
***Keywords***: *Kharif* pea, *Rabi* pea, Cost of cultivation, Break even analysis

The rapid agricultural development in the state of Himachal Pradesh during past few decades have led to transition from cereal dominated subsistence agriculture to high value cash crops including fruits and vegetables dominated commercial agriculture, especially in areas falling in the temperate agro-climatic zones. The area under vegetable crops in the state has increased from 32,000 hectares in 2000-01 to 75,000 hectares in 2018-2019 and production of vegetables which was 4.25 lakh tonnes in 1995-96 increased to 16.56 lakh tonnes. During the past two decades, District Kullu has emerged as a dominant vegetable growing district of Himachal Pradesh. In the valley areas of district Kullu, the acreage of cereal crops has continuously declined and has suitably been recompensed by vegetable crops over past decades (Bala and Sharma 2005). District Kullu occupies about 8.4 per cent of the total area under vegetables and contributes about 8.81 per cent of the total production of vegetables in the state. Garden pea is one of the major commercial crops of district Kullu. It occupies the maximum area under vegetable crops as it is cultivated not only in *Rabi* season (1020 ha) but also cultivated during *Kharif* season (911 ha) in some parts of the district. Garden pea is a highly nutritive crop containing high percentage of digestible proteins along with carbohydrates and vitamins. It is rich in minerals. Its unripe pods are used as green vegetable and the stem as fodder for the cattle. The seeds of green pea contain 25 per cent proteins and 60.3 per cent of carbohydrates. It is also rich source of lysine and tryptophan. The present study has been conducted to look into the cost and returns involved in its cultivation. The study was conducted in both the *Rabi* and *Kharif* cultivating areas and the economics was worked out for both the seasons.

## MATERIAL AND METHODS

The study was conducted in district Kullu as it is a dominant vegetable growing district of the state. Out of five development blocks, two blocks namely Kullu (*Rabi* pea dominated) and Naggar (*Kharif* pea dominated) were purposively selected for the study. For further selection of villages and pea growers, two stage random sampling technique was employed. In the first stage, a list of all pea growing villages was prepared for both the selected blocks. Then, a sample of five villages from each block was selected randomly. In second stage, a list of the pea growers in the selected villages was prepared. A sample of 60 pea growers (30 from each block) was drawn randomly from selected villages through proportional allocation method. The sampled pea growers were classified into two groups on the basis of the season i.e. Group I (*Rabi* pea growers) and Group II (*Kharif* pea growers).

Primary data were collected through survey method from the farmers. The secondary information required for the study was obtained from various published and unpublished sources and from government officials like ADOs, patwaris, pardhans, etc. The data were analysed by employing appropriate mathematical and statistical tools. Various budgeting techniques like averages, ratios, percentages, indices etc., were used to draw inferences. Standard methodology was used for computation of costs and returns and other parameters.




Selected blocks

**Fig. 1.** Map of Kullu district showing different development blocks

## RESULTS AND DISCUSSION

### Socio Economic Profile

**Family structure and size:** The family structure, size, its age-wise distribution and educational status play an important role in farming. Since, farming is a labour intensive activity, therefore, number as well as age composition of family members available for farming determines the efficiency of farm households. The average family size on Group I farms was 6.34 persons, out of which 51.09 per cent were male and 48.89 per cent were females. Whereas, in Group II farms, the average family size was 6.93 with 51.36 per cent males and 48.62 per cent females (Table1). On Group I farms, 47 per cent of farm families were nuclear while Group II farms, the proportion of nuclear farm families was 40 per cent.

The family members in the age group of 15-60 years are considered as active work-force and the members below the age of 15 and above 60 are considered as dependents on other family members. Table 1 revealed that the active work-force was more on Group II farms (75.64 per cent) than on Group I farms (68.96 per cent). The proportion of males (73.68 per cent) was higher than females (71.14 per cent) among working people. The population of the dependents was about 31 and 24.35 per cent, respectively on Group I and Group II farms. The sex-ratio was slightly higher on Group I farms (1013) than on Group II farms (950). It was observed that the proportion of the illiterates on Group II farms was 8.33 per cent as compared to 3.44 per cent on the Group I farms. About 27 and 22 per cent of the total population was educated upto senior secondary and 14 and 37 per cent was graduate, respectively on Group I and Group II farms. The literacy rate was found to be 96.56 per cent in case of Group I farms and 94.60 per cent on Group II farms. The female literacy percentage was significantly higher than the males on Group I farms.

**Land holdings and utilization:** The size of the holding that a farm household owns shows the basic strength of the farming family and its utilization reveals how efficiently this natural resource is being used by the farmers. Table 2 revealed that the total land holding on Group I and Group II farms was 1.59 and 2.67 hectares, respectively of which 92.45 and 93.63 per cent was irrigated. The main sources of irrigation were lift irrigation and government supply schemes operative in the villages.

It was observed that the cultivated land comprised of

**Table 1.** Demographic profile of farmers

| Particulars | Group I | Group II |
|---|---|---|
| Average size of family (No) | 6.34 | 6.93 |
| Number of males (%) | 3.24 (51.10) | 3.56 (51.37) |
| Number of females (%) | 3.10 (48.90) | 3.37 (48.63) |
| Sex ratio | 1013 | 950 |
| Active workforce (15 to 60 years age) (%) | 68.96 | 75.64 |
| Dependents | 31.00 | 24.35 |
| Joint families (%) | 16 (53.33) | 18 (60) |
| Nuclear Families (%) | 14 (46.66) | 12 (40) |
| Educational status | | |
| Illiterate (%) | 3.44 | 8.33 |
| Primary (%) | 18.62 | 8.69 |
| Matric (%) | 22.75 | 14.74 |
| Senior secondary (%) | 26.89 | 21.79 |
| Graduate and above (%) | 13.82 | 37.17 |
| Literacy rate (%) | 96.56 | 91.67 |

**Note:** Figures in parentheses show percentages to total

48.52 per cent and 42.67 per cent, respectively, on Group I and Group II farms. Orchards also occupied a significant proportion of the total holding which was about 44.74 and 39.60 per cent, respectively on both the groups. There was a practice of cultivating crops in orchards on both the groups of farms. It was observed that about 70 per cent of the orchard land was cultivated by Group I and 60 per cent by Group II farms. Thus, total cultivated land under Group I and Group II farms was estimated to be 1.26 hectares and 1.76 hectares, which comprised of about 85.6 and 70.5 per cent of the total holding, respectively. Agriculture was the main occupation on both the groups of farms. About 86 per cent of Group I and 82 per cent Group II farm households were engaged in agriculture as their main occupation.

**Cropping pattern:** The cropping pattern shows the spatial distribution of different crops with respect to area. From Table 3, it was seen that pea accounted for maximum area 17.6 per cent on Group II farms. Tomato was the next important crop occupying about 13.25% of the gross cropped area followed by cauliflower (12.32%), cabbage (12.22%) and maize (9.12%). On Group I farms, again pea occupied the maximum proportion (16.16 per cent) of the gross cropped area. Tomato was equally important crop on sampled farms. Cauliflower (12.80 per cent), cabbage (12%), maize (9.2%) and wheat (6%) were the other important crops of the study area. Fodder crops also accounted for 1.6 and 2.6 per cent of the cropped area, respectively on Group I and Group II farms.

**Variability in area, production and average yield:** Variability in area, production and average yield has been shown in the Table 4. Average area under pea on Group I farms was 0.40 hectares, whereas it was 0.53 hectares on Group II farms. Coefficient of variation was 33.98 per cent in case of Group I and 25.62 per cent on Group II farms. This indicated that there was more variation in the area allotted to pea by sample farmers on Group I farms as compared to Group II farms.

The average per farm production was found to be more on Group I farms (37.06 q farm$^{-1}$) as compared that on Group II farms (20.67 q farm$^{-1}$). Coefficient of variation was 33.1 per cent for Group I and 24.61 per cent for Group II farms, which again shows that the variation in per farm level of production was more on Group I farms as compared to Group II. Average

**Table 3.** Cropping pattern on sample farms (Per cent)

| Crop | Group I | Group II |
|---|---|---|
| *Kharif* Season | | |
| Maize | 9.20 | 9.12 |
| Pea | - | 17.50 |
| Tomato | 16.16 | 13.25 |
| Other vegetables | 8.32 | 7.20 |
| Pulses | 4.52 | 7.00 |
| Fodder | 1.60 | 2.60 |
| Subtotal | 39.80 | 57.24 |
| *Rabi* Season | | |
| Wheat | 6.00 | 6.80 |
| Pea | 16.16 | - |
| Cauliflower | 12.80 | 12.32 |
| Cabbage | 12.00 | 12.22 |
| Other vegetables | 11.64 | 9.12 |
| Fodder | 1.60 | 2.60 |
| Subtotal | 60.20 | 43.06 |
| Total cropped area | 100 (2.50) | 100 (3.07) |
| Net cultivated area (ha) | 1.260 | 1.764 |
| Cropping intensity (%) | 198 | 174 |

**Note**: Figures in parentheses indicate the total cropped area in hectares

**Table 2.** Land holdings and utilization on sample farms (Per cent)

| Particulars | Group I | | | Group II | | |
|---|---|---|---|---|---|---|
| | IR | UR | Total | IR | UR | Total |
| Cultivated land | 52.48 | - | 48.52 | 45.53 | - | 42.67 |
| Orchard land | 47.52 | 11.67 | 44.74 | 41.53 | 10.71 | 39.60 |
| Fallow land | - | 39.16 | 2.95 | 4.55 | 10.71 | 4.94 |
| Cultivable waste | - | 11.67 | 0.97 | - | 30.36 | 1.90 |
| Non-agricultural use | - | 37.50 | 2.82 | - | 48.22 | 3.03 |
| Land under Misc. (trees/grasses/forest) | - | - | - | 8.39 | - | 7.86 |
| Total | 100 (1.471) | 100 (0.120) | 100 (1.591) | 100 (2.504) | 100 (0.168) | 100 (2.672) |
| Total cultivated land in ha. (A+70% of B) for Group I & (A+60% of B) for Group II | 1.260 | - | 1.260 | 1.764 | - | 1.764 |

**Note:** Figures in parentheses indicate the total land holding in hectares.
IR= Irrigated, UR= Unirrigated

yield was observed to be 91.48 q ha$^{-1}$ on Group I farms which was significantly higher than the Group II farms i.e. 38.17 q ha$^{-1}$. The variability was high in area, production as well as yield on both the categories of pea growing farms and hence indicated a huge scope for enhancing production and yield.

**Cost of Pea Cultivation**

**Input use pattern:** Input use pattern is an important indicator that shows the extent to which the farmer had adopted the production technology. Pea cultivation is labour intensive so, human labour played an important role in the performance of a farm. Other important inputs included seed, fertilizers, FYM, irrigation and plant protection measures, etc. Per hectare labour requirement turned out to be 194.69 and 127.73 man days, respectively on Group I and Group II farms (Table 5). Tractor services were used to perform farm operations like ploughing and levelling of land. Table revealed that the quantity of seed used by the Group I and Group II farms was 41.40 and 38.22 kg ha$^{-1}$, respectively. Mainly two fertilizers, urea and IFFCO mixture (12:32:16) were used. Around 44 and 51 kg ha$^{-1}$ of urea was used by the Group I and Group II farms, respectively. On an average, Rs. 2,715 and Rs. 1,301 were spent by the farmers for plant protection measures which indicated that more investment on plant protection was done by Group I farms than Group II farms.

**Labour utilization pattern:** Per hectare use of the labour input for different farm operations for pea cultivation is given in Table 6. It is clear from the table that the labour use in operations such as sowing, weeding, harvesting and picking was the highest. For sowing, it took 17.5 and 9.4 man days to complete the operation, inter-cultural operations required 37.5 and 18.86 man days on Group I and Group II farms, respectively. Labour required for these two operations was significantly higher on Group I than on Group II farms, the probable reason for which may be that the quantity of seed used per hectare was more on Group I farms and thus required comparatively more labour to sow the seed at

**Table 4.** Variability in area, production and average yield

| Particulars | Group I | Group II |
|---|---|---|
| Area (ha/farm) | | |
| Average area | 0.40 | 0.53 |
| Maximum area allotted to pea | 0.64 | 0.80 |
| Minimum area allotted to pea | 0.16 | 0.24 |
| No. of farmers allocating area above average area | 13.00 | 16.00 |
| No. of farmers allocating area below average area | 17.00 | 14.00 |
| Coefficient of variation (%) | 33.98 | 25.62 |
| Production (q/farm) | | |
| Average production | 37.06 | 20.67 |
| Maximum production | 51.00 | 30.00 |
| Minimum production | 12.00 | 9.00 |
| No. of farmers getting production above average production | 20.00 | 12.00 |
| No. of farmers getting production below average production | 10.00 | 18.00 |
| Coefficient of variation (%) | 33.10 | 24.61 |
| Yield (q/ha) | | |
| Average yield | 91.48 | 38.17 |
| Maximum yield | 125.00 | 50.00 |
| Minimum yield | 75.00 | 34.37 |
| No. of farmers getting yield above average yield | 15.00 | 9.00 |
| No. of farmers getting yield below average yield | 15.00 | 21.00 |
| Coefficient of variation (%) | 13.47 | 7.90 |

**Table 5.** Input-use pattern (Per ha)

| Particulars | Group I | Group II |
|---|---|---|
| Human labour (MD) | 194.69 | 127.73 |
| Hired labour | 65.50 | 44.52 |
| Family Labour | 129.19 | 83.18 |
| Tractor services (Rs.) | 2760 | 3120 |
| Seed (kg) | 41.40 | 38.22 |
| FYM (q) | 76.82 | 52.20 |
| Fertilizer (kg) | | |
| Urea | 43.90 | 51.39 |
| IFFCO mixture | 64.82 | 65.11 |
| Irrigation (no.) | 2.28 | 1.66 |
| Plant protection measures (Rs.) | 2715.00 | 1301.00 |

**Table 6.** Labour utilization (Man days ha$^{-1}$)

| Particulars | Group I | Group II |
|---|---|---|
| Seed treatment | 2.50 | 1.80 |
| Field preparation | | |
| Ploughing | 3.25 | 2.60 |
| FYM/ Fertilizer application | 7.50 | 5.60 |
| Sowing | 17.50 | 9.40 |
| Fertilizer application (top dressing) | 8.70 | 5.47 |
| Irrigation | 18.00 | 11.32 |
| Hoeing/weeding | 37.50 | 18.86 |
| Plant protection | | |
| Fungicides | 5.07 | 3.24 |
| Insecticides | 4.67 | 3.11 |
| Harvesting and packing | 90.00 | 66.03 |
| Total man days | 194.69 | 127.43 |
| Hired labour | 65.50 | 44.52 |

narrow spacing. Again, the performance of intercultural operations on Group I farms with more number of plants and narrower spacing, was more labour intensive than Group II farms. Similar observations were reported by Bala et al (2007) in case of garlic cultivation. They opined that sowing, intercultural operations and harvesting were the major labour intensive operations in garlic cultivation where more than 65 per cent of the total labour was consumed.

The labour used for the operations such as land preparation, fertilizer application and plant protection measures was low. Around 18 and 11 man days per hectare were required for the irrigation purpose on Group I and Group II farms, respectively. Application of more number of irrigations was the reason for comparatively higher labour input for this operation on Group I farms. Harvesting and picking operation required more labour (90 man days) on Group I farms than Group II (66 man days) because of the higher production on these farms. The extent of hiring of labour was also higher (65.5 man days) on Group I farms than Group II farms (44.52 man days), which may be because of the fact that both the average family size and proportion of active work force was lower on Group I than Group II farms.

**Variable cost of pea cultivation:** Per hectare total variable cost was Rs. 85,393 on Group I and Rs. 59,277 on Group II farms (Table 7). It was observed that the cost of human labour was very high and formed major component of variable cost. The expenses on human labour were higher on Group I farms as compared to Group II because per hectare production was higher on these farms and more number of man days were required for picking and other operations. Priscilla and Singh (2015) also reported that the cost incurred on human labour was found to be major cost component in cultivation of pea, cabbage and cauliflower suggesting that vegetables are labour intensive crops. A study by Bala et al (2011) revealed that per hectare variable cost was highest for tomato followed by cabbage and it was the lowest for pea however, per quintal cost of cultivation was highest for pea followed by cauliflower, tomato and cabbage.

The expenses on FYM formed the next important component of the variable cost and were estimated to be Rs. 23,046 on Group I farms and Rs. 15,660 on Group II farms. Thus, the total variable cost also turned out to be comparatively higher on Group I farms because of the higher expenses on different components of variable costs. Bala et al (2011) also opined that plant protection costs were the major constituents of variable cost followed by expenditure on seed and fertilizers.

**Cost and returns for pea cultivation:** The cost and returns analysis on per hectare basis showed that the total fixed cost on Group I farms amounted to Rs. 24,614 and in case of Group II farms, it was Rs. 23,220 (Table 8). The total cost on Group I farms was Rs. 1,10,008 and on Group II farms it was Rs. 82,497. The total value of pea output was found to be Rs. 2,28,700 per hectare on Group I and Rs. 1,71,765 on Group II farms. Further, the net returns over total cost were Rs. 1,18,692 on Group I farms whereas the net returns over total cost on Group II farms were estimated to be Rs. 89,267. The net returns over variable cost were estimated to be Rs 1,43,306 and Rs. 1,12,487, respectively. The total cost of production per quintal on Group I farms was Rs. 1202 and on

**Table 7.** Variable costs structure of pea cultivation on sample farms (Rs $ha^{-1}$)

| Particulars | Group I | Group II |
|---|---|---|
| Seed | 4140 | 3822 |
| Human labour | 48672 | 31925 |
| Tractor | 2760 | 3120 |
| FYM | 23046 | 15660 |
| Fertilizer | 1733 | 1791 |
| Irrigation | 1065 | 781 |
| Plant protection measures | 2715 | 1302 |
| Sub total | 84132 | 58402 |
| Interest on working capital (@ 6 %) | 1262 | 846 |
| Total variable cost | 85394 | 59278 |

**Table 8.** Cost and returns from pea cultivation on sample farms (Rs $ha^{-1}$)

| Particulars | Group I | Group II |
|---|---|---|
| Total variable cost | 85394 | 59278 |
| Total variable cost excluding family labour | 53096 | 38483 |
| Total fixed cost (a+b+c) | 24614 | 23220 |
| a. Interest on fixed capital | 424 | 500 |
| b. Depreciation on fixed capital | 190 | 220 |
| c. Rental value of land for 6 months | 24000 | 22500 |
| Total cost (1+3) | 110008 | 824978 |
| Gross returns | | |
| Quantity of output(q) | 91.48 | 38.17 |
| Value of output @ Rs.2500 $q^{-1}$ in Group I and 4500 $q^{-1}$ in Group II | 228700 | 171765 |
| Net returns over total cost | 118692 | 89267 |
| Net returns over variable cost | 143306 | 112487 |
| Net returns over variable cost excluding family labour | 175604 | 133282 |
| Total cost of production (Rs $q^{-1}$) | 1202 | 2161 |
| Variable cost of production(Rs $q^{-1}$) | 933 | 1552 |
| Variable cost of production excluding family labour (Rs $q^{-1}$) | 580 | 1008 |
| Output-Input ratio | 2.07 | 2.08 |

**Table 9.** Break-even analysis of pea cultivation

| Particulars | Group I | Group II |
|---|---|---|
| Total fixed cost (Rs ha$^{-1}$) | 24614 | 23220 |
| Total variable cost (Rs ha$^{-1}$) | 85394 | 59278 |
| Average variable cost (Rs q$^{-1}$) | 933 | 1552 |
| Price realization (Rs q$^{-1}$) | 2500 | 4500 |
| Output realized (q ha$^{-1}$) | 91.48 | 38.17 |
| Break- even point (Q) | 15.71 | 7.87 |
| Break even output in monetary terms (Rs.) | 39281 | 35456 |
| Break-even output as per cent of total output (Per cent) | 17.16 | 20.61 |

Group II farms, it was Rs. 2161 while per quintal variable cost of production was estimated to be Rs. 933 and Rs. 1552, respectively. It can be concluded from the table that pea production is quite profitable proposition and the net returns were higher on Group I farms than on Group II farms. Kaur et al (2017) also revealed that green pea growing was more profitable than its major competing crop i.e. potato. Saini et al (1999) observed that the average productivity and economic potential of pea and tomato were 3 to 9 times more than maize, implying that off-season vegetable farming holds potential for the development of hill farmers.

**Break-even analysis:** Break-even analysis is useful in studying the relation between the variable cost, fixed cost and revenue. In producing and selling a certain volume of any produce, certain fixed costs and variable costs are incurred. When the volume is increased or decreased, the variable costs go up or down. The fixed costs usually remain the same. The producers are essentially concerned with the total cost which is sum of the variable and fixed costs incurred for the particular volume. At any volume and at the assumed level of price, a particular level of total revenue is generated and volume/production level at which total revenues for a product equals total expenses is called the breakeven point. An attempt has been made to compute the break-even levels for pea production in different seasons both in physical and monetary terms. The results of break-even analysis have been presented in Table 9.

It can be recorded from table that the average per quintal variable cost was Rs 933 and Rs 1,552 for Group I and Group II farms, respectively. The output realized per hectare was 91.48 q on Group I farms and 38.17 q on Group II farms. It was observed that if 15.71 q and 7.87 q pea production valued at Rs. 39,281 and Rs. 35,456 in case of Group I and Group II farms, respectively is realised, then the farms will be in no profit no loss situation under given input costs and output price structure. The break-even analysis shown in physical terms revealed that Group I and Group II farms would be at no profit no loss situation if they produce at least 17.16 and 20.61 per cent of the total output, respectively.

## CONCLUSION

The study revealed that substantial incomes in the study area can be generated through pea cultivation. Hence, pea cultivation should be encouraged to increase the income of the farmers. Price realized for *Kharif* pea was quite high, so the farmers can reap rich dividends by enhancing the production of *Kharif* pea. Farmers used the inputs lower than the recommended. The farmers should be made aware regarding the recommended doses of various inputs and their importance in improving the yield of the crop. Efficient extension advisory services should be made available to the farmers at their doorsteps to tackle various queries and problems related to package of practices for pea cultivation as many of the farmers lacked knowledge about improved pea cultivation technology. Also the inputs such as seeds, fertilizers, insecticides, fungicides etc. should be made available to the farmers at lower prices at the nearest place possible. Market-led extension should be provided to the farmers so that they can fetch better price for the crop and develop the new marketing skills to earn more profit. Farmers should also be made aware about the govt. schemes for the welfare of farmer so that they are able to take the benefit of those schemes and do not lose interest in farming.

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# Organic Produce Market in India: Production, Market Size, Growth and Potential

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***Abstract***: Organic produce has gained significant acceptance due to variety of reasons, which in turn has expanded the domestic as well as global organic produce market. This article compiles the relevant data related to organic produce market at one place and presents it from a marketing perspective. Further, it highlights the export potential along with the issues of production and per capita consumption of organic products in India. Results highlight that the market for organic products is growing exponentially in India but far behind the European countries. With increase in the area under organic farming and the production of organic products, both domestic as well as global markets are also showing an increasing trend. Per capita consumption and retail sales in India show phenomenal growth and thus, point towards the future potential of the organic produce market.

***Keywords***: Organic produce, Sustainability, Agriculture, Health issues, Organic export

The global market is witnessing many shifts in the production and consumption patterns, especially due to rising environmental awareness and growing inclination towards sustainable living choices. This encompasses taking measures to live in harmony with the environment and has encouraged or rather forced many stakeholders like governments, business organisations as well as individuals to take measures towards building a sustainable future. Many business organisations and researchers are now working towards discovering and inventing alternatives, which are environment-friendly and reduce the excessive pressure on the natural resources. Consumers across the globe are also looking for possible ways in which they can contribute at individual levels and, are thus showing interest in these sustainable alternatives (Bhattacharjee et al 2021a).

A number of sectors like energy, housing, clothing, agriculture, personal care, tourism etc. are now seeing this gradual shift towards sustainability from both sides of the market i.e., producer and consumer. One such sector is the agriculture, where the so called 'conventional' farming techniques that incorporate the use of chemical inputs like chemical fertilizers and pesticides have been proven to be harmful for the environment as well as human health (Horrigan et al 2002, Chandrashekar 2010, Pandey and Singh 2012, Saxena 2021). The rising awareness regarding these issues and the growing health consciousness among people has led to the adaptation of more sustainable techniques of agriculture like organic farming. The Indian organic produce market is growing very fast due to the favourable business environment and positive measures taken by the government with respect to organic farming. Although the organic production in India has increased but it is mostly export driven (Kumar and Ali 2011, Meek and Anderson 2020). The reason behind this could be the relatively higher demand in the developed nations, owing to high disposable income and consumer spending. Demand for organic products in India is also increasing in the upper segment of the society due to health consciousness (Bhattacharjee et al 2021a, Bhattacharjee et al 2021b). Organic products are the fastest growing product category in the global food industry (Singh 2006).

The developing nations are not far behind and are viewed as potential emerging markets for organic products. As per the Global Health and Wellness Report published by the Nielsen Company in 2015, the willingness to pay premium for health benefits was found to be highest in the developing countries. India is one such emerging market for organic products where the demand for organic food products is showing promising growth, with other organic product categories like textile and personal care. Among all the organic categories, the organic food category is witnessing the highest growth (Tyagi et al 2018). The Indian organic food market reached a value of US$ 731 Million in 2019 (IMARC, 2020) and US$ 849.5 million in 2020 (EMR Business Solutions, 2020). The organic food industry is further projected to grow at a CAGR of 20.5 percent and is expected to reach US$ 2601 million by 2026 (EMR Business Solutions, 2020). This article attempts to throw light on the global organic product market scenario, focusing on the countries which are playing major role in organic production and consumption. Further, attempt has been made to highlight the organic produce landscape in India, focusing on the production, i.e. the supply side as well as the organic produce market, both export and domestic market in India. The article compiles the relevant data at one place and presents it from a contemporary marketing perspective.

## MATERIAL AND METHODS

**Data Sources:** To achieve the objectives, this article uses the data available in the form of articles, reports and year books published in the website of international organisations like the International Federation of Organic Agriculture Movements (IFOAM), the Research Institute of Organic Agriculture (FiBL), World Health Organisation (WHO) and, United Nation's Food and Agriculture Organization (FOA). Further, information was also extracted from the websites of various government departments in India pertaining to organic agricultural standards, policies and statistics; including the Department of Agriculture, Co-operation and Farmers Welfare (http://agricoop.nic.in/) and in particular, data regarding the National Programme for Organic Production (NPOP) and Participatory Guarantee System for India (PGS-India); provided by the Agricultural & Processed Food Products Export Development Authority (http://apeda.gov.in/) and the Department of Agriculture and Cooperation (https://pgsindia-ncof.gov.in/) respectively. Information and statistics provided by the online databases run or supported by the Government of India (GOI) was also used in this article. Some of these databases are the Open Government Data (OGD) Portal India (https://data.gov.in/) and India Brand Equity Foundation (https://www.ibef.org/). Further, the information was also collected from various market reports made freely available by both international and national market research firms. Efforts have been made to collect the data for maximum time duration but it is available only after 2009 and up to 2019 and the same has been used to forecast the production, export and retail sales of organic produce in India.

**Data analysis:** The secondary data available from various sources has been consolidated in order to present a clear and holistic picture of the Global organic scenario, focusing particularly on the Indian organic landscape. Firstly, the article presents an overview for the global organic market in terms of the world-wide organic area, number of organic producers, organic exports, retail sales as well as per capita consumption of organic products. A list of top countries on various parameters has been complied to figure out the key stakeholders in organic production and consumption across the globe. Secondly, India's global positioning on various aspects is reviewed and a detailed study is carried out to present the overall performance of India on different parameters of organic landscape. Further, trend analysis has been used to forecast the production, retail sales and exports of organic produce in India.

## RESULTS AND DISCUSSION

**Organic produce market landscape: Global overview:** Although at present the global organic farmland is only 1.5% of the total farmland, but a significant number of countries are now adopting organic agriculture. The number of countries engaged in organic agriculture has increased from 141 in 2007 to 186 in 2018. The global organic farmland has also increased from a mere 11 million hectares (ha) in 1999 to 71.5 million hectares in 2018 with a CAGR of ten percent. Among all, Australia has the highest organic farmland (35,687,799 ha) which is 8.78 per cent of Australia's total farmland, followed by Argentina (3,629,968 ha) and China (3,135,000 ha) as per the 2018 data (Table 1). Australia alone has 63 per cent of the total organic farmland of top 10 countries. The number of organic producers is also increasing world-wide and at present there are 2,796,916 producers across the globe. India is leading on this parameter with 1,149,371 organic producers, which is around 41% of the global organic producers; followed by Uganda (210,352) and Ethiopia (203,602).

With increasing number of countries involved in organic production, the cross-country trade of organic products is also on the rise. Global aggregate export of organic products stands at € 13,778.97 million. United States of America (USA) stands at the first position and exports more than 21 per cent of global export of organic products and around 30 per cent of the top 10 countries export of organic products, which is € 2,981.29 Million (Table 2). Italy is on second position with € 2,266.00 Million, followed by Spain (€ 890.80 Million).

The organic produce market is growing rapidly as the growth of retail sales and per capita consumption has witnessed exponential growth over the years. The worldwide organic produce market was valued € 15.1 billion in the year 2000 and has grown at a CAGR of around eleven percent over the past 10 years, with the present estimation at € 96.7 billion in 2018. The USA is leading the global market with € 40,559 million retail sales of organic products, which is almost 48 per cent of top 10 countries' retail sales, followed by Germany (€ 10910 million), France (€ 9139 million) and China (€ 8087 million) respectively (Table 2). With increasing awareness about organic products and rising disposable income, the per capita consumption of organic products is also increasing day by day. The list of countries with higher per capita consumption of organic products is mainly dominated by the European countries as the seven countries are from Europe (Table 2). The global organic per capita consumption stands at € 12.8 per person at present, with Denmark and Switzerland are reporting the highest per capita spending on organic products of € 312 per person. Both these countries are consuming almost one-fourth of global consumption of organic products. Sweden (€ 230.71), Luxemburg (€ 221) and Austria (€ 205.16) are also not far behind on this parameter.

**Indian organic market landscape: Production, consumption and forecast:** India is at present one of the leading producer and exporter of organic products. The Government of India has launched various schemes like the *Parampragat Krishi Vikas Yojana* (PKVY), Mission Organic Value Chain Development for North Eastern Region (MOVCDNER) and the Participatory Guarantee Scheme (PGS-India) to promote organic production in India. Due to these conducive policy measures with respect to organic production, India has been able to improve its domestic as well as global trade of organic products. Moreover, according to the FiBL and IFOAM global ranking for 2020, India is performing quite well and is leading on various parameters at the global level (Table 3).

The total area under organic agriculture in India was 1,180,000 hectares in 2009, which was only 0.66 per cent of total farmland of the country (Table 4). It increased to 1,938,220.79 ha with a CAGR of 5.67 per cent in 2018. Although India ranks 9th among the countries with the highest

**Table 1.** Top ten countries with largest organic area (including in-conversion area) and organic producers

| Rank | Organic area | | | Organic producers | | |
|---|---|---|---|---|---|---|
| | Country | In hectares | Share in country's total farmland (%) | Country | Number of organic producers | Share in total global organic producers (%) |
| 1st | Australia | 35687799 | 8.78 | India | 1149371 | 41.09 |
| 2nd | Argentina | 3629968 | 2.44 | Uganda | 210352 | 7.52 |
| 3rd | China | 3135000 | 0.61 | Ethiopia | 203602 | 7.28 |
| 4th | Spain | 2246475 | 9.64 | Tanzania | 148610 | 5.31 |
| 5th | Uruguay | 2147083 | 14.86 | Peru | 103554 | 3.70 |
| 6th | France | 2035024 | 7.34 | Turkey | 79563 | 2.84 |
| 7th | USA | 2023430 | 0.59 | Italy | 69317 | 2.48 |
| 8th | Italy | 1958045 | 15.79 | Thailand | 58490 | 2.09 |
| 9th | India | 1938221 | 1.08 | France | 41632 | 1.49 |
| 10th | Germany | 1521314 | 9.09 | Spain | 39505 | 1.41 |

**Source:** Compiled by authors based on data from FiBL and IFOAM – Organics International Report, 2020

**Table 2.** Top ten countries with highest exports, retail sales and per capita consumption of organic products

| Rank | Exports | | Retail sales | | Per capita consumption | |
|---|---|---|---|---|---|---|
| | Country | Million € | Country | Million € | Country | € per person |
| 1st | USA | 2981.3 | USA | 40559 | Denmark | 312 |
| 2nd | Italy | 2266 | Germany | 10910 | Switzerland | 312 |
| 3rd | Spain | 891 | France | 9139 | Sweden | 231 |
| 4th | China | 806 | China | 8087 | Luxembourg | 221 |
| 5th | France | 707 | Italy | 3483 | Austria | 205 |
| 6th | India | 641 | Canada | 3119 | France | 136 |
| 7th | Canada | 434 | Switzerland | 2655 | Germany | 132 |
| 8th | Australia | 434 | UK | 2537 | USA | 124.5 |
| 9th | Kyrgyzstan | 418 | Sweden | 2301 | Saudi Arabia | 93 |
| 10th | Denmark | 390 | Spain | 1903 | Canada | 84 |

**Source:** Compiled by authors based on data from FiBL and IFOAM – Organics International Report, 2020 organic farmland but it uses only 1.08 per cent of its total farmland for organic farming. Annual growth rate of organic farmland was negative in 2010 and 2012 (may be due to conversion process) but from 2013 onwards, it shows positive growth. India not only has the highest number of organic producers, but holds a significant share of 41 percent of the total number of organic producers of world. Number of organic producers was 677,257 in 2009 and this number increased to 1,149,371 in 2018, with CAGR being 6.05 per cent. As per the reports of APEDA, the total area under organic certification process (cultivation & wild harvest) is 3,669,801 ha in 2019-20. Madhya Pradesh is the Indian state that accounts for one-third of total area under organic certification process with 1,161,015 ha land, followed by Rajasthan (539,245.8 ha), Maharashtra (293135.2 ha), Jammu & Kashmir (215,276 ha) and Chhattisgarh (208,392.8 ha).

Production of Organic commodities including in-conversion production has doubled during the period of 2012-13 to 2019-20 with a CAGR 10.71 per cent (Table 4). In 2012-13 total production was 1,328,999.21 million tons (MT), which increased to 2,709,119.52 MT in 2019-20. Annual growth for production has shown unpredictable trends during this period, with fluctuations in initial year's growth rate became positive in 2017-18 to 2019-20. Reduction in total production during the year 2016-17 may be due to the decrease in total area under organic certification process of agricultural land. The trend line shows that organic production may grow positively form 2020 to 2025 and the total production of organic products which is 2,709,120 MT in 2019 may increase to 4,007,714 MT by the year 2025, with a CAGR of 6.47 per cent (Fig. 1).

With increase in the area under organic farming and the production of organic products, both domestic as well as global markets are also showing an increasing trend. The per capita consumption for organic products in India was only € 0.01 in 2009 but it reached to € 0.15 in 2018, showing a constant increase at a CAGR of 35.11 per cent (Table 5). On the other hand, the organic retail sale in India has also increased during the same period. The organic retail sales

**Table 3.** India's position at global level with respect to various parameters of organic produce landscape

| Parameter | | India global ranking | Particulars |
|---|---|---|---|
| 1. | Number of organic producers | 1st | 1,149,371 |
| 2. | Organic agricultural land (Million hectares) | 9th | 1.94 |
| 3. | Increase in organic agricultural land (10 years growth) | 6th | 0.76 |
| 4. | Wild collection and further non-agricultural area (Million hectares) | 6th | 1.4 |
| 5. | Number of affiliates of IFOAM- Organics International (Affiliates) | 2nd | 55 |
| 6. | Organic retail sales (Million) | 23rd | € 186 |
| 7. | Organic per capita consumption/ person | 50th | € 0.2 |
| 8. | Organic exports (Million) | 6th | € 641 |

**Source:** Compiled by authors based on data from FiBL and IFOAM – Organics International Report, 2020

**Table 4.** Year-wise organic area, percentage share in total area and number of organic producers and organic production in India

| Year | Organic area (million hectares) | Percentage share in total area (%) | Number of organic producers | * Organic production (million tons) |
|---|---|---|---|---|
| 2009 | 1.180 | 0.66 | 677'257 | - |
| 2010 | 0.780 | 0.43 | 400'551 | - |
| 2011 | 1.084 | 0.60 | 547'591 | - |
| 2012 | 0.500 | 0.28 | 600'000 | 1.32 |
| 2013 | 0.510 | 0.28 | 650'000 | 1.23 |
| 2014 | 0.720 | 0.40 | 650'000 | 1.10 |
| 2015 | 1.180 | 0.66 | 585'200 | 1.34 |
| 2016 | 1.490 | 0.83 | 835'000 | 1.18 |
| 2017 | 1.780 | 0.99 | 1'093'288 | 1.68 |
| 2018 | 1.938 | 1.08 | 1'149'371 | 2.61 |
| 2019 | - | - | - | 2.71 |

**Source:** Compiled by authors based on data from FiBL and IFOAM – Organics International Report, 2020 and *APEDA, Government of India increased from € 13.23 million to € 185.89 million during 2009 to 2018 at a CAGR of 34.13 per cent. Per capita consumption and retail sales in India show phenomenal growth and thus, point towards the future potential of the organic produce market. Retail sale is expected to reach at € 197 million in the 2025 at a CAGR of 0.81 per cent (Fig. 2).

The demand for organic products is increasing day by day not only within the country but also at global level. The global organic market has grown from € 15.1 billion in 2000 to € 96.7 billion in 2018. The total value of export of organic products was € 13778.97 million (FiBL) in the year 2018. Organic produce export of India was just € 87.73 million in 2009, which has now grown to € 641.39 million in the year 2018 (Table 5) at a CAGR of 24.74 per cent. Annual growth rate of organic produce export remained positive from 2009 to 2018 except for the year 2015. India exported around one-fourth of its total farm production of organic products during the year 2019-20 (APEDA, 2020).

India exported 614,089.614 million tons (MT) of organic products worth $ 757.50 million in the year 2018-19, which was $ 515.44 million in 2017-18. The leading host countries for Indian organic exports are U.S.A., European Union, Canada, Switzerland, Vietnam, Israel, Australia and New Zealand. Out of the various Indian states, Madhya Pradesh is the largest exporter of organic products among 23 states & other union territories. In 2018-19 Madhya Pradesh exported around thirty per cent of total export of organic products. Telangana is the second highest exporter state with more than fifteen per cent share in India's organic produce export. Maharashtra and New Delhi both exported around 14 per cent of total organic products export. Total export quantity of organic products of India increased from 458,339.01 MT to 614,089.61 MT with an annual growth rate of around thirty-four per cent between 2017-18 and 2019-20. India exports a number of organic products like cereals & millets, coffee, dry fruits, processed foods, oil seeds, sugar, spices & condiments, tea and pulses etc. India exported 638,998.4 M.T of organic products in 2019-20. Among all the organic products categories, processed foods category has the highest share with two-third of total export quantity. Organic oil seeds are at the second place with fifteen per cent share of total organic export. Organic cereals & millets and sugar account for 14 per cent of the total export quantity. The forecasted values of organic products export up to the year 2025, shows organic exports, which stood at 88 million tons in 2009, are expected to reach 860 million tons by the year 2025 (Fig. 3).

**Table 5.** Year-wise organic exports, retail sales and per capita consumption in India

| Year | Organic exports (million €) | Organic retail sales (million €) | Organic per capita consumption (€ per person) |
|---|---|---|---|
| 2009 | 87.73 | 13.23 | 0.01 |
| 2010 | 118.68 | 20.30 | 0.02 |
| 2011 | 128.41 | 31.49 | 0.03 |
| 2012 | 291.20 | 50.89 | 0.05 |
| 2013 | 291.20 | 69.50 | 0.07 |
| 2014 | 303.00 | 101.32 | 0.10 |
| 2015 | 268.58 | 144.20 | 0.12 |
| 2016 | 268.58 | 171.65 | 0.14 |
| 2017 | 456.26 | 185.89 | 0.15 |
| 2018 | 641.39 | 185.89 | 0.15 |

**Source:** Compiled by authors based on data from FiBL and IFOAM – Organics International Report, 2020

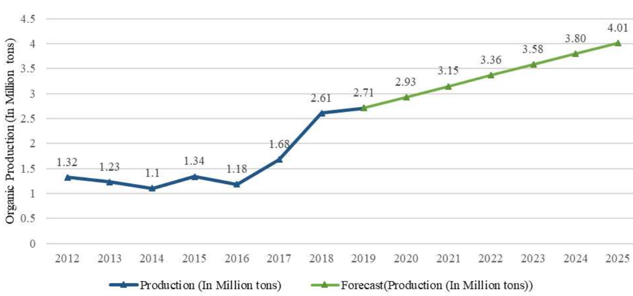


**Fig. 1.** Forecasted values of organic production for India, based on yearly data from 2009 to 2018


**Fig. 2.** Forecasted values of retail sales in India, based on yearly data from 2009 to 2018

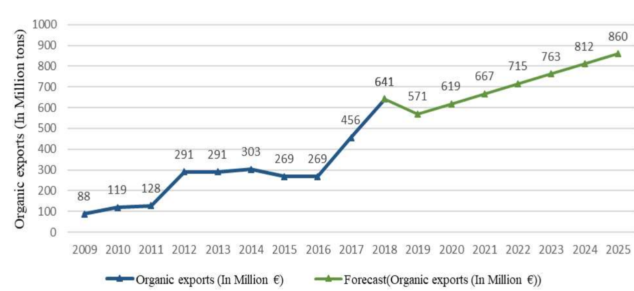


**Fig. 3.** Forecasted values of organic exports for India, based on yearly data from 2009 to 2018

The overall results show that not only the organic production but also the consumption and spending on organic products are increasing at national as well as international level. Analysis shows that Indian domestic and export market of organic products is growing rapidly. Moreover, promising growth may be expected looking at the forecasted figures for organic production, retail sales and exports, which signals a huge potential of Indian organic produce market.

## CONCLUSION

Many business organisations and researchers are now working towards discovering and inventing alternatives, which are environment-friendly and reduce the excessive pressure on the natural resources. With the increasing awareness among the masses, the global organic production and simultaneously the organic market is showing promising growth. The total area under organic agriculture in India was slightly more than half percent of total farmland of the country in 2009 and now increased to one per cent in 2018. With increase in the area under organic farming and the production of organic products, both domestic as well as global markets for organic products are also showing an increasing trend. The Indian organic produce market is growing very fast due to the favourable business environment and positive measures taken by the government with respect to organic farming. Increasing awareness about organic products and rising disposable income have contributed positively in the growth of the per capita consumption and retail sales of the organic products. Results of the study indicate that exponential growth may be expected for organic production, per capita consumption, retail sales and exports of Indian organic produce.

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# Essential Oil of Wild Marigold Induces Oxidative Stress in *Senna occidentalis*

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***Abstract***: *Tagetes minuta* L., commonly known as wild marigold is an aromatic weed belonging to family Asteraceae. The plant has naturalized more than 35 countries because of its noxious habit which may be attributed to rich phytochemistry of volatile essential oil. Hence present study was undertaken to extract essential oil from aerial parts of wild marigold and find out its effect on germination, early growth and stress related parameters. The test weed selected for the biochemical assessment was *Senna occidentalis* (L.) Link, a wasteland weed, flourishing well in almost every region of world. Growth studies under laboratory conditions showed significant inhibition of *S. occidentalis* by *T. minuta* oil. Simultaneously, an increase in hydrogen peroxide content, lipid peroxidation and proline content were also observed in root as well as leaf tissues of treated seedlings. Thus, it confirmed the allelopathic inhibition of test weed due to stress induced by essential oil of wild marigold.

***Keywords***: Wild marigold, *Senna occidentalis*, Essential oil, Oxidative stress

*Tagetes minuta* L. (Family Asteraceae), commonly known as wild marigold is an aromatic annual herb. It is native to temperate grasslands and mountain regions of southern South America (Soule 1993) and considered as a noxious weed in about 35 countries (Holm et al 1997). In India, *T. minuta* was introduced for its essential oil (Rao et al 1988), however, it escaped cultivation and naturalized near disturbed sites of deforestation, forest fires and dry areas in the North-West Himalayas between altitudes of 1250-2500 m (Walia and Kumar 2020). All aerial parts of plant bear essential oil. Rich phytochemistry of oil includes *β*-ocimene, dihydrotagetone, E-ocimenone and Z-tagetone as major compounds (Arora et al 2016, Walia and Kumar 2020). It has been reported to possess antibacterial, anti-fungal, anti-viral, anti-oxidant, anti-cancerous, nematicidal, insecticidal and allelopathic activities (Shirazi et al 2014, Gakuubi et al 2016). It also finds extensive use in flavor and perfume industry (Drennan and van Staden 1989).

*Senna occidentalis* (L.) Link (Family Fabaceae), commonly known as coffee weed, is an annual to short lived perennial smelly wasteland weed with pantropical distribution (Nassar et al 2011, Amponsah et al 2016). Many researchers have reported toxicity of its seeds for liver, heart, lungs and vascular system of domestic livestock (Haraguchi et al 1998). Plants themselves prove serious competition to main crop by forming dense infestations due to their invasive habit (Fufa et al 2017) and alternate hosts for plant pathogens (Badamasi et al 2020, Eni et al 2021). Many herbicides are found effective against the young stages of coffee weed, however, speculating the environment concerns of using chemical herbicides, natural plant products can provide eco-friendly bioherbicides to manage it. With this background, present study was planned to explore the allelopathic effect of essential oil of *T. minuta* towards *S. occidentalis* and the possible manifestation of oxidative stress induced by it in terms of $H_2O_2$ content, lipid peroxidation and proline content.

## MATERIAL AND METHODS

**Plant material collection and oil extraction:** Aerial plant parts of wild marigold were collected from various locations in Solan (Himachal Pradesh, India; 30.8885° N, 77.0572° E). Freshly collected material was chopped and hydro-distilled for two hours in Clevenger's apparatus. The oil was collected in dry glass vials and stored in refrigerator under dark conditions till used. Seeds of coffee weed (S*enna occidentalis*) were collected from wildly growing stands in Panjab University Campus, Chandigarh (30.760618° N, 76.765388° E) in the month of October. Seeds were acid treated and washed thoroughly before 24 h imbibition. Various chemicals required for estimation of lipid peroxidation (LPO), hydrogen peroxide ($H_2O_2$) and proline content were purchased from Sigma-Aldrich Chimie.

**Essential oil treatment:** Petri dishes (15 cm diameter) were lined with Whatman No. 1 filter circle which was soaked with 8 ml of double distilled water. Seeds of *S. occidentalis* were placed equidistantly on filter paper and requisite concentrations (2.5, 5, 10, 20 and 40 µg/cc) of essential oil of wild marigold was applied to lid of Petri dish. A parallel control was maintained. Five replicates each were maintained for various concentrations of *T. minuta* oil and control in growth chamber at 16/8 h photoperiod, 25 ± 2 °C temperature and 75 ± 2 % relative humidity. After one week, percent germination, seedling length and dry weight (DW) of control and treated seedlings were measured. Plant tissues were further processed for estimating LPO, $H_2O_2$ content and proline content according to the standard protocols.

**Measurement of $H_2O_2$ content***:* Hydrogen peroxide ($H_2O_2$) content was calculated according to the method given by Velikova et al (2000). For this, 100 mg of root and leaf tissues of control and treated seedlings were homogenized in 5 ml of 0.1% TCA (w/v) over ice bath. The homogenate was centrifuged at 12,000 *g* for 15 minutes. One ml of supernatant was mixed with 1 ml of 10 mM phosphate buffer (pH 7) and 1 ml of 1 M potassium iodide (KI, w/v) solution. The absorbance was read at 390 nm and expressed as $H_2O_2$ content. $H_2O_2$ level was estimated using an extinction coefficient ($\varepsilon$ = 0.28 µM $cm^{-1}$) and represented as nM/g Fresh Weight (FW).

**Measurement of proline content***:* Proline content was measured using the methods of Bates et al (1973). Hundred mg of dried roots or leaves were digested in 3 ml of 3% sufosalycylic acid for 30 min at 100°C. The contents were centrifuged at 2000 *g* for 5 min at 25°C. To 0.2 ml of supernatant, 0.4 ml of the distilled water and 2 ml of the reagent mixture (consisting of 30 ml of glacial acetic acid, 20 ml of distilled water and 0.5 g ninhydrin) were added and the contents were incubated for 1 h over a boiling water bath. After 1 h, the mixture was cooled and extracted with 6 ml of toluene. The absorbance of the toluene phase was read at 520 nm and the proline content was determined from the standard of proline (50 µg/ml) and expressed as mg/g DW.

**Estimation of lipid peroxidation***:* Lipid peroxidation was measured in the form of malondialdehyde content (MDA) according to the method of Heath and Packer (1968). For this, 100 mg of root and leaf tissue of treated and control seedlings was homogenized in 5 ml of 0.1% Tricarboxylic acid (TCA, w/v). The homogenate was centrifuged at 10,000 *g* for 15 minutes. 4 ml of 0.5% Thiobarbituric Acid (TBA, prepared in 20% TCA, w/v) was added to 1 ml of supernatant. The mixture was heated at 95°C for 30 minutes and transferred immediately to ice bath to stop the reaction. The mixture was centrifuged again at 10,000 *g* for 10 minutes. The absorbance of the supernatant was determined at 532 nm and corrected for non-specific absorbance at 600 nm. Malondialdehyde (MDA) content was measured by using extinction coefficient ($\varepsilon$ = 155 $mM^{-1}$ $cm^{-1}$) and represented as nM/g FW.

**Statistical analysis:** The experiment was repeated twice maintaining five replicates for control and various treatments. All the data were presented as mean values of both the experiments. Experimental data were subjected to one-way analysis of variance (ANOVA) followed by separation of treatment means from the control at $p \leq 0.05$ and $p \leq 0.01$ applying post-hoc Dunnett's Test using SPSS PC software.

## RESULTS AND DISCUSSION

Growth studies performed under laboratory conditions clearly showed the allelopathic inhibition of seedlings of *S. occidentalis* by *T. minuta* oil treatment (Fig. 1). Percent germination decreased significantly with increasing concentration of oil. Similar effect was observed on root and shoot length and dry weight of treated seedlings. Lower concentrations of treatment (2.5 and 5 µg/cc) promoted seedling growth to some extent, however the values were statistically insignificant (Fig. 1). The MDA content showed significant increase of 10-19% over control, however, the $H_2O_2$ content increased by almost 46% at the highest concentration (40 µg/cc). Appreciable increase in LPO, $H_2O_2$ and proline content in both leaf and root tissues confirmed the generation of oxidative stress in test weed.

Many researchers in the past have reported allelopathic inhibition of weeds by essential oils (Benvenuti et al 2017, Fagodia et al 2017, Hazrati et al 2018, Souza-Alonso et al 2018, Sharma et al 2019, Dahiya et al 2020, Issa et al 2020, Sharma et al 2021). Cheng and Cheng (2015) have attributed negative allelopathy to the interference of allelochemicals with DNA and RNA and cell cycle abnormalities that become

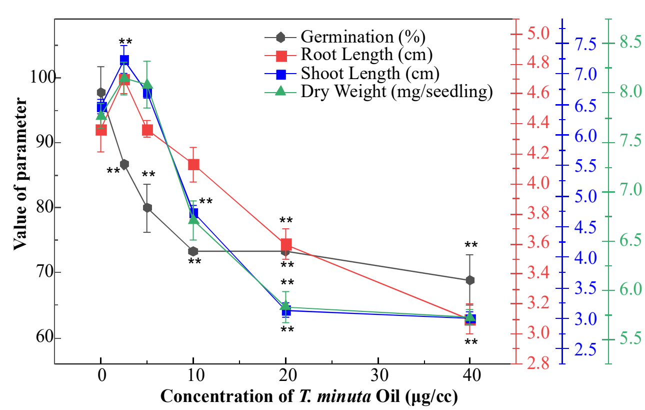


**Fig.1.** Effect of volatile essential oil of *T. minuta* on early growth of *S. occidentalis*. Data are represented as mean values. Vertical bars along each treatment indicate standard deviation of means. Asterisks indicate the statistical significance (**P ≤ 0.01; *P ≤ 0.05) applying Dunnett t-test

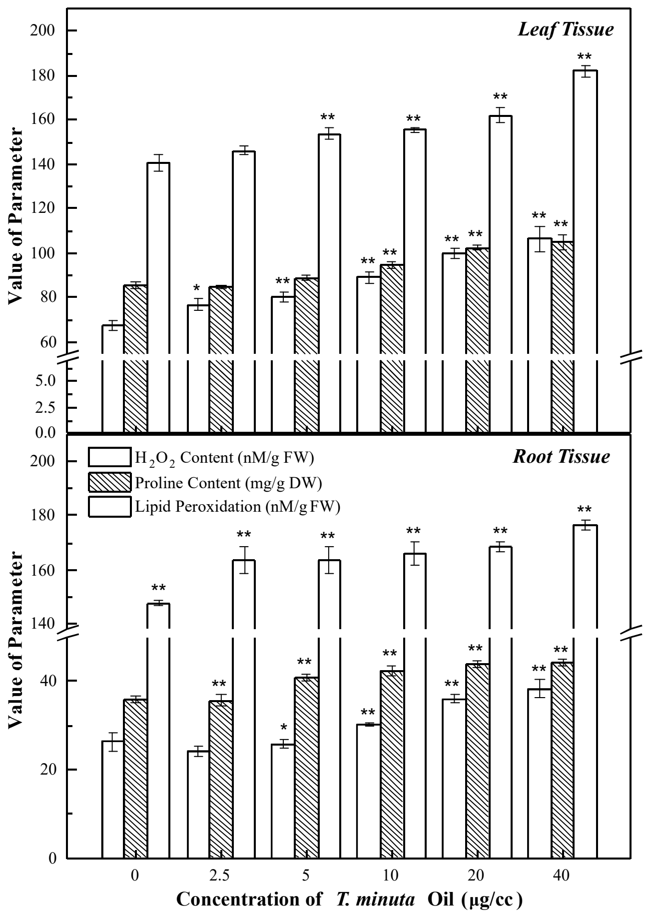


**Fig. 2.** Effect of essential oil of *T. minuta* on stress related parameters in root and leaf tissues of seedlings of *S. occidentalis*. Vertical bars along each treatment indicate standard deviation of means. Asterisks indicate the statistical significance (**P ≤ 0.01; *P ≤ 0.05) applying Dunnett t-test.

apparent in hindered germination and growth of plants. Mode of action of essential oils is a complex phenomenon due to mixture of chemicals present in them (Grana et al 2012). Oxidative burst or generation of reactive oxygen species (ROS) has long been proposed as one of the main mechanisms of action of phytotoxins (Ricci et al 2017, Raveau et al 2020). Werrie et al (2020) has summarised the action of essential oils as the myriad of photosynthesis and mitochondrial respiration inhibition, microtubule disruption, water status alteration, membrane properties and interactions, reactive oxygen species and genotoxicity, enzymatic and phytohormone regulation. In similar perspective, few reports in the past have attributed phytotoxicity of essential oil of *T. minuta* to reduced chlorophyll content, increased respiratory rates, inhibited mitotic activity and lipid peroxidation (Scrivanti et al 2003, Arora et al 2015, 2017).

## CONCLUSION

To conclude, inhibited seedling growth, decreased accumulation of dry weight, increased proline and $H_2O_2$ content and LPO clearly suggested that essential oil of *T. minuta* induced oxidative stress in seedlings of *S. occidentalis*. Thus, present study is in compliance with the reports published on allelopathic potential of essential oils, however, it is the first ever report in the context of weed management of *S. occidentalis* by essential oil of *T. minuta* and its future prospects as bioherbicide. In future, more studies are required to design formulations of essential oil of *T. minuta* for weed management under field conditions while integrating pest management strategies.

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# Integrated Effect of Azolla in Combination with Graded Doses of Nitrogen on Growth and Agronomic Parameters of Rice Crop

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***Abstract***: *Azolla pinnata*, a floating water fern, is used as green manure (bio-fertilizer) for increasing rice yields. It can fix di-nitrogen in association with *Anabaena Azollae* therefore it helps to reduce the recommended dose of chemical-nitrogen when *Azolla* is applied to the paddy fields. *Azolla* was grown in pots and trays in February, 2016 under laboratory and fieldby making trenches (2m × 2m × 0.2m) in March, 2016. A field experiment was conducted at CSKHPKV, Palampur to study and evaluate the effects of *Azolla* alone and in combination with graded doses of nitrogen in rice crop. The rice variety used was HPR-2143. The results of the experiment revealed thatthe maximum plant height was recorded in 50 kg N $ha^{-1}$ + 8 t *Azolla*, maximum number of tillers $m^{-2}$in 125 kg N $ha^{-1}$ + 8 t *Azolla*, maximum effective tillers $m^{-2}$ in 100 kg N $ha^{-1}$ + 8 t *Azolla*. The highest number of grains per panicle was recorded in 125 kg N $ha^{-1}$ +4 t *Azolla*. The application of 125 kg N $ha^{-1}$ + 8 t *Azolla* resulted in the highest grain and straw yield. It can be concluded from the experiment that integrated use of *Azolla* and nitrogen fertilizers is an effective way for increasing rice yield.

***Keywords***: *Azolla*, Rice, Grain yield, Straw yield

Rice (*Oryza sativa*) forms staple food for more than half of the world's population. About 90 per cent of total rice is grown and consumed in Asia. In the world the crop ranks first in area and second to wheat in grain production. In India rice is cultivated on an area of 43.79 million hectare with the total production of 112.91 million tonnes with the average productivity of 25.8 q $ha^{-1}$ (Anonymous 2019). Rice is also one of the important crops of our state during *Kharif* season and was cultivated on an area about 71 thousand hectare with the production of 114.8 thousand tonnes and productivity of about 16 q $ha^{-1}$ (Anonymous 2018). To safeguard and sustain the food security in India, it is therefore, imperative to explore and evaluate such technologies which may increase the productivity of rice under situations of dwindling resource base particularly when there is little scope of horizontal or lateral expansion. Thus, the increase in production has to be vertical and should come from the same cultivated land by way of increased crop productivity.

*Azolla* is a free floating water fern which fixes atmospheric nitrogen in association with the cyanobiont *Anabaena azolla*. The heterocyst of symbiont *Anabaena* is site of nitrogen fixation. Nitrogen fixation associated with high growth rate can enables *Azolla* to accumulate more than 10 kg N $ha^{-1}day^{-1}$ under optimal growth conditions. In general, a single crop of *Azolla* is known to provide 20-40 kg $ha^{-1}$ nitrogen and also increases the availability of both macro and micro nutrient but this is insufficient to meet the total nitrogen requirement of the target crop. Therefore, the use of *Azolla* in combination with chemical nitrogen affords a feasible alternative practice. Sufficient work has been done on exploitation of *Azolla* as a biofertilizer in rice in other states of country, however limited systematic studies so far has been made in Himachal Pradesh about the usefulness of *Azolla* in rice production.Keeping all this in view, the present studywas planned to find out the integrated effect of Azolla in combination with graded doses of fertilizer nitrogen on growth and agronomic parameters of rice crop.

## MATERIAL AND METHODS

The experimental farm is situated at 3206' N latitude and 76 03' E longitudes at an altitude of about 1290 meters above mean sea level. The experiment was conducted at CSKHPKV, Palampur of Kangra district in the mid hill sub humid zone of Himachal Pradesh in North-West Himalayas with three replications in a randomized block configuration during 2016. The rice variety HPR-2143 was used. A total of twelve different combination treatments were selected for the study (Table 1). An individual plot size of 10 $m^2$ (5 m x 2 m) with a spacing of 20 cm x 15 cm (PP x RR) was maintained. A non-fertilized and no Azolla used treatment (T1) was used as control.Treatments were allocated randomly to individual plot, concentrating on one replication at a time. Hand weeding was carried out in order to control weeds. The growth and agronomic parameters were recorded at the stage of panicle initiation. The parameters recorded were plant height and number of tillers $m^{-2}$. The yield attributes viz. number of effective tillers $m^{-2}$, number of grains per panicle and test weight (g) were also recorded in each plot after randomly selecting five plants from each plot. Plant height of 5 randomly selected plants was measured in cm from the base of the plant to the tip of the panicle of tillers at panicle initiation stage. One thousand fully developed and sun dried grains were counted and weight was taken in grams. Harvesting of crop was done manually when the crop was matured fully i.e. 20th October, 2016. The grain yield and straw yield was recorded plot wise. The net plot yield was then converted into kilograms per hectare.

## RESULTS AND DISCUSSION

**Plant height:** The plant height (at panicle initiation stage) was recorded highest in the treatment $T_{10}$which was significantly higher than control, $T_2$,$T_3$, $T_6$and $T_9$, whereas it was at par with the treatments $T_4$, $T_5$, $T_7$, $T_8$, $T_{11}$ and $T_{12}$(Table 2).

**Number of tillers per meter square:** The number of tillers $m^{-2}$ were highest in the treatment $T_{12}$ which is significantly at par with the treatments of $T_{11}$, $T_{10}$, $T_8$ and $T_4$, whereas it is significantly different from the rest of treatments (Table 2).

**Effective tillers per meter square:** The effective tillers per meter square were recorded highest in the treatment $T_{11}$and which was significantly higher than $T_1$ and $T_5$, whereas it was at par with rest of the treatments. Lowest numbers of tillers were recorded in the $T_1$ treatment (Table 2).

**Grains per panicle:** The highest number of grains per panicle were recorded in the treatment $T_8$ which was significantly higher than $T_1$, $T_2$, $T_3$, $T_4$, $T_5$, $T_6$, $T_7$, and $T_9$, whereas it is at par with the rest of treatments. Lowest numbers of grains were recorded in the $T_1$(Table 2).

**Table 1.** Different treatment combinations used in the field experiment

| SN | Treatments |
|---|---|
| $T_1$ | Control |
| $T_2$ | 50 kg N $ha^{-1}$ |
| $T_3$ | 100 kg N $ha^{-1}$ |
| $T_4$ | 125 kg N $ha^{-1}$ |
| $T_5$ | 4 tonnes of Azolla alone |
| $T_6$ | 50 kg N $ha^{-1}$ +4 tonnes of Azolla alone |
| $T_7$ | 100 kg N $ha^{-1}$ +4 tonnes of Azolla alone |
| $T_8$ | 125 kg N $ha^{-1}$ +4 tonnes of Azolla alone |
| $T_9$ | 8 tonnes of Azolla alone |
| $T_{10}$ | 50 kg N $ha^{-1}$ +8 tonnes of Azolla alone |
| $T_{11}$ | 100 kg N $ha^{-1}$ +8 tonnes of Azolla alone |
| $T_{12}$ | 125 kg N $ha^{-1}$ +8 tonnes of Azolla alone |

**Grain Yield:** The maximum grain yield was 5.13 t $ha^{-1}$ in $T_{11}$ followed by $T_{12}$, $T_{10}$. The increase of 0.65 per cent was observed in $T_{11}$over $T_{12}$. The increase of 512 per cent and 516 per cent was observed under these treatments respectively over $T_1$. There was increase of 11.6 per cent in grain yield in $T_1$ over $T_7$. Similarly, $T_{11}$ produced 27.27 per cent increase over $T_3$. The grain yield in all the treatments was significantly higher than $T_1$. Minimum grain yield i.e. 0.83 $tha^{-1}$ was recorded in $T_1$.

**Straw yield:** The maximum straw yield of 7.55 t $ha^{-1}$ was recorded in the treatment $T_{12}$followed by $T_{11}$, $T_{10}$. The increase of 0.70 per cent was observed in $T_{12}$ over $T_{11}$. The increase of 618.9 per cent and 613.8 per cent under these treatments respectively over $T_1$ was observed. There was an increase of 18.06 per cent in straw yield in treatment $T_{11}$ over $T_7$. Similarly $T_7$ produced 21.07 per cent increase over 100 kg N $ha^{-1}$. The straw yield obtained from all the treatments was significantly higher than $T_1$. Minimum straw yield i.e. 1.05 t $ha^{-1}$ was recorded in $T_1$.

The effect of Azolla application alone @ 4 t and 8 t was more pronounced on grain yield than the application of fertilizer nitrogen alone upto 100 kg $ha^{-1}$. Application of 8t Azolla $ha^{-1}$ gave significantly higher yield than 50 kg N $ha^{-1}$ and statistically at par but numerically lower than 100 kg N $ha^{-1}$. Treatment 125 kg N $ha^{-1}$ application alone was statistically at par with 8 t Azolla $ha^{-1}$ and gave numerically higher yield. However application of 4 t Azolla $ha^{-1}$ was statistically at par with 50 kg N $ha^{-1}$ but numerically less than 50 kg N $ha^{-1}$. Similar trend was found in straw yield. The application of 4 t and 8 t Azolla each produced 222 and 358 % more grain yield respectively than control. Similarly these treatments resulted in 240 and 413 % increase in straw yield over control. The increase in grain and straw yield could be attributed to higher and continuous supply of N, which significantly increased the yield attributes of rice. Inorganic treatment alone produced higher yield as compared to organic alone because of inability of organic sources to release N at the time of higher requirement by crop. This is in agreement with the findings earlier worker (Hossain et al 2001, Castro et al 2002, Castro et al 2003 and Riavaie et al 2013). By visualizing the grain and straw yield it is clear that 4 tonnes of Azolla per $ha^{-1}$ produced yield equivalent to 50 kg N $ha^{-1}$ and 8 tonnes Azolla $ha^{-1}$ produced yield equivalent to 100 kg N $ha^{-1}$. Although the addition of nitrogen through green manure @ 4 tonnes and 8 tonnes $ha^{-1}$ was approximately 24 and 49 kg N $ha^{-1}$ yet the contribution towards yield was much higher. This might be due to lesser leaching losses of N from Azolla green manuring than alone application of chemical N through urea. Under similar situation in Alfisol at Palampur Subehia et al (2013) reported saving of 50 % of nitrogen with the use of *Sesbania*

**Table 2.** Effect of different treatments on growth and agronomic parameters of rice

| Treatments | | Plant height (cm) | Number of tillers ($m^{-2}$) | Effective number of tillers ($m^{-2}$) | Grains per panicle | Grain yield (tonnes $ha^{-1}$) | Straw yield (tonnes $ha^{-1}$) |
|---|---|---|---|---|---|---|---|
| $T_1$ | Control | 63.0 | 407.7 | 260.7 | 121.0 | 0.83 | 1.05 |
| $T_2$ | 50 kg N $ha^{-1}$ | 76.5 | 420.7 | 358.3 | 147.0 | 2.33 | 2.99 |
| $T_3$ | 100 kg N $ha^{-1}$ | 92.7 | 435.0 | 380.0 | 154.0 | 4.03 | 5.24 |
| $T_4$ | 125 kg N $ha^{-1}$ | 101.3 | 470.0 | 363.3 | 154.0 | 4.47 | 5.90 |
| $T_5$ | 4 tonnes of Azolla alone | 101.1 | 420.0 | 325.0 | 150.0 | 2.67 | 3.57 |
| $T_6$ | 50 kg N $ha^{-1}$ +4 tonnes of Azolla alone | 93.1 | 437.0 | 370.0 | 150.0 | 3.67 | 4.99 |
| $T_7$ | 100 kg N $ha^{-1}$ +4 tonnes of Azolla alone | 100.1 | 448.0 | 375.0 | 154.3 | 4.60 | 6.35 |
| $T_8$ | 125 kg N $ha^{-1}$ +4 tonnes of Azolla alone | 102.6 | 475.0 | 390.0 | 182.0 | 4.87 | 6.81 |
| $T_9$ | 8 tonnes of Azolla alone | 99.5 | 441.7 | 391.0 | 147.0 | 3.80 | 5.40 |
| $T_{10}$ | 50 kg N $ha^{-1}$ +8 tonnes of Azolla alone | 105.7 | 475.3 | 401.7 | 172.0 | 5.00 | 7.20 |
| $T_{11}$ | 100 kg N $ha^{-1}$ +8 tonnes of Azolla alone | 104.1 | 480.0 | 403.3 | 171.0 | 5.13 | 7.49 |
| $T_{12}$ | 125 kg N $ha^{-1}$ +8 tonnes of Azolla alone | 104.2 | 492.7 | 389.0 | 174.0 | 5.10 | 7.55 |
| CD (p=0.05) | | 5.17 | 43.60 | 58.8 | 11.08 | 0.70 | 0.97 |

*aculeata* green manuring in rice wheat cropping sequence.

## CONCLUSIONS

Highest value of plant height and number of tillers per meter square during panicle initiation stage was in 125 kg N $ha^{-1}$ + 8 tonnes of Azolla. Grain yield was highest in the treatment 100 kg N $ha^{-1}$ + 8 tonnes of Azolla and straw yield was recorded highest in 125 kg N $ha^{-1}$ + 8 tonnes of Azolla. It can be concluded from the experiment that integrated use of *Azolla* and nitrogen fertilizers is an effective way for increasing rice yield.This suggests that Azolla application can be a viable option to the rice farmers for sustaining higher yields and protecting the environment as well.

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# New Distributional Record of *Holotrichia nagpurensis* Khan and Ghai 1982 and Adult Beetle Host Range in Terai Region of Uttarakhand

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***Abstract:*** In India, *Holotrichia* is the major genus of subfamily Melolonthinae of Scarabaeidae, known by its cosmopolitan nature. A survey had been conducted in Pantnagar, Uttarakhand during 2018-19 to ascertain it's occurrence of various species from the locality. Light traps and manual scouting on different host trees collection revealed the occurrence of *Holotrichia nagpurensis* Khan and Ghai. It is a new distributional record to the Pantnagar and first report, from Terai region of Uttarakhand. Their adult beetles feeding, shelter and mating activity was recorded from six host trees i.e. *Azadirachta indica* L., *Mangifera indica* L., *Artocarpus heterophyllus* Lam., *Psidium guajava* L., *Zea mays* L. and *Melia azedarach* L. from the locality. Overall, maximum occurrence and catches of *H. nagpurensis* was recorded from *A. indica*.

***Keywords***: Beetle, *Holotrichia nagpurensis*, Host plant, New distribution, Light trap

Coleoptera constitutes around one fourth of all described animal species while many are yet to be described (Grove and Stork 2000). Scarabaeidae is one of the largest families of Coleoptera that includes about 91% of all known scarabaeoids representing 27,800 species worldwide (Ratcliffe and Jameson 2004).The grubs of phytophagous scarab beetles feed on living roots, soil humus or decaying woods while adults feed on leaves, flowers and pollen (Scholtz and Grebennikov 2005). The phytophagous group of Scarabaeidae comprises mainly of four subfamilies i.e. Melolonthinae, Rutelinae, Dynastinae, and Cetoniinae. In India, of these four subfamilies, Melolonthinae is the predominant species group comprising of several genera, of which *Holotrichia* is the major genus spread across the country with several predominant species like *H. serrata, H. consanguinea*, *H. longipennis, H. sikkimensis* and *H. insularis.* The larval stage i.e. grubs of the *Holotrichia* species feed on roots, rootlets and underground portions of the crop plants and cause serious damage while adults are defoliators. As many as 27 plant species are recorded as host plants of adult *Holotrichia* under north Indian conditions (Bhadauria and Nigam 1982). Changing cropping patterns and climatic conditions contribute to shifts in species distribution and expanding host range. This necessitates continuous monitoring of species occurrence as precise documentation of the species diversity. Hence, regular surveys were taken up on the occurrence of *Holotrichia* spp. in different parts of Pantnagar, Uttarakhand.

## MATERIAL AND METHODS

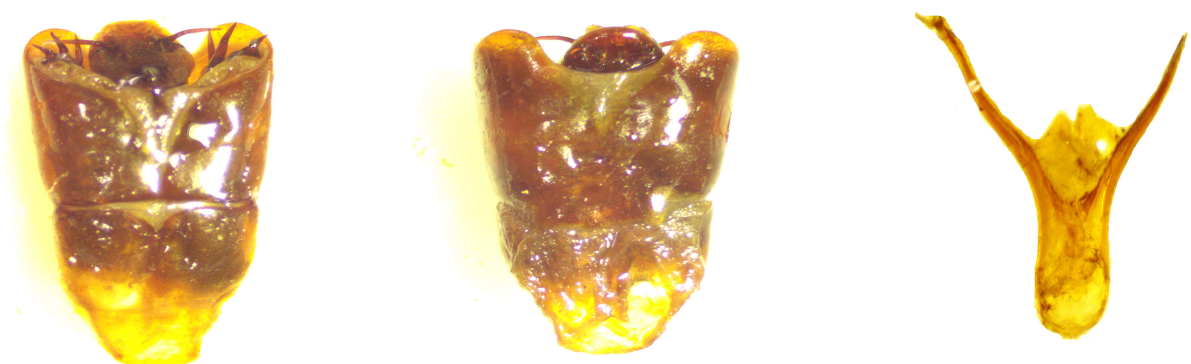
Survey and surveillance were carried out in different locations i.e. Crop Research Center (CRC), Horticulture Research Center (HRC) and Livestock Research Center (LRC) of Pantnagar, Uttarakhand, India located at 29° 02' 60.00” N to 79° 30'59.99” E for adult beetles of *Holotrichia* species during 2018-19 using light traps with fluorescent light source. As the adult beetles emerge from the soil during dusk and settle on the nearby trees for feeding and mating, the light traps were installed and operated at evening hours during March to July from 7.00 to 10.00 pm. Manual scouting for the adult beetles of *Holotrichia* was also carried out on nearby avenue trees. This has been carried out by intrinsic search on branches of host trees with the help of powerful torch during night. Finally the collected adults from light traps and from host trees, were brought to the laboratory, sorted out, cleaned and labeled properly for identification. The species were identified using keys **(**Brenske 1899, Frey, 1971, Khan and Ghai 1982).The male specimens of *Holotrichia* spp. were separated and placed in 70% alcohol for genitalia extraction. Later the genitalia and spiculum gastrale from the abdomen were extracted carefully with forceps and glued on a pointed card and pinned along with the adult male specimen. The external morphological characters of genitalia of specimens were observed through Leica M205FA stereo zoom microscope and adults images were obtained with Lumix fz300 digital camera while genitalia image with attached to the microscope using Leica automontage software. The terminology of the male genitalia was adopted from D'Hotman and Scholtz (1990) and Zorn (2006). The hosts, on which feeding activity of these beetles was observed, were further described as their preferred hosts of this area.

**(A) Dorsal view** **(B) Lateral view**

**Fig. 1.** Adult of *H. nagpurensis*

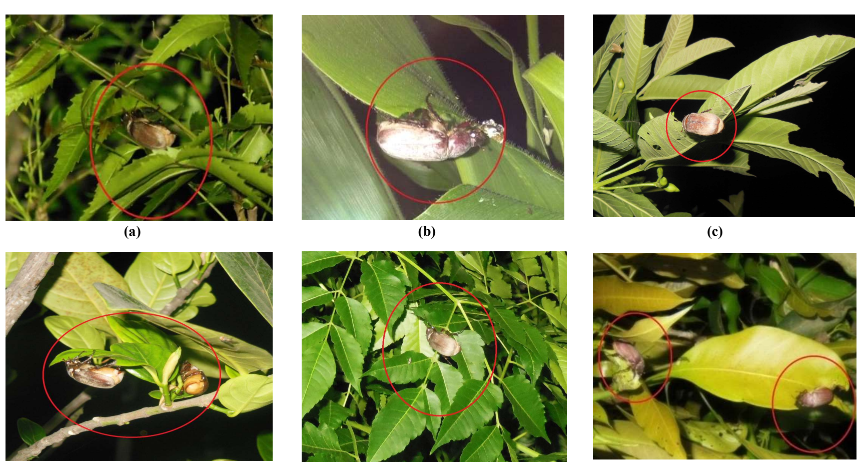
## RESULTS AND DISCUSSION

Surveys yielded new record of *Holotrichia nagpurensis* Khan and Ghai in Pantnagar region of Uttarakhand. The species *H. nagpurensis* was identified based on the external morphological and genitalia characters. The species looks morphologically similar to *H. serrata* and hence genitalia was dissected and observed. The genitalia character where the chitinous processes in the parameres of aedeagus are three needles like projections distinguishes *H. nagpurensis* from *H. serrata,* which possess two chitinous processes with knob like structures at the extremities (Khan and Ghai 1982). The study of male genitalia from the collected specimens of *Holotrichia* genus revealed the presence of *H. nagpurensis* (Fig. 1) as evidenced by the three lateral sclerotized processes (needle like) at the apex of parameres (figure 2 A) and aspiculum gastrale (Fig 2 C) and 'Y' shaped sclerite with short median fleshy structure. The external morphological characters like serrated pronotum, clypeal margin, elytral structure and most importantly genitalia characters



(A) (B) (C)

**Fig. 2**. Male genitalia of *Holotrichia nagpurensis* viz ;(A) Dorasl view ,(B) Ventral view and (C) gastrale



(a) (b) (c)

(d) (e) (f)

**Fig. 3**. Feeding of *H. nagpurensis* on different hosts. (a) *A. indica*, (b) *Z. mays*, (c) *P. guajava*, (d) *A. heterophyllus*, (e) *M. azedarach* and (f) *M. indica*

determined the species as *H. nagpurensis* (Sreedevi and Tyagi 2016). The white grub species, *H. nagpurensis* was first reported by Khan and Ghai in 1982 from Maharashtra. Further it was reported from Western Ghats, Northern Hills and Western Vidarbha plains of Maharashtra in 2014 (Dadmal and Khadakkar 2014). Apart from Maharashtra, as of now its distribution is limited to a few parts of Uttar Pradesh, Rajasthan and parts of Delhi (Dadmal et al 2013, Sreedevi and Tyagi 2016). Now, the occurrence of *H. nagpurensis* in Terai region of Uttarakhand is the first time report and thus documents a new distributional record.

Adult beetles host plants survey in respective locations for feeding, shelter and mating revealed their activity from six host plants i.e. *Azadirachta indica*, *Mangifera indica*, *Artocarpus heterophyllus, Psidium guajava*, *Zea mays* and *Melia azedarach* (Fig. 3). The host trees, *A. indica* and *M. azedarach* belonged to Meliaceae while *M. indica* and *A. heterophyllus* to Anacardiaceae and Moraceae families, respectively. *Psidium guajava* and *Z. mays* belonged to Myrtaceae and Poaceae, respectively. The monitoring of adult beetles activity of *H. nagpurensis* from March to July on different hosts revealed its maximum occurrence on *A. indica* (Table 1). Similar findings on feeding activity of *H. nagpurensis* adults was also described by Rani et al (2021) form Gazhiabad district of Uttar Pradesh with few similar hosts like *A. indica*, *P. guajava* and *M. azedarach*. Earlier this species was limited to the parts of Maharashtra, Rajasthan and a few parts of Uttar Pradesh and Delhi (Sreedevi et al 2017). The current observations indicated its presence in Uttarakhand also, which is of serious concern as the species is pestiferous on sugarcane (Sreedevi and Tyagi 2015). The yield loss due to *Holotrichia* spp. was estimated to range from 70 to 100 % in sugarcane (Chelvi et al 2011, Tyagi and Sreedevi 2014). So, in Uttarakhand, as sugarcane is grown in Terai region of Udham Singh Nagar and Haridwar, the occurrence of *H. nagpurensis* should be viewed seriously. Continuous monitoring for the species helps in advocating the farmers for timely management without much economic damage. Similarly regular surveillance and monitoring is required to document the species occurrence on other hosts trees as well as in other parts of Uttarakahand and other regions of the country.

**Table 1.** Presence of *H. nagpurensis* population on different host in different months 2018-19

| Host | *H. nagpurensis* population | | | | |
|---|---|---|---|---|---|
| | March | April | May | June | July |
| *Azadirachta indica* L. | + | + | + | + | - |
| *Zea mays* L. | + | + | - | - | - |
| *Psidium guajava* L. | + | + | + | + | - |
| *Artocarpus heterophyllus* Lam. | + | + | + | - | - |
| *Melia azedarach* L. | + | + | + | - | - |
| *Mangifera indica* L. | - | + | + | - | - |

**Note:** + present, - absent

## CONCLUSION

A light trap and manual scouting based survey conducted in Pantnagar, Terai region of Uttarakhand, India during 2018-19 revealed the occurrence of *H. nagpurensis* (Coleoptera: Scarabaeidae) from the area. This reports new distributional record of *H. nagpurensis* from Pantnagar, Uttarakhand. The adult beetles of *H. nagpurensis* were found feeding on six hosts *viz.*, *A. indica*, *M. indica*, *A. heterophyllus, P. guajava*, *Z. mays* and *M. azedarach,* of which, *A. indica* was the most preferred host. Regular monitoring is required for the documentation of the species occurrence in other unexplored parts of Uttarakhand and also other states of the country.

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# Insect-pests, Natural Enemies and Soil Micro-flora in Cabbage Grown under Subhash Palekar Natural and Conventional Farming Systems

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***Abstract*: The effect of a favourable micro-climate offered in the Subhash Palekar Natural Farming (SPNF) system in comparison to the ongoing Conventional Farming (CF) in cabbage was enumerated. Both the farming systems had same pest diversity, but, delayed their incidence by 1-3 weeks as compared to CF. The SPNF attracted relatively more natural enemies, with the occurrence of *Diadegma semiclausum* (Hellen) as an additional important bioagent. The abundance of soil microbes and enzymes activity helps stimulate plant growth. Therefore, the change in their level in a cropping season (pre-sowing to post-harvest) in both the systems was compared. Both these parameters increased, but, the increase (soil micro-flora: bacteria=3.03%; fungi=12.5%; actinomycete=12.4% and soil enzymes activity: dehydrogenase=34.29%, phosphatise=7.31% and urease=10.51%) was significantly higher in SPNF than in CF system. The SPNF, although seems a viable option for cabbage cultivation but further institutional studies on larger landscape are required.

***Keywords***: ZBNF, Diversity, Abundance, Soil microbes, Enzymatic activity

The modern agriculture has influenced the economy and way of life, resulting into the initial increase in production and farmers' earning. However, it has now become amatter of public debate for overshadowing its positive attributes with many ecological (Naresh et al 2018), economical (Lorenz and Lal 2016) and public health issues (Kumari and Sharma 2014). Furthermore, consumers are becoming more and more distrusting with respect to food safety and the credibility of the conventional farming is in doubt (Cristache et al 2018). To cope with these issues, organic farming was proposed as a better and sustainable alternative. This farming, nevertheless, has not found wide acceptance as an alternative to conventional farming by the small and marginal farmers due to various shortfalls. The intensive use of market driven inputs, depletion of natural resources, enhancing cost of cultivation and reduction in farm income emphasize the need for transition of the ongoing farm practices to a form of farming that emphasizes farmers' economic and social stability.

Subhash Palekar Natural Farming (SPNF) offers an agro-ecological farming approach, improving soil fertility through many agro-ecological principles, like diversification, nutrient recycling and increasing beneficial biological interactions. The SPNF enhances the biodiversity in the ecosystem, which helps creating local barriers for pests, creates a micro-climate to enhance pest-natural enemy interactions. Also known as Zero Budget Natural Farming, 'zero budget' implies that the need for external financing is zero, and any costs incurred can be offset by a diversified source of income which comes from diversification (APZBNF 2018). There is ample scientific evidence on the ecological benefits of the practices promoted by SPNF such as cow based microbial mixtures, mulching, improving functional on farm bio-diversity, enhancing soil microbial activity, cover cropping and management of insect- pests (Altieri 2018). However, in the absence of credible data about how the practice impacts yields, still remains unsolved. The present investigation, therefore, aims at to study the diversity and abundance of insect-pests, their natural enemies and soil micro-flora and enzymes activity in cabbage under Subhash Palekar Natural Farming (SPNF) and Conventional Farming (CF) systems.

## MATERIAL AND METHODS

The study was carried out in the YSP University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh) during 2018-19. The cabbage crop (cv. Pride of India) was raised under SPNF system following the standard recommendation (Palekar 2013), whereas, under CF, it was grown as per the University package of practices (Anonymous 2014). Under SPNF ecosystem, cabbage (main crop) was intercropped with chickpea (legume), radish (cash crop) and mustard (trap crop) (Fig. 1). The seeds of the respective crops were treated with beejamrit @ 50ml $20g^{-1}$,

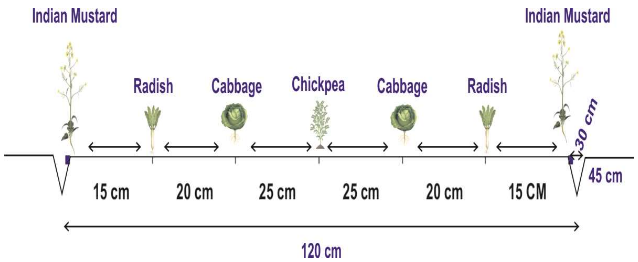


**Fig. 1.** Field crop geometry followed under SPNF system

**Table 1.** Diversity indices of insect-pests and natural enemies of cabbage under SPNF and CF systems

| Diversity indices | Insect- pests | | Natural enemies | |
|---|---|---|---|---|
| | ZBNF | CF | SPNF | CF |
| Shannon Index (H) | 0.81 | 0.89 | 0.99 | 0.93 |
| $H_{max}$ | 1.94 | 1.94 | 1.39 | 1.10 |
| Evenness (J) | 0.42 | 0.46 | 0.71 | 0.85 |
| Dominance (D) | 0.58 | 0.54 | 0.29 | 0.15 |

one day before sowing and kept overnight for drying. Before sowing, the nursery bed was applied with the ghanjeevamrit @ 100g $m^{-2}$, raked and mixed thoroughly. The ghanjeevamrit @ 10q $ha^{-1}$was mixed at the time of field preparation. The jeevamrit was sprinkled on the nursery bed @ 10%, one day before uprooting the seedlings. After uprooting, the roots of seedlings were dipped in jeevamrit for 2-3 min before transplanting. Thereafter, jeevamrit was applied through foliar application at 21 days interval. For the management of insect-pests, darekastra @ 500L $ha^{-1}$, bramhastra @ 3 per cent and agniastra @ 3 per cent were used alternatively, at 21days intervalor earlier as required, starting from one week after transplanting till one week before harvest. Different inputs used in SPNF were prepared by following standard procedures given by Palekar (2013).

**Diversity and abundance**: Different insect-pests and their natural enemies were monitored on cabbage crops at weekly interval under each cropping system, throughout the season. The observations on the total number of species and the population density of each species per sampling unit were recorded. The diversity indices were calculated as per the method of Shannon (1948).

**Soil micro-flora and enzyme activity**: The soil microflora (bacteria, fungi and actinomycetes) were enumerated using a standard technique given by Subba (1999), and enzymes *viz.* dehydrogenase and urease by Thimmaiah (1999) and phosphatase by Tabatabai and Bremner (1969).

## RESULTS AND DISCUSSION

**Diversity of insect-pests**: Both the farming systems were equally diverse, harvouring 7 pest species under 7 genera and 6 families (Table 1). The insect-pests recorded were *Brevicoryne brassicae* (L.), *Myzus persicae* (Sulzer), *Hellula undalis* (Fabricius), *Plutella xylostella* (L.), *Pieris brassicae* (L.), *Trialeurodes vaporariorum* Westwood and *Phyllotreta* sp. The Shannon index (H), species richness ($H_{max}$), species evenness (J) and species dominance (D) were 0.81, 1.94, 0.42 and 0.58, and 0.89, 1.94, 0.46 and 0.54 for SPNF and CF systems, respectively. In both the farming systems, the pest complex was dominated by four species, which were *B. brassicae, M. persicae, P. brassicae*and *P. xylostella* in SPNF and *B. brassicae, M. persicae, P. brassicae* and *T. vaporariorum* in CF. Earlier, Mahendran (2015) recorded *P. xylostella*, *P. brassicae*, *Trichoplusia ni* (Hubner), *Lipaphis erysimi* (Kaltenbach) and *M. persicae* as the dominant insect-pests of cauliflower in Delhi. Bhat (2018) also reported *P. xylostella, A. ipsilon*, *M. persicae*, *T. orichalcea*, *P. brassicae, P. rapae* and *Helicoverpa armigera* (Hubner) as the abundant insect-pests of cole crops in Kashmir, India.

**Diversity of natural enemies**: In terms of natural enemies, the SPNF system (species=7; $H_{max}$=1.39; H=0.99) was more diverse than the CF (species=6; $H_{max}$=1.10; H=0.93) (Table 1). The natural enemies recorded were *Coccinella septempunctata* L, *Hippodamia variegate* (Goeze), *Episyrphus balteatus* (De Geer), *Eupeodes frequens* (Matsumura), *Metasyrphus confrator* (Wiedemann), *Diaeretiella rapae* (Mc'Intosh) and *Diadegma semiclausum* (Hellen). All the natural enemies recorded in SPNF, except *D. semiclausum* were also recorded in CF system. The diversity indices revealed the dominance of two species (J=0.71; D=0.29) *i.e. D. rapae* and *E. balteatus* in SPNF and one (J=0.85; D=0.15) *i.e. D. rapae* in CF system. The possible reason for higher diversity of natural enemies in SPNF system could be because of non-application of chemical insecticides. The natural enemies recorded in the present study are relatable to the earlier findings (Jat et al 2017, Devi 2018). The presence of *D. semiclausum*, indicates the better hospitability of SPNF system for this important parasitoid.

**Seasonal abundance of insect-pests**: In both the farming systems, *M. persicae* was the first to appear (November end) with identical densities of 1.16±0.12 and 1.42±0.22 aphids $leaf^{-1}$ in SPNF and CF systems in cabbage, respectively (Fig. 2). Thereafter, *B. brassicae* appeared during December end, with 1.01±0.11 aphids $leaf^{-1}$ in SPNF and a significantly higher density (2.36±0.35 aphids $leaf^{-1}$) in CF grown cabbage. Subsequently, the pest density grew steadily to reach the peak in the mid-March and February end in SPNF (14.16±1.88 aphids $leaf^{-1}$) and CF (18.68±1.97 aphids $leaf^{-1}$) systems, respectively. These results indicate that the plant protection measures applied in SPNF might have repelled the alates of *B. brassicae*. Overall, the aphid density did not differ significantly between the two farming systems, except the weeks following the insecticide applications in CF. Earlier studies reported that the pest remains active throughout the cropping season with peak activity during January and February. The *B. brassicae* appeared during mid-January with low population density (8.1 aphids leaf$^{-1}$) and gradually increased to the peak (41.82-187 aphids leaf$^{-1}$) during the 1$^{st}$ fortnight of March (Pal and Singh 2012, Mishra and Singh 2015). During the study, the green peach aphid, *M. persicae* was also recorded, though low in density, on cabbage grown under both the farming systems. Mandal and Patnaik (2008) have reported the presence of *M. persicae* throughout the cropping season with highest activity during the months of December and January.

The cabbage head borer, *H. undalis* also appeared simultaneously (mid-January) in both the farming systems with the mean population of 0.01±0.01 and 0.04±0.02 larvae plant$^{-1}$ in SPNF and CF, respectively (Fig. 2). Pest density gradually increased to its peak (0.12±0.03 larvae plant$^{-1}$) during the February end in SPNF and early-March (0.20±0.05) in CF. A significant reduction in the pest population during the 2$^{nd}$ and 3$^{rd}$ week of March in CF was due to the application of insecticides. Overall, the pest density remained low throughout the season and did not differ significantly, between the two farming systems. In contrast, the incidence of this pest in other parts of the country was higher than observed in the present study (Aiswarya et al 2018, Gaikwad et al 2018), probably due to the relatively warm climate of the regions.

The incidence of the diamondback moth, *P. xylostella* in cabbage grown under SPNF (February end) was delayed by two weeks as compared to that grown in CF. Seasonally, the pest incidence remained on par in both the systems and

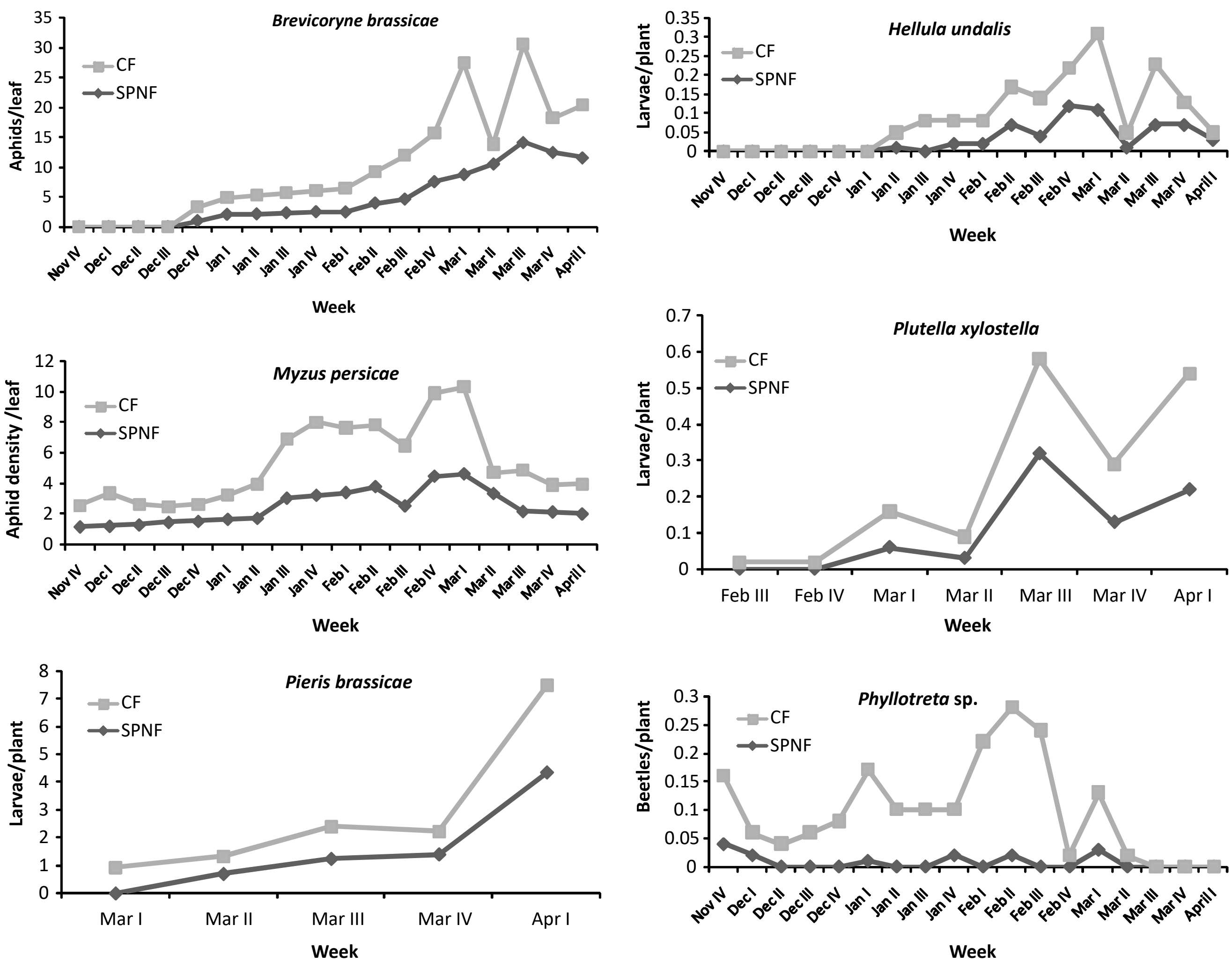


**Fig. 2.** Seasonal abundance of different insect-pests of cabbage under SPNF and CF systems

fluctuated between 0.06 to 0.32 and 0.02 to 0.32 larvae plant$^{-1}$ in SPNF and CF grown cabbage, respectively (Fig. 2). The delayed appearance of the pest hints towards the oviposition deterrence of the decoctions applied against the pest in the SPNF system. The incidence of *P. xylostella* has also been reported to persist between January end and May by earlier workers (Devi et al 2004, Sharma et al 2017b). In Rajasthan, the attack of *P. xylostella* was, however, recorded from mid-November to early January (Sharma et al 2017a).

The incidence of the cabbage butterfly, *P. brassicae* in SPNF initiated in early March, with 0.70±0.60 larvae plant$^{-1}$ and reached the peak (4.34±1.47 larvae plant$^{-1}$) during March end (Fig. 2). In the CF system, the pest incidence appeared one week earlier (February end) than on SPNF cabbage with the mean population of 0.94±0.72 larvae plant$^{-1}$, which peaked (3.14±1.34 larvae plant$^{-1}$) in March end. Seasonally, the pest density was same in both the farming systems. The delay in the pest attack in SPNF could be due to the creation of barriers and camouflaging the host plants by the intercrops (Han-ming et al 2019). Under CF conditions, however, the pest incidence in other parts of the country was reported between 9$^{th}$ to 13$^{th}$ standard weeks with its peak during 11$^{th}$ standard week (Venkateswarlu et al 2011).

The flea beetle, *Phyllotreta* sp. infestation appeared during the November end in both the farming systems, but terminated one week earlier in SPNF than the CF system (Fig. 2). The respective incidence started with 0.04±0.02 and 0.12±0.04 beetles plant$^{-1}$ in SPNF and CF and remained low in both the systems. Earlier, some workers have also reported varying appearance of this pest under chemical farming conditions i.e. from Jan end to April under Gujarat conditions and mid-September to November in Rajasthan (Sharma 2004). The incidence of the greenhouse whitefly, *Trialeurodes vaporariorum* did not occur throughout the season. The mean population fluctuated between 0.02±0.02 to 0.15±0.04 and 0.02±0.02 to 0.36±0.12 whiteflies plant$^{-1}$ in SPNF and CF grown cabbage, respectively, with non-significant differences between the two farming systems.

In SPNF system, different plant protection decoctions *viz*. darekaster, bramhaster and agniaster prepared from the indigenous cow urine and dung and locally available plants, were applied alternatively, at an interval of 21 days, or as per the requirement. The compounds of such locally available plants like drek (*Melia azedarach* L.) have a number of useful activities like toxicity, repellence, feeding and oviposition deterrence and insect growth regulator activity (Sharma and Gupta 2009). In the present study, the incidence of all the insect-pests in cabbage under SPNF system was either less or equal to that recorded in CF system. These results are in accordance with Yankit et al (2019) who reported that the occurrence of *Tuta absoluta* (Meyrick) in tomato was delayed by four weeks and the incidence was significantly less in SPNF system as compared to that grown under Organic or CF. It can, therefore, be inferred that cabbage grown under SPNF ecosystem and supplemented by periodic applications of various decoctions can be employed successfully to grow cabbage in combination with other crops with minimum reliance on local farm based resources.

**Seasonal abundance of natural enemies**: The parasitism of aphids by *D. rapae* in both the farming systems increased with the increase in aphid population (Fig. 3). The first mummification was observed in the same week (November end) the aphids appeared in SPNF system. In the CF, however, the parasitoid appeared one week later (early-December) than the aphids. Thereafter, parasitism was noticed until March, with 0.74-6.99 and 0.16-7.06 per cent parasitism in SPNF and CF system, respectively, with no significant differences. The peak parasitism was observed

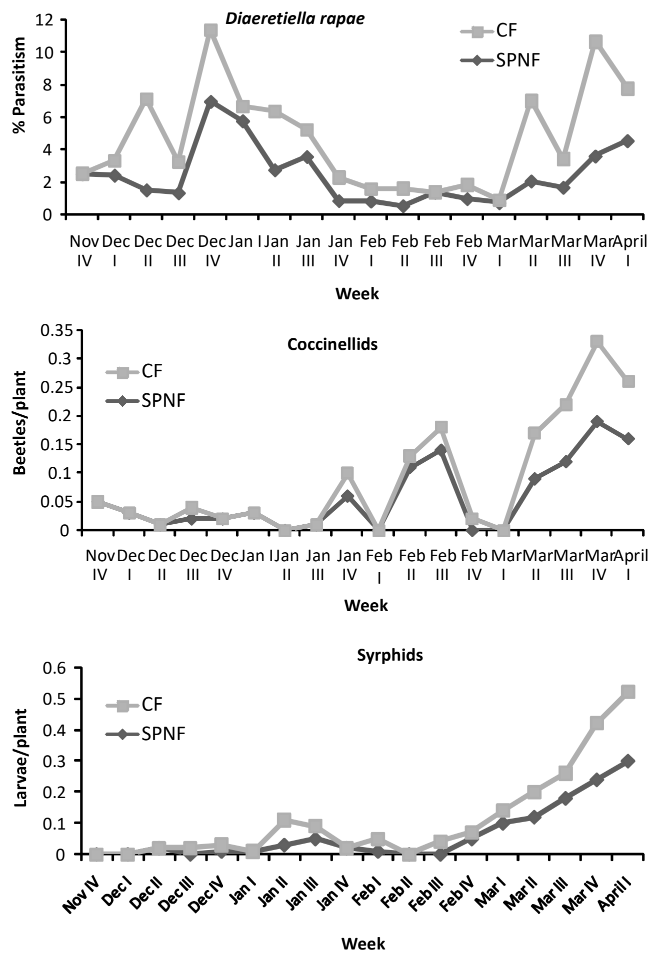


**Fig. 3.** Seasonal abundance of different natural enemies of insect-pests of cabbage under SPNF and CF systems

during the 4th and 3rd week of March in SPNF and CF systems, respectively. Bayhan and Ulusoy (2011) also recorded the population of *D. rapae* during December to January and March to April and assessed its development and parasitism rate in Balcali (Turkey). *Diadegma semiclausum*, one of the most important parasitoid of diamond back moth (*P. xylostella*), was recorded only in SPNF system during the early-April.

The aphidiphagous coccinellids appeared 3 weeks earlier in SPNF (November end) than in the CF system (Fig. 3). The peak population was observed during the third week of March, with 0.19±0.04and 0.14±0.04 beetles plant$^{-1}$ in SPNF and CF system, respectively. Seasonally, the beetles activity remained higher, though non-significantly, in SPNF system than in CF. Previous studies report divergent peak periods of activity of these beetles in different agro-climatic zones of the country, for example, March in Uttar Pradrsh (Yadav and Agrawal, 2018) and January end in Rajasthan (Sharma et al 2017a).

The predation of aphids by syrphids was observed during mid-December in SPNF, however, one week later in CF system (Fig. 3). Seasonally, the syrphid population varied from 0.01±0.01 to 0.30±0.05 larvae plant$^{-1}$ in SPNF and 0.02±0.02 to 0.22±0.06 larvae plant$^{-1}$ in CF system, with no significant differences and peaked during the March end, in the respective systems. Like other biocontrol agents, the syrphids activity was also delayed in CF system by one week (3rd week of December) indicating the negative impact of insecticides on them.

**Viable microbial count and enzymes activity in soil**: The viable count of bacteria, fungi and actinomycetes in the soil was recorded before sowing and after harvest (Table 2). Their count increased by 3.0, 12.5 and 12.4 per cent in SPNF as against 1.7, 4.4 and 6.0 per cent in CF, respectively. The SPNF system, similarly enhanced the soil enzymes activity more than CF. The per cent increase in dehydrogenase, phosphatase and urease activity was 37.3, 7.3 and 10.5 in SPNF, while a lesser increment of 4.7, 1.4 and 5.8 per cent was recorded in CF system.

Although, the soil microflora (bacteria, fungi and actinomycetes) increased (from pre-sowing to post-harvest stage) in both the farming systems, the increase was significantly higher ($p \leq 0.03$) in SPNF than in CF system. This can be attributed to the repeated use of jeevamrit and or ghanjeevamrit, which acts as a culture to enhance the microbial activity in the soil, contains macro and micro nutrients, vitamins, essential amino acids, growth promoting substances and beneficial microorganisms (Sreenivasa et al 2009, Gore and Sreenivasa 2011, Joshi 2012, Palekar 2013). Jeevamritis claimed to be a panacea for SPNF system to fulfil the nutritional requirements of crops and pest management as well. The present results are in conformity with those of Aulakh et al (2018) who observed a higher microbial population in jeevamrit treated plots as compared to chemical fertilizer and control plots.

In soil, enzymes exist intracellular or extracellular and are involved in soil bio-geochemical processes such as organic matter decomposition, humus formation and nutrient cycling (Sinsabaugh et al 1991). Dick et al (1994) reported that, the activities of soil enzymes were highly correlated to soil organic carbon, which releases energy for micro-organisms and suggested that their assay in soil provides a broad-spectrum indicator of soil biological health. In the present study, the soil enzyme activities increased in both the systems, however, the relative increment was significantly higher ($p \leq 0.02$) in SPNF than CF system. This enhancement might be attributed to the higher organic carbon content and enhanced release of root exudates in the rhizosphere soil of SPNF system. In CF system, the slight increment might be due to the recommended application of FYM and nitrogenous fertilizers. The soil enzyme activities are positively correlated with total organic carbon, active and passive pools of carbon, soil respiration, microbial biomass and soil available nitrogen

**Table 2.** Effect of the farming systems on the viable soil microbial count and enzymes activity

| Parameter | Farming systems | | | | | |
|---|---|---|---|---|---|---|
| | CF | | | SPNF | | |
| | BS (Oct 2018) | AH (Mar 2019) | Increase (%) | BS (Oct 2018) | AH (Mar 2019) | Increase (%) |
| Bacteria (× $10^8$cfu $g^{-1}$ soil) | 116±6.8 | 118±5.8 | 1.7±0.3 | 132±11.6 | 136±12.5 | 3.0±0.3 |
| Fungi (× $10^4$cfu $g^{-1}$ soil) | 9.0±1.4 | 9.4±1.6 | 4.4±1.0 | 9.6±1.6 | 10.8±1.3 | 12.5±1.2 |
| Actinomycetes (× $10^3$cfu $g^{-1}$ soil) | 28.4±2.7 | 30.2±3.0 | 6.0±0.9 | 32.2±3.3 | 36.2±2.7 | 12.4±0.9 |
| Dehydrogenase (µg TPF $g^{-1}$ $h^{-1}$) | 8.2±0.5 | 8.5±0.8 | 4.7±0.4 | 10.6±0.7 | 14.5±0.6 | 37.3±2.4 |
| Phosphatase (µg PNP $g^{-1}$ $h^{-1}$) | 65.5±0.7 | 66.4±0.4 | 1.4±0.2 | 97.8±1.9 | 104.9±1.4 | 7.3±0.4 |
| Urease (µg $NH_4^+$ $g^{-1}$ $h^{-1}$) | 6.2±1.1 | 5.8±1.8 | 5.8±0.4 | 10.0±1.0 | 11.0±2.3 | 10.5±1.0 |

CF- Conventional Farming; SPNF- Subhash Palekar Natural Farming; BS- Before sowing; AH- After harvest

(Graham and Haynes 2005). Verma et al (2018) also reported the highest enzymatic activities in SFNF soil in comparison to Conventional and Organic farming systems.

Conclusively, the SPNF system, in comparison to CF delayed the pests' incidence, attracted more natural enemies and enhanced the soil microflora and the enzyme activities in cabbage crop. The locally available plant protection decoctions managed the pests as effectively as chemical pesticides. This system can therefore, be a viable crop cultivation option, after having further systematic validation studies.

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# Variable Host-pathogen Interactions in Resistant and Susceptible Barley (*Hordeum vulgare*) Genotypes to *Ustilago hordei*

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***Abstract*:** A microscopy study involving optical research (ORM) and scanning electron microscopy (SEM) techniques was performed to compare compatible and incompatible *Hordeum vulgare-Ustilago hordei,* host pathogen interaction in susceptible (VJM 201) and resistant (DWRUB-92) barley genotypes. In compatible interaction, trailing hyphae were seen all over the host surface and all the teliospores germinated with shriveled spores being present on the host surface, while in case of incompatible interaction, the teliospores were completely intact. Some unknown biochemical substances or extracellular polysaccharides were present completely adjacent to teliospores, which might be involved in hindering the germination of teliospores.

***Keywords***: Barley, *Ustilago hordei*, Host-pathogen interaction, ORM, SEM

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop in the word after wheat, maize and rice and is one of the first crops to be domesticated for human consumption (Giraldo et al 2019). Currently in India, it is grown over an area of 672 thousand hectares with annual production of 1830 thousand tons (FAO 2018). Numerous products are being manufactured from this crop, such as malted food products like vinegar, baby food, and cocoa malt drinks. Farmers usually prefer barley over wheat under constrained conditions. Due to its ability to withstand drought conditions, it has a special significance in Indian agriculture with special reference to cultivation on arid and rain-fed agricultural-lands (Quader et al 2018). There are many constraints in barley production, among which diseases play a major threatening role. Among all biotic stresses, covered smut caused by *Ustilago hordei* (Pers.) Lagerheim is an important fungal disease responsible for a significant decrease in the yield (Mathre 1997). This disease is also significant from industrial point of view because contamination of healthy grains with teliospores, lowers grain quality of barley for food, feed, and malting, thus causing varied degree of economic losses (Grewal et al 2008). The pathogen is externally seed borne and it remains alive as teliospores on seed or in infested soil (Ali and Bakkeren 2011). The most conspicuous symptoms develop at flowering stage when the barley kernels are replaced by diploid teliospores (Hu et al 2002). Some information is available on the use of fungicides and other strategies for the control of this disease but information is also required to be generated on different aspects of host resistance, being the most economical and best method of disease control.

Resistance in many plant-pathogen interactions is accompanied by rapid development of various defense responses (Dixon and Paiva 1995). Lignification, suberisation, formation of tyloses or callose deposition are some of the characteristic changes which inhibit or isolate the pathogen from healthy tissue and is therefore associated with a very rapid, active and localized hypersensitive host cell death. This reaction is a characteristic of resistant cultivars and is correlated with a continuous series of events called programmed cell death triggered by the activation of specific genetic complement of genes. It is a main feature of many patho-systems showing race-specific resistance (Keisling 1952). The present study was aimed to compare the histopathological changes occurring in compatible and incompatible host-pathogen interaction using two barley cultivars viz., VJM 201 (susceptible) and DWRUB-92 (resistant) under optical research microscope (ORM) and scanning electron microscope (SEM).

## MATERIAL AND METHODS

Covered smut infested seeds of highly susceptible barley variety VJM 201 were sown during 2017-18 in the experimental area of Punjab Agricultural University, Ludhiana. After maturation of the crop, the infected barley heads were harvested and crushed to make powder of teliospores, which were further stored at room temperature in polythene bags for conducting experiments during 2018-19. The seeds of both the varieties were dehulled and surface sterilized for 1 min in 70% ethanol, followed by 10 min in 0.1% NaOCl solution and then washed several times with sterile, double distilled water. The seed inoculation was done by dipping the seeds in the teliospore suspension (mixing 2 g of teliospores to 1 litre of water for getting the desired strength of $10^8$ teliospores $ml^{-1}$) of *U. hordei* for 6 hours. Then, 10 seeds of each cultivar were allowed to germinate under sterile conditions in Petri dishes lined with water-soaked filter paper at 23°C in an incubator (Singh 2018). After 48 hrs, shoots and roots started emerging from the barley seedlings. At 6 days after inoculation, the root samples (1mm in length) were taken from the seedlings of both the cultivars from the portion near to the root-shoot junction.

For performing the optical research microscopy (ORM), fresh root segments were heated at 90°C for about 1 h in 10% KOH. This removed the host cytoplasm and most of the nuclei, and the roots became very clear. The roots were then rinsed in water and acidified with dilute HCl. They were then stained by simmering for 5 min in 0.05% trypan blue in lactophenol, and the excess stain was removed in clear lactophenol (Phillips and Hayman 1970). Then these stained root segments were mounted on slides temporarily in lactophenol and slight pressure was applied on the coverslip to flatten KOH-treated roots for observation under the microscope. For preparing the samples for SEM, the sampled tissue was freeze dried. The root samples were kept in the glass vials and placed in a freezer chest of the refrigerator for overnight. The roots were the freeze dried in lyophilizer (model LYOLAB ST35, Chennai, India) at -40°C for 26 hours. This process was completed in three phases; freezing, primary drying and secondary drying. The freeze dried root sections were placed on an aluminium stub and sputter coated with 10 nm gold layer in low sputter coater (model Hitachi E-1010, Japan). These samples were imaged in Field Emission-Scanning Electron Microscope (Hitachi FE-SEM, Japan) operated at 5.0 kV acceleration voltage in secondary electron imaging mode. The root surface was observed at different magnifications (1.20 to 3.50 k magnifications) to observe the mating interactions between pathogen and host.

## RESULTS AND DISCUSSION

The results obtained with optical research microscope are as shown in Figure 1. As the KOH treatment firstly removes the host cytoplasm and then the nuclei, the stain penetrates readily and there is no stained host cytoplasm to obscure the deep blue fungal tissues. In case of compatible host-pathogen interaction (Fig. 1a and 1b), whole of the root tissue is profusely covered with the mycelial growth of the fungus and there was no thickening of the host cell wall, while in case of incompatible reaction (Fig. 1c and 1d), very few mycelial strands on the host surface could be observed and moreover, the cell wall thickenings of the host root epidermis was also visualized and these epidermal thickening may also possibly curb the invasion of the internal root tissues in the resistant and incompatible host (DWRUB-92).

The results obtained with FE-SEM are illustrated in Figure 2 and 3. In the compatible interaction, there was clear germination of teliospores and the whole host surface area was profusely covered with hyphae of *U. hordei* at 6 dpi (Fig. 2a). The host root surface was studded with few teliospores which indicated that the spores have germinated and only the peridium of the spores could be visible (Fig. 3a). Therefore, the susceptible host root - *U. hordei* interaction appeared to favour germination and development of hyphae which is evident in Plate 3a. The sequence of events visible during the incompatible interaction brought about by the combination of *U. hordei* and resistant variety (DWRUB-92) indicated that the resistant genotype root surface did not favour germination of teliospores such that several teliospores could be observed on the host root surface. However, there is absence of fungal hyphae indicating germination of possibly very few teliospores (Plate 2b and 3b).

A strong correlation exists between resistance and host cell death. Structural and biochemical changes such as lignification, callose deposition, cell wall thickening, suberization, programmed cell death, hypersensitive reaction are some of the characteristic changes that isolate the pathogen from the healthy tissue in case of incompatible host-pathogen interactions as compared to the compatible ones. The results obtained in the present study clearly indicated a novel mechanism of incompatible host-pathogen interaction whereby the resistant barley cultivar DWRUB-92 resisted germination of *U. hordei* on artificial inoculation, thus, exhibiting the incompatible interaction. However, in case of susceptible barley cultivar VJM 201, the teliospores of the pathogen germinated and profuse ramification of the fungal hyphae could be observed on entire host root surface. These results corroborate with those of Kozar (1969) who have observed that the seed hulls of susceptible barley cultivar Odessa were fully covered with the mycelia of *U. hordei* after 72 hrs of infection. Further, the fungal mycelia invaded through the intercellular spaces in the epidermal walls. Another conspicuous anatomical change included thickening of the host cell wall in the resistant cultivar DWRUB-92 was observed as incompatible interaction in optical research microscopy study. Similarly, Gaudet et al (2010) have also reported cell wall thickening and callose deposition events in incompatible *U. hordei*- *Hordeum vulgare* interactions in barley cultivar Hannchenin under light and fluorescent microscopy study.

The Scanning EM study was performed to identify the predominant mechanism involved in diminished invasion by pathogenic fungus in resistant host. The SEM analysis revealed that the teliospores of *U. hordei* germinated completely and the hyphae of the pathogen grew and extended into internal tissues in the host root of susceptible cultivar, VJM 201.Thus, only the peridium of the sunken germinated spores could be visualized on the host root surface, while in case of incompatible host-pathogen interaction in cultivar DWRUB-92, intact teliospores were present on the host surface which indicated delay in germination (Fig. 3b). Further, absence or very low incidences of presence of fungal hyphae and no or very low events of germination of teliospores have been recorded. The teliospores occurred as aggregates or in bunches and appeared to be embedded or encapsulated in extracellular matricular substance (EMS) (Fig. 2b). We propose that the EMS might be involved in hindering the germination of spores or delaying their germination. These results corroborate with those of Hu et al (2003) who while studying host (*H. vulgare*)-pathogen (*U. hordei*) interactions under SEM, reported that in case of compatible interaction, the hyphae of the pathogen grew rapidly and covered the entire host parenchyma cells at 6dpi. However, in case of incompatible host-pathogen interaction, cell wall appositions occurred as soon as hyphae penetrated host root epidermal cells resulting in complete encasing of the penetrating hyphae. The growth of the hyphae seemed to be arrested and further invasion of other plant tissues was blocked. The material deposited around hyphae was thicker and granular which was involved in obstructing further invasion of the host internal root tissues. Similarly, Ali et al (2014) performed light and SEM study to analyse the compatible and incompatible infection types in barley cultivars in response to *U. hordei* and reported the accumulation of callose and some exo-polysaccharide material around the penetrating sites which led to incompatible interaction.

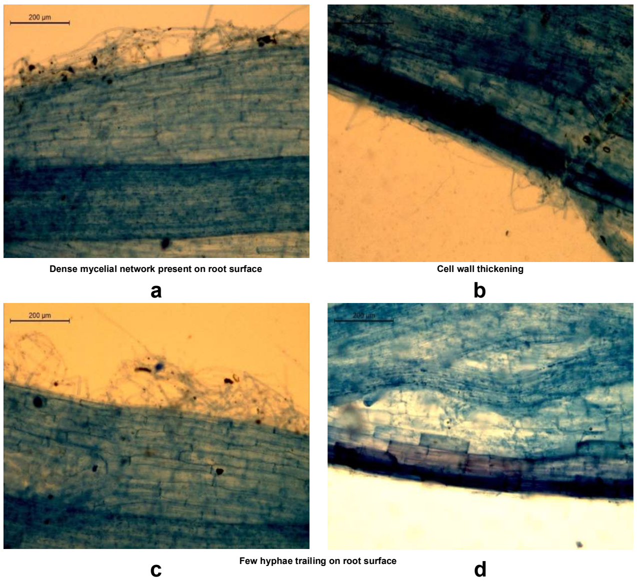
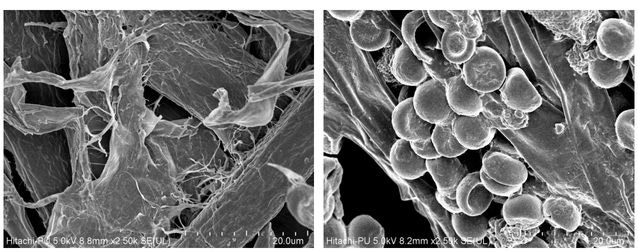


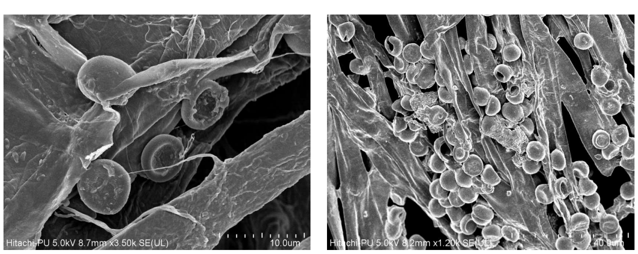

**Fig. 1.** Comparison of compatible (a,b) and incompatible (c, d) *Hordeum vulgare-Ustilago hordei,* host pathogen interaction as observed in optical research microscopy study



**Hyphae of *U. hordei*** **Extracellular**

a b

**Fig. 2.** Ultrastructural comparison of (a) compatible and (b) incompatible *Hordeum vulgare-Ustilago hordei,* host pathogen interaction using scanning electron microscope



**Hyphae trailing on root surface** **Extracellular**

a b

**Fig. 3.** Ultrastructural comparison of (a) compatible and (b) incompatible *Hordeum vulgare-Ustilago hordei,* host pathogen interaction using scanning electron microscope

## CONCLUSIONS

So, in the present study, it can be concluded that EMS present around or adjacent to the teliospores could be of host origin and might be involved in hindering the germination of spores or delaying their germination. However, absence of such interactions with the compatible host, where all the teliospores have been germinated and formed mycelium network ramifying and invading the host tissue indicates towards the possible role of root exudates of the resistant genotype to exhibit ceasing effect on germination of the teliospores.

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# Impact of Stubble Burning on Environment and Agriculture: A Review

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***Abstract*:** Stubble burning is a practice which is used by the farmers for the preparation of land for next cultivation. It is also referred as a practice of removing paddy crop residues by burning it in order prepare the filed for next crop. This practice now considered as one of the vital activities that degrade or affects the ambient air quality which in how responsible for air pollution. It also includes the removal of a large portion of the organic matter, nitrogen, phosphorus and great loss of biodiversity. It causes damage to the microorganisms present in the upper fertile layer of the soil which further affects the organic quality of soil. It has also been observed that this practice has increased from last few decades due to excessive use of combine harvester that leaves the stalk behind. Nowadays many researchers are using remote sensing and GIS technique to estimate the stubble burn area. Thus in this review an attempt is made to study the impact of stubble burning on environment agriculture and health.

***Keywords*:** Stubble, Pollution, Particulates, Impact, Emissions

India is a country which is an agricultural driven economy where 60% population is practicing in cultivation of agricultural products. Crops like rice, sugarcane and wheat not only nourish the country but they are also the major contributor to the foreign sector. In India rice is basically sown in the month of June and harvested during October-November and wheat is shortly sown after rice harvesting during the month of November and December. To make the field available for sowing of subsequent crop, crop residue is burnt to clear the land (Singh 2015). Burning of crop residue causes severe pollution of water, land and soil on large scale. Due to stubble burning there is adverse effect on the nutrient composition of soil i.e., organic carbon, Nitrogen, potassium and phosphorus. Even the minerals present in the soil are also destroyed due to burning of crop residue which in how hampers the growth of next crop (Kumar 2015). Stubble burning emits large amount of toxic pollutants in the atmosphere which consists harmful gases like $CH_4$ (Methane), $CO_2$ (carbon dioxide), CO, VOC (Volatile organic compounds) and carcinogenic polycyclic aromatic hydrocarbons. When these pollutants scatter in the surroundings they undergo chemical and physical transformation and ultimately affect the human health by causing a blanket of smog. Stubble burning also affects the soil moisture as heat generated by the burning penetrates into the soil thereby leading to the loss of moisture and useful microbes.

Punjab, Haryana and western Uttar Pradesh have been known for crop residue burning but nowadays it's spreading more commonly in the other parts of country. According to Kumar 2015, it was surveyed that stubble burning is done in two ways, one is partial burning which includes running of combine harvester followed by burning of small stalks and another is complete burning, which means entire field is set on fire. Both these ways causes air pollution but the impact of complete burning is severe. The stubble burning is known as the most easiest and economical choice for removal of stubble. Lack of awareness and non-availability of technologies often compel the farmers to take this step (Chawala 2020). Table 1 shows the crop residues produced by the major crops.

Stubble burning is also quite common as “wealth from stubble” as this crop residue can be used for electricity generation which is productive way of generating wealth from residue. Even this residue can be used for mushroom cultivation as wheat and rice straws are best substrates for the cultivation of button mushrooms and straw mushrooms. They can also be used for packaging; straw oard, mat making, paper board etc. and most importantly crop residue can be used to make biofuels which can solve our problem of oil (Yadav 2017). This review aims to generally cover the literature and existing status of stubble burning in India and most importantly to study the impacts of stubble burning on environment and agriculture.

**Effects of Stubble Burning**

**Effects on air quality:** Stubble burning brings serious threat to the air quality. Sidhu (2015) pointed out that air quality is deteriorated due to emission of Carbon monoxide, NO2, PM 10 and PM 2.5 which have surpass the permissible limits prescribed under NAAQS(National Ambient Air Quality standards) by the CPCB. To present the status of the air quality and its effects on human health, some categories are being adopted. In Table 2, the standards of WHO and NAAQS for PM2.5 and PM10 have been compared. The WHO standard for permissible levels of PM 2.5 is 10 µg $m^{-3}$in air and if we see the standard of India's NAAQS, the permissible level for PM2.5 is 40 µg $m^{-3}$ in air. However our national capital Delhi sometimes reach to 98 to 100 µg $m^{-3}$ which is ten times higher than of WHO and at least twice the standards of NAAQS.

**Effect on soil fertility:** Stubble burning also affects the soil productivity by burning some of the essential nutrients which are present inside in the soil (Singh et al 2018). Fire destroy the remains of organic matter which in how directly effects the structure of the soil (Hesammi et al 2014) reported that fire has a significant impact on soil physical, chemical, biological and biochemical properties. Plants utilize the nutrients from the soil and burning also affects the available soil nutrients. According to (Fasching 2001) burning alters the soil temperature, soil moisture and nutrient availability.

**Table 1.** Crop residues produced by major crops (Arvanitoyannis and Phonbumrung 2006)

| Source | Composition |
|---|---|
| Rice | Husk, Bran |
| Wheat | Bran, Straw |
| Maize | Stover, husk,Skins |
| Millet | Stover |
| Sugarcane | Sugarcane tops |

While burning these crop residues they cause major environmental issues but if we the other side if these crop residues are managed properly they increase the irrigation efficiency and control the soil erosion.

**Effect on agricultural productivity:** Stubble burning causes poses threat to the agriculture production or we can say directly affect the food production. Pollutants which are released in the atmosphere affect the agricultural productivity directly or indirectly. Injury to grains, leaves are the direct effects for example NO2 causes discoloration by damaging the tissue of plants similarly $SO_2$, which leads to formation of acid rain have severe effects on soil and plants which further lead to plant mortality(Augustaitis et al 2010). Indirect effects consist of growth of pests and diseases. Therefore suitable steps must be taken to deal with stubble burning to meet the increasing food demand.

**Effects on the economic development:** Stubble burning not only effects the environment and health but air pollution also hampers the growth of country's economy. Ghosh et al (2019) reported that air pollution effects cost the economy of India about 4.5 to 7.7 % of its GDP in 2018 and when he projected for 2060 there shows an increase in percentage by 15%. It has been observed that due to increase in air pollution tourist inflow in Delhi have also shown decline.

**Effect on climate:** Due to emissions from stubble fires climate is adversely effected as there is release of

**Table 2.** Source: Source: National Ambient Air Quality Standards, CPCB, 2009

| Pollutant | Time weighted average | Concentration in ambient air | | WHO |
|---|---|---|---|---|
| | | Industrial, residential, rural and other area | Ecologically sensitive area (notified by Central Government) | Standards |
| Sulphur dioxide ($SO_2$) µg $m^{-3}$ | Annual*<br>24 hours ** | 50<br>80 | 20<br>80 | 20 |
| Nitrogen dioxide (NO,). µg $m^{-3}$ | Annual*<br>24 hours ** | 40<br>80 | 30<br>80 | 40<br>200 (1-hour mean) |
| Particulate matter (size less than 10 µm) or $PM_{10}$ µg $m^{-3}$ | Annual*<br>24 hours ** | 60<br>100 | 60<br>100 | 20<br>50 |
| Particulate matter (size less than 2.5 µm) or $PM_{2.5}$ µg $m^{-3}$ | Annual*<br>24 hours ** | 40<br>60 | 40<br>60 | 10<br>25 |
| Ozone ($O_3$) µg $m^{-3}$ | Annual*<br>24 hours ** | 100<br>180 | 100<br>180 | 100(8-hour mean) |
| Lead (Pb) µg $m^{-3}$ | Annual*<br>24 hours ** | 0.50<br>1.0 | 0.50<br>1.0 | |
| Carbon monoxide mg $m^{-3}$ | Annual*<br>24 hours ** | 02<br>04 | 02<br>04 | |
| Ammonia (NH3) µg $m^{-3}$ | Annual*<br>24 hours ** | 100<br>400 | 100<br>400 | |

* Annual arithmetic mean of minimum 104 measurements in a year at a particular site taken twice a week 24 hourly at uniform intervals.
**24 hourly or 08 hourly or 01 hourly monitored values, as applicable, shall be complied with 98% of the time in a year. 2% of the time, they may exceed the limits but not on two consecutive days of monitoring.

greenhouses gases like $CH_4$ and $CO_2$, which further leads to global warming. (Bellarby et al 2008) reported that about 17 to 32 % of total greenhouse gas emissions are assisted by agricultural sector in the world. Open burning is still considered as source of black carbon, a climate pollutant which contributes to climate change, air pollution and increased melting of snow and ice. Figure 1 shows the effect of stubble burning in Delhi which further degrades the air quality.

**Impact of stubble burning on environment:** A large number of toxic pollutants are released into the atmosphere due to open burning of stubble. Many harmful gases are associated with this burning like, Carbon Monoxide, Methane, Carcinogenic Polycyclic hydrocarbons and VOCs. T these harmful gases either build clouds of ash or produce into smog which is formed due to increase in amount of smoke present in the atmosphere. Stubble burning. These harmful gases have an ability to travel thousands of kilometers which leads to increasing in air pollution in the cities nearby making the air quality more worse.

With the onset of winter, burning becomes prevalent in the Northern India mainly in Punjab, Haryana and western Uttar Pradesh .Each year farmers of Punjab burn about 7 to 8 million metric Tons of crop residues (Kedia 2020).and this crop residue burning is one of the major contributor for causing air pollution as emits Greenhouse gases.

According to (Kumar et al 2015) in Table 3 these particulates are released in the atmosphere in large quantities. Further they also added that concentration of organic pollutants was also found to be high which consists of toxic heavy metals like Iron and Zinc (Reddy et al 2019). Burning of crop residue not only degrades the air quality but it actually destroys the nutrient value of the soil which in how results in decrease in soil fertility causing loss in moisture in the soil (Chaterjee 2018) reported that burning 1 ton stubble may cause the loss of 5.5 kg nitrogen, 2.3 kg phosphorus, 25 kg Potassium and 1.2 kg Sulphur, in addition to organic carbon.

**Health implications of stubble burning:** Researchers have shown that burning of stubble or any agriculture residue is a major health hazard. Stubble burning releases fine particulate matter (PM2.5), which is an air pollutant and majorly its concerns the people's health when the concentration or levels of PM2.5 is very high. These particles get trapped inside the lungs and increase the risk of lung cancer by 36% (Kedia 2020). The gaseous emissions which are released due to burning pose risk to the health ranging from skin and eyes irritation to severe cardiovascular , neurological and respiratory diseases, asthma, chronic bronchitis and decreased lung function, COPD (Chronic obstructive pulmonary disease) , emphysema, etc.( Abdurrahman 2020). Due to prolonged exposure of pollution leads to increase in mortality rates. Even the pregnant women and infants are prone to many dangerous consequences due to pollutants released by the stubble burning (Singh et.al 2018). Other effects of exposure to burning or pollution caused by burning include Stoke, tuberculosis, lung cancer, a cardiac arrest and infections in respiratory system ( Abdurrahman 2020).

Simpson (2019) estimated that economic cost of being exposed to air pollution from stubble burning is nearly Rs. 2

**Table 3**. Particulates releasing in air

| Particulates | Range |
|---|---|
| P.M 2.5 | 146-221 μg $m^{-3}$ |
| P.M 10 | 300 μg $m^{-3}$ |
| NO2 and NH3 | 40–50 μg $m^{-3}$ |


*https://theenglishpost.com/public-health-emergency-declared-delhi-ncr/*

**Fig. 1.** Pollution of Delhi after the stubble burning, November 2019 (A) and image of Delhi in July 2019

**Table 4**. IND-AQI category and range (CBCB )

| AQI category | AQI range | Possible health impacts |
|---|---|---|
| Good | 0 – 50 | Minimal Impact |
| Satisfactory | 51 – 100 | Minor breathing discomfort to sensitive people |
| Moderate | 101 – 200 | Breathing discomfort to the people with lungs, asthma and heart diseases |
| Poor | 201 – 300 | Breathing discomfort to most people on prolonged exposure |
| Very Poor | 301 – 400 | Respiratory illness on prolonged exposure |
| Severe | 401 - 500 | Affects healthy people and seriously impacts those with existing diseases |

lakh crore annually or the three north Indian states of Punjab, Haryana and Delhi. These air quality standards (Table 4) are set by the individual countries to protect the public health of their citizens. Though Indian AQI standard are less stringent as compared to WHO. Air quality degradation results in increase in respiratory problems (Like asthma, cough, bronchitis), eye and skin diseases. Stubble burning sometimes also causes poor visibility which further leads to increase of road accidents (Sidhu 2015).

**Management practices:** Farmers can manage the crop residues very effectively. An holistic approach is required to tackle this problem from the base by adopting various precautionary and preventive methods or techniques. The techniques are incorporating the stubble in to the soil, generating fuel from husk (Biofuel production), Composting, bio char, Fodder (Abdurrahman 2020). Instead of burning of the stubble this can be used in different ways like mushroom cultivation, biomass energy, packing materials and in industrial production also.

In ancient times when the agriculture produce was less or limited, some old methods were adopted by the farmers instead of burning the fields like stubble mostly used as fodder, Rice straw had been used to bind clay in built up wall construction and in the manufacturing of fired brick, for packing the materials, Bedding for livestock, poultry for built up litter system. But now these methods are considered as insignificant in front of new technologies or modernization. Technologies like making pellets out of stubble replace the coal in industries. These pellets can be widely used for heating, industrial furnace, hot water boiler, life stove etc. Fuel from pellets have high efficiency and are easy to store they can also solve the problem of environment as replacing coal will be beneficial for environment (Verma 2014).Agriculture waste or we can say stubble can generate energy vi acombustion, gasification, or methanation (Shafie 2016) This stubble can be combusted mixing up with the other biomass or can be combusted directly also to generate heat and electricity (Kumar et al 2015).

Composting is also one of the method which proved to be essential in the management of stubble. A very popular method of composting is vermicomposting that produces compost with the use of earthworms which is known to improve the productivity of soil (Singh et al 1996). Stubble composting can be done in two stages in which anaerobic proves is followed by aerobic process for 40 days (Gummert et al 2020). In a study conducted by (Ravindra et al 2018), they founded that stubble from millet, sugarcane, wheat, pulse etc. generates valuable vermicomposting when mixed with Cow dung. It was also reported by (Zhao et al 2019) that rice straw decomposition restores the fertility of the soil. There are some other approaches which can be considered as other alternatives for the management of stubble which includes production of bio-lubricants, pulp manufacturing, production of nano silica, Paper manufacturing (Zhang et al 2017). Further this Nano silica can be used in solar sells, cosmetics and Nano medicines (Zhang et al 2016).

## CONCLUSION

Burning of stubble is mostly taken as an easiest and cost effective alternative therefore states like Haryana, Punjab, Uttrakhand and Uttar Pradesh should adopt or initiate new technologies. Stubble burning is one of the major environmental hazard so it is extremely important to understand the causes situations as why farmers burn the stubble. Alternative approaches can be taken to solve the problem of stubble burning by encouraging and making the farmers aware by trainings, workshops, or through Kisan camps etc. While adopting these new technologies of agriculture conservation it can help in improving the soil health, quality, reducing pollution and enhancing the sustainability.

**Strategies to be adopted in future to mitigate the effects of stubble burning:** Following strategies for the management of stubble burning are appropriate to adopt

1. The foremost step should be spread awareness among the farmers to enlighten them about th environmental concerns, economic benefits by managing the stubble.
2. Government can provide stubble collecting machinery to farmers on rent or subsidy basis.
3. All the pulp, paper, biomass, construction and power

industries should be forced by the government to use stubble as raw materials.

4. Biomass fuel can be set up to generate fuel.
5. Some packaging industries can be contacted to collect the stubble for packaging the boxes.
6. Machine like happy seeder can be used by the farmers.
7. Farmers should be encouraged for diversification of crops.

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# Inter-linkage of Climate and Streamflow Dynamics in Kashmir Himalayas

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***Abstract*****:** The markers of the changing climate over the availability and distribution of water resources are explicit evidently in the densely populated parts of the Himalayas. Decreasing precipitation, warming air temperatures and depleting ice reserves are sinking the region into a serious hydrological stress vis-à-vis burgeoning population growth. Changes in the hydrological regimes are projected to accentuate further, resulting in a range of serious implications on the socio-environmental and bio-physical fronts affecting directly or obliquely the local and downstream populace. Based on the historical records of temperature, precipitation and streamflow for a period of 36 years (1980-2015), this study focuses on the multi-site intra-region micro analysis investigating the influence of the variability in the climatic parameters (temperature and precipitation) on the hydrological regimes in terms of annual and seasonal streamflow. The study reveals that, coupled with decreasing precipitation there has been an overall warming trend in the average temperatures (minimum and maximum) in the Upper Jhelum Catchment of the Kashmir Himalayas. The streamflow records are in conformity with the climate variation and exhibit decreasing trends. The streamflow response was found to be more correlated with precipitation than temperature apart from being correlated positively with precipitation and negatively so with the temperatures.

***Keywords*****:** Climate variability, Streamflow, Mann-Kendall's trend test, Sen's slope estimator

In the current global scenario water availability is one of the most essential and emerging challenge, and the gravity of this concern hinders the progress of sustainable development. Hydrological circulation depends on complex and intricately related physical processes being sensitive to offsets within the system. With reference to the changing climate, hydrologic regime has a close dependence on climate and vice versa. Climate elements such as the precipitation, temperature, solar radiation, wind speed, humidity etc. influence the direct as well as indirect processes governing runoff behavior. Studies related to the impact of climate variability on hydrologic regimes and fluxes though incipient are of undeniable importance in present context of the population growth and the incumbent stress on hydrologic resources. Climate change and its overriding repercussions being a global concern are well-studied problems (IPCC 2014). Recent studies have shown that variations in the climate parameters affect the terrestrial water resource systems (Arnell et al 2001). Based on the climate studies in the Himalayas, it is inferred that there is a rise in the air temperature and decline in snowfall (Bhutiyani et al 2010, Shekher et al 2010). Whereas some studies suggest that in Himalayas runoff is bound to be increased as a result of increasing precipitation and the subsequent increase in net glacier melt (Lutz et al 2014) others infer that the streamflow in the Himalayan region has decreased over the years (Tahir et al 2011). These contrasting findings in the results further suggest that climate-induced hydrologic stresses of a region particularly in glaciered highlands like Himalayas need region specific investigation (Li and Jin 2017).

Kashmir Himalayas are experiencing prolonged summers with rise in temperatures (Immerzeel et al 2010). Increasing trends in maximum, minimum and mean temperatures have been reported in a number of studies (Bhat et al 2007). The investigation of classical trends of streamflow in relation to that of the climatic indicators is a useful tool to study the behavior of the hydro-climatic dynamics of the region and to further strategize the sustainable use and adaptation to available water resource as well as ground-water resources (Mir et al 2021). This study as a way forward attempts to establish the linkages between variability in climate parameters and the streamflow regimes, and to find the correlations thereof by studying a thirty six years long dataset which is agreeable to study and establish stable trends. The analysis has been micro-scaled to an upstream catchment, for the representation of the internal dynamics of its hydro-climatic apparatus through a temporal outline.

## MATERIAL AND METHODS

**Study area:** The study area (Fig. 1.) henceforth referred to as the Upper Jhelum catchment (UJC) is nestled in the upstream area of the Jhelum catchment and forms the Southern arc of the tectonic valley of Kashmir. UJC being located in the southern terminus of the valley of Kashmir shares the climatic pattern of the valley. Despite its latitudinal location, the valley being surrounded by Himalayan ranges has a modified subtropical climate with marked seasonality. The valley of Kashmir experiences a fairly cold and wet spring, humid and dry summer, dry autumn and rigorously cold winters with widespread snow and low temperatures dominated by depressions known as western disturbances. UJC forms the upper (originating) part of the Jhelum sub-basin with River Jhelum as the major river, originating at Verinag, and its tributaries viz. Right bank: Lidder, Arapat kol, Arapal, and Bringi. Left Bank: Sandran, Vishow and Rambi ara, draining the area (Fig. 2). Almost all the streams originate from the glacial melt-off from the adjoint mountain ranges with few stream sites such as Sandran having their origin in springs.

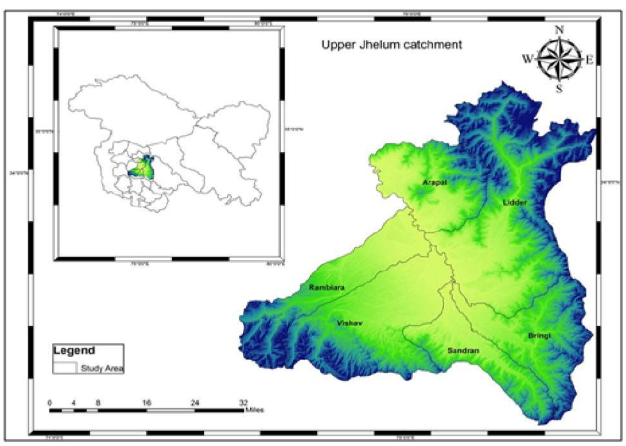


**Fig. 1.** Study area map

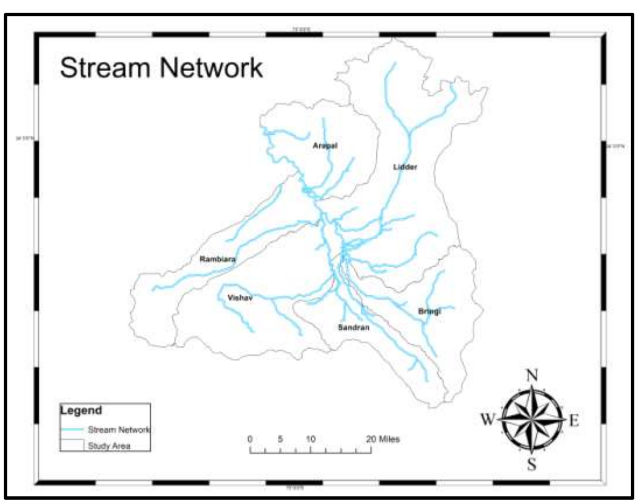


**Fig. 2.** Stream network

**Climate data:** Quality controlled monthly observed climate data (precipitation and temperature) was obtained for a period of 36 years (1980-2015) from three stations of the India Meteorological Department viz. Kokernag, Pahalgam and Qazigund (Fig. 3) located within UJC. This data includes monthly averages of maximum, minimum air temperatures and the total monthly rainfall. The datasets were classified into annual and seasonal averages; Winter (December, January and February) Spring (March, April and May) Summer (June, July and August) and Autumn (September, October and November).

**Streamflow data:** Streamflow regimes were studied in a multi-site assessment in six watersheds, namely Arapal, Brengi, Lidder, Rembiara, Sandran and Vishow. Tail gauges which better represent the outlets of the watersheds were selected for the analysis of streamflow regimes. The streamflow data was obtained from the Flood and Irrigation Department, Kashmir for the above mentioned sub watersheds. A thirty-six years long study interval (matching the climatic analysis study period) was chosen to study the annual and seasonal streamflow regimes. To represent the streamflow changes area-wise in terms of the runoff, a volumetric conversion of the calculated annual change into runoff depth was carried out (Liu et al 2014) and further the values were categorized into decadal change for identifying and assessing climate-induced variations more purposefully.

**Mann-Kendall's test:** The Mann-Kendall test statistic is calculated according to:

$$S = \sum_{k=1}^{n-1} \sum_{j=k+1}^{n-1} \operatorname{sgn}(Xj - Xk)$$

Where, *S* = Mann-Kendall trend statistic

*n* = Number of data points

*Xj, Xk* = Generic sequential data values

The function sign ($x_i$-$x_j$) assumes the following values:

$$\operatorname{sgn}(Xj - Xk) = \begin{cases} +1, if\ (Xj - Xk) > 0 \\ 0, if\ (Xj - Xk) = 0 \\ -1, if\ (Xj - Xk) < 0 \end{cases}$$

Under the hypothesis of independent and randomly distributed variables when n≥10, the statistic S is approximately normally distributed with zero mean and the variance Var(S) as follows

Var (S) = 1/18 [n(n-1) (2n+5)]

Where *n* is the length of the time series.

In the case where the values of x and y are tied the formula for Var(S) must be modified, as;

$$Var\ (S) = \sqrt{\frac{n(n-1)(2n+5) - \Sigma_{i=1}^{n} t_i(i)(i-1)(2_i+5)}{18}}$$

(Kendall, 1975)

The standardized test statistic Z is given by:

$$Z_s = \begin{cases} \frac{S-1}{\sqrt{Var\ (S)}} if\, S > 0 \\ 0 if\, S = 0 \\ \frac{S+1}{\sqrt{Var\ (S)}} if\, S < 0 \end{cases}$$

The presence of a statistically significant trend is evaluated using the Z value. This statistic is used to test the null hypothesis such that no trend exists. The null hypothesis can be rejected at significance level α if

$$Z_s > Z_{crit}$$

Where $Z_{crit}$ is the value of the standard normal distribution with a probability of exceedance of α/2. The critical values of Z at various significance levels are:

$\alpha = 0.1$; $Z_{crit} > 1.645$,

$\alpha = 0.05$; $Z_{crit} > 1.96$ and;

$\alpha = 0.01$; $Z_{crit} > 2.576$

The *p*-value (local significance level or probability value, *p*) for the Mann-Kendall trend test can be obtained from:

$p = 0.5 - \phi\,(|Z_s|)$

Where the cumulative distribution function of a standard normal variable is given by:

$$\phi\,(|Z_s|) = \frac{1}{\sqrt{2x}} \int_0^{|Z_s|} e \frac{t^2}{2} dt$$

At significance level (α), if $p < \alpha$, then a trend is considered to be statistically significant.

**Sen's slope estimator:** To estimate the magnitude of the trend the Sen's Slope estimator (Sen 1968) was used. This test computes both the slope (i.e. linear rate of change) and intercept according to Sen's method. First, a set of linear slopes is calculated as follows:

$$d_k = \frac{X_j - X_i}{j - 1}$$

for ($1 \geq i < j \geq n$), where *d* is the slope, X denotes the variable, *n* is the number of data, and i, j are indices.

Sen's slope is then calculated as the median from all slopes: $b$ = Median $d_k$. The intercepts are computed for each time step t as given by

$a_t = X_t - b \times t$

And, the corresponding intercept is as well the median of all intercepts. This function also computes the upper and lower confidence limits for Sen's slope.

Further, to understand the type and magnitude of the correlation between streamflow and the climatic variables (temperature and precipitation), the locational data of the IMD stations and the watershed area were integrated in a GIS environment using the theissen polygons in order to determine the area of influence (Subramanya, 2013) and subsequently correlation coefficients (Pearson and Kendall) were also worked out.

## RESULTS AND DISCUSSION

### Climate Parameters

**Mean maximum temperature:** The trends corresponding to the seasonal as well as annual, average maximum temperature in all the IMD stations of the UJC have been registering an increasing trend. The annual trends in Pahalgam (0.4ºC/10yr.) and Kokernag (0.4ºC/10yr) are statistically significant. Maximum air temperature shows an upward inclination in the spring season in UJC and the results are statistically significant. The spring temperatures have been increasing in Pahalgam (0.7ºC/10yr.), Qazigund (0.5ºC/10yr.) and Kokernag (0.6ºC/10yr.) stations significantly. The average winter maximum temperatures are also increasing in Pahalgam (0.5ºC/10yr). Table 1 provides more information on the seasonal and annual trends in Mean Maximum Temperature in UJC.

**Mean minimum temperatures:** The mean minimum

**Table 1.** Mann Kendall's trend test and Sen's slope estimator for the seasonal average maximum temperature in the UJC for (1980-2015)

| T-Max | | | |
|---|---|---|---|
| Kokernag | | | |
| Series\Test | Kendall's tau | p-value | Sen's slope |
| Annual | 0.285 | 0.016 | 0.044 |
| Spring | 0.232 | 0.050 | 0.063 |
| Summer | 0.127 | 0.293 | 0.012 |
| Autumn | 0.227 | 0.055 | 0.040 |
| Winter | 0.191 | 0.105 | 0.051 |
| Qazigund | | | |
| Series\Test | Kendall's tau | p-value | Sen's slope |
| Annual | 0.163 | 0.168 | 0.024 |
| Spring | 0.282 | 0.017 | 0.056 |
| Summer | -0.155 | 0.194 | -0.012 |
| Autumn | -0.140 | 0.241 | -0.022 |
| Winter | 0.156 | 0.186 | 0.042 |
| Pahalgam | | | |
| Series\Test | Kendall's tau | p-value | Sen's slope |
| Annual | 0.298 | 0.011 | 0.039 |
| Spring | 0.289 | 0.014 | 0.072 |
| Summer | 0.021 | 0.870 | 0.003 |
| Autumn | 0.065 | 0.586 | 0.007 |
| Winter | 0.293 | 0.013 | 0.057 |

temperatures in the annual and seasonal time series show a significant increase in the Pahalgam station where the seasonal as well as the annual temperatures have been spiking over the years. In Pahalgam the annual and all seasonal mean minimum temperatures have shown significant increasing trends. The increase in the average minimum temperatures in annual (0.4ºC/10yr.), spring (0.3ºC/10yr.), summer (0.5ºC/10yr.), autumn (0.2ºC/10yr.) and winter (0.8ºC/10yr.) in Pahalgam station are statistically significant. More information on mean minimum trends in UJC can be seen in Table 2.

**Precipitation:** Only the spring (40.7mm/10yr.) precipitation in the Pahalgam station shows a statistically significant decreasing trend. All other time series (annual or seasonal) though show a decreasing trend but none of the trends are statistically significant (Table 3).

**Streamflow**

**Right bank tributaries:** The right bank tributaries of the Upper Jhelum Catchment (UJC) comprise Brengi, Arapal and Lidder. While Brengi and Lidder register overall decreasing trends in streamflow, Arapal does not. The Brengi watershed is represented by Brengi and Arapat kol. The streamflow trends for the Brengi Stream are significant for summer (-10.4mm/yr.) and autumn (3.68mm/yr.). For Arapat-kol the trends for annual (-40mm/10yr.), spring (-10.87 mm/10yr.), summer (-15.92mm/10yr.), and winter (-8.12mm/10yr.) time-series. The Lidder gauges also statistically significant decreasing trends are observed in annual (-49.21mm/10yr.), spring (-20.25mm/10yr.), summer (-19.34 mm/10yr.) and autumn (-3.97 mm/10yr.) stream-flow time series. The annual and seasonal trends in the streamflow of the Arapal stream are of the increasing order, although only the spring season (9.46 mm/10yr) trend is statistically significant. Table 4 provides more information on the streamflow trends in UJC.

**Left bank tributaries:** Rambi ara, Sandran and Vishow streams comprise the left bank tributaries of UJC. All these streams show major decline in stream-flow in annual or seasonal time series. All the trends in the Rambi ara stream are statistically significant. The highest decrease is seen in the annual (-123.43 mm/10yr.) time-series. Spring (-32.41mm/10yr.) summer (-35.18mm/10yr.) autumn (-28.82 mm/10yr.) and winter (-26.87 mm/10yr.) time-series also show significant decline. The trends in the Sandran stream are statistically significant for the annual (-52.64 mm/10yr.),

**Table 2.** Mann Kendall's trend test and Sen's slope estimator for the seasonal average minimum temperature in the UJC for, (1980-2015)

| | | | |
|---|---|---|---|
| T-Min | | | |
| Kokernag | | | |
| Series\Test | Kendall's tau | p-value | Sen's slope |
| Annual | 0.208 | 0.083 | 0.018 |
| Spring | 0.210 | 0.078 | 0.022 |
| Summer | 0.047 | 0.702 | 0.003 |
| Autumn | 0.191 | 0.110 | 0.019 |
| Winter | 0.107 | 0.368 | 0.024 |
| Qazigund | | | |
| Series\Test | Kendall's tau | p-value | Sen's slope |
| Annual | 0.039 | 0.753 | 0 |
| Spring | 0.13 | 0.28 | 0.008 |
| Summer | 0.014 | 0.913 | 0 |
| Autumn | -0.044 | 0.722 | -0.002 |
| Winter | 0.107 | 0.368 | 0.024 |
| Pahalgam | | | |
| Series\Test | Kendall's tau | p-value | Sen's slope |
| Annual | 0.495 | < 0.0001 | 0.041 |
| Spring | 0.305 | 0.009 | 0.030 |
| Summer | 0.331 | 0.005 | 0.050 |
| Autumn | 0.253 | 0.031 | 0.025 |
| Winter | 0.355 | 0.002 | 0.083 |

**Table 3.** Mann Kendall's trend test and Sen's slope estimator for the intra annual total precipitation in the UJC for (1980-2015)

| Series\Test | Kendall's tau | p-value | Sen's slope |
|---|---|---|---|
| Kokernag | | | |
| Annual | -0.063 | 0.595 | -1.685 |
| Spring | -0.168 | 0.153 | -3.324 |
| Summer | -0.035 | 0.775 | -0.329 |
| Autumn | 0.016 | 0.902 | 0.191 |
| Winter | -0.089 | 0.454 | -1.479 |
| Qazigund | | | |
| Series\Test | Kendall's tau | p-value | Sen's slope |
| Annual | -0.140 | 0.236 | -5.144 |
| Spring | -0.225 | 0.055 | -5.318 |
| Summer | -0.010 | 0.946 | -0.069 |
| Autumn | 0.121 | 0.307 | 1.812 |
| Winter | -0.124 | 0.294 | -2.423 |
| Pahalgam | | | |
| Series\Test | Kendall's tau | p-value | Sen's slope |
| Annual | -0.035 | 0.775 | -1.709 |
| Spring | -0.232 | 0.048 | -4.729 |
| Summer | 0.057 | 0.634 | 0.510 |
| Autumn | 0.143 | 0.225 | 1.485 |
| Winter | 0.025 | 0.838 | 0.272 |

**Table 4.** Mann Kendall's trend test and Sen's slope estimator for the seasonal averages of the streamflow data (1980-2015) for various streams of the UJC

| Arapal | Series\Test | Kendall's tau | Z-values | p-value | Sen's slope |
|---|---|---|---|---|---|
| | Annual | 0.139 | 1.165 | 0.482 | 1.058 |
| | Spring | 0.382 | 3.210 | 0.006 | 2.487 |
| | Summer | 0.039 | 0.321 | 0.856 | 0.676 |
| | Autumn | 0.045 | 0.369 | 0.843 | 0.286 |
| | Winter | -0.331 | -2.783 | 0.081 | -1.209 |
| Brengi | Series\Test | Kendall's tau | Z-values | p-value | Sen's slope |
| | Annual | -0.180 | -1.505 | 0.339 | -1.013 |
| | Spring | -0.230 | -1.931 | 0.223 | -1.423 |
| | Summer | -0.398 | -3.352 | 0.040 | -3.086 |
| | Autumn | 0.173 | 1.449 | 0.075(0.1) | 1.106 |
| | Winter | -0.284 | -2.386 | 0.210 | -0.808 |
| Arapat-Kol | Series\Test | Kendall's tau | Z-values | p-value | Sen's slope |
| | Annual | -0.405 | -3.408 | 0.006 | -3.042 |
| | Spring | -0.355 | -2.982 | 0.016 | -3.233 |
| | Summer | -0.435 | -3.664 | 0.020 | -4.736 |
| | Autumn | -0.230 | -1.931 | 0.200 | -1.893 |
| | Winter | -0.412 | -3.465 | 0.012 | -2.471 |
| Rembiara | Series\Test | Kendall's tau | Z-values | p-value | Sen's slope |
| | Annual | -0.613 | -5.169 | 0.002 | -9.120 |
| | Spring | -0.506 | -4.260 | 0.006 | -9.501 |
| | Summer | -0.576 | -4.857 | 0.002 | -10.313 |
| | Autumn | -0.724 | -6.107 | 0.000 | -8.540 |
| | Winter | -0.741 | -6.249 | < 0.0001 | -8.053 |
| Sandran | Series\Test | Kendall's tau | Z-values | p-value | Sen's slope |
| | Annual | -0.297 | -2.499 | 0.040 | -2.851 |
| | Spring | -0.324 | -2.727 | 0.07(0.1) | -4.680 |
| | Summer | -0.543 | -4.573 | 0.005 | -6.444 |
| | Autumn | 0.039 | 0.312 | 0.816 | 0.222 |
| | Winter | 0.079 | 0.653 | 0.651 | 0.817 |
| Lidder(Gur) | Series\Test | Kendall's tau | Z-values | p-value | Sen's slope |
| | Annual | -0.385 | -3.238 | 0.042 | -2.329 |
| | Spring | -0.311 | -2.613 | 0.152 | -1.785 |
| | Summer | -0.576 | -4.857 | 0.001 | -5.704 |
| | Autumn | -0.405 | -3.408 | 0.037(0.1) | -1.593 |
| | Winter | -0.439 | -3.692 | 0.013 | -2.913 |
| Vishow | Series\Test | Kendall's tau | Z-values | p-value | Sen's slope |
| | Annual | -0.304 | -2.556 | 0.138 | -6.654 |
| | Spring | -0.274 | -2.301 | 0.114 | -7.591 |
| | Summer | -0.308 | -2.585 | 0.168 | -8.211 |
| | Autumn | -0.139 | -1.165 | 0.514 | -3.787 |
| | Winter | -0.254 | -2.130 | 0.260 | -6.731 |
| Lidder_Kirkadal | Series\Test | Kendall's tau | Z-values | p-value | Sen's slope |
| | Annual | -0.785 | -6.600 | < 0.0001 | -7.069 |
| | Spring | -0.852 | -7.186 | < 0.0001 | -11.541 |
| | Summer | -0.771 | -6.504 | < 0.0001 | -11.022 |
| | Autumn | -0.341 | -2.869 | 0.084(0.1) | -2.288 |
| | Winter | -0.304 | -2.556 | 0.188 | -0.791 |

spring (-31.78 mm/10yr) and the summer (-30 mm/10yr) seasons all of which show major declines. After taking the auto-correlation into account (Hamed 2008), none of the trends for Vishow stream could qualify to be significant. The pattern of all the residual trends can be seen in Table 4.

**Correlation of the streamflow with precipitation:** Correlation (Kendall and Pearson) was computed for the streamflow and the climate variables based on Theissen polygons (Fig. 4.). The correlation reveals that a strong association exists between precipitation and streamflow regimes (Fig. 5a.). The basin-wide correlation with precipitation is significantly high (r=0.80) in the entire UJC (Table 5). The annual precipitation shows significant correlation with the streamflow for entire UJC (Table 5). The Vishow (r=0.71) stream shows the maximum degree of correlation with precipitation in the spring season. Brengi (r=0.63) exhibits the highest correlation with the spring precipitation. Streamflow from almost all other streams also show significant positive correlation with the spring precipitation (Table 6). The Vishow (r=0.66) stream shows the highest correlation with summer precipitation. Autumn stream-flows in almost all the streams are significantly correlated with autumn precipitation. Vishow stream (r=0.79) has the highest correlation with autumn precipitation (Table 6).

**Table 5.** Catchment-wide correlation coefficients between climate and discharge for annual time-series (1980-2015) in UJC

| Precipitation | Mean maximum temperature | Mean minimum temperature |
|---|---|---|
| 0.802 | -0.663 | -0.238 |

**Correlation of the streamflow with minimum and maximum temperatures:** The correlation between the temperature and streamflow is typically negative (Fig. 5b.). With respect to the mean maximum temperatures the annual time series shows a strong inverse correlation (r=-0.66) with the basin-wide streamflow (Table 5). All the streams within

**Table 6.** Annual and seasonal correlation coefficients (Pearson and Kendall) between various climate parameters (temperature and precipitation) and streamflow data (1980-2015) in UJC

| Seasons | Parameters | Arapal | Arapat kol | Lidder Kirkadal | Lidder Gur | Brengi | Sandran | Vishow | Rambiara |
|---|---|---|---|---|---|---|---|---|---|
| Annual | Tmax | (-0.332) - 0.270 | (-0.534) - 0.381 | (-0.648) - 0.444 | (-0.480) - 0.416 | (-0.390) - 0.449 | (-0.411) - 0.414 | (-0.577) 0.452 | (-0.284) - 0.308 |
| | Tmin | (0.080) - 0.105 | (0.203) 0.075 | (-0.328) - 0.222 | (-0.174) - 0.162 | 0.128 -0.052 | (-0.139) - 0.43 | (-0.220) - 0.167 | (-0.362) - 0.118 |
| | ppt | (0.602) 0.411 | (0.606) 0.352 | (0.577) 0.422 | (0.558) 0.406 | (0.696) 0.473 | (0.481) 0.403 | (0.705) 0.549 | (0.365) 0.337 |
| Spring | Tmax | (-0.144) - 0.048 | (-0.370) - 0.239 | (-0.496) - 0.391 | (-0.230) - 0.191 | (-0.467) - 0.517 | (-0.255) - 0.226 | (-0.667) - 0.528 | (-0.385) - 0.308 |
| | Tmin | (-0.033) - 0.085 | (-0.047) - 0.078 | (-0.323) - 0.359 | (-0.138) - 0.155 | (-0.102) - 0.157 | (-0.108) - 0.109 | (-0.459) - 0.289 | (-0.398) - 0.329 |
| | ppt | (0.337) 0.186 | (0.474) 0.263 | (0.381) 0.346 | (0.334) 0.187 | (0.639) 0.435 | (0.276) 0.225 | (0.567) 0.443 | (0.465) (0.403) |
| Summer | Tmax | (-0.300) - 0.249 | (-0.300) - 0.227 | (-0.369) - 0.228 | (-0.305) - 0.247 | (-0.184) - 0.146 | (-0.26) - 0.195 | (-0.459) - 0.155 | (0.083) 0.079 |
| | Tmin | (-0.035) - 0.084 | (0.182) 0.050 | (-0.228) - 0.150 | (-0.089) - 0.093 | (0.046) 0.000 | (0.015) 0.118 | (-0.051) - 0.121 | (-0.052) - 0.103 |
| | ppt | (0.422) 0.234 | (0.596) 0.359 | (0.299) 0.229 | (0.286) 0.171 | (0.473) 0.403 | (0.477) 0.327 | (0.667) 0.371 | (-0.022) 0.087 |
| Autumn | Tmax | (-0.411) - 0.293 | (-0.167) - 0.269 | (-0.153) - 0.265 | (-0.185) - 0.293 | (-0.099) 0.079 | (-0.107) 0.221 | (-0.220) - 0.092 | (-0.018) - 0.129 |
| | Tmin | (0.250) 0.149 | (0.622) 0.094 | (0.021) 0.006 | (0.038) - 0.077 | (-0.682) 0.167 | (0.436) - 0.049 | (0.102) 0.190 | (0.074) 0.218 |
| | ppt | (0.549) 0.237 | (0.618) 0.175 | (0.396) 0.263 | (-0.133) - 0.022 | (0.730) 0.417 | (0.544) 0.178 | (0.792) 0.425 | (0.390) 0.125 |
| Winter | Tmax | (-0.540) - 0.363 | (-0.380) - 0.386 | (-0.267) - 0.165 | (-0.109) - 0.074 | (-0.27) - 0.159 | (-0.254) - 0.254 | (-0.215) - 0.062 | (-0.135) - 0.099 |
| | Tmin | (-0.155) - 0.076 | (0.010) 0.086 | (-0.159) - 0.088 | (-0.175) - 0.051 | (-0.042) - 0.020 | (-0.17) - 0.089 | (-0.069) 0.087 | (-0.117) - 0.111 |
| | ppt | (0.232) 0.035 | (0.435) 0.415 | (-0.089) - 0.008 | (0.406) 0.287 | (0.167) 0.148 | (0.346) 0.291 | (0.349) 0.213 | (-0.212) - 0.173 |

The values in parenthesis are the Pearson's correlation coefficient

UJC also show significant negative correlation with temperature annually. The Lidder (Kirkadal, r=-0.64) shows the highest negative correlation with annual mean maximum temperature. The basin wide correlation exhibited by the minimum temperature and stream-flow annually (Fig. 5b) reflects a very poor correlation (r = -0.238) in the catchment (Table 5). No significant correlation between minimum temperature and streamflow whether annual or seasonal, could be confirmed in individual streams as well (Table 6). The overall climatic impressions in the Upper Jhelum basin are of warming temperatures (both minimum and maximum) and decreasing precipitation. What follows from both the trend examinations and the correlation tests is that the period (1980-2015) in the UJC has been getting conspicuously warmer.

The warming trend is more pronounced in annual and spring time series. Precipitation does not show any significant change though it is in a decreasing continuum. Climatic variables are important determinants of hydrologic regimes. The reduction or enhancement in the amount of precipitation has a direct impact on the streamflow which reflects in its volume. Temperature has a more convoluted relation with streamflow, particularly in high mountainous regions which have a perpetual glacial cover. Rise in temperature increases the melting rate in glacial reserves but at the same time co-accelerates the evapotranspiration processes and enhances the consumptive use which consequently causes a decrease in the streamflow. Hydrological processes are complex interrelated phenomena affected by a variety of factors (quantifiable and non-quantifiable). However at the risk of oversimplification, it can be stated that the above analysis shows that the increasing temperature is negatively correlated with the depleting streamflow. Mean maximum temperature shows a stronger correlation with streamflow than mean minimum temperature. Evapotranspiration through the temperature rise seems to out-weigh its ablative facilitation of the ice reserves.

The degree of the positive correlation that exists between precipitation and streamflow in UJC must also not be overlooked. The positive correlation that exists between streamflow and precipitation and their identical demeanor with regards to trend analysis, is significantly strong to rule out the possibility of a co-incidental existence. In fact historical stream-flows have been found to be more correlated to precipitation and less with temperature (Chen et al 2014, Bawden et al 2013) besides being positively

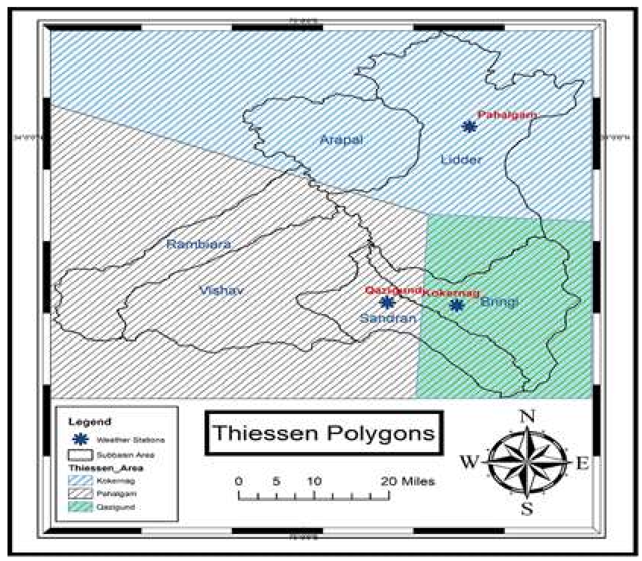


**Fig. 3.** Location and Theissen Polygons of IMD stations in UJC

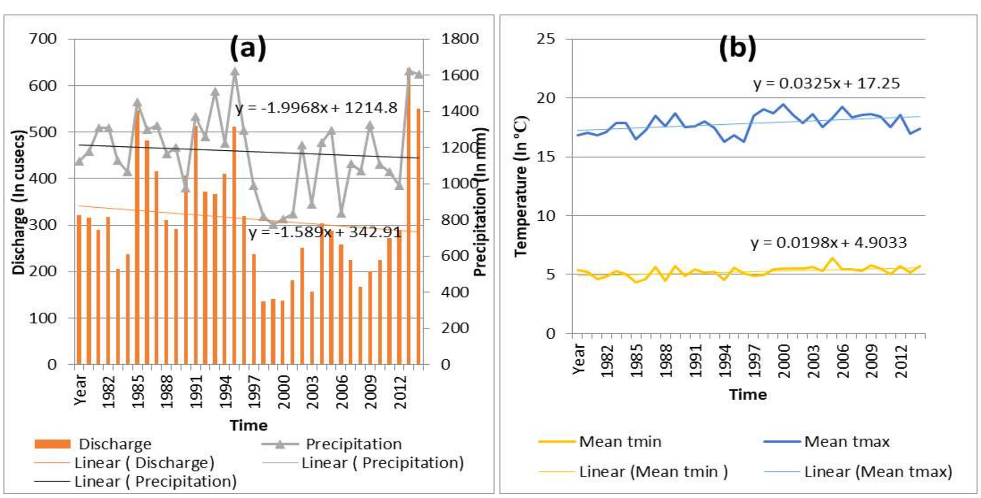


**Fig. 4.** Annual variations of streamflow, precipitation, mean minimum and maximum air temperature for the entire upper Jehlum Catchment

correlated with precipitation and negatively with temperature (Li and Jin 2017, Archer and Fowler 2008) and the present study is in line with above observations. Rise in temperature as reported by several studies (Shekhar et al 2010, Negi et al 2018) has been found out to be in conjunction with an increase in the discharge volumes (Miller et al 2012, Cavalcante et al 2019, Nepal et al 2015). However, the behavior of the streamflow in UJC with respect to the trends depicted is both contradictory and alarming, calling for immediate action as well as further research.

## CONCLUSION

Going by the preceding discussion it may be followed that there is enough statistical evidence pointing to the depletion of the streamflow in the UJC. This is significantly related to the increasing temperature and declining precipitation. The study although spanning over a long period of time and adopting data from multiple streams and weather stations may have inherent limitations on account of the agency mediated for database construction as well as the inherent uncertainties entailed in hydrologic analysis. Nevertheless, UJC is an important upstream catchment, and depletion of streamflow in such a catchment warrants widespread water stress in the catchment as well as the drown-stream areas. Growing population and un-checked water regulation systems in place may put the population and water crisis in a direct face-off. The most probing facet of the study is the rapid recession of glaciers and yet the lack of expression of the melt-down in the streamflow particularly summer flows. The results of the study call for integrated watershed development strategies that would encompass sustainable water resources usage and also guarantee support to the catchment as well as downstream population. An effective water regulation mechanism which involves structural as well as non-structural procedures should be devised for the planned and controlled flow pathways in the system and to sustain heavy flows hyphenated with judicious and sustainable usage of this precious natural resource.

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# Study on Mollusc *Teredo navalis* Linnaeus 1758 in Mangrove Vegetation Environment of East Halmahera, Indonesia

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***Abstract***: The aim of this study was to determine the ideal osmoregulation and salinity pattern for the growth of *T. navalis* in the mangrove trunk habitat. The research location consists of site I in the northern part of Wailukum Village and station II in the southern part of the mangrove waters, each station is 50 meters long. The study was conducted from June 2019 to December 2020. A sampling of 45 *T. navalis* measuring 70 cm on the mangrove trunks of *Rhizophora* sp and *Avicennia* sp. Samples of mangrove stems were taken with a size of 8 cm as many as 24 pieces, drinking water samples were 4 bottles. Determination of patterns based on osmotic workload using Automatic micro osmometer Roebling type 13/13 DR with three repetitions. Salinity was measured using a refractor meter. The osmotic workload varied, the highest was in *Avicennia* sp (294 mOsm/l $H_2O$) with media salinity of 25 ppt and the lowest was of *Rhizophora* sp (3 mOsm/l $H_2O$) with media salinity of 16 ppt. The ideal media salinity for *T. navalis* is 16 ppt. The pattern of osmoregulation of *T. navalis* in the mangrove trunks of *Rhizophora* sp and *Avicennia* sp is hyperosmotic, iso-hyperosmotic, and isoosmotic. To maintain the presence of *T. navalis* in the stem habitat, it is necessary to pay attention to the isoosmotic range of media salinity.

***Keywords***: Osmotic work level, *Teredo navalis*, Mangrove stem habitat, East Halmahera

The issue of the environment and nature conservation is currently an important theme in the international world. Indonesia is part of the international community which has abundant natural resources. On a state scale, the implementation of obligations and awareness of environmental sustainability is synchronized with sustainable development policies. Sustainable development is a conscious and planned effort that integrates environmental aspects into development strategies to ensure the integrity of the environment. The environmental protection and management plan (RPPLH) contains the potential, environmental problems and efforts to protect and manage them (KLHK Law 2009). Currently, most research topics in Indonesia are directed at developing coastal and tropical areas with the aim of making scientific contributions to areas that have the potential for coastal resources, especially mangrove ecosystems (Akbar et al 2018). Coastal resources that can be managed and developed according to environmental principles are mangroves (Utami et al 2017). Coastal and tropical areas are one of the mangrove vegetated environments that can be managed and developed sustainably (Endah Widiyanti et al 2018), especially in relation to the sustainability of organisms that live in mangroves (Puryono et al 2019). Mangroves in East Halmahera Regency are very potent and have a mangrove area of 5,389 hectares. The area has high biodiversity and forms an ecosystem that is still natural, one of which is fauna diversity. The East Halmahera mangrove resource that has the potential to be managed and utilized by local communities is the mollusk *T. navalis* Linnaeus 1758 (Sinyo et al 2020). *T. navalis* is an invertebrate animal belonging to the phylum Mollusca class Bivalvia which is also called mangrove worm ((Didžiulis 2011, Pati 2014, Swaim D 2017). *T. navalis* was first discovered in the 1930s to 1950s in coastal waters of Denmark, Sweden, and Germany. Furthermore, in 1969 until now *T. navalis* was found in Thailand, Australia and Indonesia (Lippert et al 2017). *T. navalis* belongs to a group of marine wood-boring organisms, known as worms. ships and spread all over the world from the poles to the tropics (Borges et al 2014, Treneman et al 2018). The physiological activity of *T. navalis* in wood was assisted by a unique endosymbiosis, namely bacteria (Teredinibacter turnerae) which produce cellulolytic enzymes (Distel et al 2002). In 2011 the presence and distribution of *T. navalis* in the Baltic sea was ecologically strongly influenced by the main environmental parameter, namely salinity (Hamdani, et al 2011). In 2014 the growth, reproductive development and fecundity of *T. navalis* (shipworm) in tropical Australia collected over a 12 month period, showed very rapid growth, precocious development and high reproductive yield with average body length in the spawning phase. 23.13 mm and reaches sexual maturity within 2 months, with a body length of 2-4 mm. It has a fecundity below 40 mm in length, and after the spawning phase *T. navalis* grows more fertile by a factor of ten, reaching a clutch size of 3 × 106 eggs with a length of 100 mm (Macintosh et al 2014). In 2019 the research was conducted in the Wailukum mangrove area, East Halmahera Regency. *T. navalis* was found to have three morphological characteristics based on body size, namely body length of 60 cm, 30 cm and 15 cm and the population density at the four research stations was found to vary between 1-2 ind/$m^2$. The highest population density is at site II (2 ind/m2) and the lowest at site III is 1 ind/$m^2$ (Sinyo et al 2019).It is interesting to study *T. navalis* in East Halmahera because this biota is very well known and favored by the people of East Halmahera and is used as food, as a substitute for side dishes, and as a source of medicines. Preserved *T. navalis* meat contains 13.30% protein, 44.73% carbohydrates, 1.057% fat content, and 0.056% crude fiber content (BBTPPI Testing and Calibration Laboratory, 2020). *T. navalis* is known as a euryhaline osmoregulator or organism that can adapt to salinity levels. Development and growth are influenced by the osmotic balance between body fluids and media. The effect of salinity on *T. navalis* can be direct or indirect. In most euryhaline animals, the direct effect of salinity acts by osmosis. Its effect on osmoregulation, food digestion, and absorption by animals (Anggoro and Nakamura 2009). In a wider salinity condition, it can result in mass death of *T. navalis* and its larvae caused by disturbances in the osmolarity balance (Sahri et al 2014); this will also affect the osmoregulation and bioenergy of *T. navalis.* Therefore to maintain its sustainability, *T. navalis* needs a good place to live and environmental factors that support especially salinity to carry out the reproductive process (Weigelt et al 2017). *T. navalis* has a high reproductive ability and is widely distributed according to its physiological tolerance (Appelqvist and Havenhand 2016). Reproduction of *T. navalis* requires osmoregulation to balance its osmotic pressure (Samudra and Anggoro 2020).

Salinity is the main variable in influencing the osmotic pressure of the body and the environment/media (Anggoro 2017). *T. navalis* requires regulation of the osmolarity of the medium and the balance of body fluids (hemolymph osmolarity) through the osmotic work level so that the ion content in the body of *T. navalis* will be the same as the ion content of the medium (Appelqvist et al 2015). The high salinity range causes the osmoregulation of the body of aquatic organisms to experience pressure and cause death (Anggoro, Suprapto et al 2018). Conversely, if the salinity level decreases, the organism will again experience osmotic pressure so that it must expend additional energy (Lestari et al 2017). The basic problem of this research is that the presence of *T. navalis* is very abundant in the mangroves of East Halmahera, but data on the osmoregulation mechanism of *T. navalis* has not been revealed so that a study is needed to become scientific information for the local community. The purpose of this study was to determine the effect of the osmotic level of salinity on the osmotic action and osmoregulation pattern of *T. navalis* in the stem habitat. The final objective of this research is to determine the ideal osmoregulation and salinity pattern that is suitable for the growth of *T. navalis* in the mangrove trunk habitat.

## MATERIAL AND METHODS

**Area of study:** Wailukum mangrove waters are productive mangrove areas and most of them are still natural. Administratively, the Wailkukum mangrove waters are located in the District of Maba City, East Halmahera Regency, North Maluku Province. Wailukum is about 9.4 kilometers from the center of Maba City and is located at an intersection between Buli Bay, Maba City, South Maba, Central Halmahera, and South Wasile. The geographical location is at latitude 0 37'25" - 0 55'10" north latitude and 1270 57'45" - 128'27'20" east longitude. Determination of research site using purposive sampling method based on water conditions at high tide and low tide. The research location was divided into two sites, namely site 1 in the northern part of Wailukum Village and site 2 in the southern part of the Wailukum mangrove waters (Fig. 1).

**Sample collection:** The research was carried out from June to December 2019. This research method is a case study with a descriptive analysis approach. Purposive sampling was done. The research location consists of two stations, namely in the North and South with a length of each station 50 meters (Fig. 1) which are determined based on water conditions at high and low tide. *T. navalis* was taken from each mangrove stem of *Rhizophora* sp and *Avicennia* sp cut 70 cm in size as many as 45 tails were taken using tongs, while samples of mangrove stem for analysis of media osmolarity were taken measuring 8 and water samples for measuring salinity were taken at two research stations (Fig. 2). Determination of the osmoregulation pattern based on the value of the osmotic work level obtained from the measurement of hemolymph osmolarity (body fluids) and media (stem) osmolarity using Automatic micro osmometer Roebling type 13/13 DR, with three repetitions. Salinity was measured using a refractor meter. All samples (*T. navalis*, mangrove stems, and samples of tidal water) into a coolbox and brought to the laboratory for examination of the osmolarity of the media and hemolymph following the instructions (Anggoro and Nakamura 2005).

**Data analysis:** Observations of osmotic work level carried out by measuring the osmolarity of the hemolymph and the osmolarity of the medium using an Automatic Micro Osmometer Roebling. The osmotic work level can be determined using the formula (Anggoro and Nakamura 2005):

TKO = (P osmo haemolymph – P osmo)

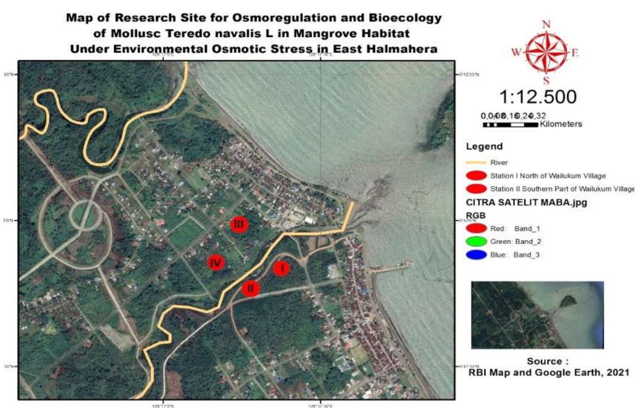


**Fig. 1.** Research site in the Wailukum mangrove forest area, East Halmahera, Indonesia

Where: TKO = osmotic work rate (mOsm/l $H_2O$);

P osmo hemolymph = osmotic pressure of body fluids (mOsm/l $H_2O$);

P osmo medium = osmotic pressure of the medium (mOsm/l $H_2O$).

## RESULTS AND DISCUSSION

**Osmotic regulation:** Osmotic work level (OWL) is the response or workload carried out by *T. navalis* to maintain a balance between the internal osmolarity of body fluids and the media/environment. The osmolarity of the *Rhizophora* sp medium ranged from 420 to 421 mOsm/l $H_2O$. The osmolarity of hemolymph *T. navalis* 450 to 452 mOsm/l $H_2O$ and salinity value of 15 ppt. From the osmolarity measurement, the osmotic workload range was 29 to 32 mOsm/l H2O. The osmolarity of the medium water is lower than the osmolarity of hemolymph, *T. navalis* has an iso-hyperosmotic osmoregulation pattern. The osmolarity of *Avicennia* sp media ranged from 420 to 421 mOsm/l $H_2O$ and hemolymph osmolarity 702 to 706 mOsm/l $H_2O$, the salinity was 24 ppt and an osmotic workload range of 282 mOsm/l $H_2O$ was obtained. The osmolarity of the medium is lower than the osmolarity of hemolymph. The osmoregulation pattern of *T. navalis* is hyperosmotic. The osmolarity value of *Rhizophora* sp media at site II of *Rhizophora* sp media's osmolarity ranged from 450 to 452 mOsm/l $H_2O$. The osmolarity of hemolymph *T. navalis* was at 455 mOsm/l $H_2O$, the salinity value was 16 ppt. The osmotic workload ranges from 3 to 5


Site I. In the northern part of Wailukum Village

Site II in the southern part of the mangrove waters

**Fig. 2.** Research sample site I and II

mOsm/l $H_2O$. The osmolarity of the medium and the osmolarity of the hemolymph are almost the same and the level of osmotic work is close to 0 so that *T. navalis* has an isoosmotic osmoregulation pattern. Meanwhile, the osmolarity of *Avicennia* sp media ranged from 450 to 452 mOsm/l $H_2O$ and the osmolarity of *T. navalis* 745 mOsm/l $H_2O$, salinity 25 ppt and an osmotic work level ranged from 206 to 294 mOsm/l $H_2O$. The osmolarity of the hemolymph is higher than the osmolarity of the medium. *T. navalis* has a hyperosmotic osmoregulatory pattern.

The study at low tide conditions at the site I, the osmolarity value of *Rhizophora* sp media ranged from 398 to 399 mOsm/l $H_2O$ and hemolymph osmolarity 416 to 417 mOsm/l $H_2O$. The calculation results obtained that the osmotic workload ranged from 17 to 19 mOsm/l $H_2O$ and a salinity of 14 ppt. The osmoregulation pattern of *T. navalis* is iso-hyperosmotic. Meanwhile, the osmolarity of *Avicennia* sp media was in the range of 396 to 397 mOsm/l $H_2O$ and the osmolarity of hemolymph *T. navalis* was 452 mOsm/l $H_2O$. The osmotic work level was obtained to 56 mOsm/l $H_2O$ and a salinity of 15 ppt with an iso-hyperosmotic *T. navalis* osmoregulation pattern. The osmolarity of the *Rhizophora* sp media at station II ranged from 417 to 418 mOsm/l $H_2O$ and the hemolymph osmolarity 449 to 451 mOsm/l $H_2O$, osmotic work level was 31 to 34 mOsm/l $H_2O$ and salinity 15 ppt. Iso-hyperosmotic osmoregulation pattern. The osmolarity of *Avicennia* sp media ranged from 418 to 421 mOsm/l $H_2O$ and hemolymph osmolarity 648 to 650 mOsm/l $H_2O$ and the osmotic work level ranged from 228 to 230 mOsm/l $H_2O$ and salinity of 21 ppt. Hyperosmotic *T. navalis* osmoregulation pattern. The measurement of the osmolarity of the media and the osmolarity of the hemolymph of *T. navalis* in each water condition are different so that the osmotic work level (OWL) is also varied (Fig. 3).

The differences in the osmolarity of the media and hemolymph in *Rhizophora* sp, both under high and low tide conditions at site I and II, resulted in varying osmotic work level (Fig. 4). The osmolarity *T. navalis* hemolymph is higher than the osmolarity of the medium (rod). The osmotic work level is highly dependent on the osmolarity of the hemolymph and the medium. The highest workload at high tide conditions was found at the site I, which was 32 mOsm/l $H_2O$ and the lowest was at site II, which was 3 mOsm/l $H_2O$. For low tide conditions, the highest osmotic workload was found at site II, namely 34 mOsm/l $H_2O$ and the lowest was at the site I, namely 17 mOsm/l $H_2O$. The osmolarity of the medium affects the body's osmoregulation system and determines the osmotic work level (Fitria 2012). At low tide conditions, the salinity decreases so that it affects the osmolarity of the media and the osmotic workload. Likewise with water conditions at high tide, in this condition, the salinity is in the range that gives life tolerance to *T. navalis*, while the osmolarity of the medium has a range that is almost the same as the osmolarity of hemolymph. This is in line with the statement that the range of salinity and osmolarity of media that is close to zero will result in an osmotic workload with an isoosmotic pattern (Riza et al 2020, Maulana et al 2013, Anggoro et al 2018). The movement of seawater due to tides affects salinity. The distribution of varying salinity is influenced by tidal currents (Septiani et al 2015). The osmotic work level is the workload to balance the total concentration of electrolytes dissolved in the body fluids of *T. navalis* with electrolytes dissolved in water media (Yuliani et al 2018). Differences in the osmotic work level can also occur in *Avicennia* sp mangrove species (Fig. 4).

The results of measurements of the osmolarity of *Avicennia* sp media and the osmolarity of hemolymph *T. navalis* in each water condition at site I and II resulted in

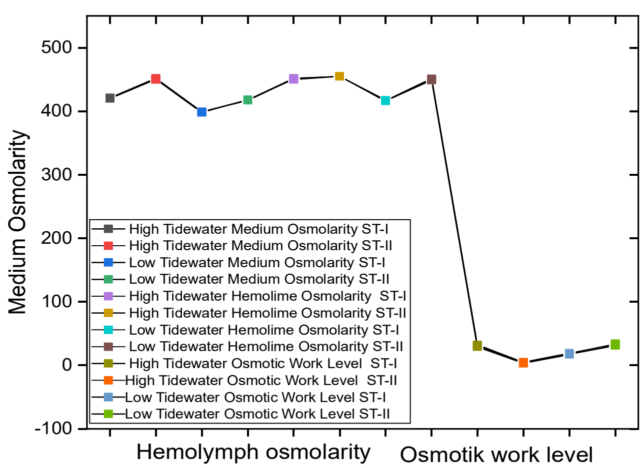


**Fig. 3.** Osmotic work level (OWL) *T. navalis* on stems of *Rhizophora* sp

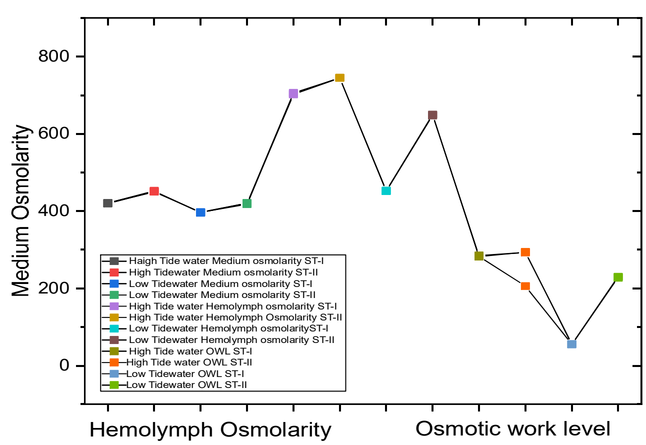


**Fig. 4.** Osmotic work level action *T. navalis* at *Avicennia* sp stems

different osmotic workloads. The highest and lowest working (load) levels for water conditions at high tide were at site II, 294 mOsm/l $H_2O$ and low tide 206 mOsm/l $H_2O$). For low tide conditions, the highest osmotic work level was at site II (230 mOsm/l $H_2O$) and the low tide was at site I (55 mOsm/l $H_2O$). The osmotic workload results from the osmolarity of the medium and the osmolarity of the hemolymph and are influenced by the salinity range. This shows that salinity decreases at low tide conditions so that it affects the osmolarity of the media and the workload of the osmotic level while high salinity is always in water conditions at high tide (Anggoro 2010). In line with the statement that the regulation of osmotic pressure (osmoregulation) is a very important and necessary process for the physiological activity of *T. navalis*. It is necessary because, firstly, the physiological process or biosynthesis requires osmotic pressure, secondly, the osmotic pressure of the environment is different from the osmotic pressure of body fluids which sometimes it is quite extreme, and thirdly there are parts of the body of *T. navalis* that allow the flow of material (fluid) into and out of the body through the gills (Taufik and Kusrini 2006).

Based on the osmotic workload on *Rhizophora* sp and *Avicennia* sp, *T. navalis* has an osmoregulatory pattern that is osmoconformer. Osmoconformers are aquatic organisms that are osmotically unstable and the osmotic pressure of their body fluids often changes to adjust to the osmotic pressure of their living medium. *T. navalis* is an osmoconformer determined by the value of its osmotic work rate which is less than 500 mOsm/l $H_2O$. This is in line with the statement if the range of TKO values <500 mOsm/l $H_2O$ is osmoconformer. if the OWL value> 500 mOsm/l $H_2O$ is expressed as an osmoregulator pattern (Anggoro et al 2018). The osmotic pressure of the environment that is close to the osmotic pressure in the body will produce relative ions with balanced concentrations (Saptiani and Mulawarman 2017). The osmotic work level serves to evaluate the osmotic response based on the need for osmoregulation in ecophysiological mechanisms (Romano and Zeng 2012). *T. navalis* has permeable properties to the environment and saline solutions (Lippert et al 2017). Differences in the physical properties of the environment cause the process of osmoregulation of *T. navalis* with its habitat (Rachmawati and Ka 2012). The osmosis process showed that the osmolarity of the hemolymph in the body of *T. navalis* was higher than the salinity of the medium (Palanti et al 2015). When salinity is in normal conditions, the osmoregulation process runs in balance (Yurisma 2013). Salinity affects the osmotic workload (Widodo et al 2011) (Table 1).

The salinity has a significant effect on the osmotic work level (Table 1) and difference in salinity affects the osmotic work level (OWL). *T. navalis* can survive if the salt concentration in body fluids is in the low range according to metabolic needs. (Widodo et al 2011). This is related to the salinity value of the media, the higher the salinity of the media (environment) will affect the osmolarity value of the media so that it becomes higher (Karim and Trijuno 2017). The higher the hemolymph osmolarity value, the lower the osmolarity and salinity values of the medium (Pamungkas 2012). The osmolarity of the medium is lower than the osmolarity of the hemolymph *T. navalis* causing the osmotic to work greater (Yulan et al 2013). This shows that *T. navalis* has the ability to regulate the concentration of body fluids against the concentration of the media (Sinyo et al 2019). *T. navalis* requires adaptation of osmotic regulation to increase salt and water ion uptake through gills (Barbieri et al 2019). *T. navalis*

**Table 1.** Values osmotic work level and salinity on stem media *Rhizophora* sp and *Avicennia* sp

| No | Sample code | Osmotic work level | Salinity (ppt) |
|---|---|---|---|
| | High tidewater (P):ST-1 | | |
| | *Rhizophora*-1 ( ST-1) | 32 | 15 |
| A | *Rhizophora*-2 (ST-1) | 29 | 15 |
| | *Rhizophora*-3 (ST-1) | 31 | 15 |
| | *Avicennia*-1 (ST-1) | 282 | 24 |
| | *Avicennia*-2 (ST-1) | 285 | 24 |
| | *Avicennia*-3 (ST-1) | 283 | 24 |
| | Haigh tidewater (P) ST-2 | | |
| | *Rhizophora*-1 (ST-2) | 5 | 16 |
| B | *Rhizophora*-2 (ST-2) | 3 | 16 |
| | *Rhizophora*-3 (ST-2) | 4 | 16 |
| | *Avicennia*-1 (ST-2) | 294 | 25 |
| | *Avicennia*-2 (ST-2) | 293 | 25 |
| | *Avicennia*-3 (ST-2) | 206 | 25 |
| | Low tidewater (S) ST-1 | | |
| | *Rhizophora*-1 (ST-1) | 19 | 14 |
| C | *Rhizophora*-2 (ST-1) | 17 | 14 |
| | *Rhizophora*-3 ( ST-1) | 18 | 14 |
| | *Avicennia*-1(ST-1) | 55 | 15 |
| | *Avicennia*-2 (ST-1) | 55 | 15 |
| | *Avicennia*-3 (ST-1) | 56 | 15 |
| | Low tide water (S) ST-2 | | |
| | *Rhizophora*-1 (ST-2) | 33 | 15 |
| D | *Rhizophora*-2 (ST-2) | 31 | 15 |
| | *Rhizophora*-3 (ST-2) | 34 | 15 |
| | *Avicennia*-1 (ST-2) | 230 | 21 |
| | *Avicennia*-2 (ST-2) | 230 | 21 |
| | *Avicennia*-3 (ST-2) | 228 | 21 |

when absorbs large amounts of seawater, the salt content will also be absorbed more and enter the body, causing the body to experience excess salt. Excretion of salt in large quantities is carried out by the gills through chlorite cells. Chlorite cells are cells that function to remove NaCl from plasma into seawater (Septiani et al 2015). The osmosis process causes differences in osmoregulation patterns (Fig. 5).

Different osmoregulation patterns are determined based on the high and low osmotic workload. The highest osmotic work level of *T. navalis* in the waters of East Halmahera was in the water at high tide at site II (*Avicennia* sp-1) 294 mOsm/l $H_2O$), followed by (*Avicennia* sp-1 (site-2), 293 mOsm/l $H_2O$) and the lowest was at *Rhizophora* sp-2 (site-2) 3 mOsm/l $H_2O$. The optimal level of osmotic work in this study was in media with ideal salinity of 16 ppt and a range of TKO was 3 to 5 mOsm/l $H_2O$. Meanwhile, the highest media salinity was in water at high tide at site II (*Avicennia* sp-1 (site-2) 25 ppt, followed by the site I (*Avicennia* sp-2 (site-1) 24 ppt and the lowest at low tide at site I (*Rhizophora* sp-1 (site-1) 14 ppt. The salinity of *T. navalis* in East Halmahera is high compared to the salinity of *T. navalis* in the Danish Baltic Sea, which is 8 ppt (Appelqvist et al 2015), but higher salinity in European coastal waters ranging from 7 to 39 ppt (Borges et al 2014) and salinity at the port of Visakhapatnam State of Andhra Pradesh along the Bay of Bengal on the east coast of India which ranges from 20 to 36 ppt (Pati 2014).

Salinity has a significant effect on the value of the level of osmotic work. The salinity range of the media that is close to isoosmotic can minimize the osmotic work level of *T. navalis*. Therefore, *T. navalis* requires an isoosmotic environment in order to live and grow normally (Sobirin et al 2014). The osmotic work level action can determine the pattern of osmoregulation. If the OWL is close to 0 then the osmoregulation pattern is isoosmotic, if the OWL value is greater then the osmoregulation pattern is hyperosmotic. There are two factors that influence the low osmotic work level, namely the first activity of the Na-K-ATPase enzyme is at a minimum level. Second, active ion transport and exchange of Osmo effector ions are at a low level (Novian et al 2013). The osmoregulation pattern of *T. navalis* in stem media of *Rhizophora* sp and *Avicennia* sp was mostly hyperosmotic, then Iso-hyperosmotic, and isoosmotic. The osmoregulation pattern that is hyperosmotic and iso-hyperosmotic aims to maintain a balance system between body fluids and media fluids. In this property, electrolyte exchange is carried out by means of active transport through the gills. The greater the difference in osmotic pressure between body fluids and the environment, the more metabolic energy needed to perform osmoregulation (Saptiani and Mulawarman 2017). If the osmolarity of the medium is the same as the osmolarity of hemolymph, then energy can be used optimally in the osmoregulation process and energy allocation for growth becomes more optimal (Maulana et al 2013). Novian et al (2013) observed that hyperosmotic and Iso-hyperosmotic regulation occurs when the osmolarity of body fluids is higher than the osmolarity of the living medium. To maintain relatively constant body fluids, *T. navalis* must carry out osmotic regulation and have the ability to undergo osmoregulation mechanisms in balancing osmotic pressure inside and outside the body (Maghfiroh et al 2019). *T. navalis* is a euryhaline species that can survive in various salinity media through an osmoregulation mechanism. However, the osmotic effect of water media on the osmoregulation of *T. navalis* still has a significant effect on its survival and vitality (Anggoro 2002). Meanwhile, hemolymph osmolarity always moves and changes according to the salinity of the medium. These changes were caused by the osmoregulation mechanism in *T. navalis* (Anggoro et al 2018).

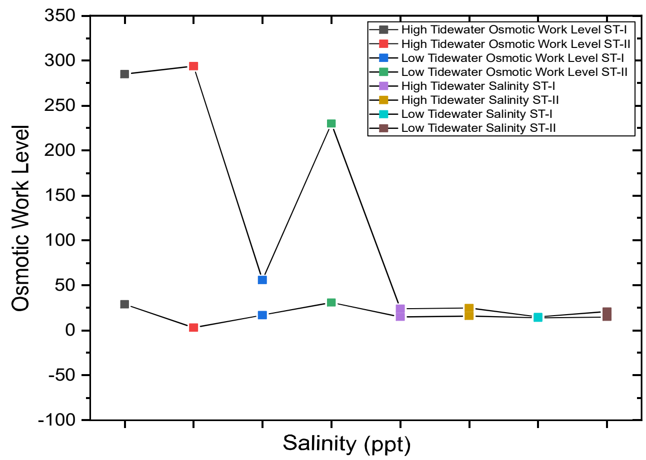


**Fig. 5.** Osmotic work level and media salinity

## CONCLUSIONS

*The isoosmotic media sa*linity range of 16 ppt has a significant effect on the level of osmotic work (TKO). The salinity range of the media that is close to isoosmotic can minimize the level of osmotic work of *T. navalis*. The highest osmotic workload is found in water conditions at station II tide of *Avicennia sp* (294 mOsm/l $H_2O$) with media salinity of 25 ppt and the lowest is *Rhizophora sp*. (3 mOsm/l $H_2O$) with a media salinity of 16 ppt. The ideal media salinity for *T. navalis* is 16 ppt. The osmoregulatory pattern of *T. navalis* inhabiting the mangrove trunks of *Rhizophora sp* and *Avicennia sp* was hyperosmotic, iso-hyperosmotic, and isoosmotic.

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# Chemical Composition and in Vitro Evaluation of Antioxidant and Antibacterial Activity of *Rosmarinus officinalis* L. Extract

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***Abstract*:** The present study was on extracting and identifying the secondary metabolites in the leaves of *Rosmarinus officinalis* L and evaluating their biological activities. Four compounds were identified in the methanolic extract: which include total polyphenols, anthocyanins, C -glycosides and aglycones. The content of total polyphenols were 12.87 mg EAG $g^{-1}$ dry matter. The anthocyanins, C-glycosides and aglycones were estimated as 1.13, 0.07 and 0.045 mg $g^{-1}$ respectively. The antioxidant activity of the compounds contained in the methanolic extract was evaluated in vitro by several tests. In the case of the DPPH assay, IC50 are 0.122, 0.130, 0.350 and 0.370 mg $ml^{-1}$ for antocyanins, total polyphenols, C-glycosides and aglycones respectively. For the FRAP test, at a concentration of 1 mg $ml^{-1}$, the reducing power of iron showed ODs of 2.54, 2.52, 1.98 and 1.62 for total polyphenols, anthocyanins, aglycones and C-glycosides, respectively. The antibiogram shows that anthocyanins and C-glycosides significantly inhibited the growth of two bacterial strains, causing zones of inhibition of 12, 25 and 14.5 mm in diameter for *Escherichia coli* and 10.5 and 13.75 mm for *Staphylococcus aureus,* respectively. The microdilution method in liquid medium conclude that the best minimum inhibitory concentration (MIC) for anthocyanins and C-glycosides on *Staphylococcus aureus* is 62.5 mg $ml^{-1}$ and 11.5 mg $ml^{-1}$respectively. MIC of 31.25 mg $ml^{-1}$ and 45.84 mg/ml was obtained for anthocyanins and c-glycosides against *Escherichia coli.* The study indicate that anthocyanins and total polyphenols show a good antioxidant activity. Anthocyanins and C-glycosides have an inhibitory effect on the growth of *Escherichia coli* and particularly on *Staphylococcus aureus* which is a sensitive bacterium. These results can be considered as very promising and justify further research, among others, on the identification of antioxidant and antimicrobial components in the active extracts

***Keywords***: Antimicrobial activity, Antioxidant activity, Chemical composition, Methanolic extract, *Rosmarinus officinalis* L.

Plants have long presented a very important role for mankind, as they can synthesize a large number of complex organic molecules often with potential biological activities. They contain secondary metabolites that can be defined as molecules indirectly essential to plant life. Polyphenols are one of the main classes of secondary metabolites that are generally located in different parts of the plant. These compounds are of great interest due to their numerous health benefits, prevention of the spread of the COVID-19 pandemic and treatment of certain cancers, treatment of inflammatory, cardiovascular and neurodegenerative diseases (Sharma et al 2021, Mir and Sehgal 2021). Some of them are also used as additives for the food, pharmaceutical and cosmetic. Polyphenols are commonly subdivided into tannins, lignins, flavonoids and anthocyanins, which are all derived from the assembly of phenolic units. In particular, flavonoids are known for their antioxidant, antibacterial, antiviral, anti-inflammatory, antiproliferative, enzymatic systems regulating properties etc. These activities are very often related to their antioxidant activity and in particular their ability to scavenge free radicals, chelate metal ions or inhibit enzymes responsible for radical formation (Leplat 2017). *Rosmarinus officinalis* L., belong to family Lamiaceae known as rosemary. This aromatic plant is native to the Mediterranean basin. Due to their strong antioxidant and antimicrobial properties, plants of the Lamiaceae family are one of the most frequently used botanical families worldwide (Casanova and Tomi 2018). In Algeria, this family is considered as one of the most important of the local flora in terms of diversity and representation. The aim of this study is to evaluate the antioxidant activity by the FRAP test and the DPPH test and antibacterial activity

## MATERIAL AND METHODS

**Study area:** The study area is located in the central part of the Tellian Atlas, in the north of Algeria, at a distance of 100km east of Algiers and west of the Djurdjura mountain range, between latitudes 36°20'N and 36°40'N and longitudes 3°40'E and 4°35'E. The species *Rosmarinus officinalis* L. was collected in April 2018, in Bastos in the region of TiziOuzou (Algeria) far from any pollution impact. The leaves of the plant were dried in a well-ventilated place and protected from light, crushed and stored in glass bottles in the dark. Preparation of the methanolic extract: 20g of leaf powder is macerated in 200 ml of methanol, for 24h at room temperature and under stirring. After filtration and evaporation of the solvent using a rotary evaporator, at 55°C, the extract is recovered with 3ml of demethyl sulfoxide (DMSO) and placed in the refrigerator at 4°C (Rebaya et al 2015).


**Source:** https://www.viamichelin.fr/web/Cartes-plans/Carte_plan-Tizi_Rached-_-Tizi_Ouzou-Algerie

**Fig. 1.** Location map of the sample collection area

### Determination of Total Phenolic Compounds

**Determination of total polyphenols:** The total phenol was determined with the Folin-Ciocalteu reagent according to the technique of Singleton (1999) using gallic acid as standard. This determination is based on the quantification of the total concentration of hydroxyl groups present in the extract. The Folin-Ciocalteau reagent is an acidic yellow solution containing a polymeric complex of ions (heteropolyacids). In alkaline medium, this reagent oxidizes phenols into phenolate ions and partially reduces its heteropolyacids resulting in the formation of a blue complex. The absorbance of the samples and standards is measured with a spectrophotometer at 760 nm. The total phenol content is given in mg gallic acid equivalent (GAE)/g dry matter.

**Determination of flavonoids:** The determination of flavonoids is performed according to the method described by Hertog et al (1992) using quercetin as standard. The flavonoids were quantified by a colorimetric method with aluminum trichloride ($AlCl_3$) and soda (NaOH). Aluminum trichloride forms a yellow complex with flavonoids and soda forms a pink complex. The absorbance of the samples and standards is measured with a spectrophotometer at 430nm. The flavonoid content is expressed per mg quercetin equivalent/g dry matter.

**Determination of proanthocyanins:** The reading of the extract is established by spectrophotometry at 520 nm and absolute content of anthocyanins was calculated by the following formula with a corrective coefficient of 6 (Lebreton et al 1967).

$$T \text{ (en mg/g)} = 5.2 \times 10^{-2} \times DO \times V \times d/p$$

**Determination of C-glycosyl flavones:** The reading of the extract is also established by spectrophotometry at 340 nm and the absolute content is calculated as:

$$T \text{ (en mg/g)} = 2.37 \times 10^{-2} \times DO \times V \times d/p$$

**Determination of flavonica glycones:** To determine the content of aglycones, method of differential dosage, which is based on two dilutions was used. The 1st dilution is done in ethanol (ethereal extract + 95° ethanol). The 2nd dilution is done in $AlCl_3$ solution (ethereal extract + $AlCl_3$ in 95° ethanol). The $AlCl_3$ solution is prepared by mixing 1g of aluminum chloride in 100ml of 95° ethanol. From the ethereal dry residue in 95° ethanol, 1ml of 1% aluminum chloride solution is added, after incubation for 15 min, at room temperature the absorbance of the aglycones is read between 400 and 435nm with a spectrophotometer. The estimation of flavonica glycones (420nm) and flavonols (435nm) was calculated as follow:

$$T \text{ (mg/g)} = 1{,}3 \times 10^{-2} \times \Delta DO \times V \times d/p$$

### Evaluation of the Antioxidant Activity of the Extract

**DPPH free radical scavenging test**: In the presence of free radical scavengers, the purple-colored DPPH (2, 2 diphenyl) was reduced to the yellow-colored 2.2 Diphenyl 1 picryl hydrazine). The DPPH radical scavenging activity is measured according to the protocol described by Lopes-lutz et al (2008). 1 ml of the extract at different concentrations (0.2, 0.4, 0.6, 0.8 and 1.2 mg/ml) was added to 1ml of the methanolic solution of DPPH. At the same time, a negative control is prepared by mixing 1ml of methanol with 1ml of the methanolic solution of DPPH. The positive control is represented by a solution of a standard antioxidant; ascorbic acid and after 30 min of incubation of the samples in the dark at room temperature, the absorbance is read against a blank prepared for each concentration at 517nm.The test is repeated three times and the results are expressed as percentage reduction of DPPH (I %) according to the following formula:

$$I\% = [1- (\text{Abs control} - \text{Abs test})/\text{Abs control}] \times 100$$

The concentrations to inhibit 50% of the initial DPPH concentration (IC50) were determined graphically by linear or logarithmic regression of the inhibition percentages as a function of the different concentrations of the tested compounds (Fabri et al 2009, Scherer and Godoy 2009).

**Iron reduction test FRAP:** The method is based on the reduction reaction of Fe3+ present in the potassium ferrocyanide complex to Fe2+. The reaction is revealed by the yellow color change of the ferric iron (Fe3+) to the blue-green color of ferrous iron (Fe2+) (Jayanthi and Lalitha 2011). One ml of the extract at different concentrations is mixed with 1ml of a 0.2 M phosphate buffer solution (pH= 6.6) and 1ml of a 1% potassium ferricyanide K3Fe (CN)6 solution. The whole is incubated in a water bath at 50°C for 20 min, then cooled to room temperature. 1mld of trichloroacetic acid (10%) is added to stop the reaction. The mixture is centrifuged at 3000rpm for 10 min at room temperature. To 1 ml of supernatant is added 1 ml of distilled water and100µl of 0.1% iron chloride solution (FeCl3, 6H2O) (Yildirim 2001). The absorbance of the reaction medium determined at 700 nm against a similarly prepared blank (distilled water). The positive control is composed of a standard antioxidant: ascorbic acid whose absorbance is measured under the same conditions.

**Determination of antibacterial activity:** The disc diffusion (antibiogram) and the microdilution method are used to evaluate the antibacterial effect of the different compounds contained in the crude extract of *Rosmarinus officinalis* L. leaves. Two reference bacterial strains from the Pasteur Institute (Algeria) are tested: *Escherichia coli* ATCC 25922 Gram negative and *Staphylococcus aureus* ATCC 43300 (Gram positive). From young colonies, from 18 to 24 h, a bacterial suspension is made in sterile distilled water for each strain. After homogenization of the bacterial suspension with a vortex, standardization to 106 CFU/ml was performed by spectrophotometer at a wavelength of 620nm. The OD obtained is between 0.08 and 0.1 which corresponds to a concentration of 107 to 108 CFU/ml (Colony Forming Unit). The two bacterial strains are subcultured by the streak method, then incubated at 37°C for 24 hours to obtain isolated colonies that will be used for inoculum preparation. Standard antibiotic discs (gentamicin at 10 µg/disc) are used as positive control.

**Diffusion method from disks-antibiogram:** Bacterial suspensions are prepared from media subcultured with the corresponding standardized strain corresponding standardized strain. Inoculation with previously prepared suspensions is performed by the swabbing method on the surface of petri dishes in which nutrient agar is poured. A few drops of the bacterial suspension (inoculum) are spread with a swab on the surface of the solidified agar. Discs impregnated with plant extract (10ml) are placed on the surface of the agar. Petri dishes are then kept at +4°C for 15 to 30 minutes to allow the extract to diffuse into the agar before the bacteria start to multiply. The plates are then placed in an oven at 37°C, for 24 h. Zones of inhibition around the point of application of the discs are measured in millimeters after 24h of incubation (Kosalec et al 2013). Methanol was used as a negative control in antibacterial tests. Gentamicin is used as a positive control. Bacterial susceptibility is estimated by measuring the diameter of the inhibition zone around the discs:-resistant bacteria: no inhibition zone, - sensitive bacteria: the diameter of the inhibition zone is equal or superior to 10mm, bacteria (intermediate): the diameter of the inhibition zone is less than 10mm

**Determination of the MIC (minimum inhibitory concentration):** The MIC is determined by the standardized method of micro-dilution in liquid medium. The concentration range of the tested extract was prepared by two dilution method (1/2, 1/4, 1/8, 1/16, 1/32) (Oussou et al 2004) from a stock solution of initial concentration of 500 mg /ml. All tests are performed twice. The different concentrations are deposited in the wells of a micro plate which is incubated for 18 hours at 37°C under aerobic conditions. The MIC in general is the lowest concentration of antimicrobial capable of inhibiting any visible growth after an incubation time of 18 to 24h (Basli et al 2012).

**Statistical analysis:** The statistical study is performed by EXCEL.STAT software

## RESULTS AND DISCUSSION

**Total polyphenol content:** The content of phenolic compounds is estimated using a linear calibration curve (Fig. 2). The determination of total polyphenols in the methanolic crude extract of *Rosmarinus officinalis L* reveals 12.87 mg gallic acid equivalent per g dry matter. The total polyphenols concentration in leaves (12.87 mg GAE/g dry matter) shows some similarity (12.6 g 100 $g^{-1}$ dry matter) with that evaluated by Sánchez-Vioque (2015) on *Rosmarinus officinalis* L., but remains relatively low compared to the Tawaha et al (2007), Tsai et al (2007), Erkan et al (2008) and Ho et al (2008). This difference is likely a result of the fact that the Folin-Ciocalteu

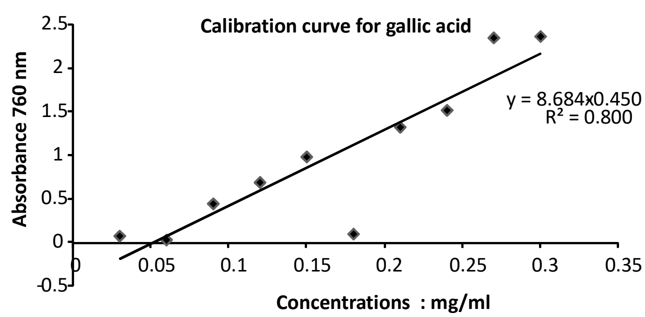



**Fig. 2**. Gallic acid standard curve for the determination of total polyphenols

reagent assay is not specific to polyphenols, but many compounds can react with this reagent, giving an apparent high phenolic level (Tawaha et al 2007). The total polyphenol concentration in the leaves (12.87 mg GAE/g dry matter) is not similar to Lamiaceae. The total polyphenol concentration in *Leonurus cardiaca*, *Lamium album Lamium purpureum* and *Salvia officinalis* was motherwort (0.200 g GAE /g dry matter), white nettle (0.192 g GAE /g dry matter), purple loosestrife (0.180 g GAE /g dry matter) and sage (0.132 g GAE /g dry matter) (Matkowski and Piotrowska 2006). Podsedek (2007) and Falleh et al (2008) observed that variability in results could be related to a number of intrinsic (genetic) and extrinsic (climatic conditions of the species' biotope) factors.

**Flavonoid content:** The anthocyanins represent the most important class of flavonoids with a value of 1.13 mg/g (91%). C-glycosides and aglycones present the lowest values with 0.07 mg/g (6%) and 0.045 mg/g (3%) respectively. The methanolic extract of rosemary is rich in total polyphenols (12.87 mg EAG/g dry matter) and poor in flavonoids and is in agreement with Tsai et al (2007) and Ho et al (2008),

**Table 1.** Contents of the three classes of flavonoids

| OD/T Extracts | | Optical density (OD) | Content (T) |
|---|---|---|---|
| Anthocyanins | | 0.310 | 1.13 mg $g^{-1}$ (91 %) |
| c-glycosides | | 0.088 | 0.07 mg $g^{-1}$ (6.0%) |
| Aglycones | Aglyconesflavoniques | 0.655 | 0.045 mg $g^{-1}$ (3 %) |
| | Flavonols | 1 | |

Athmena et al (2010) and Rao (2012) reported that the total flavonoid content in methanolic extracts of 28 plants is related to the content of total phenolic compounds. Bilusic Vundac et al (2007) also described a negative correlation between the content of total polyphenols of 7 species of *Stachys taxa* and flavonoids.

**Antioxidant Activity**

**DPPH test:** The percentage of free radical inhibition is proportional to the concentration of the compounds studied. DPPH test indicated concentration 1mg/ml, the total polyphenols exert a power of trapping the DPPH free radical at 70.25% while the anthocyanins inhibit this free radical at 60.63%. C-glycosides and aglycones is 58.26% and 59.90%, respectively. Ascorbic acid showed the highest activity (about 87% at a concentration of 0.5mg/ml) compared to total polyphenols, anthocyanins, C-glycosides and aglycones (Fig. 3, 4, 5, 6 and 7). IC50 expresses the quantity of antioxidants necessary to decrease the concentration of the free radical by 50%. The lower the IC50 value, the higher the antioxidant activity of a compound, which indicates the effectiveness of the compound (Bastos et al 2007).

The IC50 obtained by anthocyanins, total polyphenols, C-glycosides and aglycones was 0.122, 0.130, 0.350 and 0.370 mg/ml, respectively. Anthocyanins and total polyphenols showed the highest antioxidant. The IC50 level

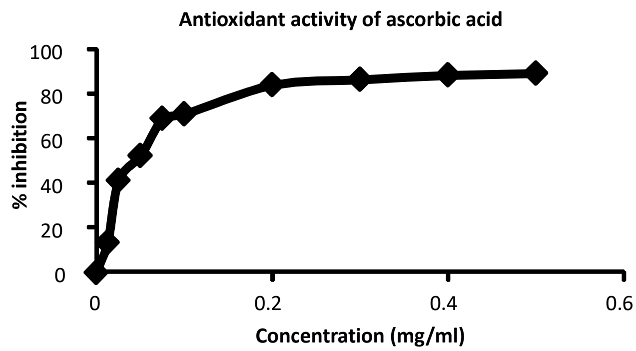



**Fig. 3.** Percentage of free radical inhibition as a function of ascorbic acid concentrations

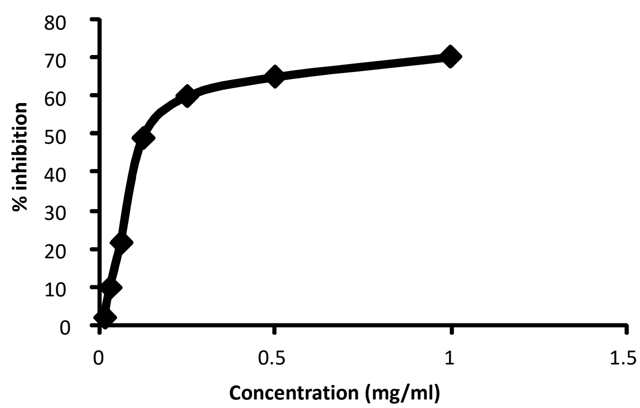



**Fig. 4**. Percentage of free radical inhibition as a function of total polyphenol concentrations

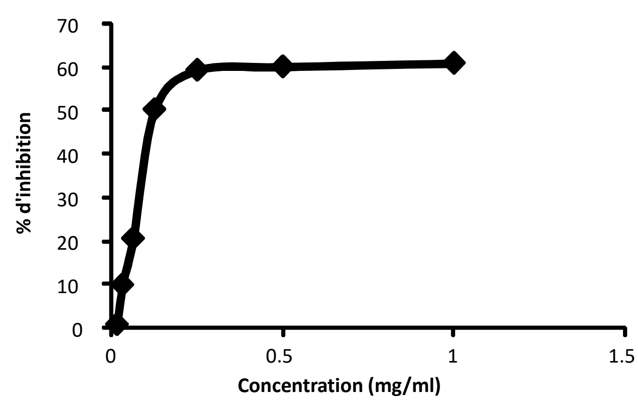



**Fig. 5.** Percentage of free radical inhibition as a function of anthocyanin concentrations

for all the compounds was significant, but remains low compared to that of ascorbic acid, (0.045 mg ml$^{-1}$) (Fig. 8). The results are in agreement with earlier workers (Torres de Pinedo et al 2007, Mariod et al 2009, Ba et al 2009, Locatelli et al 2010) where a positive correlation between the concentration of polyphenols in extracts of medicinal plants and the antioxidant activity, confirms that polyphenols are powerful antioxidants capable of inhibiting the formation of free radicals and oppose the oxidation of macromolecules. Falleh et al (2008) also showed that the antioxidant activity does not only depend on the concentration of polyphenols but also on the nature and structure of antioxidants in the extract.

**FRAP test:** The iron reduction capacity is proportional to the increase in the concentration of the compounds (Fig. 10). These results are in agreement with Ozturk et al (2007), Su et al (2008) and Liu et al (2009) which indicated any increase in sample concentration leads to the increase in iron reduction capacity. The a concentration of 1 mg/ml, total polyphenols show a high reducing power, with a maximum OD of 2.54 followed by anthocyanins with an OD of 2.517. C-glycosides and aglycones present the lowest OD of 1.62 and 1.98 respectively. But the data indicate that all our compounds

**Table 2.** IC 50 values of the different compounds (mg ml$^{-1}$)

| Extracts | Anthocyanins | Total polyphenols | C-glycosides | Aglycones |
|---|---|---|---|---|
| Ascorbic acid | 0.122<br>0.045 | 0.130 | 0.350 | 0.370 |

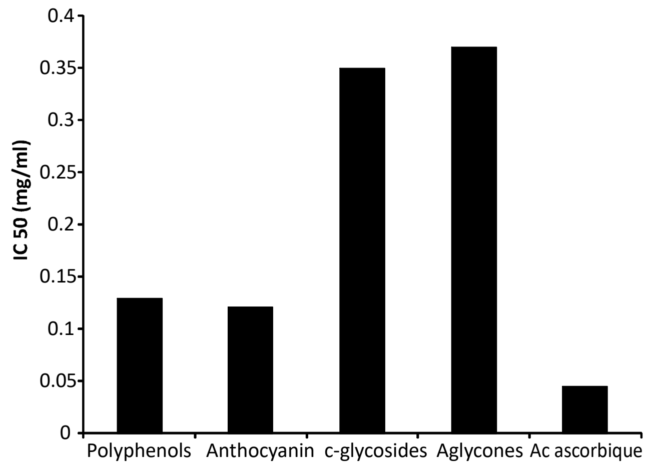



**Fig. 8.** DPPH radical IC50 inhibitory concentrations of the different compounds (mg ml$^{-1}$)

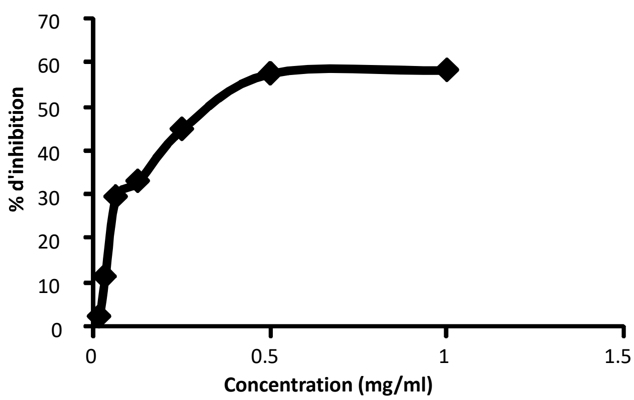



**Fig. 6.** Percentage of free radical inhibition as a function of C-glycosideconcentrations

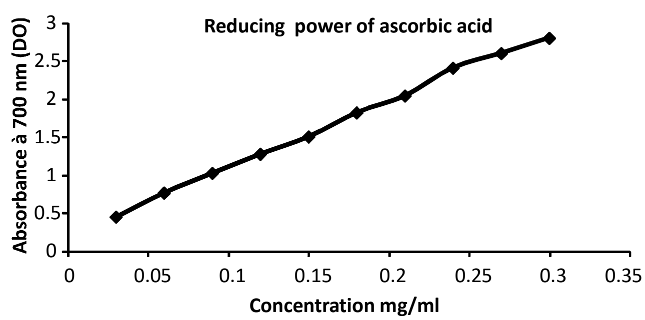



**Fig. 9**. Reducing power of ascorbic acid

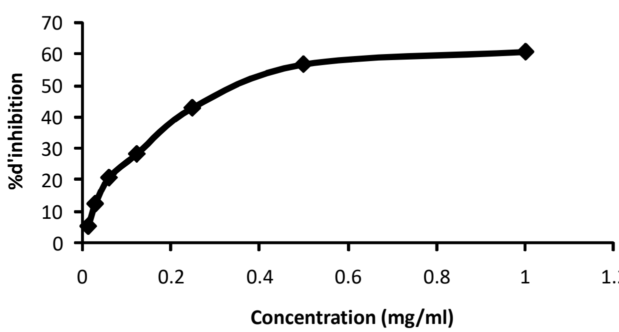



**Fig. 7.** Percentage of free radical inhibition as a function of aglycone concentrations

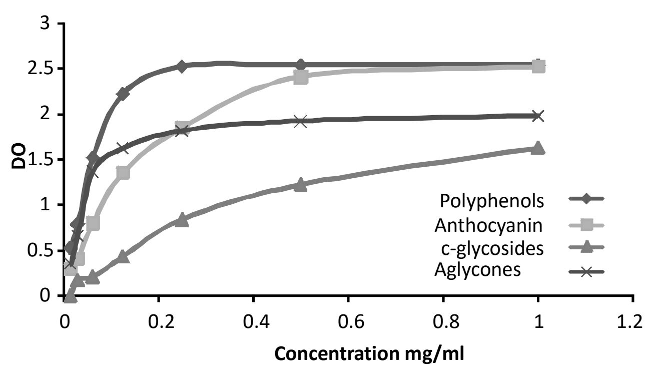
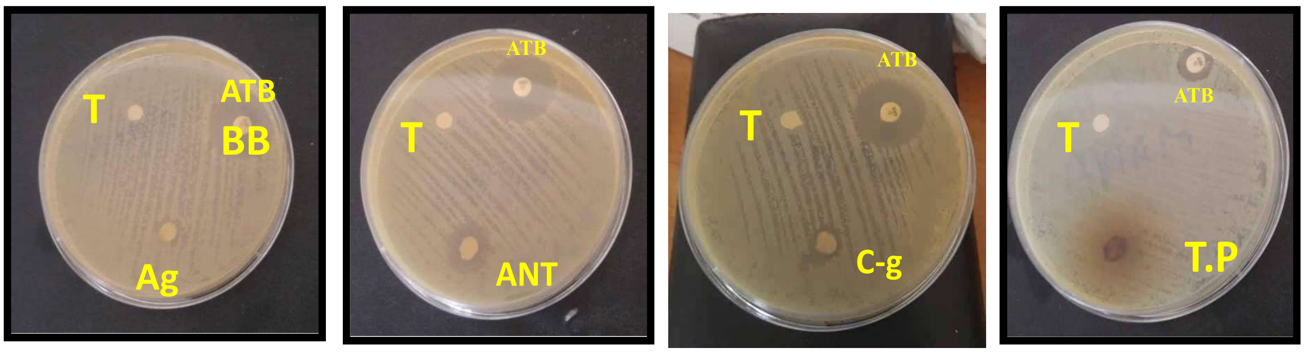



**Fig. 10.** Reducing power of Iron at the different compounds

have the ability to reduce iron and antioxidant activity remains low compared to ascorbic acid, which has an OD of 2.809 (almost complete reduction) from a concentration of 0.3 mg $ml^{-1}$ (Fig. 10). The reducing power of the different compounds is probably due to the presence of hydroxyl group in the phenolic compounds which can serve as electron donors. Therefore, antioxidants can be considered as oxidant reducers and inactivators of oxidants (Siddhuraju and Becker 2007).

**Disc diffusion method (Antibiogram):** The results of the antibacterial activity tested on the two bacterial strains *E. coli and S. aureus* are given in Figure 11 and 12. The results of the antibacterial activity revealed the effectiveness of all the compounds against the 2 tested strains, except for the total polyphenols (Tables 3, 4, Fig. 13, 14, 15). The presence of a weak activity on the bacterial strain *E. coli* whose diameter of inhibition is 6mm and a strong activity against the strain *S. aureus* (13,75mm). Aglycones develop medium inhibition zones (8.75 mm for *E. coli* and 10.5mm for *S. aureus*). High activity of anthocyanins and C-glycosides is observed on *E.coli* (14.5 and 10.5 mm, respectively) and on *S.aureus* (inhibition diameters were12.25 and 13.75 mm, respectively). This inhibition activity is quite close to that of the reference antibiotic whose inhibition diameters are 14.25




Ag: Aglycones, ANT: Anthocyanins, C-g: C-glycosides,T.P.: Total polyphenols, T: Negative control (Methanol), ATB: Antibiotic (gentamicin)

**Fig. 11.** Zones of inhibition of the different compounds tested on the *E.coli* strain

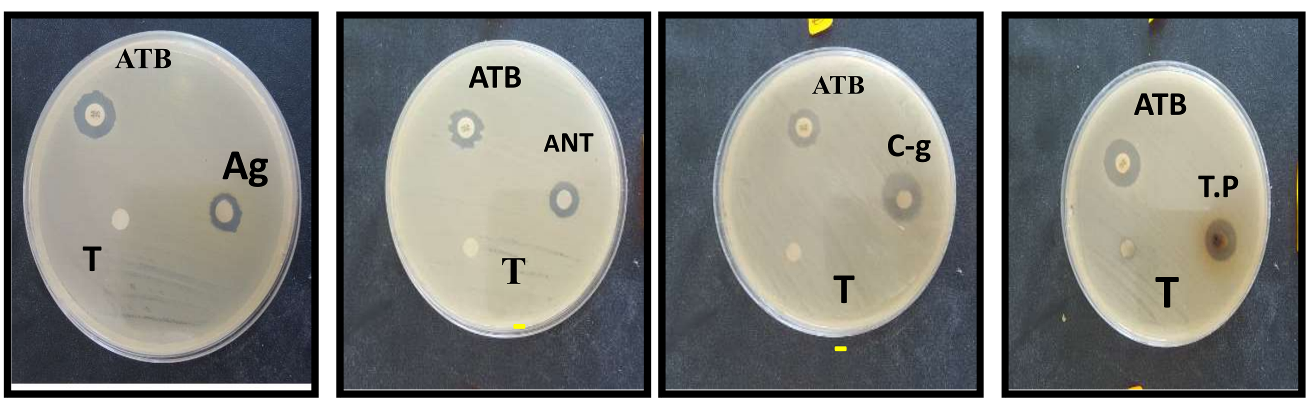



**Fig. 12.** Zones of inhibition of the different compounds tested on the *S. aureus strain*

**Table 3.** Antibacterial activity of the compounds on *E coli*

| Extracts/ATB | Total polyphénols | Anthocyanins | c-glycosides | Aglycones | Antibiotic ATB |
|---|---|---|---|---|---|
| Average +ES | 6±0 | 14.5±0.32 | 10.5±0.32 | 8.75±0.24 | 13±1.08 |

**Table 4.** Antibacterial activity of compounds on *S. aureus*

| Extracts /ATB | Total polyphénols | Anthocyanins | c-glycosides | Aglycones. | Antibiotic ATB |
|---|---|---|---|---|---|
| Average diameters | 13.75 | 12.25 | 13.75 | 10.5 | 14.25 |
| Standard deviation | 0.9574211 | 0.957 | 1.50 | 1.290 | 1.707 |
| Average +ES | 13.75±0.24 | 12.25±0.24 | 13.75±0.37 | 10.5±0.32 | 14.25±0.43 |

mm for *S. aureus* and 13 mm for *E. coli*. The anthocyanins are the most active against *S.aureus* and *E. coli*. The C-glycosides also showed an interesting inhibitory activity against both strains (Table 5). Moreover, a higher activity of rosemary compounds was observed against *Staphylococcus aureus*. The hypersensitivity of the *Staphylo coccusaureus* (gram+) strain can be attributed to the structural difference between gram-positive and gram-negative bacteria (David and Sudarsanam 2013).

**Determination of the minimum inhibitory concentration:** The MIC shows variability in the sensitivity of strains to different concentrations of compounds. The bacterial growth decreases with the increase of the concentration of the compounds. The lower the MIC, the better the antibacterial activity of the compounds. (Tables 6 and 7).

The screening of the antibacterial activity indicated that 1/8 is the minimal inhibitory concentration gives a response classified as sensitive for the *Staphylococcus aureus* strain to anthocyanins and to the 1/32 concentration for C-glycosides. *E.coli* strain is considered as sensitive to the 1/16 concentration for anthocyanins and to the 1/8 concentration for C-glycosides. This indicate that *S aureus* is more

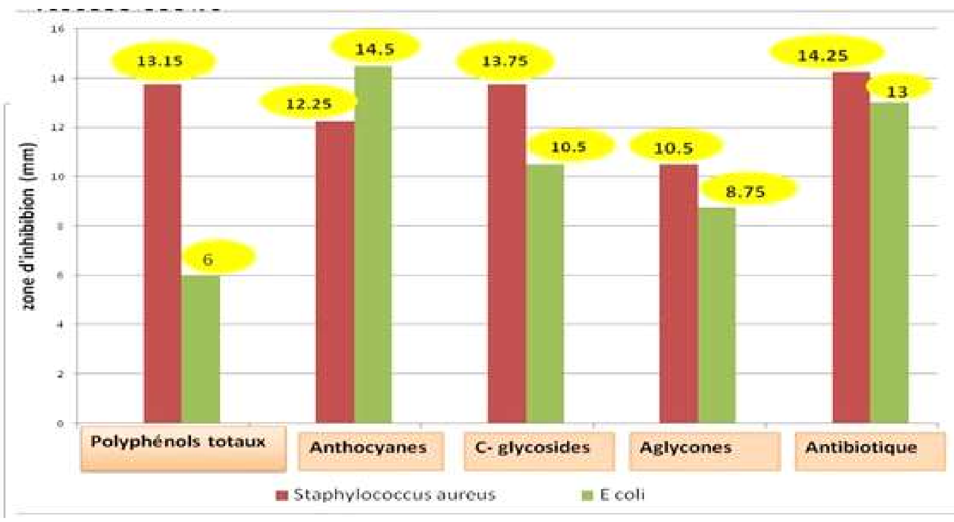



**Fig. 13.** Diameters of inhibition of the different compounds tested on the two bacteria

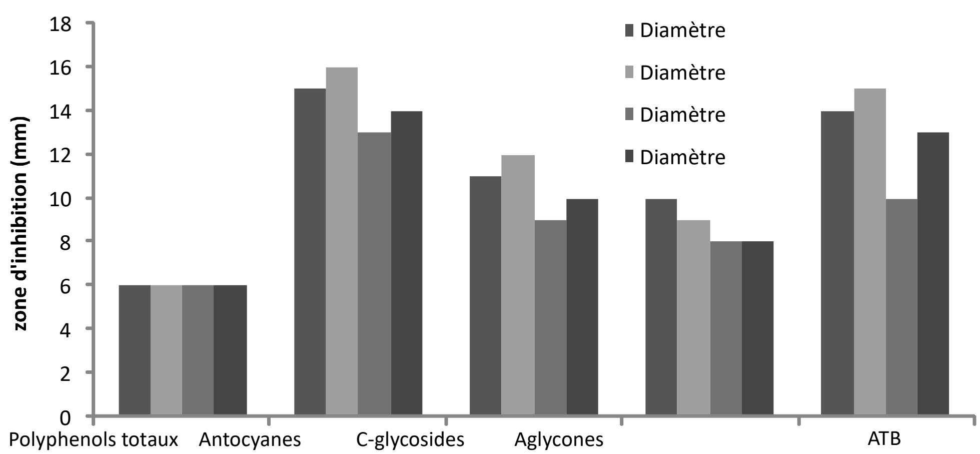



**Fig. 14.** Antibacterial activity of the different compounds on *Escherichia coli*

**Table 5.** Sensitivity of the two bacterial strains to the different *Rosmarinus officinalis L.* compounds

| Extracts/ATB Strains\bacterial | Total polyphénols | Anthocyanes | C -glycosides | Aglycones |
|---|---|---|---|---|
| *Staphylococcus aureus* | ++ | ++ | ++ | + |
| *Escherichia coli* | - | +++ | + | - |

Not sensitive (-), Sensitive (+), Very sensitive (+ +), Extremely sensitive (+++)

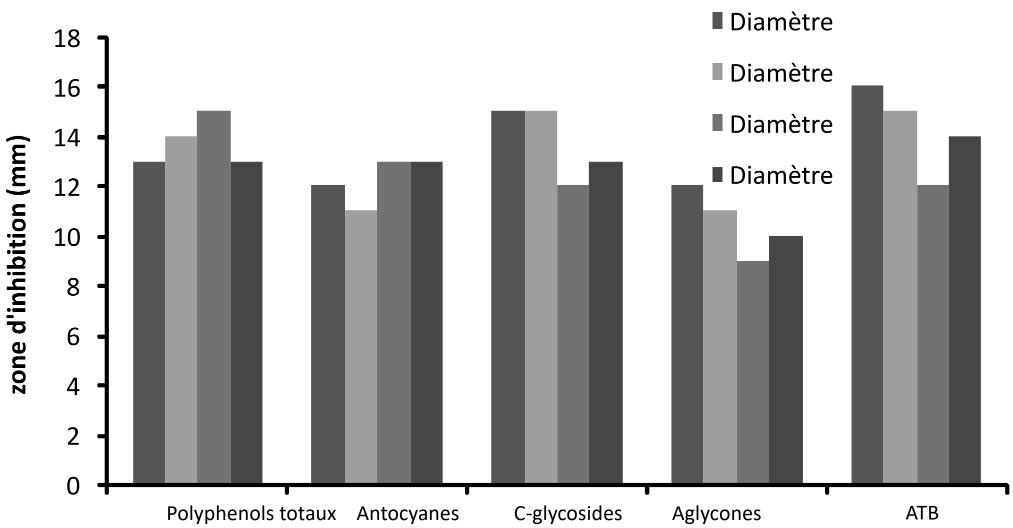



**Fig. 15.** Antibacterial activity of the different compounds on *S. aureus*

**Table 6.** MIC results for *Rosmarinus officinalis L.* compounds

| Extracts | Anthocyanins | | | | | | | c-glycosides | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dilutions mg $ml^{-1}$ | 1 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1 | 1/2 | ¼ | 1/8 | 1/16 | 1/32 | 1/64 |
| *S.aureus* | - | - | - | + | + | + | + | - | - | - | - | - | + | + |
| *E.coli* | - | - | - | - | + | + | + | - | - | - | + | + | + | + |

**Table 7.** MIC results in liquid medium (expressed in mg $ml^{-1}$)

| Extracts | *Escherichia coli* | *Staphylococcus aureus* |
|---|---|---|
| Anthocyanins | 31,25 | 62,5 |
| c-glycosides | 45,84 | 11,5 |

sensitive to anthocyanins (62.5 mg/ml) and *E.coli* to C-glycosides (45.84 mg/ml). Anthocyanins and C-glycosides could exert excellent antibacterial activity as long as they are not used as pure products but crude extracts (Sanogo 2014).

## CONCLUSION

The phytochemical study of the methanolic extract of *Rosmarinus officinalis* L leaves shows the presence of total polyphenols, flavonoids mainly anthocyanins, C-glycosides and aglycones. The antioxidant activity by DPPH and FRAP tests revealed that total polyphenols and anthocyanins show significant anti-radical activities. *Escherichia coli* and *Staphylococcus aureus* were sensitive to all compounds. However, the most active compounds against *S. aureus* and *E. coli* are the anthocyanins. The study concluded that anthocyanins could be a potential source for new antibiotics.

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# Rare Species of *Asteraceae* from Tlemcen Region (Western Algeria)

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***Abstract:*** This research aims to highlight the rare species of Asteraceae in the Tlemcen region and precisely the two stations Zarifet and Beni-Saf, based on the flora of Quezel and Santa (1962-1963). The presence of rare species in the two stations with degrees which vary from quite rare (AR) to rare (R) or even very rare (RR). The biological type Therophytes and Hemi-cryptophytes dominate the two stations and the total absence of Geophyte mainly due to various degradation factors (climatic and anthropogenic) leading to the almost complete disappearance of geophytes in the field. To better conserve rare and / or threatened species. need to better protect their natural biotopes.

***Keywords*:** Rarity, *Asteraceae*, Tlemcen, biological type, Protection

Vegetation is one of the main components in the structure and functioning of the ecosystem. The biodiversity crisis since the second half of the twentieth century is related to very high species extinction rates is of serious concern. The current rate of extinction of species is 100 to 1000 times higher than the casing rates of paleontological given (Hamel et al 2013). Rare species pose a high risk of extinction. The spatial distribution of threats to biodiversity is not random. Specific wealth and endemism rates are very high in some parts of the world known as "biodiversity hotspots".In Algeria 1670 species (or 53.20% of total wealth) are relatively infrequent aout of these 314 relatively rare species 590 rare species 730 very rare species and 35 rare species and nearly 700 species are endemic (Quezel 2000 and babali 2010). The 226 species are threatened with extinction and are legally protected. An integrated conservation strategy for taxa threatened or deemed of heritage interest must be based on a good knowledge of the autoecology and biology of rare species (Quézel and Médail 2003). In order to effectively protect these plants, governments and conservation agencies need to know which plant species are threatened and/or where can be found. The International Union for the Conservation of Nature and Its Resources established its Endangered Plants Committee in 1974 to gather this kind of information on all the world's flora.The criteria of the World Conservation Union are the internationally adopted benchmark are generally used to assess the vulnerability of plants at regional scales. Much of the work is being carried out in Natural Ecosystem Ecology and Management Laboratory on comprehensive vegetation inventories in the Tlemcen region. The objective of of present study is to determine the rare species of the Asteraceous family of the Tlemcen region and compare them with that of the flora of Quezel and Santa 1962-1963.

## MATERIAL AND METHODS

The study area is located in the western part of Algeria's northwest and largely covers the wilaya of Tlemcen (Zarifet Station) and a station in the wilaya of Ain Témouchent (Beni-Saf) (Fig. 1). It is located between 34-25' and 35'25' north latitude and 0-55' and 2-30' west longitude, with an area of 9017.69 km². It is geographically limited in the north by the Mediterranean Sea the northeast by the wilaya of Ain Témouchent, east by the wilaya of Sidi Bel-Abbés, west by the Algerian-Moroccan border and south by the wilaya of Naama

**Station 1 : Beni-Saf (Sid Safi) :** Located north of the Traras Mountains near the Beni-Saf cement plant has a fairly varied vegetation with a recovery rate of 50-60%, on a slope of 10 to 15%. The substrate is silica. The plant species that dominate this station are: *Centaurea aspera* (*Astéraceae*). *Brachypodium retosum* (*Poaceae*). *Phillyrea angustifolia* (*Oleaceae*).et *Quercus coccifera* (*Fagaceae*). This station is marked by the presence of species indicating a degraded matorral on limestone substrate : *Centaurium umbellatum. Teucrium pollium. Daucus carota* et *Asparagus acutifolius, Rosmarinus officinalis, Ononis spinosa.*

**Station 2 : Zarifet :** This matorral is located in the southwest of the city of Tlemcen in an area of 931 hectares and is bounded to the north by the territory of Mansourah and Beni-Mester, to the south by the municipality of Terny, to the east by the municipality of Mansourah and to the west by the Hafir State Forest. (Haffaf 2011). Its substrate is silica marked by a micro relief with outcrops of the parent rock. The slope is 30%


**Fig. 1.** Geographical location of study stations

lower and the recovery rate is 70-80% with a tree layer of 20-25% of its total area. This station is marked by the old stands of *Quercus suber* from mediocre tailings strains of *Quercus faginea* and *Quercus ilex*. The vegetation is composed by a floristic mosaic where the following species dominate: *Quercus suber, Phillyrea angustifolia, Erica arborea, Globularia alypum, Lavandula stoechas, Ampelodesma mauritanicum and Asphodelus microcarpus.*

From a bioclimatic point of view, the rain-thermal clima gram (shows that the Zarifet station characterized by a semi-arid climate superior to cool winter to temperate while the Beni-Saf station is under stage semi-arid bioclimatic in temperate winter.

The seasonal study indiacte the dominance of winter followed by autumn, spring and finally summer. To calculate the frequency of each species and deduce the degree of rarity, 100 floristic surveys for the stations (Beni-Saf; Zarifet) were carrid out in March 2019. The survey area is 100 m² and each survey was carried out using the stigmatic method (Braun Blanquet 1951). The frequency of each species in the 100 surveys was calculated, divided the taxa in rarity classes (Table 1) and finally compare them with the rare species of flora of Quezel and Santa (1962-1963).

E = Endangered: Taxing on the way to extinction or whose survival is unlikely if the factors responsible continue to act.

VU = Vulnerable taxons, which are expected to be in the "endangered" category in the near future if risk factors persist.

A = Rare taxon that is not currently in danger or vulnerable, X=taxon presumed missing.

The regional assessment is based on the analysis of plant distribution within the UTM 10 km x 10 km mesh (10 kilometre side mesh) that simplifies the representation of a species' distribution by its presence or absence within each of the 388 mesh. (Gérard and Pascal 2008). The current relative frequency of plants within the UTM 10 x 10 network allows taxa to be distributed in rarity classes (Table 1). *Rare species* :A comparative study of the rare species of Zarifet with the Flora of Quezel and santa 1962-1963 shows a decrease of these species in Zarifet and a total absence of common species. The majority of rare species (R) of the flora of Quezel and Santa are *Evax argentea; Bellis sylvestris var papullosa et Sanchus arvensis* becomes Very Rare (VR) in the resort of Beni-saf.

**Common species:** Fairly Common and/or Very Common (FC; CC; VC) of the flora of Quezel and Santa include *Senecio vulgaris ; Picris duriaei ; Reichardia picrioides ; Pallenis spinosa ; Inula montana* are currently Quite Rare; rare and/or very rare with a recovery rate ranging from 3.12% to 6.25% according to the CBNB Red Lists of Threatened Species (2001) (Table 1).

This change in the distribution of species over two different periods can be explained by:

- The station is subject to several environmental degradation factors (grazing, grazing, crops and repeated fires).
- Climate change is reflected in the decrease in precipitation rates and rising temperatures are reducing or even disappearing the majority of hygrophile species and the installation of xerophyte species.
- The advanced degradation of this forest formation towards sparse matorrals training explains the absence of common species in this station.

The Zarifet station has characteristics of a forest formation. These species, which are common according to the flora of Quezel and Santa 1962-1963, are currently

**Table 1**. Rarity categories selected by the CBNB for the development of red lists (2001)

| Category of Rarity | Relative frequency of taxa (Asa % of the taxon mesh) |
|---|---|
| Very Common (VC) ≥ 75 % | ≥ 75 % |
| Common (C) | ≥ 50 et < 75% |
| Fairly Common (FC) | ≥ 25 et < 50% |
| Unusual (U) | ≥ 12.5 et < 25% |
| Fairly Rare (AR) | ≥ 6.25 et < 12.5% |
| Rare (R) | ≥ 3.12% et < 6.25% |
| Very Rare (TR) | < 3.12% |
| Not Reported Recently (NRR) | 0% |

becoming very rare and rare due to the intense anthropogenic action on forest formation including the arson and involuntary fires on them. For Beni-Saf, the AR degree remains constant only for the station as well as the flora of Quezel and Santa with 14% while the very common species (CCC), (CC) and (AC) have become AR in 2019 as (*Asteriscus maritimus*); rare as: *Centaurea pullata* , *Micropus bombycinus* and very rare as: *Rhagadiolus stellatum* , *Reichardia picrioides. The* very rare species decrease in the number. The transformation into a species: AR, R , RR is explained by:

The position of the station next to the Sid-Safi cement plant removes from a very high number of the demanding species of Asteraceae and the persistence of some tolerant species.- Species that have a degree of rarity "RR" and become "R" can be explained either by the presence of some stational ecological factors favouring the installation of these (a high rate of precipitation acquired by clearing phenomena) and/or where are protected by the thorny shrub stratum and the tree stratum.

For their biological types the dominance of therophytes in both stations with a percentage of 39 to 59% was observed (Table 4 and Fig. 4). Therophytes are represented by species very common to quite common for the flora of Quezel and Santa 1962-1963 and quite rare to very rare for data recently

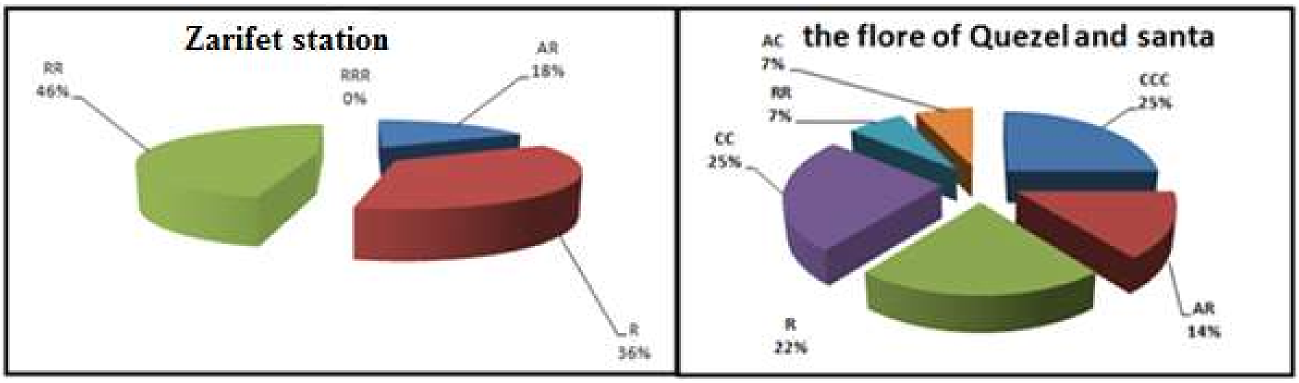


**Fig. 2.** Degree of rarity of the resort of Zarifet and the Flora of Quezel and Santa 1963

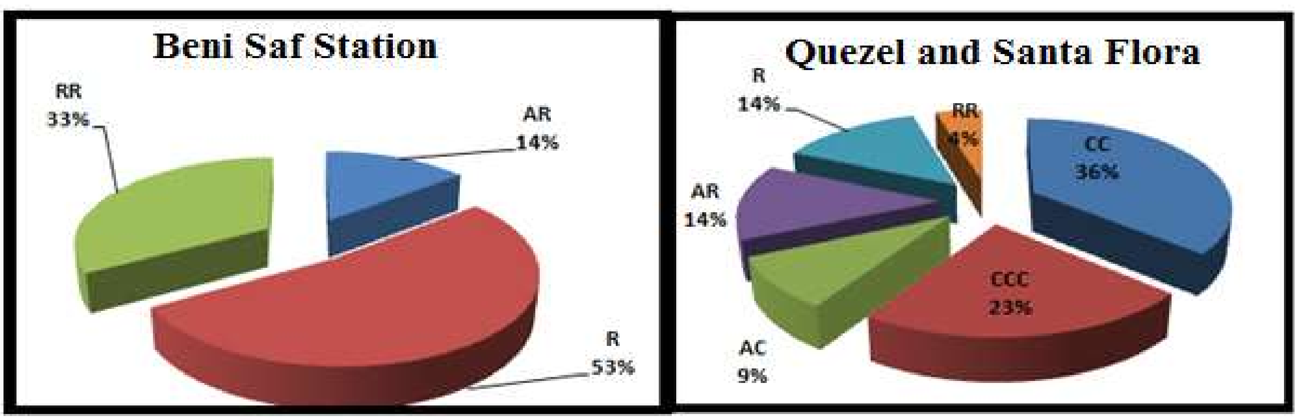


**Fig. 3**. Degree of rarity of the resort of Beni-Saf and the Flora of Quezel and Santa 1963

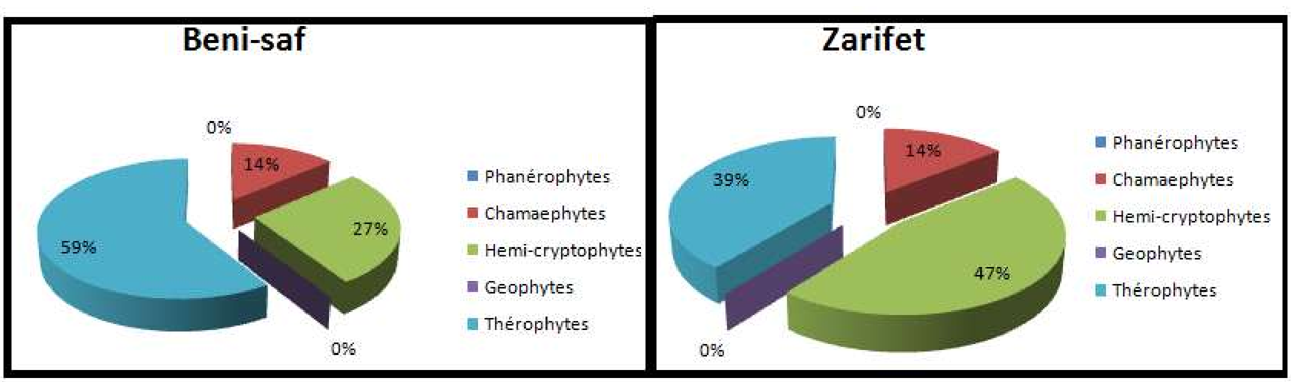


**Fig. 4.** Biological types of study stations

**Table 2**. Rare species inventory of the Beni Saf station

| Species genres | Frequency of Rarity | Category of Rarity | Rarity according to Quezel and Santa 1962-1963 | Biological Type | Morphological Type |
|---|---|---|---|---|---|
| *Anacyclus radiatus* L. | 3.10 | RR | AR | Therophyte | Annual Herbaceous |
| *Asteriscus maritimus* (L.) Less. | 6.31 | AR | CCC | Chamaephyte | Perennials |
| *Bellis annua* L. | 6.31 | AR | R | Therophyte | Annual Herbaceous |
| *Carduus pycnocephalus* L. | 2.45 | RR | CC | Hemicryptophyte | Annual Herbaceous |
| *Catananche coerulea* L. | 5.26 | R | R | Hemicryptophyte | Perennial Herbaceous |
| *Centaurea incana* L. | 4.56 | R | CC | Hemicryptophyte | Annual Herbaceous |
| *Centaurea pullata* L. | 3.50 | R | CCC | Therophyte | Annual Herbaceous |
| *Chrysanthemum coronarium L.* | 5.26 | R | CC | Therophyte | Annual Herbaceous |
| *Chrysanthemum grandiflorum* L. Batt | 2.80 | RR | CC | Hemicryptophyte | Perennial Herbaceous |
| *Gnaphalium luteo-album* L. | 4.56 | R | AC | Therophyte | Annual Herbaceous |
| *Micropus bombycinus* L | 5.61 | R | CCC | Therophyte | Annual Herbaceous |
| *Pallenis spinosa* (L) *Cass* | 2.10 | RR | AC | Therophyte | Perennial Herbaceous |
| *Rhagadiolus stellatus* L | 3.10 | RR | CCC | Therophyte | Annual Herbaceous |
| *Reichardia picrioides* (L) *Roth* | 2.80 | RR | CCC | Therophyte | Perennial Herbaceous |
| *Reichardia tingitana* (L) *Roth* | 5.51 | R | CC | Therophyte | Annual Herbaceous |
| *Senecio vulgare* L | 4.21 | R | CC | Therophyte | Annual Herbaceous |
| *Taraxacum officinalis* L | 3.50 | R | TR | Hemicryptophyte | Annual Herbaceous |
| *Tolpis barbata* L | 4.91 | R | CC | Therophyte | Annual Herbaceous |
| *Xeranthemum inapertum* L | 1.40 | RR | CC | Therophyte | Annual Herbaceous |
| *Artemisia herba alba* L | 6.66 | RR | AR | Chamaephyte | Perennial Herbaceous |
| *Serratula cichoracea var propinqua* (pomel)M | 3.5 | R | R | Hemicryptophyte | Perennial Herbaceous |
| *Catananche caespitosa* L | 5.26 | AR | AR | Chamaephyte | Perennial Herbaceous |

inventoried in 2019. The Phanerophytes are totally absent for the two study stations including the flora of Quezel and Santa 1962-1963. Chamaephytes are represented by either common species where many species are quite rare and which have become rare and/or very rare for the two selected stations. Hemi-cryptophytes dominate the field as perennial species are, even if conditions are unfavourable; the sub-field part keeps the species alive slowed down and waits for the conditions of the environment to return favourable. The majority of these Hemi-cryptophytes are very common or even common in the flora of Quezel and Santa while currently become quite rare; rare or even very rare. Executive Decree 12-03 of SAFAR 1433, corresponding to 4 January 2012, determined the list of uncultivated plant species protected. Among the species cited in this decree and which are present in the Tlemcen region are *Filago fuscescens, Filago pomelii , Centaurea phaeolepsis*Among the species quoted in the decree and which present in stations are :*Bellis annua, Centaurea dimorpha.* The genera cited in the decree and are present in our region include *Atractylis, Centaurea ;Chrysanthemum , Crepis , Sartula , Euvax , Senecio and Taraxacum.*

## CONCLUSION

Despite the influence of various ecological, climatic and anthropogenic factors on the Tlemcen region. The latter remains a very important pole and model in terms of biodiversity and spatial and climatic floristic heterogeneity. This work was carried out with the aim of inventing the rare species of the Ateracaea family between 1962-1963 and the 2019 years at the Tlemcen Mountains. Scarcity increases in almost all types. On the other hand, the extinction rate shows a marked increase only among the most vulnerable types (Geophytes). This conservation and protection of endangered taxa requires ecological planning and rigorous management. Given the seriousness of this ecological situation in the Tlemcen region, it is necessary to establish an action plan for the preservation of the plant mat and biodiversity.We would the likely to see more enthusiastic support from the national authorities, particularly with regard to species protected by law. Similarly, regional authorities (forest conservation - National Park) are called upon to support scientists and

**Table 3.** Rare species inventory of the Zarifet station

| Species genres | Frequency of Rarity | Category of Rarity | Rarity according to Quezel and Santa 1962-1963 | Biological Type | Morphological Type |
|---|---|---|---|---|---|
| *Asteriscus maritimus* (L) *Less.* | 1.23 | RR | CCC | Chamaephyte | Perennials |
| *Atractylis cancellata* L | 5.55 | R | CCC | Therophyte | Perennial Herbaceous |
| *Atractylis gummifera* L | 2.46 | RR | AR | Hemicryptophyte | Perennial Herbaceous |
| *Atractylis humilis* L | 5.55 | R | CC | Chamaephyte | Perennial Herbaceous |
| *Bellis annua* L | 1.85 | RR | R | Therophyte | Annuel Herbaceous |
| *Calendula arvensis* L | 8.64 | AR | CCC | Therophyte | Annuel Herbaceous |
| *Carduus pycnocephalus* L | 7.40 | AR | CC | Hemicryptophyte | Annuel Herbaceous |
| *Carlina racemosa* L | 5.55 | R | CCC | Hemicryptophyte | Annuel Herbaceous |
| *Carthamus caeruleus* L | 1.85 | RR | CCC | Hemicryptophyte | Perennial Herbaceous |
| *Centaurea dimorpha Viv.* | 3.08 | RR | CC | Hemicryptophyte | Annuel Herbaceous |
| *Centaurea parviflora Desf.* | 3.70 | R | AR | Hemicryptophyte | Annuel Herbaceous |
| *Centaurea pungens Pome*L | 4.93 | R | RR | Therophyte | Annuel Herbaceous |
| *Chrysanthemum coronarium* L | 7.40 | AR | CC | Hemicryptophyte | Perennial Herbaceous |
| *Chrysanthemum grandiflorum* (L) *Batt.* | 3.08 | RR | CC | Hemicryptophyte | Perennial Herbaceous |
| *Echinops spinosus* L | 1.23 | RR | R | Hemicryptophyte | Perennial Herbaceous |
| *Hypochoeris radicata* L | 1.23 | RR | CC | Hemicryptophyte | Perennial Herbaceous |
| *Inula montana* L | 0.61 | RR | AC | Therophyte | Perennial Herbaceous |
| *Pallenis spinosa* (L) *Cass.* | 4.32 | R | AC | Therophyte | Perennial Herbaceous |
| *Reichardia picroides* (L) *Roth* | 6.17 | R | CCC | Therophyte | Perennial Herbaceous |
| *Reichardia tingitana* (L) *Roth.* | 3.70 | R | R | Hemicryptophyte | Perennial Herbaceous |
| *Senecio vulgaris* L | 9.25 | AR | CCC | Therophyte | Annuel Herbaceous |
| *Sonchus arvensis L* | 2.46 | RR | R | Therophyte | Perennial Herbaceous |
| *Taraxacum officinalis* L | 6.17 | R | RR | Hémi cryptophyte | Annuel Herbaceous |
| *Bellis silvestris*(*var pappulosa* L | 0.61 | RR | R | Hemicryptophyte | Perennial Herbaceous |
| *Centaurea eriophora* L | 0.61 | RR | AR | Therophyte | Annuel Herbaceous |
| *Evax argentea* L | 1.23 | RR | R | Therophyte | Annuel Herbaceous |
| *Catananche caespitosa* L | 6.55 | AR | AR | Chamaephyte | Perennial Herbaceous |
| *Picris duriaei* L | 3.61 | R | CC | Chamaephyte | Perennial Herbaceous |

**Table 4.** Biological types of study stations

| Biological type | Phanerophyte | Chamaephyte | Hemicryptophyte | Geophyte | Therophyte |
|---|---|---|---|---|---|
| Beni-saf | 0% | 14% | 27% | 0% | 59% |
| Zarifet | 0% | 14% | 47% | 0% | 39% |

environmentalists who undertake such actions.

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# Design and Development of Smart Biomonitoring System using Internet of Things

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***Abstract***: The quality of water bodies has deteriorated over the years with the rapid growth of urbanization and industrialization, resulting in increased emission of pollutants into the ecosystem. This study proposes an effective technological solution to obtain the number of fishes in a water body and also the degree of pollution in the water body based on pH, turbidity and the total dissolved solids. The study area focuses on the Buckingham Canal, Chennai (Okkiyam Maduvu & Neelankarai stretch) which has turned into a main focal point of industrial and domestic sewage dumping. Water samples from the study area were collected and analysed for significant physio-chemical parameters to assess its suitability as a source of water for fish farming. The results of the analysis compared with optimum range values revealed that the pH was 6.63 within the acceptable range for fish farming. However, the pH value of 5.95, turbidity of 87 and 111 NTU and total dissolved solids of 954 and 1007 mg/L differ from the standard prescribed values. During the study, a web application was developed using Internet of Things (IoT) to effectively display the fisheries count and also the pH, turbidity and TDS values to the government officials and policy makers. This study will aid on the necessary treatment to improve the water quality in the Okkiyam Maduvu stretch so that the water can be used for fish farming.

***Keywords***: Aquatic pollution, Domestic sewage, pH, Turbidity, Total dissolved solids, Internet of things, Web application

The burning issue of today's environmental problem is the release of toxic contaminants from various man-made sources resulting in the contamination of natural resources of the earth and leading to scarcity of freshwater (Sharma and Pandey 2014). Some 80 percent of the world's wastewater is dumped, largely untreated, back into the environment, polluting rivers, lakes, and oceans. More than 70 percent of sewage in developing countries is discharged untreated, polluting rivers, lakes, and coastal areas. Deteriorating water quality has a negative impact on the climate, human health, and the global economy. Water pollution has a greater effect on marine life because their survival is dependent on water quality and any change in their environment has the greatest impact on their ecosystem. In polluted water, due to the abundant growth of algae, the oxygen content becomes lesser, causing the death of fishes, seaweeds, mollusks, marine birds, crustaceans, and other sea organisms that serve as food for human. Concentration of heavy metals (Cu, Zn, Cd, Pb and As) were studied from the estuary of Tinto and Odiel rivers in Huelva (Spain) Martorell et al (2009). According to the author, the concentration was measured in water, sediment, and two fish species, Sparus aurata and Solea senegalensis. Assessment and Biomonitoring of aquatic pollution by heavy metals (Cd, Cr, Pu, Pb and Zn) for Hammam Dam in Mila (Algeria) was studied by Aissaoui et al (2017). It is necessary to monitor the state of the water bodies to prevent pollution. Vandhana Devi and Nagendran (2017) assessed the water quality in Kondurupalem lagoon inlet, southeast coast of India. A review on concepts, current use, and anticipated future directions of bio-monitoring approaches and bio-indicators used for the river ecosystem have demonstrated the efficiency of molecular techniques on enhancing the taxonomic resolution and detecting the genetic diversity in river bio-monitoring (Li and Lusan 2010, Ezeanya et al 2015). The design and development of a low-cost system for real-time monitoring of the water quality monitoring system based on IoT (Internet of Things) consist of several sensors to measure both physical and chemical parameters of the water, such as temperature, pH, turbidity, flow sensor (Vaishnavi et al 2017). The objective of the present study is to design and develop a bio-monitoring system to monitor the aquatic life count and physiochemical properties such as pH, turbidity and total dissolved solids (TDS) of polluted water using sensors and Internet of Things.

## MATERIAL AND METHODS

**Study area:** The Buckingham Canal is a freshwater navigation canal with a length of 796 kilometres. Most of the natural backwaters along the coast are linked to the Chennai port by this canal. South Buckingham Canal has its length of 110 km from Chennai to Marakanam. Two sampling sites were selected. The first location Okkiyam Maduvu - (80°14'18.27"E; 12°55'17.94"N) gets water from the

Pallikaranai Wetland and drains into the Buckingham canal (Fig. 1). The second location is located between Kottivakkam and Neelankarai (80°14'54.01"E; 12°56'57.57"N) canal which is severely polluted by sewage and industrial effluents. Okkiyam Maduvu lake serves as a livelihood for many people engaged in inland fishing in some parts of the channel. Currently, the channel is filled with hyacinth and has turned into a dumping garbage area and hence the aquatic life has been deteriorated. The silting up of the canal has left the water stagnant, creating an attractive habitat for mosquitoes (Fig. 2). The selection of sampling sites was based on the contamination vulnerability and pollution from degradable and non-degradable wastes disposal. The physiochemical parameters like temperature, pH, turbidity, total suspended solids, Total dissolved solids, Dissolved Oxygen, Biological Oxygen Demand and Chemical Oxygen Demand of Buckingham Canal were investigated from March 2011 to February 2012 by Vinodkumar et al, 2018.

**Methodology**

The surface and bottom water samples from the study area were collected using a Van Dorn sampler. Temperature was measured using a 0-50°C precision thermometer. The transparency of water was measured using a Secchi Disc. Standard methods were adopted for estimating pH, dissolved oxygen, total dissolved solids and turbidity. During the study, technology that uses sensors and the Internet of Things to track the properties of contaminated water, such as pH, turbidity, and total dissolved solids (TDS) was developed. The methodology adopted during the study is depicted in Figure 3. Sensors like pH sensor, turbidity sensor, and TDS sensors along with the microcontroller are fabricated to a board. This process will be followed by connecting the Wi-Fi MODULE to the microcontroller to facilitate the development of IoT. The image processing aspect of this study is software that is designed using python code. It runs the video of the aquatic life that was shot under water and divides it into grids to count the aquatic life in the water body. The software identifies the aquatic species using an in-built library which

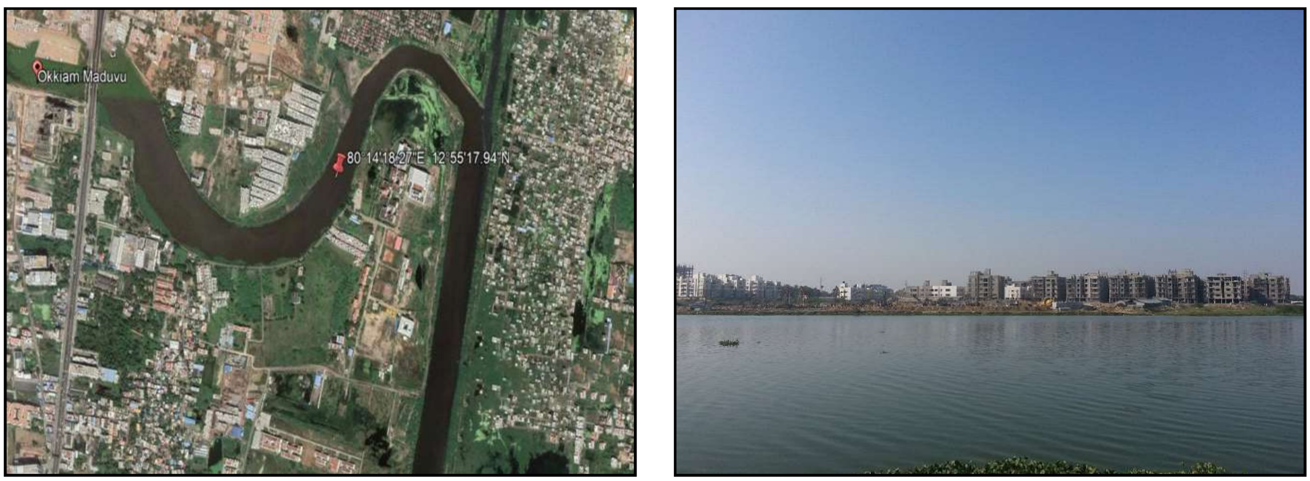


**Fig. 1.** Study area - Okkiyam Maduvu Lake


**Fig. 2.** Breeding of mosquitoes in the Neelankarai Canal stretch

will be coded in to the software during its development. The input values from the sensors will be collected through the microcontroller and these input values will be transmitted wirelessly to a web application or a mobile application using the Wi-Fi Module. The developed aquatic bio-monitoring system once integrated with IoT will be ready to be tested in a glass tank containing water that was collected from Buckingham canal at two sample points. A web application has been created to count the number of fishes in a water body. This is a practical way to keep track of the number of fishes in the water as well as the level of pollution. Individuals will be able to access real-time data through the Android application. The values can be noticed depending on the samples in the proposed hardware module.

**Hardware requirements:** From the block diagram in Fig 4.a, pH sensor, turbidity sensor, and TDS sensor will be used to detect the water quality in the water body. The ESP8266 is a microcontroller that will collect all the data in this system, which will connect with embedded C and will share the data through the Wi-Fi gateway to the system in the first part. The microprocessor-based system is used for the fish count (object detection) data which will be updated to a web server (Fig. 4b). In this study, some hardware devices are used such as a Power supply module, Microcontroller, pH sensor, Turbidity sensor, and TDS sensor. The hardware is connected with a Wi-Fi gateway which enables the system to connect and share the information through the internet. Arduino is an open-source electronic platform that is simple to use and implement and is based on the connection of hardware and software. They are built in such a way that they read the input of water quality parameters and convert them into an output by sending it to the web application. There are three different types of sensors used in this study viz. pH sensor, Turbidity sensor and TDS sensor. Each type of pH sensor (Fig. 5) works differently to measure the quality of the water samples. In the pH sensor, as the value goes below 6, it is predicted that the water is acidic, whereas if it increases above 8 it is considered basic and when it lies between 6 and

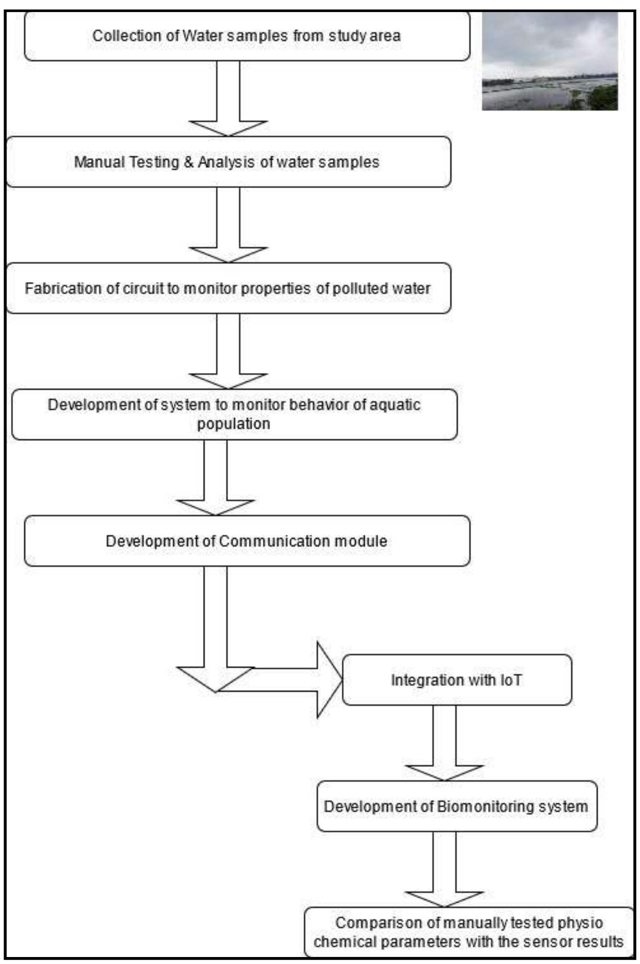


**Fig. 3.** Methodology

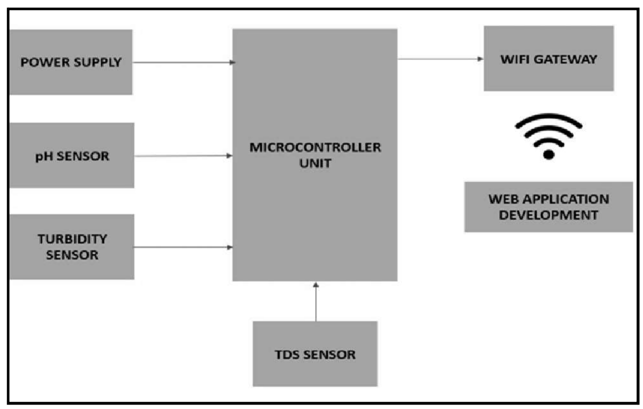


**Fig. 4a.** Hardware block diagram

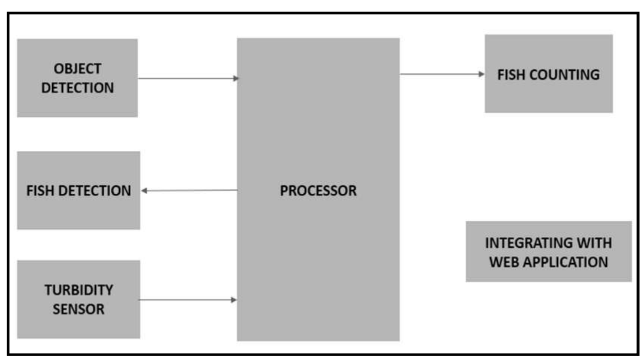


**Fig. 4b.** Software block diagram

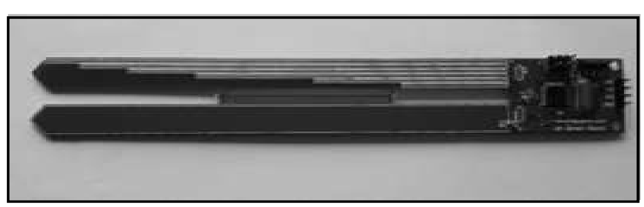

**Fig. 5.** pH sensor

7 it is considered to be neutral, which is the optimal range for most speices in the water. A turbidity sensor was designed (Fig. 7) with a scope to determine the pollution of the water so that the water quality can be determined. Turbidity is a key measure of the amount of dissolved sediment in a body of water, and it can have a variety of negative consequences for marine life. An LDR sensor is used in the turbidity sensor which acts as a light detecting sensor depending on the reflection of light through the water when light falls upon it, then the resistance changes. Values of the resistance of the LDR may change over many orders of magnitude, the value of the resistance falling as the level of light increases. An LED is used in the opposite direction to fetch the light intensity. Whenever the water is poured inside the container, depending on the water quality the reflection of light varies and hence the value fetched from the LDR sensor also varies. Turbidity Sensor and the microcontroller unit, takes care of turbidity measurements, uses light to convey information about the turbidity content in water. The value from the sensor decreases when pure water is poured whereas the value increases when the quality of water is less. Thus the turbidity of the water is determined. The TDS sensor (Fig.8) has two electrodes that generate voltage while kept inside the water body by which the presence of ions can be detected. The output comes as an ADC value with which the higher level of voltage is been guessed which in turn indicates the abnormal presence of ions in the water body.

**Software requirements:** The visual studio is integrated with build and scripting tools to perform common tasks making everyday workflows faster. VS Code has support for GitHub, hence can work with source control without leaving the editor including viewing pending changes. VS Code also includes great tooling for web technologies such as HTML and CSS.

**Node.js:** VS Code includes enriched built-in support for Node.js development with JavaScript, powered by the same underlying technologies that drive Visual Studio. Node.js is an open-source and cross-platform runtime environment for executing JavaScript code outside a browser. During the study, for the development of bio-monitoring system, Node.js for building back-end services like APIs like Web App or Mobile App has been used. Frontend and Backend are the two most popular terms used in web development. During the study, an application has been developed using this, to show the values. HTML, CSS, and JavaScript are the languages used for frontend development. The frontend and backend development are quite different from each other, but still, they are two aspects of the same situation. The frontend is what users see and interact with and the backend is how everything works.

**MQTT:** Message Queuing Telemetry Transport Protocol (MQTT) makes the whole procedure fast, reliable, and secure. Wi-Fi gateway from the hardware part will push the

**Fig. 7.** Turbidity sensor

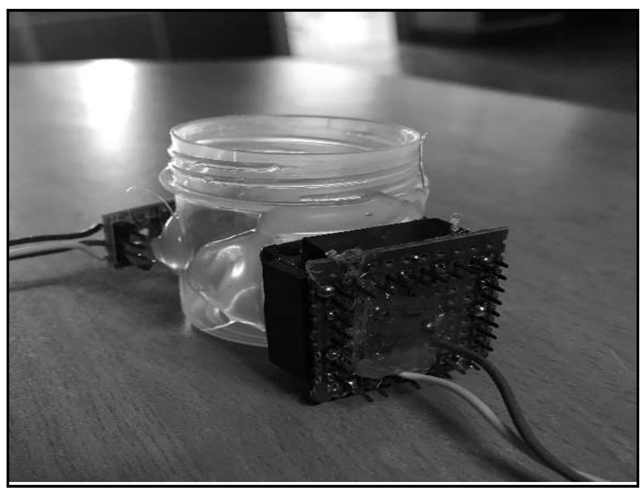

**Fig. 8.** TDS sensor

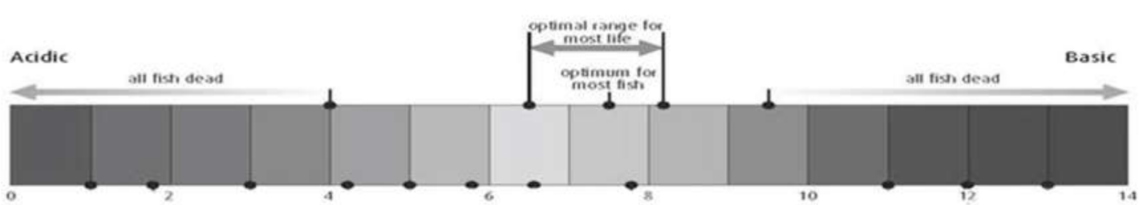


**Fig. 6.** pH range for fishes

data to this cloud document.

**JavaScript:** MQTT grabs the numerical value information. This will be achieved by various methods. Java is object-oriented and it is designed to have as few implementation dependencies as possible.

**Python:** Python programming is used to develop software on the web and in application form. Efficient Net is used to train the model for effective fish detection which helps in counting the number of fishes.

**Working:** The implementation of the system includes all the wiring that is to be installed on the breadboard along with Arduino and other sensors. The coding of the water quality which comprises embedded C that is needed to be called in the code. Sensor values are wirelessly transmitted to cloud storage using the MQTT protocol. A web application using node JS is developed in this project to interactively fetch and display the live sensor values obtained by the pH sensor, turbidity sensor and TDS sensors. Object detection method has been employed to detect the number of fishes in the water body. The fishes in the water body were identified using a deep learning object detection algorithm efficient net (Fig. 9).

## RESULTS AND DISCUSSION

The results obtained from analysis of water samples of the two sampling station are plotting in the graphs shown in Figure 10 and Table 1. The reported values refer to the mean value of water samples collected in different seasons at different areas along the stretch of the Ogiyam Maduvu and Neelankarai water body. The water quality in the study area was monitored during January 2021 to May 2021. The results indicate that the quality of water varies slightly from location to location. A summary of the findings is given below. The value of the pH of Okkiyam Maduvu Lake (sample 1) was found to be 6.63 and for Neelankarai stretch 5.95. Marked variations were observed in pH between the samples collected from the two stations. The pH was slightly acidic in station 2 compared to station 1 and this could be due to the tidal influence from the adjoining backwaters. Similar observations were made from Muthupettai mangroves in Tamil Nadu. The value of total dissolved solids of Okkiyam Maduvu land Neelankarai stretch was 954 and 1007 mg/l respectively and is greater than the standard of 400 mg/l which is recommended for most fresh water fishes (Khan et al 2017). This implies that both the study areas should first be treated to reduce the quantity of dissolved solids to the acceptable range before it could be used as a source of water for fish farming.

The physio-chemical parameters of the samples collected from both the sampling stations were tested manually and also using sensors. The obtained results shows a sensor accuracy of 95% when the parameters are analysed using sensors. Development of water quality monitoring system based on wireless sensor network presents a system framework taking the advantages of WSN for the real-time monitoring on the water quality in

**Table 1.** Water quality in the study area

| Water quality parameters | Study area | | Optimal range value for fishes |
|---|---|---|---|
| | Okkiyam Maduvu (Station 1) | Neelankarai (Station 2) | |
| pH | 6.63 | 5.95 | 6.0 – 9.0 |
| Turbidity (NTU) | 87 | 111 | < 8 NTU |
| TDS (mg $L^{-1}$) | 954 | 1007 | 400 mg/L |
| Dissolved oxygen (mg $L^{-1}$) | 2.3 | 2.1 | <5 mg/L |

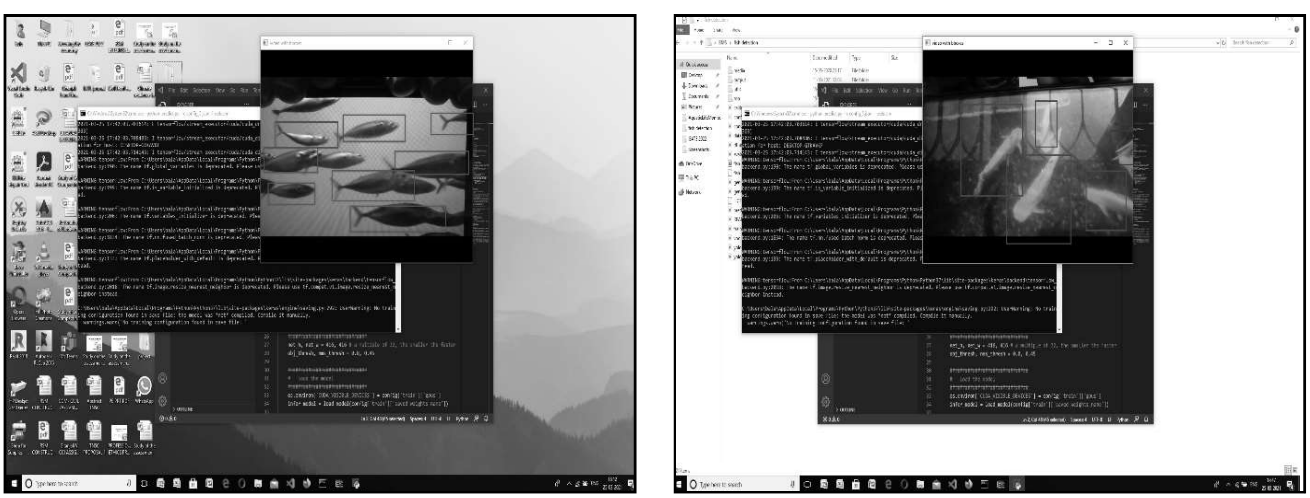

**Fig. 9.** Fish count detection

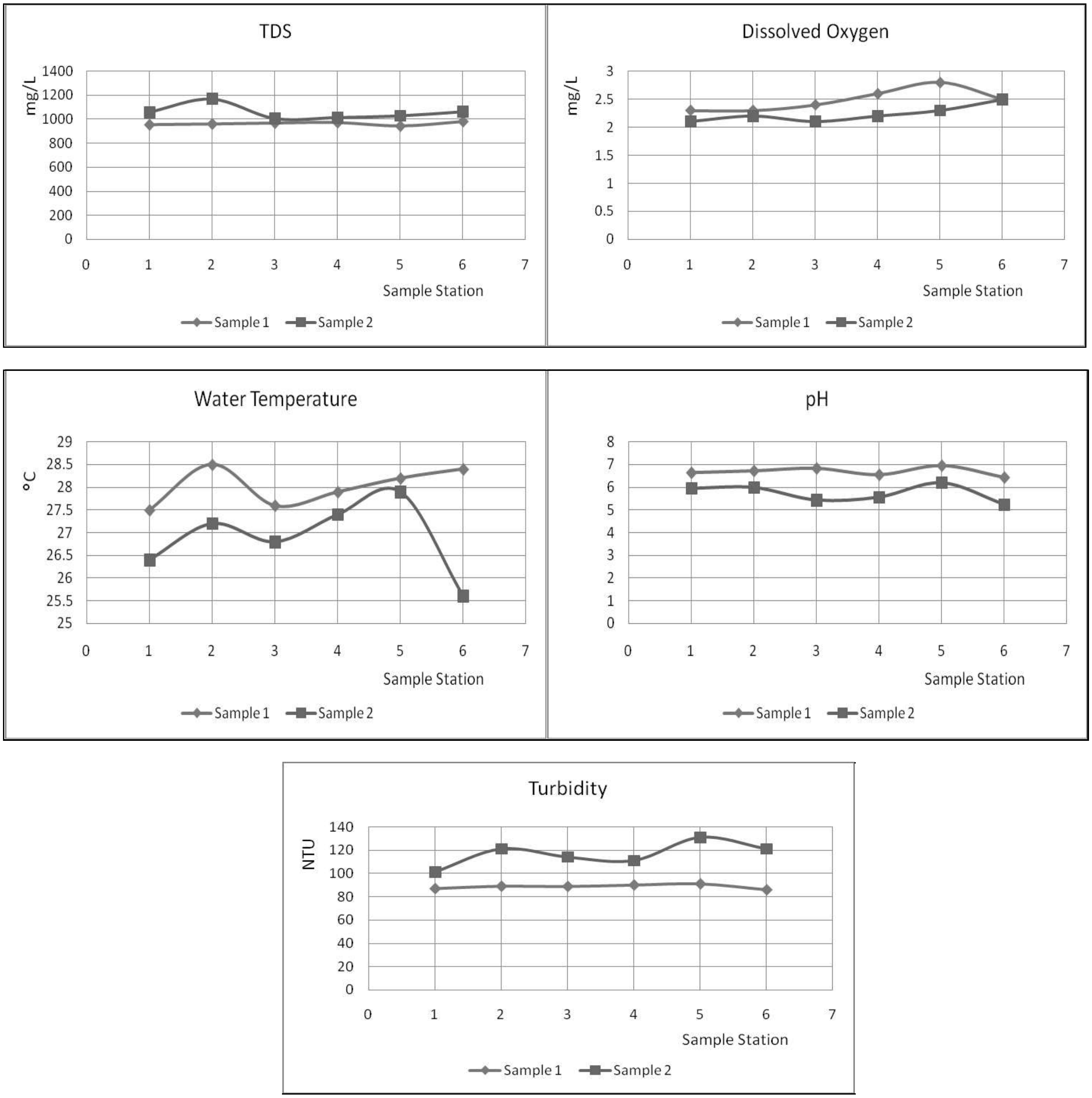


**Fig. 10.** Physio chemical parameter results obtained for the sampling stations

aquaculture (Zhang et al 2011). Neelankarai stretch is highly polluted by dumping of sewages and effluents, all the parameters tested shows that the water body will not support for the growth of aquatic species. Changes in the water quality affect the biotic community of the aquatic ecosystem which ultimately reduces the primary productivity. This system enables authorities to monitor the quality of water using the collected data through mobile and web. Designing successful biomonitoring and bioassessment programs are an important basis for the rehabilitation and restoration of aquatic ecosystems. Developed countries have great progress in this regard, meanwhile, developing countries still have much to do to improve the ecological status of their aquatic systems (Abdulkarim 2020).

## CONCLUSION

Based on the results of the current study, the results of physio chemical parameters tested manually and with the help of sensors were compared and found to be almost the same. The amount of turbidity and TDS are much higher in the sample stations. In this study, a technological solution for effectively monitoring the fishes and also the degree of pollution in water body using the appropriate sensors and web application has been successfully developed. Moreover, this system can be utilized by the general public and is also very promising for real-time applications because of the fast-processing time. The application for the aquatic life monitoring system implemented in this project can be further developed to use as a product in real-time it can be further analysed for higher accuracy. Thus, implementing this in real-time will help the concerned officials to effectively determine the degree of pollution at a very early stage and also identify fishes with a separate hardware part development for image detection sensor. Hence, the study gives an effective solution to monitor the number of fishes and also the degree of pollution in water.

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# Fortification of Food: Achieving Zero Hunger in Sub-Saharan Africa

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***Abstract***: Hidden hunger or malnutrition is a form of undernutrition that occurs due to the deficiencies of vitamins and minerals. It is mainly as a result of the lack of food quality, which is deficient in micronutrients that are necessary for growth and development of human body. Sub-Saharan Africa is known to be a major developing country where micronutrient deficiency continues to bedevil. The prevalence of hidden hunger is alarmingly high level which, therefore, requires consideration of the potential for fortified food to fight hidden hunger. Hence, this article aims to present ways to combat hunger and the insidious deficiencies in the form of hidden hunger, or micronutrient deficiencies, which prevails in Sub-Saharan Africa. It talks about achieving food and nutritional security through sustainable agriculture, fortification of food and post handling of food. It also suggests solutions to tackle undernourishment and hunger problems in SSA, keeping in mind rural development and protection of the environment.

***Keywords***: Hidden hunger, Fortification, Staple food, Nutritional security

The Sub-Saharan Africa region is highly dependent on Agriculture as it provides employment to about 60 percent of its population. It contributes approximately 15.6 percent in total GDP (The World Bank 2019). Agriculture in SSA is extremely reliant on rain (Grace et al 2012) and must adapt to water scarcity to meet future food requirements (Hadebe et al 2017). The negative influence of climate change, especially the droughts in SSA in 2015-16 induced by El Niño (FSIN 2018) has increased stress on agricultural productivity of land (Chivenge et al 2015) and degraded the nutritional quality of staple food crops (Hummel et al 2018). As a result, these adverse environmental conditions forces shift from cultivation of more climate-resilient crops to less dependent options such as millets, for example, sorghum, which is high in protein and can help cure hidden hunger (Hadebe et al 2017). Sub-Saharan Africa (SSA) has one of the highest proportions of undernourished people, accounting for 32 percent of the 820 million undernourished people in the world (FAO et al 2019) and the number is increasing rapidly (Abegaz 2018). According to the percentage of undernourishment is on the rise for the third year in a row, from 22 in 2016 to 22.7 in 2017 and is estimated to be at 22.8 in 2018 (FAO et al 2019) (Fig. 1). However, achieving the Zero Hunger target by 2030 in Sub- Saharan Africa, remains virtually static (Chopra and Darnton-Hill 2006, Otekunrin et al 2019) with a decreasing trend below 11 percent (Fig. 2). However, the rapid rate of population growth and undernourishment raises alarms for urgent need to improve the food supply chain, which involves processes of production, processing, distribution, and consumption, whilst reducing food losses and waste.

**Factors Affecting Food Security**

**Environmental*:*** The continuous climate change variability has become a severe threat to agricultural production (Ayinde et al 2011), distribution and consumption of staple food crops which directly impacts food security and nutrition challenges. It not only creates food shortage but reduces nutritional value of the crops, especially due to increased carbon emissions (Cohen et al 2008, Gleadow et al 2009).

**Socio-economic:** High food prices remain a major issue in the accessibility of food to meet nutritional requirement for poor sections of the society, as Temple and Steyn (2011) reports that buying healthy food items resulted in a 69 percent rise in average costs.

**Technical:** The unavailability of food is mainly due to a lack of post-harvest management and technology, and low development of food-processing industry sector (Kitinoja et al 2011), which therefore contributes to food and nutrition insecurity (Aworh 2008).

**Status of food production and population:** With the increasing population in Sub-Saharan Africa (SSA), there is a visible uptrend in food production index (Fig. 3 and 4). Sub-Saharan Africa's population grew steadily from 917.7 million in 2012 to 968.9 million in 2014, an overall increase of 5.6 percent is observed. Similarly, there has been an increase of approximately 9 percent in agricultural production index

(World Bank 2019). Thus, it can be assumed that production is stable and increasing over the years and hence the region remains self-sufficient to feed expanding population. These figures highlight that adequate food quantity is produced but that it is not available to the population for consumption due to insufficiencies in supply and distribution. Since most of the food commodities are highly perishable in nature, which leads to degradation of quantity and quality of the produce (Kiaya 2014). Hence, the production and consumption trend signifies that increasing agricultural productivity is not probably necessary to achieve food security in SSA.

**Addressing sustainability development goal 2 (SDG 2):** The principle target of SDG 2 comprises combating hunger, ending micronutrient deficiencies, doubling productivity and income of small farmers, and achieving food and nutritional security through sustainable agriculture. Combating approaches need to be built on ensuring food access, food sustainability and also create opportunities for the poor. Since problems like hunger and malnutrition are a result of lack of availability or access to a diversified diet, it can be solved by fortification of food (Verma 2015, Pardeep et al 2018) which could play a vital role to meet nutritional needs.

**Food Fortification:** Food fortification refers to the addition of one or more micronutrients to foods which subsequently provide a higher level of micronutrient than the normal routine diet. It has been recognized as an effective and highly cost-efficient method for reducing micronutrient malnutrition (Chadare et al 2019, Dary et al 2006). The strategy is a food-based approach, where present food supplies and restricted access fail to provide adequate nutrient levels in the diet, which has shown subsequent improvement in nutritional status of a population. Fortification can be categorized into two based on legal considerations as mandatory and voluntary fortification. Mandatory fortification involves addition of certain micronutrients to a specified food, while in voluntary fortification, it is choice of the food manufacturers to select the micronutrients they want to add to the food items (WHO 2006). It is important to fortify food which is locally available and consumed by maximum portion of target population. Fortifying staples and condiments like flour, salt with essential nutrients could also balance the nutrients lost due to rising carbon emissions. This shall increase productivity in adults, leading to an improved agricultural workforce which can contribute to help encounter hunger (Biesalski et al 2016)

**Food fortification vehicles:** Fortification requires appropriate vehicle and a fortificant that delivers the micronutrient to the consuming population. Usually, fortification vehicles are basic commodities that are regularly consumed and centrally processed to reach either large proportion of the population or targeted groups, aiming at specific population groups, to meet their dietary gaps. For example, fortification of staples and condiments is population-based fortification (Friesen et al 2017) whereas fortification of complementary food for infants and young children is a targeted strategy (Preedy et al 2013). Dwyer et al (2014) reported that selection of an appropriate fortification vehicle, and identification of target and non-target groups are some of the challenges that countries face. In addition, monitoring the health and nutritional status is also challenging in an attempt to combat hunger and ensure nutritional security (Gillespie and van den Bold 2017, Tavares et al 2015). To survey effectiveness of fortification initiatives, the Global Alliance for Improved Nutrition (GAIN) developed the Fortification Assessment Coverage Toolkit (FACT) in 2013, which assesses data on quality, coverage, consumption and effectiveness of fortified food by various

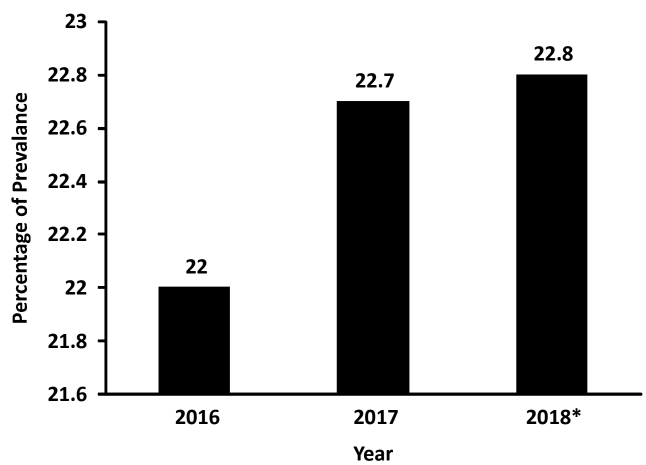



**Fig. 1.** Prevalence of undernourishment in Sub-Saharan Africa

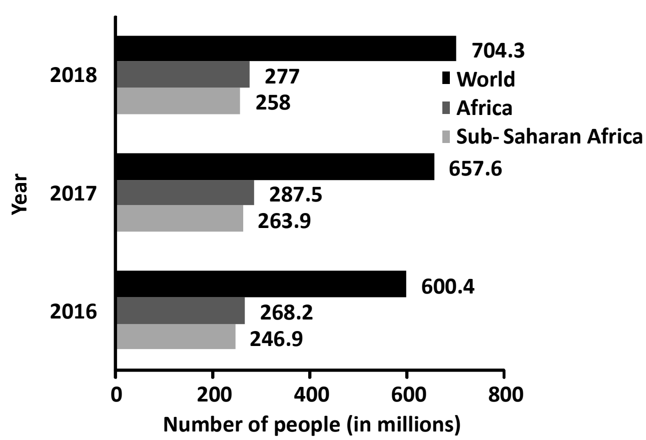



**Fig.** 2. Number of people experiencing severe food insecurity, 2016-2018 population groups. The vehicle selection for success of fortification program depends on various key factors which involve accessibility, affordability, consumption pattern, palatability of the food, and stability and bioavailability of the nutrients added to the food (de Lourdes Samaniego-Vaesken et al 2012, Huma et al 2007, Öztürk 2017). A study of three regions in Central Africa reported that consumption of wheat flour, sugar, and bouillon cubes was about 75 percent, whereas cooking oil consumption accounted for 54 percent (Klassen-Wigger et al 2018). Hence, flour, sugar and cooking oil, are some common and effective vehicles for fortification in improving micronutrient intake.

**Bouillon cubes:** The prevalence of anemia in the world was estimated to be about 24.8 percent of the total population, in which women, pregnant women (41.8%) and non-pregnant women (30.2%), and children, pre-school (47.4%) and school-age (25.4%), are reported as most vulnerable population groups (World Health Organization. (2008). Deficiency of iron is also caused by Schistosomiasis, disease caused by infection with freshwater parasitic worms (Chaparro and Suchdev 2019) and malaria (World Health Organization 2019). In Sub-Saharan region, consumption of bouillon cubes is high as 79 to 99 percent of women reveal bouillon cube consumption over the last 7 days (Hess et al 2013). In 2012, Nestle produced iron-fortified bouillon cubes focusing on accessibility and affordability for the low-to-middle socioeconomic groups (Klassen-Wigger et al 2018, Mejia et al 2015). Multinational companies like Unilever and GB Foods also produced fortified bouillon cubes under the brand name Knorr and Jumbo respectively (Mejia et al 2015). Hence, Bouillon cubes have been reported as a promising vehicle for micronutrient fortification in the Sub-Saharan Africa region (Eilander et al 2019, Mkambula et al 2020).

**Flour:** Sub-Saharan Africa shows an unchanging trend in subclinical vitamin A, i.e., the concentration of serum retinol in the blood lower than 0.70 μmol/L, and mineral deficiencies (Van et al.2007). According to WHO, the prevalence of vitamin A deficiency is the highest in Sub-Saharan Africa (48%), followed by South Asia (44%). Due to the deficiency, more than 95 percent deaths were reported from both the regions (Stevens et al 2015). Fortification of flour has helped to deliver sufficient concentration of micronutrients and target specific health conditions. Maize, wheat, and rice flour fortified with vitamin A, iron and zinc shows improvement in the growth (Steyn et al 2008) and nutritional status of undernourished children. In 2003, this led to a remarkable decline in birth defects in South Africa (Fanzo 2012, van Jaarsveld et al 2015). Being a main dietary staple and highly affordable, flour is commonly used fortification vehicle. Moreover, folic acid was added to flour on a mandatory basis in over 60 countries to prevent neural tube birth defects (Oakley and Tulchinsky 2010).

**Milk:** Deficiency of vitamin D is particularly evident in dark skinned children and obese children and adults, primarily in South America and Africa (Holick and Chen 2008, Palacios and Gonzalez 2014, Method and Tulchinsky 2015). Fortified vitamin D products can be cost-effective intervention in prevention of vitamin D deficiency, along with optimum exposure to sun (Holick 2010). For this, milk has been extensively used as a vehicle for fortification, especially with vitamin D, vitamin A, iron and calcium.

**Oil:** Over the years, fortification of oil with Vitamin A has increased in Sub-Saharan Africa. Palm oil, being an important source of edible oil in SSA, is identified to be significant for fortification as about 21 percent of the people in West Africa consume it daily (Tagoe et al 2012, Wang et al 2018). Consumption of fortified margarine is also recognized in Ghana (Galbete et al 2017). Fortification of unrefined sunflower oil is reported to be feasible and acceptable in Tanzania, but the packaging increases its cost by 20 percent (Walters et al 2017). Mandatory legislation has implemented the production of fortified oil and other staples in various

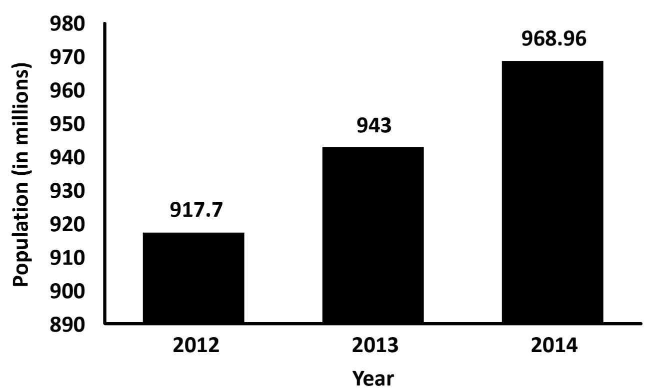
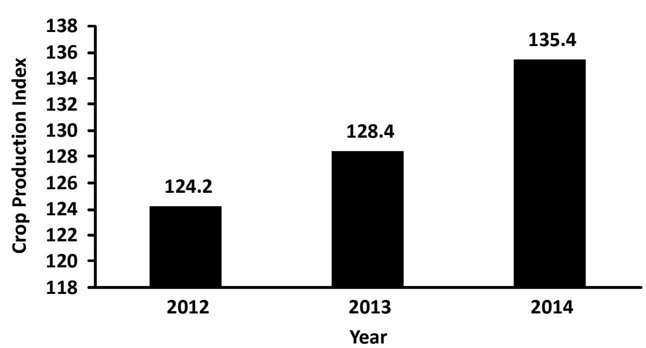



**Fig. 3.** Population in Sub-Saharan Africa (2012-14)




Crop Production Index indicates the agricultural production each year relative to the base period 2004-2006, which includes all crops expect fodder crops

**Fig. 4.** Crop production index in Sub-Saharan Africa (2012-14)

countries of Sub-Saharan Africa.

**Salt:** Iodized salt was the first food to be fortified and was introduced in the 1920's, initially in Switzerland and U.S. (Leung et al 2012) to prevent goiter (Mannar and Hurrell 2018) and other iodine deficiency disorders. Global success of universal salt iodization program confirms its adequacy in controlling iodine deficiency.

**Sugar**: Sugars act as a primary source of fuel for the human body and are consumed daily. Consumption of sugar has increased in urban and rural Sub-Saharan Africa for which Vitamin A is proven to be a successful fortification combination (Dary and Hurrell 2006). In Zimbabwe, Vitamin A supplementation had a major positive impact in reducing stunting in children of low socioeconomic groups (Kairiza et al 2020). Fortification of sugar with vitamin A has allowed many countries to successfully tackle micronutrient deficiencies in populations. In Central and South America, the biological efficacy and fortification program effectiveness of vitamin A fortified sugar is well known (Rodriguez-Amaya et al 2015). Jamison et al (2006) reports the biological effectiveness of fortifying monosodium glutamate with vitamin A but reveals no program has been developed for the same. Overall, sugars fortification with micronutrients could be potent public health intervention.

**Effectiveness**

**Bioavailability and nutritional impact:** The evidence of food fortification program effectiveness is quite less and weak. Remarkable reduction of anaemia, 2.4 percent annually, was reported from the countries that fortified flour with micronutrients (Nair 2019). Wheat and maize flour fortified with iron helped in preventing neural tube defects (Tablante et al 2019). In case of bouillon cubes, Nestle uses ferric pyrophosphate, which is known to be the fortificant with highest iron bioavailability (Klassen-Wigger et al 2018). Also, research on fortification of ferric pyrophosphate in salt, rice and complementary cereals have proved its efficacy in preventing anemia (Waller et al 2020). In Indonesia, vitamin A fortified oil intake reported reduction in vitamin A deficiency amongst infants and children (Jus'at et al 2015, Laillou et al 2013). Similarly, (Raghavan et al 2019) studies the effectiveness of vitamin A fortified palm and soyabean oil and confirms the potential of the vehicles to prevent vitamin A deficiency. Fortification of sugar in Sudan has reduced the prevalence of vitamin A deficiency in school children (Shommo et al 2008). Assessment results from eastern Uganda also states potential of sugar as a promising vehicle for fortification. The consumption of sugar in Central America has increased which contributes to about 45-180 percent of vitamin A Reference Daily Intake for people more than 3 years of age, Vitamin D fortified milk helped in eradication of rickets in children in Europe and North America (Mkambula et al 2020) and showed results of increased height and weight of Mongolian school-age children (Ganmaa et al 2008). In addition, folic acid and iron fortified milk is only produced in Costa Rica and reveals significant reduction of iron deficiency in children from 19.3 percent to 4 percent, and in women from 18.4 percent to 10.2 percent at national level (Martorell et al 2015). In Sub-Saharan Africa, Ethiopia was the first country to achieve 80 percent iodized salt coverage (Adish et al 2013), whereas, in Ghana, mandatory legislation for iodization of salt was implemented in 1996 with a target to achieve 90 percent population coverage (Nyumuah et al 2012). Studies found that children who consumed iodized salt had higher IQ and prevent risk of goiter and iodine deficiency (Aburto et al 2014).

**Cost and feasibility:** Cost-effectiveness is usually measured by comparison of health benefits and cost of fortification, for example, in cost per disability adjusted life-year (DALY), is the number of years lost due to health issues. Vitamin A fortification of vegetable oil (US $18 per DALY averted) was revealed as more cost-effective than fortification of sugar ($82 per DALY averted) (Fiedler et al 2010). Furthermore, for mild iron and iodine deficiencies, fortification of sugar, salt and cereal is more cost-effective than supplementation (Jamison et al 2006). Feasibility is vital to ensure availability of adequate fortified food for the population and monitoring of its quality. But the cost and feasibility of fortification as a mechanism to overcome micronutrient deficiencies is not well documented. However, the results of various food fortification initiatives in low-income countries have been reported as cost-effective and feasible (Fiedler et al 2014, Fiedler et al 2009) by partnership of public and private food producing and marketing agencies.

**Fostering environment sustainability and rural development**: Fortification of food can help to prevent stress on existing land holdings and reliance on new infrastructure to produce new food that prevents additional emissions of greenhouse gases. Food system accounts for approximately 26 percent of global anthropogenic greenhouse gas emissions (Poore and Nemecek 2018), whereas the overall impact of fortification to carbon footprint of food system is negligible as it uses existing food chain supply system (Garrett et al 2019).

Furthermore, hunger and food insecurity are major constraints to economic growth and development. Improvement in nutritional status of the population enables them to participate in socio-economic activities and development (Péter et al 2015). Therefore, food fortification can be an effective intervention in combating poverty and promoting rural development by making population healthier and productive (Chaudhary and Gupta, 2019; Drewnowski 2020, GAIN 2016).

**Major challenges:** There are various challenges and gaps faced in implementing any food fortification initiative and monitoring the quality of fortified foods. Cost implications are one of the major challenges and an important aspect for any food fortification initiative as they facilitate production and distribution and bear various costs of intervention. Similarly, fortified food requires adequate distribution system and thus often fails to reach the target population groups due to underdeveloped system of distribution (Lalani et al 2019). There are other technological issues with use of nonmaterial for fortification. Some nonmaterial can produce reactive oxygen species and potentially increase toxicity (Dwyer et al 2015). Furthermore, as reported by (Neufeld et al 2017), the lack of quality control of fortification leads to significant nutrients' loss. In addition, due to the lack of basic consumer awareness about micronutrients and the benefits of fortified foods (Pambo et al 2014), the goal of ensuring food access to end hidden hunger remains unsolved. Thus, to address current nutritional issues, The Food and Drug Administration's fortification policy needs significant update (Dwyer et al 2015) and proper enforcement and monitoring is required to access effectiveness of the program.

## CONCLUSION

The aim of this essay was to highlight self-sufficiency of SSA in food production, discuss the major reasons for food and nutritional insecurity and identify significance of food fortification to combat hidden hunger in the Sub-Saharan Africa region. The study provides a comprehensive assessment of major factors affecting efficacy and effectiveness of food fortification and alludes that availability of food supplies does not guarantee nutritional security. There is, therefore, a definite need of continuous production and regulatory monitoring of fortified food, along with promotion of its consumption and utilization rather than increasing agricultural intensity which generates pressure on land resources and environment as a whole. Overall, this study strengthens the idea of promoting fortification of common staple food to optimize the availability and affordability of micronutrients to a larger population. Hence, Fortification of food is a cost-effective approach in combating micronutrient deficiencies, improving public health with minimal risk and addressing the Sustainability Development Goal 2 with negligible environmental effects.

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# Colour and Viscosity Attributes of Twin-Screw Extrusion Processed Potato Starch

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***Abstract***: The effect of twin screw extrusion processing on colour and viscosity characteristics of potato starch was studied using response surface methodology (RSM). Experiments were designed using central composite rotatable design with three-independent variables- feed moisture (14-18%), screw speed (400-550 rpm) and barrel temperature (125-175°C). Extrusion processing significantly affected the colour and viscosity of starch. Increase in feed moisture reduced redness, yellowness, but enhanced luminosity. Increase in screw speed significantly increased all the colour characteristics (luminosity, redness and yellowness) of potato starch extrudates. Increase in barrel temperature decreased luminosity, yellowness and increased redness of the extrudates. The hue angle and chroma values for potato starch extrudates ranged between 74.64 to 86.04$^{0}$ and 17.23 to 33.75 respectively. Viscosity decreased during extrusion cooking of starch. Regression coefficient analysis indicated a lower viscosity with increase in screw speed, whereas an increased viscosity with increasing feed moisture and barrel temperature was observed during processing of potato starch. The study is important for the processed food industry with respect to reformulation and development of food products with desired consistency and improved functional properties using potato starch.

***Keywords***: Colour, Extrusion, Potato starch, Response surface methodology, Viscosity

Starch is a versatile carbohydrate which is used in food processing industry as a food component in the form of gelling agent, water absorbent, thickening agent, texture modifier, dusting agent and nutrient (provide up to 80% of the calories) etc. The physico-chemical characteristics and functional properties of starch vary in different food products with respect to its biological origin. Potato starch has received extensive attention in relation to its various functional properties. These are popularly used as thickening agents in liquid based foods (e.g. soup, sauces, gravies, custards etc.) because of its bland flavour and viscosity characteristics. The potato starch granules are large (40µm) and its high molecular weight amylose and phosphate groups esterified to amylopectin contribute to high transparency, water-binding capacity, swelling power and freeze-thaw stability (Craig et al 1989). The use of native potato starch in processed food and other industries is limited due to low shear and thermal resistance, thermal decomposition and high tendency of amylase towards retrogradation (Meister 2000). Processing or modification of starch, which involves the alteration of the physicochemical properties of native starch to improve its functional characteristics, generally used to adapt starch to specific food applications. Starch can be modified by different methods such as physical, chemical, and enzymatic. Out of these, Extrusion is a high temperature short time (HTST) method which can be successfully used for gelatinization/modification of starch (Gandhi et al 2018). In extrusion processing, flour/starch is passed through the length of barrel in the presence of water using heat and shear forces over a certain time above the gelatinizing temperature of starch to achieve the desired functional product attributes. Important parameters for product quality in extrusion cooking include moisture content of the ingredient, residence time, which is influenced by feeding rate, screw speed and configuration, die geometry, time and temperature (Obatolu et al 2005). The results of extrusion include gelatinization of starch, denaturation of proteins, inactivation of antinutritional factors and many native enzymes, reduction of microbial counts, and improvement in digestibility and biological value of proteins. Different extrusion processing conditions result in different type of meshwork structures, and there is improvement in the functional properties of starch including colour profile and viscosity (Shimelis et al 2006), which can be utilized as functional ingredient in food products. High energy density flour produced by extrusion cooking had the added advantage that the extrudate has a low water activity, and is cheaper to handle, transport and store and is less susceptible to microbial spoilage. The present investigation was proposed to assess the effect of twin screw extrusion on colour profile and viscosity potato starch. The study was attempted using Response Surface Methodology (RSM) as it helps in unbiased study of experiment.

## MATERIAL AND METHODS

**Raw material:** Commercial Potato starch, used for the study, was procured from the local market Ludhiana, Punjab.

**Proximate analysis:** The moisture, protein, fat and ash contents were analysed according to AACC methods (AACC 2000). Crude fibre was estimated using Fibertec (Foss Company, Hillerod, Denmark). The proximate analysis of the potato starch was 7.19% moisture, 0.13% crude protein, 0.08% fat, 2.90% crude fibre, 0.39% ash and 89% carbohydrates.

**Extrusion:** Extrusion experiments were performed on a co-rotating intermeshing twin screw extruder model BC-21 (Clextral, Firminy, France). The barrel diameter and its length to diameter ratio (L/D) were 2.5 mm and 16:1, respectively. The extruder barrel is divided into four zones. Temperature of first, second and third zone was maintained at 40, 70 and 100°C, respectively throughout the experiment, while in the fourth zone the temperature was varied according to the experimental design. The extruder was powered by 8.5 kW motor with speeds variable from 0 to 682 rpm. However, over entire experiment, the screw speed of the extruder was varied from 400- 550 rpm. Raw material was metered into the extruder with a single screw volumetric feeder (DS and M Modena, Italy). The feed rate was varied for optimum fill according to screw speed. The moisture content of the feed was adjusted by injecting water (approximately 30 °C) into the extruder with a pump. The die plate with four openings (1.5 mm diameter each) was used. A variable speed die face cutter with four bladed knives was used to cut the extrudates (Fig. 1).

**Experimental design:** Twenty experimental runs for potato starch were generated using central composite rotatable design (CCRD) for three independent variables i.e. feed moisture (14-18%), screw speed (400-550 rpm) and barrel temperature (125-175°C). Experimental design was generated using commercial statistical package, Design-Expert Version 7 (Statease Inc., Minneapolis, MN, USA). A feed of around 500g was used for each processing condition. Response variables include colour profile and viscosity of starch. Response surface methodology (RSM) was used to investigate the effects of extrusion conditions on product quality. Multiple linear regression method was used to analyze the results. This method describes the effects of variables in the models derived. Regression coefficients were obtained by fitting the experimental data to the selected models. The individual effect of each variable and also the effects of interaction term in coded levels of variables were determined.

**Colour profile:** The colour of the extruded starch samples was measured using colour meter, CR-300 (Minolta Camera Co. Ltd., Japan). Three random readings were taken on the leveled surface. Results were expressed in the CIE L* a* b*. The colour psychophysical parameters chroma (C*) and hue angle (h*) were obtained by transformation of the Cartesian coordinates (a* and b*) to polar coordinates (C* and h*) according to the following equations (Gonnet 1998).

$$\text{Hue Angle } (h^*) = \arctan\left(\frac{b^*}{a^*}\right)$$

$$\text{Chroma } (C) = \sqrt{a^2 + b^2}$$

**Viscosity:** Viscosity of extruded starch samples were determined using Rapid Visco Analyser (RVA) model StarchMaster2 (Newport Scientific, Warrie Wood, Australia) at 01.30 minute of RVA cycle (Gandhi et al 2018).

## RESULTS AND DISCUSSION

During extrusion processing, starch in the presence of water, heat and shear forces cause substantial changes in the physical composition and functional properties of the starch granules which lead to the modification of colour characteristics and viscosity of starch.

**Colour profile:** Colour is a very important quality parameter of food products since it influences consumer acceptability. The effect of extrusion conditions (feed moisture, screw speed and barrel temperature) on luminosity (L*), redness (a*) yellowness (b*), hue angle and chroma of potato starch extrudates was observed (Table 1). The L* value ranging between 0 to 100 gives an idea about the lightness of the product. The maximum for L is 100 which depicts a perfect reflecting diffuser. The minimum is 0 which indicates black. Positive value for a* denotes redness of product and negative value for a* depicts greenness. Positive b* value is yellow and negative b* is blue. Regression analyses for all the parameters were carried out to fit mathematical models to the experimental data. The quadratic model for L*, a*, b*, hue angle and chroma values can be described by the following equation in terms of coded values i.e. A= feed moisture, B= screw speed and C= barrel temperature

$$L = +70.06 + 1.84A + 1.09B - 1.99C - 0.23AB + 0.67AC + 0.085BC + 2.49A^2 + 0.25B^2 + 0.75C^2$$

$$a^* = +8.20 - 1.63A + 0.16B + 0.17C + 0.010AB - 0.17AC + 0.14BC - 1.64A^2 - 0.72B^2 - 0.48C^2$$

$$b^* = +31.64 - 3.57A + 0.65B - 0.17C - 0.41AB - 0.29AC + 0.28BC - 3.32A^2 - 0.66B^2 - 1.11C^2$$

$$\text{Hue angle} = +75.47 + 2.26A - 0.086B - 0.32C - 0.27AB + 0.29AC - 0.18BC + 2.24A^2 + 0.96B^2 + 0.34C^2$$

$$\text{Chroma} = +32.69 - 3.81A + 0.66B - 0.12C - 0.40AB - 0.32AC + 0.30BC - 3.58A^2 - 0.81B^2 - 1.19C^2$$

The significance of the fitted quadratic model was evaluated by using F- test and P- value. The measured values of the L* (lightness) parameter of potato extrudates varied from 67.54 to 80.28 (Table 1, 2). The $R^2$ (coefficient of determination) and adjusted $R^2$ for L* value were 0.999 and 0.998 respectively. The measured a* value, which indicates the redness of the sample, ranged from 1.19 to 8.71. The $R^2$ and adjusted $R^2$ for a* value were 0.967 and 0.937 respectively. The b* of the potato extrudates ranged from 17.19 to 32.71. The coefficient of determination ($R^2$) and adjusted $R^2$ for b* value were 0.979 and 0.960 respectively. The measured hue angle values (which depicts all colour parameters of the sample) for potato extrudates ranged from 74.64 to 86.04. The coefficient of determination ($R^2$) and adjusted $R^2$ for hue angle were 0.947 and 0.899 respectively. Chroma values varied from 17.23 to 33.75 under different conditions of feed moisture, screw speed and barrel

**Table 1.** Effect of extrusion conditions on colour profile and viscosity (n=3) of potato starch extrudates

| Extrusion conditions (Actual and coded values) | | | Colour characteristics | | | | | Viscosity (cP) |
|---|---|---|---|---|---|---|---|---|
| A: Feed moisture (%) | B: Screw speed (Rpm) | C: Barrel temperature (°C) | L* | A* | B* | Hue angle (°) | Chroma | |
| 14 (-1) | 400 (-1) | 125 (-1) | 73.04 | 7.03 | 29.61 | 76.64 | 30.43 | 49.0 |
| 18 (1) | 400 (-1) | 125 (-1) | 75.86 | 3.77 | 23.76 | 80.98 | 24.06 | 116.0 |
| 14 (-1) | 550 (1) | 125 (-1) | 75.51 | 7.12 | 31.28 | 77.18 | 32.08 | 46.0 |
| 18 (1) | 550 (1) | 125 (-1) | 77.44 | 3.94 | 22.59 | 80.11 | 22.93 | 62.0 |
| 14 (-1) | 400 (-1) | 175 (1) | 67.54 | 6.79 | 29.54 | 77.05 | 30.31 | 140.0 |
| 18 (1) | 400 (-1) | 175 (1) | 73.06 | 2.91 | 21.31 | 82.22 | 21.51 | 142.0 |
| 14 (-1) | 550 (1) | 175 (1) | 70.37 | 7.46 | 31.12 | 76.52 | 32.00 | 71.0 |
| 18 (1) | 550 (1) | 175 (1) | 74.96 | 3.58 | 22.46 | 80.94 | 22.74 | 64.0 |
| 12.64 (-1.682) | 475 (0) | 150 (0) | 74.14 | 6 | 27.53 | 77.70 | 28.18 | 39.0 |
| 19.36 (+1.682) | 475 (0) | 150 (0) | 80.28 | 1.19 | 17.19 | 86.04 | 17.23 | 136.0 |
| 16 (0) | 348.87 (-1.682) | 150 (0) | 69.09 | 6.02 | 28.24 | 77.97 | 28.87 | 64.0 |
| 16 (0) | 601.13 (+1.682) | 150 (0) | 72.69 | 6.39 | 31.56 | 78.55 | 32.20 | 78.0 |
| 16 (0) | 475 (0) | 107.96 (-1.682) | 75.64 | 5.87 | 28.46 | 78.35 | 29.06 | 61.0 |
| 16 (0) | 475 (0) | 192.04 (+1.682) | 68.95 | 7.89 | 28.79 | 74.67 | 29.85 | 128.0 |
| 16 (0) | 475 (0) | 150 (0) | 70.05 | 8.2 | 31.63 | 75.47 | 32.68 | 54.0 |
| 16 (0) | 475 (0) | 150 (0) | 70.11 | 8.71 | 31.71 | 74.64 | 32.88 | 59.0 |
| 16 (0) | 475 (0) | 150 (0) | 69.99 | 7.69 | 31.55 | 76.30 | 32.47 | 53.0 |
| 16 (0) | 475 (0) | 150 (0) | 70.16 | 8.31 | 32.71 | 75.75 | 33.75 | 61.0 |
| 16 (0) | 475 (0) | 150 (0) | 69.94 | 8.09 | 30.55 | 75.17 | 31.60 | 55.0 |
| 16 (0) | 475 (0) | 150 (0) | 70.06 | 8.21 | 31.65 | 75.46 | 32.70 | 57.0 |

**Table 2.** Analysis of variance for the fit of experimental data to response surface model

| Term | Colour characteristics | | | | | Viscosity (cP) |
|---|---|---|---|---|---|---|
| Factor | L* | a* | b* | Hue angle (°) | Chroma | |
| Model F value | 1441.18* | 32.54* | 52.61* | 19.90* | 53.15* | 5.96* |
| Lack of fit | 4.23** | 4.15** | 2.17** | 4.53** | 2.54** | 1.60** |
| Mean | 72.44 | 6.26 | 28.16 | 77.89 | 28.88 | 76.75 |
| Standard deviation | 0.13 | 0.53 | 0.86 | 0.92 | 0.92 | 26.15 |
| C V (%) | 0.18 | 8.46 | 3.06 | 1.19 | 3.18 | 34.08 |
| $R^2$ | 0.9992 | 0.9670 | 0.9793 | 0.9471 | 0.9795 | 0.5117 |
| Adjusted $R^2$ | 0.9985 | 0.9373 | 0.9607 | 0.8995 | 0.9611 | 0.4201 |
| Predicted $R^2$ | 0.9951 | 0.7887 | 0.8799 | 0.6566 | 0.8776 | 0.1797 |
| Adequate precision | 138.28 | 19.76 | 25.27 | 15.50 | 25.44 | 8.493 |

*Significant at $P < 0.01$, **non-significant at $P > 0.05$

temperature. The values for $R^2$ and adjusted $R^2$ for chroma under quadratic model were found out to be 0.979 and 0.961 respectively (Table 3). L* value of the potato extrudates increased gradually with increase in feed moisture and screw speed but decreased with respect to elevated temperature of barrel (Fig. 2 a,b). Sun and Muthukumarappan (2002) also reported insignificant effect of barrel temperature on L value but found increase in L* value with increase in feed moisture. The redness (a*) showed positive correlation with barrel temperature, screw speed and negatively correlated to feed moisture content. The positive effect of barrel temperature on a* value could be due to heat induced degradation of feed material which may also have lowered the L* value of samples after extrusion. Takahashi et al (2005) reported the similar colour changes of milled rice flour heat treated in an autoclave (120°C for 60 min) and oven (160°C for 60 min). A decrease in b* value (yellowness) of potato extrudates was observed with an increase in barrel temperature which is in opposite with the change in a* value (redness). Non-enzymatic browning and pigment destruction are the two different reactions which mainly induced the changes in yellowness (b*) during extrusion processing. During extrusion cooking of starch in this study, changes in yellowness (b*) were markedly lower than the changes reported for other cereal based extrudates (Ilo and Berghofer 1999). Both hue angle and chroma of modified starch decreased with an increase in barrel temperature indicating more redness and darkness of the product. High barrel temperature causes heat induced degradation of feed material. This might be the reason for increased darkness of product after extrusion. These results are validated by the findings of earlier workers (Fratianni et al 2005, Lamberts et al 2007, Sandhu et al 2*007*). In general, extrusion variables affected the colour of potato extrudates and an overall increase in luminosity, increase in redness and yellowness was observed.

**Viscosity:** The type of starch (granule size, amylose/amylopectin ratio and chain-length distribution of amylopectin) is responsible for viscosity profile of the product. During gelatinization, in the presence of heat and moisture, starch granules swell several times to their initial volume. Swelling of starch granules is accompanied by granule rupture, crystallinity loss and leaching of granule constituents, predominately amylose and the formation of gel (a three dimensional network) (Zeng et al 2011). After extrusion processing, maximum viscosity occurs at the equilibrium point between swelling and polymer leaching which causes an increase in viscosity and can be measured at 1.30 min of rapid visco analyzer (RVA) cycle. It also indicates the water binding capacity of starch and dependent on the rigidity of starch granules. Potato starch exhibits

**Fig. 1.** Potato starch extrudates

**Table 3.** Regression coefficients and model fit parameters for each response surface equation

| Parameter | L* | a* | b* | Hue angle (°) | Chroma | Viscosity (cP) |
|---|---|---|---|---|---|---|
| Intercept of model | 70.06 | 8.20 | 31.64 | 75.47 | 32.69 | 76.75 |
| Feed moisture (A) | 1.84** | -1.63** | -3.57** | 2.26** | -3.81** | 17.66* |
| Screw speed (B) | 1.09** | 0.16 | 0.65* | -0.09 | 0.66* | -13.21 |
| Barrel temperature (C) | -1.99** | 0.17 | -0.17 | -0.32 | -0.12 | 18.79* |
| Feed moisture x Screw speed (AB) | -0.23** | 0.01 | -0.41 | -0.27 | -0.40 | - |
| Feed moisture x Barrel temperature (AC) | 0.67** | -0.17 | -0.29 | 0.29 | -0.32 | - |
| Screw speed x Barrel temperature (BC) | 0.09 | 0.14 | 0.28 | -0.18 | 0.30 | - |
| Feed moisture x Feed moisture ($A^2$) | 2.49** | -1.64** | -3.32** | 2.24** | -3.58** | - |
| Screw speed x Screw speed ($B^2$) | 0.25** | -0.72** | -0.66* | 0.96** | -0.81** | - |
| Barrel temperature x Barrel temperature ($C^2$) | 0.75** | -0.48** | -1.11** | 0.34 | -1.19** | - |

*Significant at $P < 0.05$, ** Significant at $P < 0.01$

unique viscosity behaviour with change in temperature, moisture and shear rate which varied from 39 to 142 cp (Table 1). Equation for linear model obtained for viscosity of extruded potato starch as follows:

$$\text{Viscosity} = +76.75 + 17.66\,A - 13.21\,B + 18.79\,C$$

Regression model fitted to experimental results of viscosity showed the p-value for lack of fit is non-significant (Table 2). Extrusion conditions showed marked influence on the viscosity of potato starch extrudates. Screw speed has inverse relation with the viscosity of potato starch when assessed on RVA. It might be due to the greater mechanical action which results in degradation of the starch granules and therefore lead to lower viscosity value (Hernandez-Nava et al 2011). Similar trend was observed by Leonel et al (2011) at higher screw speed. The extent of breakdown depends on the temperature and shear stress or degree of mixing applied to the mixture and the nature of material itself. However, Lai (2001) documented that viscosity reduced upon thermal processing. Similar results were also reported by Hussain et al (2013) for reconstituted rice. Himmelsbach et al (2008) observed that pregelatinized flours have fewer amounts of native granules available for hydration and subsequently lower viscosity. The lower value of viscosity of potato extruded starch indicated that the extrusion process affected the ability of starch to form a viscous paste because of disruption of starch granules by gelatinization.

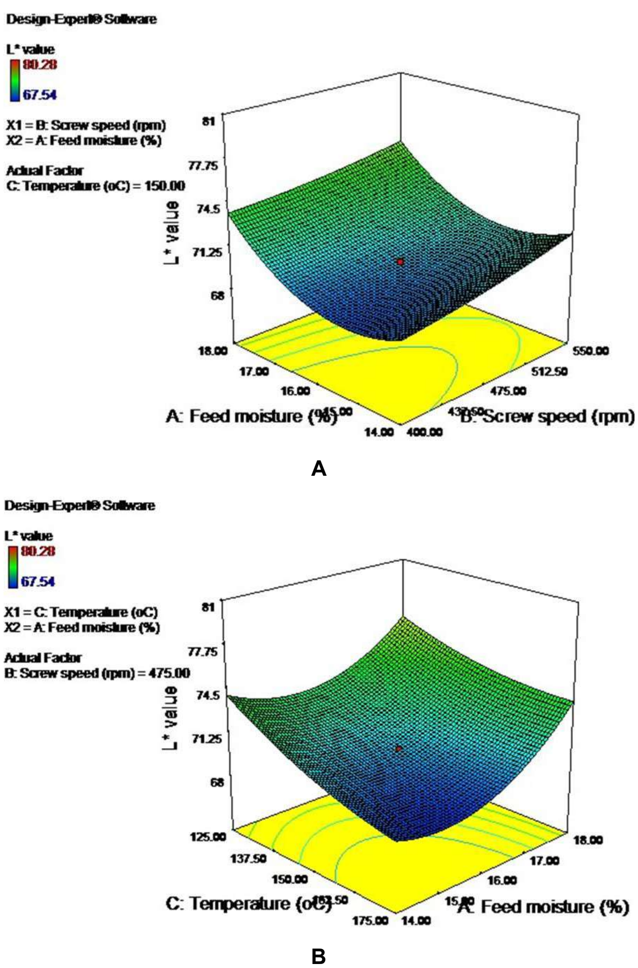


**Fig. 2.** Effect of (a) feed moisture and screw speed (b) barrel temperature and feed moisture on L* value of potato starch

## CONCLUSION

The study showed significant changes in colour and viscosity characteristics of potato starch with respect to different extrusion processing conditions. A positive effect of screw speed on luminosity (L*), redness (a*) and yellowness (b*) of potato starch was observed. Application of higher barrel temperature leads increase redness and decrease in yellowness and luminousity of potato extrudates. Increase in feed moisture content enhanced luminosity but lowered redness and yellowness. Maximum viscosity was observed at lower extrusion conditions. Higher screw speed resulted in degradation of the starch which lead to reduction in viscosity. Understanding the relationship between starch structure and processing conditions will help the food technologists in the preparation of instant food products with the desired consistency.

## DECLARATION

The authors declare that they do not have any conflict of interest.

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# Application of Fuzzy Logic for Sensory Evaluation of Pre-treated Vacuum Fried Carrot Chips

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***Abstract***: The five various pre-treatments of vacuum fried (VF) carrot chips were evaluated using the sensory evaluation by consumers. Sensory analysis of the samples was conducted based on fuzzy logic model in terms of their ranking. Based on general sensory attributes color and appearance, texture, flavor, taste and overall acceptability of the VF chips five different pre-treatments selected were named and coded as Blanching (C1), Blanching cum drying(C2), Blanching cum freezing (C3), Freezing(C4) and Guar gum (C5). These five pre-treatments were compared with control samples (C6) and atmospheric frying (C7). The output of the fuzzy logic model samples were ranked as excellent, good, medium, fair and not satisfactory. The results of sensory analysis using the fuzzy logic showed order of ranking of the vacuum fried carrot chips follows as C4>C3=C1>C6>C2>C7>C5.

***Keywords***: Fuzzy logic, Sensory study, Pre-treatments, Vacuum frying, Carrot chips

Fuzzy logic is an important decision-making tool for comparing a developed product with similar products available in market and is possible to find out the reasons for low and high ranking of products. The fuzzy model can be used to determine the importance of individual factors to the overall quality of a product. This can be useful for product development and improvement because the most important factors for a particular market can be identified and improved. In most cases the experts opinion rather comes in linguistic form, which contains a lot of subjectivity, vagueness and ambiguity. Fuzzy logic is an important tool by which indistinct and vague data can be analyzed and important conclusions regarding acceptance, rejection, ranking, strong and weak attributes of food can be drawn. In fuzzy modelling, linguistic variables (not satisfactory, good, excellent, medium and fair) are used for developing relationship between independent (taste, color and appearance, flavor, texture and overall acceptance) and dependent (acceptance, rejection, ranking, strong and weak attributes of food) variables (Das 2005, Routray and Mishra 2011). Fuzzy sets can be used for analysis of sensory data instead of average scores to compare the samples attributes, since fuzzy sets are not confined to deterministic value and have a merit in sensory evaluation because human expressions on filling for foods are fuzzy rather than deterministic. The developed fuzzy mathematical models perform remarkably well in the evaluation and ranking of food products (Fatma et al 2016). In fuzzy theory, a subject can be represented by fuzzy sets with a series of elements and their membership degrees compared to crisp sets without membership. Such fuzzy sets provide the mathematical methods that can represent the uncertainty of human's expressions attributes of ready to eat (RTE) food that are evaluated by human senses are color and appearance texture, flavour, taste and overall acceptance (Lazim and Suriani 2009). The objective of this research was to conduct a sensory analysis using fuzzy logic model in order to analyze the acceptability of five various pre-treated vacuum fried carrot chips.

## MATERIAL AND METHODS

**Preparation of vacuum fried carrot chips:** The orange colour and matured carrot was procured from the local market at Tavanur, Kerala. Carrots were cleaned manually, peeled with peeler and made into strips by using Dicer. The average thickness and diameter carrot strips were less than 4 mm and 39.07 mm respectively.

**Sensory evaluation:** The data available from subjective evaluation of five different pre-treated VF carrot chips sample were analyzed by using fuzzy logic model and Matlab software (Das 2005). The samples were coded and named as blanching (C1), blanching cum drying (C2), blanching cum freezing (C3), freezing(C4) and guar gum (C5). These five pre-treatments were compared with control (C6) and atmospheric frying (C7). Judges were selected for sensory evaluation from the staffs and students of the Department of Processing and Food Engineering (Kelappaji College of Agricultural Engineering and Technology, Tavanur, Kerala) in the age group between 23 and 56 years, included 5 female

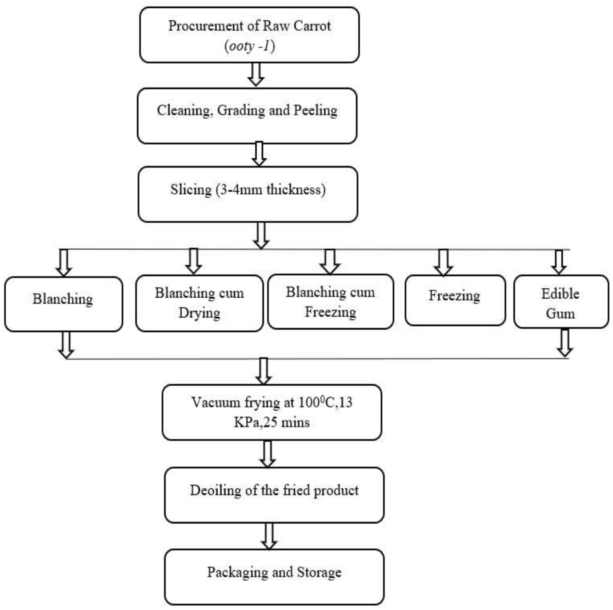


**Fig. 1.** Flow chart for vacuum frying of carrot chips using various pre-treatments

and 10 male. Judges were acquaint with the colour and appearance, texture, flavour, taste and overall acceptance of vacuum fried carrot chips prepared from fresh 'Ooty-1' variety of carrot before the actual sensory evaluation. The judges were asked to rate the pre-treated vacuum fried carrot chips for these sensory attributes the samples were labelled as C1, C2, C3, C4, C5, C6 and C7. Judges were instructed to give tick (√ ) mark in the respective fuzzy scale factor for each of the quality attributes of the sample after evaluating the samples (Jaya and Das 2003). The fuzzy scale factors were assigned as Excellent: EX, Good: GD, Medium: MD, Fair: FR and not satisfactory: NS. The judges were asked to give a score in the fuzzy scale based on their own opinion and likings regarding chips. After evaluating the samples, were asked to give marks for each of the quality attributes out of 100 based on their own taste regarding chips. These marks were called as 'weightage' of each of the attributes. Average of weightages of each attribute given by all the judges were found and called 'average weightage' of that quality attribute. The results were analyzed by using fuzzy comprehensive model to find out the best of the seven samples.

**Fuzzy comprehensive model for sensory scores:** The fuzzy comprehensive model was used for the analysis of sensory data. Fuzzy model for the present problem was having three sets: (i) Factor set $U_f$, (ii) Evaluation set $V_f$ and (iii) Fuzzy transformation $T_f$. The factor set, $U_f$ contains all of the quality attributes such as hardness, taste, smell and mouth feel of the products. The evaluation set, $V_f$ includes the scale factor for each of the quality attributes, such as Excellent, Good, Medium, Fair and Not satisfactory. For the fuzzy transformation ($T_f$) of the factor set ($U_f$) into evaluation set ($V_f$), numerical values assigned to the scale factors were: Excellent (EX) = 1, Good (GD) = 0.9, Medium (MD) = 0.7, Fair (FR) = 0.4 and Not satisfactory (NS) = 0.1.

**Evaluation of Analysis (Jaya and Das 2003)**

**Fuzzy membership function (FMF), $M_f$:** It was calculated by adding the individual scale factor given to each of the quality attribute of the product and dividing it by the number of judges who tasted the product.

$$M_f = \sum V_f / \text{total of judges} \ldots\ldots\ldots\ldots(1)$$

**Normalized fuzzy membership function (NFMF), Nf :** NFMF was calculated by multiplying each of the fuzzy membership function with the assigned numerical value of the respective 'scale factor'.

$$N_f = M_f \times S_f \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

**Normalized fuzzy membership function matrix, $O_f$:** Addition of the normalized fuzzy membership function of individual linguistic term of respective quality attributes for each the products given for sensory evaluation formed the elements of the normalized fuzzy membership function matrix. Like this, all the element of the normalized matrix were form and written in the form of a matrix called normalized fuzzy membership function matrix having its row as quality attributes and the column as samples number.

$$O_f = \sum N_f \text{ for each quality attribute} \ldots\ldots\ldots(3)$$

**Judgment membership function matrix, Xf :** The column values of a sample were then added and the individual values of the same column were divided by the "Maximum" of the added value. These values formed the elements of the judgment membership function matrix. Thus, the matrix decided the rank of the chips.

$$X_f = O_f / \max \sum O_f \ldots\ldots\ldots\ldots\ldots(4)$$

**Judgment subset, Yf**: The average of numerical weightage (as fraction) given by the judges for individual quality attributes: çolor and appearance, texture, flavour, taste and overall acceptance formed the judgement subset, Yf (Ranaselva 2017).

$$Yf\ ,= \left[\frac{0.2}{C\ \&\ A}, \frac{0.3}{T}, \frac{0.1}{F}, \frac{0.2}{TA}, \frac{0.2}{0A}\right]$$

**Quality-ranking subset, Zf:** Finally a comparison was made between the individual elements of the judgment membership function matrix (Xf) and the respective elements of the judgment subset (Yf). Thus, the minimum of them was taken to form the quality-ranking subset, Zf.

**Ranking of the sample:** Rank one was assigned to the sample, which had the maximum value in the quality ranking subset Zf. Then the quality attribute, which gave the highest value, was considered as the reason for that sample to get the highest rank. Likewise, all the seven samples were assigned a rank based on its overall acceptability.

**Calculations for determination of different membership functions:**

$$\textbf{Fuzzy membership function (FMF), } M_f = \frac{\text{Individual scores of samples}}{\text{Total no. of judges}}$$

Eg : FMF for sample C1 (Color & Appearance) = 3/15 = 0.2

= 9/15 = 0.6

**Normalized fuzzy membership function (NFMF)**, $N_f$ = FMF × Scale factor

Eg : NFMF for sample C1(Color &appearance) = 0.2 × 1 =0.2

= 0.6 × 0.9 = 0.54

**Table 1a.** Scale factor, fuzzy membership function (FMF) and normalized membership function (NFMF) for quality attributes of first three samples of VF carrot chips

| Sensory attributes | Sensory quality factor | Scale factor | Blanching (C1) | | | Blanching cum drying (C2) | | | Blanching cum freezing (C3) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. of judges rated | FMF (Mf) | NFMF (Nf ) | No. of judges rated | FMF (Mf) | NFMF (Nf ) | No. of judges Rated | FMF (Mf) | NFMF (Nf ) |
| Color and Appearance | EX | 1 | 3 | 0.2 | 0.2 | 0 | 0 | 0 | 4 | 0.266 | 0.266 |
| | GD | 0.9 | 9 | 0.6 | 0.54 | 6 | 0.4 | 0.36 | 7 | 0.466 | 0.42 |
| | MD | 0.7 | 2 | 0.133 | 0.093 | 4 | 0.266 | 0.186 | 4 | 0.266 | 0.186 |
| | FR | 0.4 | 1 | 0.066 | 0.026 | 5 | 0.333 | 0.133 | 0 | 0 | 0 |
| | NS | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | | | 15 | | 0.86 | 15 | | 0.68 | 15 | | 0.873 |
| Texture | EX | 1 | 4 | 0.266 | 0.266 | 0 | 0 | 0 | 3 | 0.2 | 0.2 |
| | GD | 0.9 | 7 | 0.466 | 0.42 | 5 | 0.333 | 0.3 | 6 | 0.4 | 0.36 |
| | MD | 0.7 | 3 | 0.2 | 0.14 | 7 | 0.466 | 0.326 | 5 | 0.333 | 0.233 |
| | FR | 0.4 | 1 | 0.066 | 0.026 | 3 | 0.2 | 0.08 | 1 | 0.066 | 0.026 |
| | NS | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | | | 15 | | 0.853 | 15 | | 0.706 | 15 | | 0.82 |
| Flavor | EX | 1 | 2 | 0.133 | 0.133 | 0 | 0 | 0 | 4 | 0.266 | 0.266 |
| | GD | 0.9 | 9 | 0.6 | 0.54 | 6 | 0.4 | 0.36 | 6 | 0.4 | 0.36 |
| | MD | 0.7 | 4 | 0.266 | 0.186 | 5 | 0.333 | 0.233 | 5 | 0.333 | 0.233 |
| | FR | 0.4 | 0 | 0 | 0 | 3 | 0.2 | 0.08 | 0 | 0 | 0 |
| | NS | 0.1 | 0 | 0 | 0 | 1 | 0.066 | 0.006 | 0 | 0 | 0 |
| Total | | | 15 | | 0.86 | 15 | | 0.68 | 15 | | 0.86 |
| Taste | EX | 1 | 4 | 0.266 | 0.266 | 1 | 0.066 | 0.066 | 5 | 0.333 | 0.333 |
| | GD | 0.9 | 8 | 0.533 | 0.48 | 5 | 0.333 | 0.3 | 6 | 0.4 | 0.36 |
| | MD | 0.7 | 3 | 0.2 | 0.14 | 6 | 0.4 | 0.28 | 4 | 0.266 | 0.186 |
| | FR | 0.4 | 0 | 0 | 0 | 3 | 0.2 | 0.08 | 0 | 0 | 0 |
| | NS | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | | | 15 | | 0.886 | 15 | | 0.726 | 15 | | 0.88 |
| Overall acceptability | EX | 1 | 3 | 0.2 | 0.2 | 0 | 0 | 0 | 5 | 0.333 | 0.333 |
| | GD | 0.9 | 9 | 0.6 | 0.54 | 6 | 0.4 | 0.36 | 5 | 0.333 | 0.3 |
| | MD | 0.7 | 3 | 0.2 | 0.14 | 6 | 0.4 | 0.28 | 5 | 0.333 | 0.233 |
| | FR | 0.4 | 0 | 0 | 0 | 3 | 0.2 | 0.08 | 0 | 0 | 0 |
| | NS | 0.1 | 0 | 0 | 0 | 2 | 0.133 | 0.013 | 0 | 0 | 0 |
| Total | | | 15 | | 0.88 | 15 | | 0.733 | 15 | | 0.866 |
| $O_f$ | | | | | $O_{f1}$ = 4.34 | | | $O_{f2}$ =3.52 | | | $O_{f3}$ =4.30 |

**Note:** EX- Excellent; GD-Good; MD- Medium; FR -Fair; NS- Not satisfactory; $O_f$- Normalized fuzzy membership function matrix
$O_{f1}$, $O_{f2}$, $O_{f3}$–Normalized fuzzy membership function matrix of blanching, blanching cum drying and blanching cum drying samples

**Normalized fuzzy membership function matrix, $O_f$**

$O_f$ = Total of NFMF (Color & appearance + Texture + Flavor +Taste + overall acceptability) where n =1,2,….and 7.

Eg: sample C1 ($O_{f1}$) = 0.86+0.853+0.86+0.886+0.88 = 4.34

Sample C2 ($O_{f2}$) = 0.68+0.706+0.68+0.726+0.733=3.52

Repeated the same procedure for all samples and sensory attributes

From above step, find the maximum of the total of normalized fuzzy membership function. Eg : Sample C4($O_{f4}$ = 4.68) .

**Judgement membership function (JMF), Xf=** $\dfrac{\text{Total of NFMF}}{\text{Maximum of total NFMF}}$

Eg: $X_f$ for sample C1 - C&A = 0.86 / 4.68 = 0.183

Texture = 0.853 / 4.68 = 0.182

**Table 1b.** Scale factor, fuzzy membership function (FMF) and normalized membership function (NFMF) for quality attributes of next four samples of VF carrot chips

| Sensory attributes | Sensory quality factor | Scale factor | Freezing (C4) | | | Guar Gum (C5) | | | Control (C6) | | | Atmospheric fried (C7) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. of judges rated | FMF (Mf) | NFMF (Nf) | No. of judges rated | FMF (Mf) | NFMF (Nf) | No. of judges rated | FMF (Mf) | NFMF (Nf) | No. of judges rated | FMF (Mf) | NFMF (Nf) |
| Color & appearance | EX | 1 | 6 | 0.4 | 0.4 | 0 | 0 | 0 | 1 | 0.066 | 0.066 | 0 | 0 | 0 |
| | GD | 0.9 | 9 | 0.6 | 0.54 | 0 | 0 | 0 | 8 | 0.533 | 0.48 | 0 | 0 | 0 |
| | MD | 0.7 | 0 | 0 | 0 | 8 | 0.533 | 0.373 | 3 | 0.2 | 0.14 | 1 | 0.066 | 0.046 |
| | FR | 0.4 | 0 | 0 | 0 | 7 | 0.466 | 0.186 | 2 | 0.133 | 0.053 | 7 | 0.466 | 0.186 |
| | NS | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.066 | 0.006 | 7 | 0.466 | 0.046 |
| Total | | | 15 | | 0.94 | 15 | | 0.56 | 15 | | 0.746 | 15 | | 0.28 |
| Texture | EX | 1 | 7 | 0.466 | 0.466 | 0 | 0 | 0 | 1 | 0.066 | 0.066 | 0 | 0 | 0 |
| | GD | 0.9 | 6 | 0.4 | 0.36 | 0 | 0 | 0 | 8 | 0.533 | 0.48 | 0 | 0 | 0 |
| | MD | 0.7 | 2 | 0.133 | 0.093 | 6 | 0.4 | 0.28 | 2 | 0.133 | 0.093 | 0 | 0 | 0 |
| | FR | 0.4 | 0 | 0 | 0 | 6 | 0.4 | 0.16 | 3 | 0.2 | 0.08 | 8 | 0.533 | 0.213 |
| | NS | 0.1 | 0 | 0 | 0 | 3 | 0.2 | 0.02 | 0 | 0 | 0 | 7 | 0.466 | 0.046 |
| Total | | | 15 | | 0.92 | 15 | | 0.46 | 15 | | 0.72 | 15 | | 0.26 |
| Flavor | EX | 1 | 5 | 0.333 | 0.333 | 0 | 0 | 0 | 1 | 0.066 | 0.066 | 0 | 0 | 0 |
| | GD | 0.9 | 8 | 0.533 | 0.48 | 0 | 0 | 0 | 9 | 0.6 | 0.54 | 3 | 0.2 | 0.18 |
| | MD | 0.7 | 2 | 0.133 | 0.093 | 7 | 0.466 | 0.326 | 4 | 0.266 | 0.186 | 8 | 0.533 | 0.373 |
| | FR | 0.4 | 0 | 0 | 0 | 8 | 0.533 | 0.213 | 1 | 0.066 | 0.026 | 4 | 0.266 | 0.106 |
| | NS | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | | | 15 | | 0.906 | 15 | | 0.54 | 15 | | 0.82 | 15 | | 0.66 |
| Taste | EX | 1 | 8 | 0.533 | 0.533 | 0 | 0 | 0 | 1 | 0.066 | 0.066 | 0 | 0 | 0 |
| | GD | 0.9 | 7 | 0.466 | 0.42 | 0 | 0 | 0 | 8 | 0.533 | 0.48 | 2 | 0.133 | 0.12 |
| | MD | 0.7 | 0 | 0 | 0 | 3 | 0.2 | 0.14 | 6 | 0.4 | 0.28 | 9 | 0.6 | 0.42 |
| | FR | 0.4 | 0 | 0 | 0 | 8 | 0.533 | 0.213 | 0 | 0 | 0 | 2 | 0.133 | 0.053 |
| | NS | 0.1 | 0 | 0 | 0 | 4 | 0.266 | 0.026 | 0 | 0 | 0 | 2 | 0.133 | 0.013 |
| Total | | | 15 | | 0.953 | 15 | | 0.38 | 15 | | 0.826 | 15 | | 0.606 |
| Overall acceptability | EX | 1 | 9 | 0.6 | 0.6 | 0 | 0 | 0 | 1 | 0.066 | 0.066 | 0 | 0 | 0 |
| | GD | 0.9 | 6 | 0.4 | 0.36 | 0 | 0 | 0 | 7 | 0.466 | 0.42 | 2 | 0.133 | 0.12 |
| | MD | 0.7 | 0 | 0 | 0 | 5 | 0.333 | 0.233 | 5 | 0.333 | 0.233 | 8 | 0.533 | 0.373 |
| | FR | 0.4 | 0 | 0 | 0 | 6 | 0.4 | 0.16 | 2 | 0.133 | 0.053 | 3 | 0.2 | 0.08 |
| | NS | 0.1 | 0 | 0 | 0 | 4 | 0.266 | 0.026 | 0 | 0 | 0 | 2 | 0.133 | 0.013 |
| Total | | | 15 | | 0.96 | 15 | | 0.42 | 15 | | 0.773 | 15 | | 0.586 |
| Of | | | | | $O_{f4}$=4.68 | | | $O_{f5}$=2.36 | | | $O_{f6}$=3.88 | | | $O_{f7}$=2.89 |

**Note** - EX- Excellent; GD-Good; MD- Medium; FR -Fair; NS- Not satisfactory; $O_f$ - Normalized fuzzy membership function matrix
$O_{f4}$, $O_{f5}$, $O_{f6}$ and $O_{f7}$- Normalized fuzzy membership function matrix of Freezing, Guar gum, Control and Atmospheric fried samples

Repeat the same procedure for all samples and sensory attributes. The values of the JMF, were then compared with the average weightage given by the judges for each of the quality attributes. Based on this, the quality ranking sub set values were calculated and (Table 4).

**Quality ranking subset (QR):** Comparing the weightage average of quality attributes and the judgment membership function formed, the minimum of these two was assigned as the quality ranking subset value.

## RESULTS AND DISCUSSION

**Quality ranking on the basis of sensory attributes of VF carrot chips:** The obtained data were analyzed using a fuzzy decision making an approach to determine the ranking of the seven pre-treatment of chips. The data collected from scorecard were analysed using fuzzy decision making. The results are presented as follows. Fuzzy Membership Function ($M_f$) and Normalized Fuzzy Membership Function ($N_f$) were calculated using the Equations (1) and Equation (2). The results are presented in Table 1a and 1b. The seven different samples of various sensory attributes of normalised fuzzy membership function for color and appearance, texture, flavor, taste and overall acceptability varied from 0.56 (C5) to 0.94 (C4), 0.26 (C7) to 0.94 (C4), 0.54 (C5) to 0.906(C4), 0.38(C5) to 0.953(C4), 0.42(C5) to 0.96 (C6), respectively. The results shown that pre-treated freezing sample (C4) having good sensory attributes compared with other samples. This was mainly due to frying of frozen treated samples, free water between the cells would vaporize, and the interspaces left by water evaporation expanded resulted an increase in porosity and reduction in hardness and provide good sensory attributes. These two membership functions ($M_f$ and $N_f$) led to calculation of Normalized Fuzzy Membership Function Matrix ($O_f$) using Equation (3). The maximum and minimum of NFMF matrix ($O_f$) value was 4.68 and 2.36 which was obtained for freezing(sample-C4) and edible gum coated sample(sample-C5). The increasing order of the other samples as follows: sample C1 - 4.34, sample C3 - 4.30, sample C6 - 3.88, sample C2- 3.52 and sample C7- 2.86. The variations were mainly due to application of pre-treatments to the carrot strips which provides good sensory attributes. The matrix $O_f$ was converted to Judgment Membership Function Matrix $X_f$ by using Equation (4). The value of $X_f$ and their corresponding quality attributes are shown in Table 2. Out of seven samples, the freezing sample (C4) has the highest $O_f$ value which was useful for calculation of Judgement membership function (JMF). Judgment subset $Y_f$ was formed using the step in Equation (5). The values of the judgment membership function $X_f$ were then compared with the weights Yf given by the judges for each of the quality attributes. The highest and lowest Xf value of all sensory attributes was obtained for pre-treatment of freezing and

**Table 2.** Evaluation of judgment membership functions (JMF) of seven samples of VF carrot chips

| Sensory attributes | Judgment Membership Functions (JMF), $X_f$ | | | | | | |
|---|---|---|---|---|---|---|---|
| | Blanching (C1) | Blanching cum drying (C2) | Blanching cum freezing (C3) | Freezing (C4) | Guar gum coating (C5) | Control (C6) | Atmospheric fried (C7) |
| Color & acceptability | 0.183 | 0.145 | 0.186 | 0.200 | 0.109 | 0.159 | 0.119 |
| Texture | 0.182 | 0.150 | 0.175 | 0.196 | 0.098 | 0.153 | 0.147 |
| Flavour | 0.183 | 0.145 | 0.183 | 0.193 | 0.105 | 0.175 | 0.141 |
| Taste | 0.187 | 0.153 | 0.188 | 0.202 | 0.081 | 0.175 | 0.129 |
| Overall acceptability | 0.188 | 0.156 | 0.185 | 0.205 | 0.089 | 0.165 | 0.125 |

**Table 3.** Evaluation of quality ranking (QR) of all seven samples of VF carrot chips

| Sensory attributes | Weightage average, $Y_f$ | Quality Rating (QR), ($Z_f$) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | C1 | C2 | C3 | C4 | C5 | C6 | C7 |
| C&A | 0.2 | 0.183 | 0.145 | 0.186 | 0.20 | 0.10 | 0.15 | 0.05 |
| Texture | 0.3 | 0.182 | 0.150 | 0.175 | 0.19 | 0.09 | 0.15 | 0.05 |
| Flavour | 0.1 | 0.183 | 0.145 | 0.183 | 0.19 | 0.10 | 0.17 | 0.14 |
| Taste | 0.2 | 0.187 | 0.153 | 0.188 | 0.20 | 0.08 | 0.17 | 0.12 |
| OA | 0.2 | 0.188 | 0.156 | 0.185 | 0.20 | 0.08 | 0.16 | 0.12 |
| Total | | 0.188 | 0.156 | 0.188 | 0.20 | 0.1 | 0.17 | 0.14 |
| | RANKING | II | IV | II | I | VI | III | V |

C&A – Color and acceptability; OA – Overall acceptability edible gum coated, respectively. Based on $X_f$ values, the quality ranking sub set values were calculated and tabulated in the Table 4. Comparing the weightage average ($Y_f$) of quality attributes and the JMF formed, the minimum of these two was assigned as the quality ranking subset value. The ranks of the samples were assigned from the maximum of quality ranking subset value of the sample. Based on this quality ranking subset ($Z_f$) value, the overall ranking to the chips were given and which follows as: C4(0.2) > C3 (0.188) = C1 (0.188) > C6 (0.17) > C2 (0.156) > C7 (0.14) > C5 (0.10).

## CONCLUSION

The fuzzy logic technique is used to find the order of ranking of ready to eat VF carrot samples. All the samples were good but sample 4 (C4-Freezing) was better than others and was highly acceptable in the market. The results of sensory analysis by using fuzzy logic showed the order of ranking of chips follows as: C4>C3=C1>C6>C2>C7>C5.

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# Food Consumption Pattern and Dietary Intake among Young Punjabi Women

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***Abstract*:** Diet plays an important role in determining the body composition. Dietary pattern of young women is disturbed due to their faulty eating habits. Improvements in eating behavior at younger age can help preventing the metabolic diseases at a later stage. The present investigation was conducted on 473 young adult women in the age group of 20-30 years residing in Punjab Agricultural University hostels to study their food consumption pattern and dietary intake. Majority of the subjects were vegetarians with 96% subjects not in a habit of fasting. Skipping of one of the meals regularly was common in the studied group. The young women had higher preference for health promoting foods with higher consumption frequency score for milk and its products (6.71) followed by salad (5.20) and fruits (3.22). There was quantitative inadequacy of protein in their diets as the average daily protein intake met 72% of the Recommended Dietary Allowance of protein. The subjects were consuming high carbohydrate diet with carbohydrate to protein ratio varying between a narrow range of 4.5 to 4.6. The diets were high in fat and inadequate in many vitamins and minerals. The daily calcium intake was only 50% of the recommended level. The daily intake of Vitamin $D_3$ was negligible. The study recommends an urgent need to provide nutrition education and create awareness to the young population of the country to select foods on the basis of their nutritional benefits which possess a potential to provide better health in later life

***Keywords*:** Young women, Diets, Food habits, Dietary intake, Food consumption score

India is facing a dual burden of malnutrition. Overweight and obesity rates are on rise all over the world. The most crucial time of life for the development of obesity is college years. The young students living in hostels and away from their families are more prone to have an unhealthy lifestyle (Hull et al 2006). Youngsters like to consume processed and convenient foods and fast foods, which are often associated with negative aspects. Diets of many young people of the country offers poor quality protein, saturated fats, lack of vitamins and excess of salt. Moreover, faulty food-related behaviours developed by young adults including irregular meal patterns, such as meal skipping and frequent snacking(Al-Rethaiaa et al 2010), and frequent consumption of commercially prepared meals, such as, restaurant or pre-packaged meals, takeaway food are mostly associated with a poorer quality of diet (Burns et al 2002).

Obesity can arise in early years due to sedentary lifestyle with lack of exercise irregular diet and addiction to junk foods or drugs**.** Physical inactivity and unhealthy dietary habits are key factors that potentially have adverse effects on body composition in young adults and consequently, the future health of adults. These two risk factors have been associated with raised blood glucose and blood pressure, abnormal blood lipids, major chronic diseases like ischemic heart disease, cancer and diabetes (Hutchesson et al 2015). An unhealthy lifestyle among young people is unnoticed and a serious problem. Majority of these young adults study in educational institutes and know the type of food they eat. They need constant motivation for making better food choices. So, there is an urgent need of studying the dietary pattern of these young adults and providing them nutrition education so that they can make better food choices and may remain healthy. Therefore, the present study was undertaken to determine the food consumption pattern and dietary intake of the young adult women living in Punjab Agricultural University hostels.

## MATERIAL AND METHODS

**Sample selection:** The study was conducted in the hostels of Punjab Agricultural University (PAU), Ludhiana district of Punjab. A total of 473 young adult women in the age group of 20-30 years residing in PAU, hostels were selected randomly for the study.

**Dietary habits and food regularity:** The information on food habits i.e. vegetarian, non-vegetarian and ovatarian was collected. For food regularity, the frequency of skipping of three major meals was studied.

**Food consumption frequency:** Information regarding food consumption frequency of the key foods associated with body composition of the selected subjects was collected. The foods were divided into two categories. Category I included foods related to obesity like fried foods, modern snacks, Indian sweets and carbonated beverages. The Category II included health promoting foods like fruits, salads, milk and milk products, dry fruits, eggs and chicken/meat. The consumption frequency for above mentioned foods was recorded over a week. The weekly consumption frequencies Scores (CFS) were calculated. A score of 7 was given to a food if it was consumed in all the 7 days of the week. Similarly, a score of 2 was given to foods consumed twice a week and 3 for a food consumed thrice a week.

**Dietary assessment:** A questionnaire cum interview schedule was used to collect general information and socio-economic profile of the subjects. Dietary intake was assessed using 24-Hour Recall method for 3 consecutive days. An interactive, multiple pass, 24-hour recall method was adopted; the interactive approach incorporates several tools to aid participant's memories in recalling details of foods and beverages consumed, including preparation methods and portion sizes. The nutrient intakes were determined using DietCal software (Kaur 2014). The daily nutrient intake was compared with the Recommended Dietary Allowances (RDA) given by ICMR (2010) to assess the adequacy of nutrients in diet.

## RESULTS AND DISCUSSION

A total number of 473 subjects were divided into two groups according to their age i.e. Group I: 20-24.9 yr and Group II: 25-30yr. Seventy three per cent of the subjects belonged to Group I while 27% of the subjects belonged to Group II. The average age of the subjects was 21.82 and 26.65 yr in Group I and Group II, respectively with an overall mean age of 23.12 yr. The subjects belonged to the families with an average monthly income of Rs. 62,832 with an average per capita income of Rs.11,486. Majority of the subjects i.e. 41% were perusing their master's degree, followed by graduation (34%) and doctorate degree (25%) (Table 1).

**Food habits and food regularity:** Forty six per cent subjects in Group I and 47% of subjects in Group II were vegetarians. Custom of fasting was not common in both the groups as only 6% of the subjects in both the groups reported fasting once a week. Skipping of one or other meal was found in 42% of the subjects in Group II and by 38% of the subjects in Group I. Most commonly skipped meal in both the groups was breakfast as 21 and 22% of the subjects in Group I and II, respectively used to skip breakfast twice a week. Skipping of lunch once a week was observed in 17% in Group I, while only 6% of the subjects used to skip their lunch in Group II (Table 2). The constricted involvement in study assignments by the students was the reason for skipping their meals frequently. Similar findings were observed by Sehrawat (2019) as 30% of the girls living in a hostel in Rohtak, Haryana skipped their breakfast once a week. In another

**Table 1.** General Information and economic status of selected young adult women

| Parameters | Group I (n= 346) | Group II (n=127) | Overall (n=473) |
|---|---|---|---|
| Age (yr) | | | |
| 20.0-22.9 | 228 (66) | - | 228 (48) |
| 23.0-24.9 | 118 (34) | - | 118 (25) |
| 25.0-25.9 | - | 33 (26) | 33 (7) |
| 26.0-30.0 | - | 94 (74) | 94 (20) |
| Average age, yr | 21.82±1.26 | 26.65±1.33 | 23.12±2.50 |
| Monthly family income, Rs. | | | |
| <50,000 | 103 (30) | 29 (23) | 132 (28) |
| 50,001-75,000 | 118 (34) | 40 (32) | 158 (33) |
| 75,001-1,00,000 | 37 (11) | 17 (13) | 54 (12) |
| >1,00,000 | 88 (25) | 41 (32) | 129 (27) |
| Average monthly family income (Rs.) | 57,667±29,110[a] | 76,264±66,448[b] | 62,832±43,582 |
| Per capita monthly income (Rs.) | 10,334±7156[a] | 14,666±10,341[b] | 11,486±8055 |
| Education level | | | |
| Graduating student | 159(46) | 2(1.57) | 161(34) |
| Master's student | 178(51.45) | 15(11.81) | 193(41) |
| Doctorate student | 9(2.60) | 110(86.61) | 119(25) |

Group I: 20-24.9 y; Group II: (25-30 years)
Values in parenthesis indicate percentage
Values in columns followed by different superscripts differ significantly (p≤0.05)

study by Shekar et al (2016) 54.5, 52.4 and 55.6% students in a nursing college in Bihar from urban, semi- urban and rural areas skipped their breakfast.

**Consumption frequency of key foods:** The consumption frequency of key foods which may determine the body composition has been shown in Table 3. The foods included in category I were those which may promote adiposity. The consumption frequency score (CFS) of fried snacks foods such as *samosa, tikki, poori,* etc. was the highest (1.94) in Group I followed by carbonated drinks (0.93), Indian sweets (0.58) and modern snacks (0.55) which included biscuits, pizza, burger pasta etc. by Group I. Similarly, consumption frequency of fried snacks foods was the highest (2.02) followed by carbonated drinks, modern snacks and Indian sweets by the subjects in Group II, the CFS being 2.02, 0.74, 0.54 and 0.53, respectively. The lower consumption frequency of adiposity promoting foods could be due to awareness regarding health risks associated with high consumption of these foods by the subjects. Kaur (2012) reported that majority of the young women (21-30yr) i.e. 34% showed highest preference for sweet snacks followed by fried foods and modern snacks such as pizza, noodles, burgers, etc.

The category II included the foods which may restrict adiposity and improve lean body mass. In Group I, maximum CFS was for milk and its products (6.63) followed by salad (5.28), fruits (3.20) and dry fruits (1.53). CFS among subjects from Group II was also highest for milk and its products (6.91) followed by salad (4.96), fruits (3.27) and dry fruits (1.61). The CFS of egg was only 0.66 and 0.75, while for chicken, meat and fish, it was negligible i.e. 0.22 and 0.17 in Group I and II, respectively. The data revealed that the subjects had higher consumption frequency of foods from category II in comparison to foods in category I as they were aware of health promoting effects of milk and its products, salad, fruits and dry fruits. However, very low consumption of egg, chicken, meat and fish might be attributed to lesser preference, religious or cultural reasons or non-availability of these foods in hostel diets. Kaur (2012) asked young adult women (21-30 yr) for their preferences for healthy food

**Table 2.** Food habits and food regularity among the selected young adult women

| Parameters | Group I (n= 346) | Group II (n=127) | Overall (n=473) |
|---|---|---|---|
| Food habits | | | |
| Vegetarian | 159(46) | 60(47) | 219(46) |
| Non-vegetarian | 131(38) | 46(36) | 177(38) |
| Ovatarian | 56(16) | 21(17) | 77(16) |
| Custom of fasting | | | |
| Yes | 27(8) | 33(26) | 60(13) |
| No | 319(92) | 94(74) | 413(87) |
| Frequency of fasting | | | |
| Once a week | 8(2) | 18(14) | 26(6) |
| Twice a week | 4(1) | 2(2) | 6(1) |
| Once a month | 15(5) | 13(10) | 28(6) |
| Skipping of meals | | | |
| Yes | 131(38) | 53(42) | 184(39) |
| No | 215(62) | 74(58) | 289(61) |
| Breakfast | | | |
| Never | 223(65) | 77(61) | 300(63) |
| Daily | 10(3) | 4(3) | 14(3) |
| Once a week | 14(4) | 2(2) | 16(3) |
| Twice a week | 74(21) | 28(22) | 102(22) |
| Thrice a week | 25(7) | 16(12) | 41(9) |
| Lunch | | | |
| Never | 240(69) | 111(87) | 351(74) |
| Daily | 7(2) | 0(0) | 7(2) |
| Once a week | 59(17) | 8(6) | 67(14) |
| Twice a week | 29 (9) | 6(5) | 35(7) |
| Thrice a week | 11(3) | 2(2) | 13(3) |
| Dinner | | | |
| Never | 275(79) | 104(82) | 379(80) |
| Daily | 9(3) | 2(1) | 11(2) |
| Once a week | 33(9) | 13(10) | 46(10) |
| Twice a week | 20(6) | 6(5) | 26(6) |
| Thrice a week | 9(3) | 2(2) | 11(2) |

Values in parenthesis indicate percentage

**Table 3.** Food consumption frequency of selected foods associated with obesity by selected young adult women

| Parameters | Group I (n= 346) | Group II (n=127) | Overall (n=473) |
|---|---|---|---|
| Fried foods | $1.94±2.25^a$ | $2.02±2.46^a$ | 1.96±2.31 |
| Carbonated drinks | $0.93±1.72^a$ | $0.74±1.46^a$ | 0.88±1.65 |
| Indian sweets | $0.58±1.21^a$ | $0.53±1.18^a$ | 0.57±1.21 |
| Modern snacks | $0.55±1.10^a$ | $0.54±1.05^a$ | 0.55±1.08 |
| Milk and milk products | $6.63±1.45^a$ | $6.91±0.75^b$ | 6.71±1.31 |
| Salad | $5.28±2.66^a$ | $4.96±2.73^a$ | 5.20±2.68 |
| Fruits | $3.20±2.63^a$ | $3.27±2.56^a$ | 3.22±2.61 |
| Dry fruits | $1.53±2.53^a$ | $1.61±2.55^a$ | 1.56±2.53 |
| Eggs | $0.66±1.51^a$ | $0.75±1.36^a$ | 0.69±1.47 |
| Chicken/meat | $0.22±0.91^a$ | $0.17±0.47^a$ | 0.21±0.82 |

Values are Mean±SD
Values in columns followed by different superscripts differ significantly ($p≤0.05$)

options and found that fruits were the top preference followed by milk and milk products. Salads got the 3$^{rd}$rank, while dry fruits were the least preferred food.

**Macronutrient Intake:** The overall mean daily carbohydrate and protein intake of the subjects in both the groups was 180.43g and 39.53 g, respectively. The protein intake of the subjects was 71.88% of the recommended value of 55 g day$^{-1}$ (ICMR, 2010). Kaur and Bains (2013) reported that the daily protein intake ranged between 21.0 -58.0 g day$^{-1}$ with the mean value of 37.7 g day$^{-1}$ and the mean adequacy being 69.6% among 21-30 yr old women. On calculating the carbohydrate: protein ratio, it was found to vary between a narrow range of 4.5 to 4.6 among both groups. Carbohydrate: protein ratio higher than 2.5 indicates a high carbohydrate diet which is associated with increased risk of obesity.

The diets of both groups were rich in total fat with an average intake of 48.45 g per day and percent adequacy of 108 percent. On the contrary, all the subjects of the study were consuming an energy deficit diets (1332 Kcal day$^{-1}$), which was 30% below the RDA (ICMR 2010). Kaur and Bains (2013) reported that the daily fat and energy intake ranged between 28.0 -83.0g and 732-1743 Kcal with the mean value of 49.1 g and 1307 Kcal, respectively among 21-30 yr old women. The diets of the selected population were found to be adequate in dietary fibre with a mean intake of 24.50 g day$^{-1}$. On the whole, the subjects of the present study were consuming high carbohydrates and fat and low protein diet which may increase the risk of adiposity at a younger age. Diets consumed by female Indian students from Jaipur, Rajasthan were found to be significantly inadequate in energy while fat intake was significantly higher than the RDA(Monika et al 2017) whereas diets of female students residing in a University hostel of Pantnagar, Uttarakhand was adequate in terms of protein and fat (Joshi and Kushwaha 2019).

**Vitamins and minerals:** The daily intake of thiamine, folic acid and ascorbic acid was marginally adequate (>70% of RDA), the percent adequacy being 73, 71 and 94%, respectively, for the subjects in the two groups (Fig. 2). On the other hand, riboflavin and niacin were inadequate with the percent adequacies less than 50%. The daily intake of Cholecalciferol (D3) was negligible by the subjects whereas ergocalciferol (D2) was 25.70 µg. As mentioned by Laird et al (2010), apart from sunlight, Vitamin D is also obtained to a limited extent from the diet, although few dietary sources naturally contain the vitamin in sufficient quantities to make a significant contribution to requirements. Vitamin $D_2$ is derived from plants and fungi. Vitamin $D_3$ is produced from 7-dehydrocholesterol and is obtained in the diet from animal products with oily fish, fish oils, eggs and dairy produce providing the best dietary sources. The higher effectiveness of vitamin $D_3$ over $D_2$ is owing to a possible increased affinity for the vitamin D binding protein.

The total calcium intake by the subjects in both the groups was found to be almost the same with the an overall

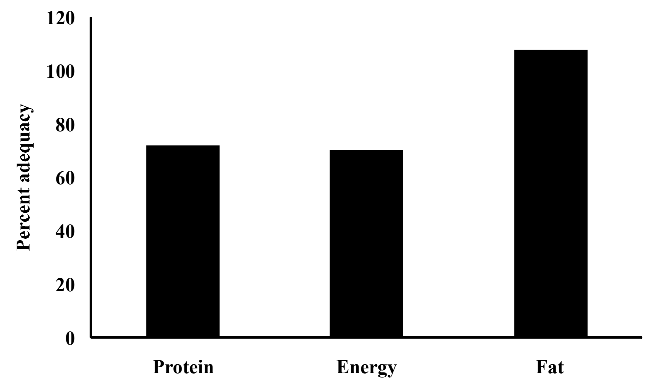


**Fig. 1.** Per cent adequacy of macronutrients against the recommended dietary allowances of ICMR (2010) among the selected young adult women

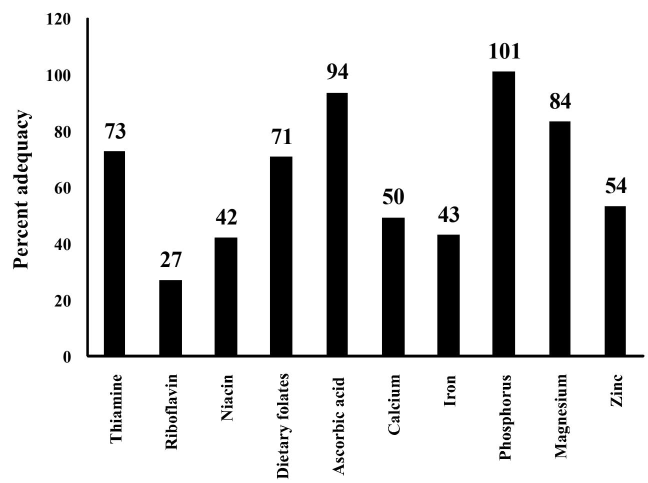


**Fig. 2.** Per cent adequacy of micronutrients against the recommended dietary allowances of ICMR (2010) among the selected young adult women

**Table 4.** Average daily intake of macronutrients by selected young adult women

| Nutrient | Group I (n= 346) | Group II (n=127) | Overall (n=473) |
|---|---|---|---|
| Carbohydrates | 180.44±78.57$^{a}$ | 181.80±73.64$^{a}$ | 180.43±77.69 |
| Protein | 39.22±7.73$^{a}$ | 40.39±8.46$^{a}$ | 39.53±7.94 |
| Fat | 47.98±7.05$^{a}$ | 50.08±8.23$^{b}$ | 48.45±7.86 |
| Dietary fibre | 24.64±6.41$^{a}$ | 24.34±6.52$^{a}$ | 24.50±6.47 |
| Energy | 1332.09±102.35$^{a}$ | 1360.89±98.56$^{b}$ | 1337±101.58 |

Values are Mean±SD
Values in columns followed by different superscripts differ significantly ($p \leq 0.05$)

**Table 5.** Average daily intake of vitamins and minerals by selected young adult women

| Nutrient | Group I (n= 346) | Group II (n=127) | Overall (n=473) | RDA** |
|---|---|---|---|---|
| Vitamins | | | | |
| Thiamine, mg | 0.75±0.21[a] | 0.71±0.27[a] | 0.73±0.23 | 1.0 |
| Riboflavin, mg | 0.30±0.09[a] | 0.30±0.09[a] | 0.30±0.10 | 1.1 |
| Niacin, mg | 5.11±1.26[a] | 5.00±1.34[a] | 5.07±1.29 | 12 |
| Folic acid, µg | 143.93±52.63[a] | 149.87±50.47[a] | 142.11±54.37 | 200 |
| Ascorbic acid | 37.23±13.64[a] | 38.29±11.48[a] | 37.44±13.51 | 40 |
| Ergocalciferol µg (Vitamin $D_2$)* | 25.84±6.51[a] | 25.45±6.63[a] | 25.70±6.34 | - |
| Minerals | | | | |
| Calcium, mg | 300.68±149.43[a] | 307.57±152.67[a] | 301.91±150.23 | 600 |
| Iron, mg | 9.12±3.24[a] | 9.10±3.29[a] | 9.09±3.34 | 21 |
| Phosphorus, mg | 607.67±118.43[a] | 608.72±116.55[a] | 608.02±115.24 | 600 |
| Magnesium, mg | 259.13±67.74[a] | 258.27±68.87[a] | 258.90±68.21 | 310 |
| Zinc, mg | 5.38±1.38[a] | 5.33±1.41[a] | 5.36±1.39 | 10 |

Values are Mean±SD
Values in columns followed by different superscripts differ significantly (p≤0.05)
*Cholecalciferol (Vitamin $D_3$) intake was negligible, **ICMR(2010)

mean value of 301.91 mg which was only 50.31% of the RDA of 600 mg (ICMR, 2010). Indian rural and urban populations consume a lower daily calcium intake in comparison to the recommended dietary allowances (Harinarayan et al 2007). In a recent study, Harinarayan and Akhila (2019) reported that dietary calcium intake (g/CU/day) (grams per consumption unit per day) shows a decline from 606 (1975-79) (RDA-400) to 433 in year 2011-2012 (RDA-600) with median intake of 331 g/CU/day among rural areas of India. The daily intake of phosphorus by the subjects was found to be adequate followed by magnesium which had marginal adequacy (>75% of RDA). The subjects of the study used to take diets with iron and zinc content about 50% of the recommended level (ICMR 2010).

## CONCLUSION

The diets of the Punjabi women were poor in quality as they were low in protein, vitamins and minerals but were high in carbohydrates and fat. The study shows an alarming situation where people belonging to average to high middle-class families of Punjab having lesser money restriction consume a diet which may satisfy hunger and provide sufficient energy, but fails to provide the required quantities of essential nutrients. The studied group of population has the awareness about the benefits of eating health promoting foods. But still their diets lack in many of the vital nutrients. This can be due to the peer pressure of being fashionable and having so called zero figure among girls. The consumption of this type of diet by the young women for a longer duration will further increase country's dual burden of malnutrition. Hence, there is an urgent need to provide nutrition education to rural as well as educated population of the country to select foods on basis of their nutritional benefits. They must be made aware that there is a need to have good body composition with lesser fat mass and more of muscle mass which has a potential to keep various metabolic activities in balance for sustainability of healthy human life.

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# Scheduling of Planting Dates for Maximum Profitability in Carnation (*Dianthus caryophyllus* L.) under Mid Hills of Solan, Himachal Pradesh

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***Abstract*:** An experiment was conducted to evaluate the economics and profitability of carnation (*Dianthus caryophyllus* L.) production as influenced by planting dates and cultivars. Three commercial carnation cultivars, viz. 'Dumas', 'Kiro' and 'Master' and seven planting dates starting from 15th October to 15th April at monthly intervals were undertaken in split-plot design for investigation at the Department of Floriculture and Landscape Architecture, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan. Highest gross returns were obtained from cultivar 'Dumas' when planted in the months of April 2015 (Rs. 210856.70 500m$^{-2}$) and March 2015 (Rs. 206801.50 500m$^{-2}$) planting of the same cultivar. It also resulted in the maximum benefit cost ratio of 4.05:1 and 4.16:1, respectively. The March 2015 planting was identified as the best planting time over other planting dates with highest cumulative returns to produce desired quantity and quality flowers to meet the growing domestic as well as international demand under mid hills of Solan, Himachal Pradesh.

***Keywords:*** Profitability, Carnation, Scheduling, Planting dates

The nature has endowed the state of Himachal Pradesh with a variety of agro-climatic conditions ranging from sub-tropical to temperate. The farmers of the state can harness the niche based advantages of growing off-season flowers and producing the quality disease free planting material of ornamental plants for better livelihood. Flowers, if properly grown can yield 15-20 times more returns than cereals and other crops. In the states like Himachal Pradesh, where land holdings are small and 87.03 per cent of the farmers are marginal and small, having average size holding of 1.04 hectares (Economic Survey of Himachal Pradesh 2013-14). The flower cultivation can be taken up on a small piece of land may be in open or in polyhouse. These enterprises being labour intensive provide gainful employment to rural artisans and other seeking employment. Floriculture has been a major thrust area for diversification of horticulture industry in the state. The mid-hills of Himachal Pradesh have a potential for the year round production of this crop, particularly in the months when carnation flowers cannot be produced in the plains. Its flowers are demanded in the market throughout the year. Carnation is considered as a long day crop for its flowering and requires short day for its vegetative growth. It naturally flowers under the natural long days in March-April under Solan conditions and thereby causing huge gluts, often depressing the market and interest of the grower. Extending the harvesting date of carnation is possible through staggered planting and is very helpful for regulating flowering in carnation (Anonymous 1968, Bhatt 1989). Succession planting is a strategy to get flowers for over a longer period of time. One of the important aspects of marketing is to get a higher price for the produce by selling it out of the growing season. Carnation has a huge potential in Himachal Pradesh in terms of returns and employment generation for the small and marginal flowers. Keeping in view the higher profit per unit area and the bright prospects of carnation cultivation, the present investigation was undertaken to explore the best planting time of carnation cut flower production under Nauni (Solan) conditions to get maximum profit, which will enable the farmers for making regular supplies of carnation cut flowers to the plains and even to foreign markets.

## MATERIAL AND METHODS

The experiment on the effect of staggered planting on growth and flowering of carnation (*Dianthus caryophyllus* L.) was carried out at the experimental farm of Department of Floriculture and Landscape Architecture, University of Horticulture and Forestry, Nauni, Solan during 2014-2016. The experimental farm is located 1276 m above mean sea level at the latitude of 32°51′0″ N and longitude of 77°11′30″ E. The experiment was conducted under polyhouse conditions fitted with fan and pad cooling systems. Experiment on staggered planting of carnation was conducted in split-plot design considering seven planting dates viz. $P_1$ (October 15), $P_2$ (November 15), $P_3$ (December 15), $P_4$ (January 15), $P_5$ (February 15), $P_6$ (March 15) and $P_7$ (April 15) as main plot and three commercial cultivars namely; $V_1$ (Dumas), $V_2$ (Kiro) and $V_3$ (Master) as sub plot. The growing medium used was prepared by mixing soil, well rotten farm yard manure and cocopeat in the ratio of 2:1:1 (v/v). The mother block was raised and maintained by applying a basal fertilizer dose of by 20:5:5 g $m^{-2}$ NPK followed by fertigation twice a week by applying 150 ml of fertilizer solution in the root zone of a plant manually. The cost of cultivation was calculated by taking into account the man power involved for land preparation, planting, intercultural operations, irrigation and harvesting; planting material, plant protection, staking materials, transportation and packaging expenses with the labor charge as Rs 300 manday$^{-1}$. The sale price of cut flowers of carnation was adopted from the current rate list of Department of Floriculture and Landscaping of University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh which varied from time to time according to their quality and demand. Gross monetary return and net return (Rs 500 $m^{-2}$) was worked out using the following formula.

Gross monetary return (Rs500 $m^{-2}$) = value of cut flower

Net returns (Rs 500$m^{-2}$) = Gross monetary returns – Total cost of production

$$\text{Benefit cost ratio} = \frac{\text{Net returns}}{\text{Total expenditure}}$$

## RESULTS AND DISCUSSION

The cost of establishment, management including packing and transportation of carnation crop under 500 meter square area of polyhouse have been worked out taking into consideration the prevailing market prices (Table 1). Once planted, the carnation crop remains in the field for three cycles and based on this, cost analysis for three flushes have been estimated. It was calculated separately for different flushes and has been presented in Tables 2 and 3, respectively. The economic analysis revealed that the initial establishment cost of carnation cultivation was Rs. 106696.20/500$m^2$ and thereafter, a total cost of Rs. 43547.32/500$m^2$was involved for 2$^{nd}$ and 3$^{rd}$ flush. The cost of cultivation in 2$^{nd}$ and 3$^{rd}$ flush is lesser owing to the exclusion of cost required for planting material, man-power required for bed preparation and planting. The major factors responsible for carnation growing in terms of input costs is planting material which was Rs. 56250 (52.72%) for the first flush which is a recurring contingency (for a life span of three years) followed by labour charges which was Rs. 30900 (28.96%) for the first flush and Rs. 24300 (55.8%) for the second flush. Other factors like manures and fertilizers, plant protection chemicals and staking material contributed Rs. 13515.60 (12.67%) and 30.35%, Rs. 2645.60 (2.48%) and 6.08% and Rs. 3385.00 (3.17%) and 7.77% during first and second flush; respectively. In a very recent study, Chahal et al (2020) also worked out the average cost of cultivation of carnation in 500 square metre areas under mid hills of Himachal Pradesh which was estimated to be Rs. 1,38,309.

The perusal of data presented in Table 2 revealed that amongst the different cultivars studied in the first flush, the highest net returns were obtained from cultivar 'Dumas' consecutively for two months when planted in $P_7$ (April 15)(Rs.147707.80/500$m^2$) and $P_6$(March15)(Rs.143652.60 500$m^{-2}$), respectively. This in turn also resulted in maximum benefit cost ratio of 1.28:1 and 1.27:1 in $P_7$ (April 15) and $P_6$(March 15)respectively, indicating an economically sound and remunerative returns from cultivar Dumas ($V_1$). In contrast, lowest net returns were obtained in $P_4$ (January 15) planted crop (Rs. 45081.43 500$m^{-2}$) in the same cultivar with minimum benefit cost ratio of -0.16:1. This may be attributed to the fact that the carnation plants raised during March and April months performed better in respect to growth and flowering parameters like maximum plant height, stem length, bud length, bud width, flower diameter, number of cut flower stems per plant and cut flower stems per meter square. As carnation is a quantitative long day plant and the vegetative growth of plants planted in the earlier mentioned months fall under short day condition; thus it is not congenial for favourable growth and development in many important parameters whereas the ones planted in later months had their vegetative growth in synchronization with long day condition resulting into the increasing trend thereof. Further, the overall maximum returns were also obtained from cultivar Dumas ($V_1$) when planted in the month of March while, least in January planting of same cultivar. It may be due to the reason that March planted crop flowered in the month of October which gained highest market price (Rs. 6.31 flower$^{-1}$) whereas, January planted crop flowered in the month of August fetching comparatively lower market price (Rs. 4.50/flower) hence resulted in lower gross returns. Under similar conditions of mid hills of Himachal Pradesh, Chahal et al (2020) reported that the benefit cost ratio of carnation cultivation on an area of 500 square metre was 1.45:1. Sudha et al (2005), Sengar and Kothari (2008), Singh et al (2013), Sharma et al (2014), Taranum et al (2014), Punera et al (2017), Gamanagatti and Patil (2018) also reported similar

**Table 1.** Cost of carnation cultivation at different planting dates in an area of 500 sq.m from planting to marketing of produce

A. Input Cost

| Particulars | Manday (s) required (No.) | Rate (Rs.) | Total cost (Rs.) |
|---|---|---|---|
| i) Labour | | | |
| a. Bed preparation, mixing of farm yard manure and basal dose of fertilizers | 20 | 300 manday$^{-1}$ | 6000.00 |
| b. Planting | 2 | 300 manday$^{-1}$ | 600.00 |
| c. Irrigations | 18 | 300 manday$^{-1}$ | 5400.00 |
| d. Pinching | 1 | 300 manday$^{-1}$ | 300.00 |
| e. Weeding and hoeing | 15 | 300 manday$^{-1}$ | 4500.00 |
| f. Fertigation | 18 | 300 manday$^{-1}$ | 5400.00 |
| g. Drenching and Spraying | 6 | 300 manday$^{-1}$ | 1800.00 |
| h. Disbudding and deshooting | 5 | 300 manday$^{-1}$ | 1500.00 |
| i. Staking | 6 | 300 manday$^{-1}$ | 1800.00 |
| j. Harvesting of flowers, grading, packaging and loading for transportation | 12 | 300 manday$^{-1}$ | 3600.00 |
| Total 103 30900.00 | | | |
| ii) Planting material | Quantity required (No.) | Rate (Rs.) | Total cost (Rs.) |
| Rooted cuttings considering total planting area as 375 m$^2$ and planting density of 25 plants/m$^2$ | 25 x 375 = 9375 | 6 plant$^{-1}$ | 56250.00 |
| iii) Manures and fertilizers | | | |
| a. Farm yard manure | 1875 kg | 1.50 kg$^{-1}$ | 2812.50 |
| b. Vermicompost | 375 kg | 5.00 kg$^{-1}$ | 1875.00 |
| c. Urea | 38 kg | 320/50 kg | 243.20 |
| d. Single Super Phosphate (SSP) | 62.48 kg | 300/50 kg | 374.88 |
| e. Muriate of Potash (MOP) | 10.4 kg | 800/50 kg | 166.40 |
| f. Multi-K | 58.5 kg | 4750/50 kg | 5557.50 |
| g. Calcium nitrate | 47.81 kg | 1300/25kg | 2486.12 |
| Total 13515.60 | | | |
| iv) Plant Protection chemicals | | | |
| a. Insecticides | | | |
| Cypermethrin ( 1ml/l) | 90 x 2= 180 ml | 450 litre$^{-1}$ | 81.00 |
| Imidachloprid ( 0.5 ml/l) | 45 x 2= 90 ml | 1500 litre$^{-1}$ | 135.00 |
| Decis (Deltamethrin) (1ml/l) | 90 x 2= 180 ml | 700 litre$^{-1}$ | 126.00 |
| Simba (1ml/l) | 90 x 2= 180 ml | 890 litre$^{-1}$ | 160.20 |
| Total | 502.20 | | |
| b. Fungicides | | | |
| Dithane M-45 (2g/l, drenched three times) | 2.25 kg | 360 kg$^{-1}$ | 810.00 |
| Bavistin (1g/l, drenched three times) | 1.13 kg | 1180 kg$^{-1}$ | 1333.40 |
| Total | 2143.40 | | |
| v) Staking material | | | |
| a. Iron poles (cost for 6 months considering total life for 15 years) | 200 poles | 150 pole$^{-1}$ | 1000.00 |
| b. Nets | | | |
| 4x4 inch size of mesh , 2 Bottom rows (cost for 6 months) considering total life for 5 years | 750 m$^2$ | 16.80 m$^{-2}$ | 1260.00 |
| 5x5 inch size of mesh, 2 Upper rows (cost for 6 months) considering total life for 5 years | 750 m$^2$ | 15.00 m$^{-2}$ | 1125.00 |
| Total | 3385.00 | | |

**Cont...**

**Table 1.** Cost of carnation cultivation at different planting dates in an area of 500 sq.m from planting to marketing of produce

| Particulars | Manday (s) required (No.) | Rate (Rs.) | Total cost (Rs.) |
|---|---|---|---|
| vi) Transportation and packaging cost (cultivar-wise for different planting dates depending upon yield of cut flowers) | | | |
| a) Boxes required (Box size = 95 cm x 40 cm x 22 cm) | | | |
| Carnation cv. 'Dumas' ($V_1$) | No. | Rate/Box (Rs.) | Total cost (Rs.) |
| $P_1$ (October 15) | 32 | 80 | 2560.00 |
| $P_2$ (November 15) | 37 | 80 | 2960.00 |
| $P_3$ (December 15) | 31 | 80 | 2480.00 |
| $P_4$ (January 15) | 20 | 80 | 1600.00 |
| $P_5$ (February 15) | 40 | 80 | 3200.00 |
| $P_6$ (March 15) | 16 | 80 | 1280.00 |
| $P_7$ (April 15) | 22 | 80 | 1760.00 |
| Carnation cv. 'Kiro' ($V_2$) | | | |
| $P_1$ (October 15) | 23 | 80 | 1840.00 |
| $P_2$ (November 15) | 41 | 80 | 3280.00 |
| $P_3$ (December 15) | 23 | 80 | 1840.00 |
| $P_4$ (January 15) | 21 | 80 | 1680.00 |
| $P_5$ (February 15) | 33 | 80 | 2640.00 |
| $P_6$ (March 15) | 12 | 80 | 960.00 |
| $P_7$ (April 15) | 16 | 80 | 1280.00 |
| Carnation cv. 'Master'($V_3$) | | | |
| $P_1$ (October 15) | 25 | 80 | 2000.00 |
| $P_2$ (November 15) | 34 | 80 | 2720.00 |
| $P_3$ (December 15) | 23 | 80 | 1840.00 |
| $P_4$ (January 15) | 21 | 80 | 1680.00 |
| $P_5$ (February 15) | 33 | 80 | 2640.00 |
| $P_6$ (March 15) | 13 | 80 | 1040.00 |
| $P_7$ (April 15) | 16 | 80 | 1280.00 |
| b) Cellophane paper required | Kilo gram | Rate (300 Rs. $kg^{-1}$) | Total cost (Rs.) |
| Carnation cv. 'Dumas' ($V_1$) | | | |
| $P_1$ (October 15) | 0.8 | 300 | 240.00 |
| $P_2$ (November 15) | 0.9 | 300 | 270.00 |
| $P_3$ (December 15) | 0.8 | 300 | 240.00 |
| $P_4$ (January 15) | 0.5 | 300 | 150.00 |
| $P_5$ (February 15) | 1.0 | 300 | 300.00 |
| $P_6$ (March 15) | 0.4 | 300 | 120.00 |
| $P_7$ (April 15) | 0.5 | 300 | 150.00 |
| Carnation cv. 'Kiro'($V_2$) | | | |
| $P_1$ (October 15) | 0.6 | 300 | 180.00 |
| $P_2$ (November 15) | 1.0 | 300 | 300.00 |
| $P_3$ (December 15) | 0.6 | 300 | 180.00 |
| $P_4$ (January 15) | 0.5 | 300 | 150.00 |
| $P_5$ (February 15) | 0.8 | 300 | 240.00 |
| $P_6$ (March 15) | 0.3 | 300 | 90.00 |
| $P_7$ (April 15) | 0.4 | 300 | 120.00 |

**Cont...**

**Table 1.** Cost of carnation cultivation at different planting dates in an area of 500 sq.m from planting to marketing of produce

| Particulars | Manday (s) required (No.) | Rate (Rs.) | Total cost (Rs.) |
|---|---|---|---|
| Carnation cv. 'Master'($V_3$) | | | |
| $P_1$ (October 15) | 0.6 | 300 | 180.00 |
| $P_2$ (November 15) | 0.9 | 300 | 270.00 |
| $P_3$ (December 15) | 0.6 | 300 | 180.00 |
| $P_4$ (January 15) | 0.5 | 300 | 150.00 |
| $P_5$ (February 15) | 0.8 | 300 | 240.00 |
| $P_6$ (March 15) | 0.3 | 300 | 90.00 |
| $P_7$ (April 15) | 0.4 | 300 | 120.00 |
| c) Vehicle charges up to Delhi flower market | | | |
| Carnation cv. 'Dumas' ($V_1$) | | | |
| $P_1$ (October 15) | 32 | 300 | 9600.00 |
| $P_2$ (November 15) | 37 | 300 | 11100.00 |
| $P_3$ (December 15) | 31 | 300 | 9300.00 |
| $P_4$ (January 15) | 20 | 300 | 6000.00 |
| $P_5$ (February 15) | 40 | 300 | 12000.00 |
| $P_6$ (March 15) | 16 | 300 | 4800.00 |
| $P_7$ (April 15) | 22 | 300 | 6600.00 |
| Carnation cv.'Kiro'($V_2$) | | | |
| $P_1$ (October 15) | 23 | 300 | 6900.00 |
| $P_2$ (November 15) | 41 | 300 | 12300.00 |
| $P_3$ (December 15) | 23 | 300 | 6900.00 |
| $P_4$ (January 15) | 21 | 300 | 6300.00 |
| $P_5$ (February 15) | 33 | 300 | 9900.00 |
| $P_6$ (March 15) | 12 | 300 | 3600.00 |
| $P_7$ (April 15) | 16 | 300 | 4800.00 |
| Carnation cv. 'Master'($V_3$) | | | |
| $P_1$ (October 15) | 25 | 300 | 7500.00 |
| $P_2$ (November 15) | 34 | 300 | 10200.00 |
| $P_3$ (December 15) | 23 | 300 | 6900.00 |
| $P_4$ (January 15) | 21 | 300 | 6300.00 |
| $P_5$ (February 15) | 33 | 300 | 9900.00 |
| $P_6$ (March 15) | 13 | 300 | 3900.00 |
| $P_7$ (April 15) | 16 | 300 | 4800.00 |

results which showed the economic feasibility of carnation cultivation under protected conditions. The effect of staggered planting on the yield of different flowers have also been reported by Dahiya et al (2007), Jindal et al (2018) in chrysanthemum, Meena Ravi et al (2018) in tuberose, Chaudhary and Dhatt (2017) and Khutiya et al (2018) in gladiolus.

The performance of cultivar $V_1$ (Dumas) was also superior in terms of yield and other growth parameters as compared $V_2$ (Kiro) and $V_3$ (Master) which may be because of the difference in their genetic makeup and their interaction with climate particularly temperature which was variable during different months.The difference in the performance of carnation cultivars have also been reported by Dalal et al (2009), Gharge et al (2009), Dalawai and Naik (2014), Momin et al (2015) and Singh et al (2016) in carnations when grown under protected cultivation. The data recorded for second and third flush (Table 3) also revealed similar results where cultivar Dumas ($V_1$) recorded the maximum benefit cost ratio of 4.16:1 in March (2016) followed by April flush (2016) with

**Table 2.** Net Returns from 1st flush on an area of 500 $m^2$ during different planting times of the year

| Yield of cut flowers Cultivars wise and planting date wise | | Market price for cut stem during the flowering time (Rs.) | Total returns Cultivars wise and planting date wise (Rs.) | |
|---|---|---|---|---|
| Carnation cv. 'Dumas' ($V_1$) | Yield | | | |
| $P_1$ (October 15) | 50936.25 | 5.75 | 191010.90 | |
| $P_2$ (November 15) | 39375.00 | 3.75 | 147656.30 | |
| $P_3$ (December 15) | 44062.50 | 3.75 | 165234.40 | |
| $P_4$ (January 15) | 32126.25 | 3.00 | 96378.75 | |
| $P_5$ (February 15) | 48123.75 | 4.50 | 216556.90 | |
| $P_6$ (March 15) | 40657.5 | 6.31 | 256548.80 | |
| $P_7$ (April 15) | 41666.25 | 6.31 | 262914.00 | |
| Carnation cv. 'Kiro' ($V_2$) | | | | |
| $P_1$ (October 15) | 35748.75 | 5.25 | 187680.90 | |
| $P_2$ (November 15) | 42780.00 | 3.75 | 160425.00 | |
| $P_3$ (December 15) | 36468.75 | 3.75 | 136757.80 | |
| $P_4$ (January 15) | 40623.75 | 3.00 | 121871.30 | |
| $P_5$ (February 15) | 33967.50 | 3.37 | 114470.50 | |
| $P_6$ (March 15) | 47655.00 | 4.20 | 200151.00 | |
| $P_7$ (April 15) | 47917.50 | 4.20 | 201253.50 | |
| Carnation cv. 'Master'($V_3$) | | | | |
| $P_1$ (October 15) | 39063.75 | 5.25 | 205084.70 | |
| $P_2$ (November 15) | 36686.25 | 3.75 | 137573.40 | |
| $P_3$ (December 15) | 33532.50 | 3.75 | 125746.90 | |
| $P_4$ (January 15) | 33273.75 | 3.00 | 99821.25 | |
| $P_5$ (February 15) | 39907.50 | 4.50 | 179583.80 | |
| $P_6$ (March 15) | 31717.50 | 4.20 | 133213.50 | |
| $P_7$ (April 15) | 30206.25 | 4.20 | 126866.30 | |
| Gross Returns (Rs.) | | | | |
| Production cost cultivar wise and planting date wise | | Gross returns (Rs.) | Net return (Rs.) | Benefit cost ratio |
| Carnation cv. 'Dumas' ($V_1$) | | | | |
| $P_1$ (October 15) | 55947.32 | 191010.90 | 135063.62 | 2.41 |
| $P_2$ (November 15) | 57877.32 | 147656.30 | 89778.93 | 1.55 |
| $P_3$ (December 15) | 55567.32 | 165234.40 | 109667.06 | 1.97 |
| $P_4$ (January 15) | 51297.32 | 96378.75 | 45081.43 | 0.88 |
| $P_5$ (February 15) | 59047.32 | 216556.90 | 157509.56 | 2.67 |
| $P_6$ (March 15) | 49747.32 | 256548.80 | 206801.51 | 4.16 |
| $P_7$ (April 15) | 52057.32 | 262914.00 | 210856.72 | 4.05 |
| Carnation cv. 'Kiro'($V_2$) | | | | |
| $P_1$ (October 15) | 52467.32 | 187680.90 | 135213.58 | 2.58 |
| $P_2$ (November 15) | 59427.32 | 160425.00 | 100997.68 | 1.70 |
| $P_3$ (December 15) | 52467.32 | 136757.80 | 84290.48 | 1.61 |
| $P_4$ (January 15) | 51677.32 | 121871.30 | 70193.98 | 1.36 |
| $P_5$ (February 15) | 56327.32 | 114470.50 | 58143.18 | 1.03 |
| $P_6$ (March 15) | 48197.32 | 200151.00 | 151953.68 | 3.15 |
| $P_7$ (April 15) | 49747.32 | 201253.50 | 151506.18 | 3.05 |
| Carnation cv. 'Master'($V_3$) | | | | |
| $P_1$ (October 15) | 53227.32 | 205084.70 | 151857.40 | 2.85 |
| $P_2$ (November 15) | 56737.32 | 137573.40 | 80836.08 | 1.42 |
| $P_3$ (December 15) | 52467.32 | 125746.90 | 73279.58 | 1.40 |
| $P_4$ (January 15) | 51677.32 | 99821.25 | 48143.93 | 0.93 |
| $P_5$ (February 15) | 56327.32 | 179583.80 | 123256.50 | 2.19 |
| $P_6$ (March 15) | 48577.32 | 133213.50 | 84636.18 | 1.74 |
| $P_7$ (April 15) | 49747.32 | 126866.30 | 77118.98 | 1.55 |

**Table 3.** Net returns from 2nd and 3rd flowering flush on an area of 500 $m^2$ during different planting times of the year

| Yield of cut flowers Cultivars wise and planting date wise | | Market price for cut stem during the flowering time (Rs.) | Total returns Cultivars wise and planting date wise (Rs.) | |
|---|---|---|---|---|
| Carnation cv. 'Dumas' ($V_1$) | Yield | | | |
| $P_1$ (October 15) | 50936.25 | 5.75 | 191010.90 | |
| $P_2$ (November 15) | 39375.00 | 3.75 | 147656.30 | |
| $P_3$ (December 15) | 44062.50 | 3.75 | 165234.40 | |
| $P_4$ (January 15) | 32126.25 | 3.00 | 96378.75 | |
| $P_5$ (February 15) | 48123.75 | 4.50 | 216556.90 | |
| $P_6$ (March 15) | 40657.5 | 6.31 | 256548.80 | |
| $P_7$ (April 15) | 41666.25 | 6.31 | 262914.00 | |
| Carnation cv. 'Kiro' ($V_2$) | | | | |
| $P_1$ (October 15) | 35748.75 | 5.25 | 187680.90 | |
| $P_2$ (November 15) | 42780.00 | 3.75 | 160425.00 | |
| $P_3$ (December 15) | 36468.75 | 3.75 | 136757.80 | |
| $P_4$ (January 15) | 40623.75 | 3.00 | 121871.30 | |
| $P_5$ (February 15) | 33967.50 | 3.37 | 114470.50 | |
| $P_6$ (March 15) | 47655.00 | 4.20 | 200151.00 | |
| $P_7$ (April 15) | 47917.50 | 4.20 | 201253.50 | |
| Carnation cv. 'Master' ($V_3$) | | | | |
| $P_1$ (October 15) | 39063.75 | 5.25 | 205084.70 | |
| $P_2$ (November 15) | 36686.25 | 3.75 | 137573.40 | |
| $P_3$ (December 15) | 33532.50 | 3.75 | 125746.90 | |
| $P_4$ (January 15) | 33273.75 | 3.00 | 99821.25 | |
| $P_5$ (February 15) | 39907.50 | 4.50 | 179583.80 | |
| $P_6$ (March 15) | 31717.50 | 4.20 | 133213.50 | |
| $P_7$ (April 15) | 30206.25 | 4.20 | 126866.30 | |
| C. Gross Returns (Rs.) | | | | |
| Production cost cultivar wise and planting date wise | | Gross returns (Rs.) | Net return (Rs.) | Benefit cost ratio |
| Carnation cv. 'Dumas' ($V_1$) | | | | |
| $P_1$ (October 15) | 55947.32 | 191010.90 | 135063.62 | 2.41 |
| $P_2$ (November 15) | 57877.32 | 147656.30 | 89778.93 | 1.55 |
| $P_3$ (December 15) | 55567.32 | 165234.40 | 109667.06 | 1.97 |
| $P_4$ (January 15) | 51297.32 | 96378.75 | 45081.43 | 0.88 |
| $P_5$ (February 15) | 59047.32 | 216556.90 | 157509.56 | 2.67 |
| $P_6$ (March 15) | 49747.32 | 256548.80 | 206801.51 | 4.16 |
| $P_7$ (April 15) | 52057.32 | 262914.00 | 210856.72 | 4.05 |
| Carnation cv. 'Kiro' ($V_2$) | | | | |
| $P_1$ (October 15) | 52467.32 | 187680.90 | 135213.58 | 2.58 |
| $P_2$ (November 15) | 59427.32 | 160425.00 | 100997.68 | 1.70 |
| $P_3$ (December 15) | 52467.32 | 136757.80 | 84290.48 | 1.61 |
| $P_4$ (January 15) | 51677.32 | 121871.30 | 70193.98 | 1.36 |
| $P_5$ (February 15) | 56327.32 | 114470.50 | 58143.18 | 1.03 |
| $P_6$ (March 15) | 48197.32 | 200151.00 | 151953.68 | 3.15 |
| $P_7$ (April 15) | 49747.32 | 201253.50 | 151506.18 | 3.05 |
| Carnation cv. 'Master' ($V_3$) | | | | |
| $P_1$ (October 15) | 53227.32 | 205084.70 | 151857.40 | 2.85 |
| $P_2$ (November 15) | 56737.32 | 137573.40 | 80836.08 | 1.42 |
| $P_3$ (December 15) | 52467.32 | 125746.90 | 73279.58 | 1.40 |
| $P_4$ (January 15) | 51677.32 | 99821.25 | 48143.93 | 0.93 |
| $P_5$ (February 15) | 56327.32 | 179583.80 | 123256.50 | 2.19 |
| $P_6$ (March 15) | 48577.32 | 133213.50 | 84636.18 | 1.74 |
| $P_7$ (April 15) | 49747.32 | 126866.30 | 77118.98 | 1.55 |

4.05:1. In comparison to the first flush, the yield of the cut flowers increased during the second and third flush in 2015-16 as the crop planted during first year planting (2014-15) were headed back resulting in more number of shoots.

The first year plants diverted the photosynthates into overall vegetative development of the plant including root development as well. In contrast, the second year plants already had well established root system which acted as a source and the vegetative as well as flower developed acted as sink (Taiz and Zeiger 2002). Thus the second year plants saved the energy which was required in root development in first year thus diverting the same energy in flowering resulting into better yield and quality of the flowers. In a recent study, Singh et al (2019) also reported similar findings where the gross returns per hectare in carnation crop under Kumaun hills of Uttrakand was INR 9,42,500 in the first year which later increased to INR 11,78,125 in the second year indicating the feasibility of the crop.

## CONCLUSION

Based upon the results obtained from the experiment, it can be concluded that the cultivar 'Dumas'($V_1$) planted in the month of March was identified as the best planting time over other plantings and cultivars with highest cumulative returns during both the years under mid hills of Solan, Himachal Pradesh. Further, this planting date can be adopted by the farmers to meet the demand of carnation flowers during off season and to get maximum returns.

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# Seed Quality of CGMS Based Chilli (*Capsicum annuum* L.) Hybrid Induced by Time of Pollination and Number of Fruit Pickings

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***Abstract***: Cytoplasmic genetic male sterility (CGMS) based chilli hybrid UARChH42 was developed and seeds were assessed for quality attributes. The objects of research were to optimize time of pollination and number of picking of fruits in this newly released hybrid in Hyderabad Karnataka (H-K) region of Karnataka (2016-17and 2017-18). In current study it was proven that pollinating A line (male sterile) plants at 10-1100 hour with pollens collected from of R line (male fertile) yielded seeds with maximum seed germination (86.91 %), seedling vigour – length (2369), speed of germination (14.07), dehydrogenase enzyme activity (0.275), α-amylase enzyme activity (15.18 mm) and minimum electrical conductivity (0.094 dS $cm^{-1}$). Picking red dry chilli pods at second picking i.e. fruits developed from crossing female flower in mid-October to mid-November, and harvesting in the month of December resulted in maximum. seed germination (78.62 %), seedling vigour – length (1988), speed of germination (12.17), dehydrogenase enzyme activity (0.202), α-amylase enzyme activity (12.86 mm) and minimum electrical conductivity (0.114 dS $cm^{-1}$), respectively. The 10 to 11 am pollination time for crossing female line and harvesting red ripe dried fruits at second picking (crossing female flower in mid-October to mid-November and harvesting fruits in December) can be recommended.

***Keywords***: CGMS, Chilli, Pollination, Seed quality

Chilli bears bisexual, small sized, white coloured, pentapetalous, pentasepalous flowers at the axis of leaves and flowers are indeterminate and the crop bears it throughout the season. Chilli may also persist as perennial crop and can be pruned during harsh winters. This solanaceous vegetable is the one crop where parthenocarpy is a disorder and is non profitable for hybrid seed production. Basically being self pollinated with natural crossing ranging from 4 to 36 per cent and falls under the category of often cross pollinated crop. Therefore for successful fruit and seed set demands availability of good quantity of viable pollens at right time of the day when stigma is also receptive. For most of the solanaceous vegetables viz. tomato, brinjal and chilli 9000 AM to 1200 hours is considered as best time of pollination for obtaining maximum fruit set (Priya et al 2009, Vidyadhar et al 2015, Tembhurne et al 2016). In current study CGMS lines were used for production of hybrid seeds as it eliminated the strenuous and moderate procedure of emasculation. The main object is to optimize best time of pollination and to ascertain the best order of number of fruit picking for seed purpose

## MATERIAL AND METHODS

The study was conducted in experimental field for two consecutive years (2016 and 2017) in factorial randomized complete block design in at UAS Raichur, Karnataka. The parents of hybrid UARChH42 were sown in 75 female: 25 male ratio in the seed production plot. Pollens collected from R line (male line) were transferred on to flowers of A line (female line) at six different times of the day starting from 9.00 AM to 5.00 PM. The fruits were set and harvested at the red ripe dried stage from plant 60 days after pollination. The best order of fruit picking was determined out of the three different picking. First picking fruits were those developed from crossing female flower in mid-September to mid-October and harvested in November. Second picking fruits that are formed after crossing female flower in mid-October to mid-November, were harvested in December and third picking was done in January for those fruits that were developed from crossing female flower in mid-November to mid-December. All cultural practices were followed as per package of practice. The various seed quality and biochemical parameters observed were germination (%) (Anonymous 2013), root length (cm), shoot length (cm), seedling vigour index- length (Abdul-Baki and Anderson 1973), speed of germination (Maguire, 1962), electrical conductivity (dS $cm^{-1}$) (Anonymous 2013), dehydrogenase enzyme activity (OD value) (Kittock and Law 1968) and α amylase enzyme activity (mm) (Simpson and Naylor 1962). The statistical analysis was carried out for each observed character under the study using MS-Excel, OPSTAT software.

**Table 1.** Effect of time of pollination and fruit picking on seed quality parameters in CGMS based chilli hybrid UARChH42 (JCH42)

| Treatments | Germination (%) | | | Root length (cm) | | | Shoot length (cm) | | | Seedling vigour index- length | | | Speed of germination | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2016 | 2017 | Pooled mean | 2016 | 2017 | Pooled mean | 2016 | 2017 | Pooled mean | 2016 | 2017 | Pooled mean | 2016 | 2017 | Pooled mean |
| Pollination time- T | | | | | | | | | | | | | | | |
| $T_1$ | 75.66 (60.45)* | 76.88 (61.27) | 76.27 (60.86) | 12.86 | 13.65 | 13.25 | 9.98 | 10.82 | 10.40 | 1729 | 1881 | 1805 | 11.75 | 11.84 | 11.80 |
| $T_2$ | 86.59 (68.60) | 87.22 (69.17) | 86.91 (68.87) | 14.49 | 15.33 | 14.91 | 12.07 | 12.50 | 12.29 | 2305 | 2433 | 2369 | 13.98 | 14.16 | 14.07 |
| $T_3$ | 75.31 (60.23) | 76.62 (61.11) | 75.96 (60.67) | 12.86 | 13.08 | 12.97 | 9.26 | 10.06 | 9.66 | 1682 | 1773 | 1727 | 10.19 | 10.34 | 10.27 |
| $T_4$ | 82.14 (65.05) | 83.72 (66.23) | 82.93 (65.63) | 13.55 | 14.38 | 13.97 | 10.65 | 10.89 | 10.77 | 1989 | 2111 | 2050 | 13.15 | 13.08 | 13.12 |
| $T_5$ | 70.47 (57.11) | 72.09 (58.15) | 71.28 (57.62) | 11.64 | 12.05 | 11.85 | 8.97 | 9.59 | 9.28 | 1453 | 1562 | 1508 | 10.30 | 10.32 | 10.31 |
| $T_6$ | 67.27 (55.11) | 67.11 (55.03) | 67.19 (55.07) | 11.33 | 12.01 | 11.67 | 8.20 | 8.22 | 8.21 | 1315 | 1355 | 1335 | 9.86 | 9.84 | 9.85 |
| CD (p=0.01) | 1.41 | 1.56 | 1.32 | 0.73 | 0.62 | 0.50 | 0.74 | 0.47 | 0.51 | 82.05 | 68.12 | 62.90 | 0.40 | 0.14 | 0.23 |
| Fruit picking- P | | | | | | | | | | | | | | | |
| $P_1$ | 75.91 (60.77) | 77.65 (61.97) | 76.78 (61.37) | 12.25 | 12.96 | 12.61 | 9.62 | 10.17 | 9.90 | 1681 | 1804 | 1743 | 11.36 | 11.44 | 11.40 |
| $P_2$ | 78.24 (62.53) | 79.00 (63.11) | 78.62 (62.82) | 14.00 | 14.58 | 14.29 | 10.44 | 11.09 | 10.76 | 1930 | 2046 | 1988 | 12.14 | 12.20 | 12.17 |
| $P_3$ | 74.56 (59.96) | 75.17 (60.39) | 74.87 (60.17) | 12.12 | 12.72 | 12.42 | 9.51 | 9.78 | 9.65 | 1625 | 1707 | 1666 | 11.12 | 11.16 | 11.14 |
| CD (p=0.01) | 1.00 | 1.11 | 0.93 | 0.51 | 0.44 | 0.36 | 0.52 | 0.33 | 0.36 | 58.02 | 48.17 | 44.48 | 0.28 | 0.10 | 0.16 |
| Interaction effect (T×P) | | | | | | | | | | | | | | | |
| $T_1P_1$ | 75.09 (60.07) | 76.55 (61.04) | 75.82 (60.55) | 12.44 | 13.03 | 12.74 | 9.61 | 10.27 | 9.94 | 1655 | 1784 | 1720 | 11.78 | 11.81 | 11.80 |
| $T_1P_2$ | 76.81 (61.22) | 77.99 (62.02) | 77.40 (61.62) | 14.55 | 15.62 | 15.09 | 10.34 | 11.45 | 10.89 | 1912 | 2105 | 2009 | 11.85 | 11.98 | 11.92 |
| $T_1P_3$ | 75.07 (60.05) | 76.11 (60.74) | 75.59 (60.40) | 11.57 | 12.30 | 11.94 | 10.01 | 10.74 | 10.38 | 1619 | 1753 | 1686 | 11.62 | 11.72 | 11.67 |
| $T_2P_1$ | 85.37 (67.52) | 86.28 (68.27) | 85.83 (67.89) | 13.66 | 14.81 | 14.24 | 11.20 | 11.85 | 11.53 | 2122 | 2301 | 2212 | 13.79 | 14.03 | 13.91 |
| $T_2P_2$ | 89.54 (71.14) | 90.38 (71.98) | 89.96 (71.55) | 16.93 | 17.32 | 17.13 | 13.59 | 13.61 | 13.60 | 2733 | 2795 | 2764 | 14.98 | 15.18 | 15.08 |
| $T_2P_3$ | 84.87 (67.13) | 84.99 (67.25) | 84.93 (67.19) | 12.87 | 13.87 | 13.37 | 11.43 | 12.05 | 11.74 | 2061 | 2203 | 2132 | 13.18 | 13.28 | 13.23 |
| $T_3P_1$ | 76.33 (60.91) | 78.13 (62.13) | 77.23 (61.51) | 11.89 | 12.09 | 11.99 | 8.69 | 10.19 | 9.44 | 1622 | 1741 | 1681 | 9.84 | 9.94 | 9.89 |
| $T_3P_2$ | 77.31 (61.56) | 78.33 (62.26) | 77.82 (61.91) | 13.36 | 13.69 | 13.52 | 9.24 | 10.37 | 9.81 | 1747 | 1885 | 1816 | 10.86 | 11.19 | 11.02 |
| $T_3P_3$ | 72.27 (58.23) | 73.39 (58.95) | 72.83 (85.59) | 13.33 | 13.45 | 13.39 | 9.85 | 9.62 | 9.73 | 1676 | 1693 | 1685 | 9.87 | 9.90 | 9.89 |
| $T_4P_1$ | 79.34 (62.98) | 82.14 (65.00) | 80.74 (63.97) | 12.66 | 13.92 | 13.29 | 10.04 | 11.19 | 10.62 | 1829 | 2044 | 1937 | 12.97 | 13.01 | 12.99 |
| $T_4P_2$ | 84.49 (66.82) | 85.35 (67.50) | 84.92 (67.16) | 15.25 | 15.69 | 15.47 | 11.99 | 11.57 | 11.78 | 2265 | 2327 | 2296 | 13.83 | 13.54 | 13.69 |
| $T_4P_3$ | 82.59 (65.34) | 83.68 (66.18) | 83.14 (65.76) | 12.75 | 13.55 | 13.15 | 9.92 | 9.90 | 9.91 | 1872 | 1962 | 1917 | 12.66 | 12.68 | 12.67 |
| $T_5P_1$ | 70.38 (57.04) | 72.70 (58.55) | 71.54 (57.78) | 11.50 | 11.57 | 11.54 | 9.49 | 9.32 | 9.41 | 1477 | 1519 | 1498 | 9.70 | 9.95 | 9.82 |
| $T_5P_2$ | 73.50 (59.02) | 74.71 (59.81) | 74.11 (59.41) | 11.92 | 12.61 | 12.27 | 9.43 | 10.61 | 10.02 | 1570 | 1734 | 1652 | 11.14 | 11.01 | 11.07 |
| $T_5P_3$ | 67.53 (55.27) | 68.85 (56.08) | 68.19 (55.67) | 11.48 | 11.98 | 11.73 | 7.98 | 8.83 | 8.41 | 1313 | 1433 | 1373 | 10.08 | 10.01 | 10.04 |
| $T_6P_1$ | 68.95 (56.14) | 70.09 (56.85) | 69.52 (56.49) | 11.35 | 12.35 | 11.85 | 8.67 | 8.21 | 8.44 | 1381 | 1438 | 1409 | 10.06 | 9.88 | 9.97 |
| $T_6P_2$ | 67.82 (55.45) | 67.24 (55.09) | 67.53 (55.27) | 11.95 | 12.55 | 12.25 | 8.03 | 8.91 | 8.47 | 1355 | 1431 | 1393 | 10.21 | 10.28 | 10.25 |
| $T_6P_3$ | 65.03 (53.75) | 64.01 (53.14) | 64.52 (53.44) | 10.68 | 11.13 | 10.91 | 7.90 | 7.54 | 7.72 | 1209 | 1196 | 1202 | 9.30 | 9.35 | 9.32 |
| Mean | 76.24 | 77.27 | 76.76 | 12.79 | 13.42 | 13.10 | 9.86 | 10.35 | 10.10 | 1746 | 1852 | 1799 | 12 | 12 | 12 |
| CD (p=0.01) | 2.44 | 2.71 | 2.28 | 1.26 | 1.08 | 0.87 | 0.64 | NS | NS | 142.11 | 117.98 | 108.95 | NS | 0.25 | 0.39 |

*Figures in parenthesis represent Arcsine transformation; See details Table 2

## RESULTS AND DISCUSSION

There was a significant effect of different time of pollination on seed quality parameters (Table 1). Pooled mean data of two years (2016 & 17) indicated the maximum germination (86.91), root length (14.91), shoot length (12.29), seedling vigour index (2369) and speed of germination (4.07) in T2 (10 to 11 am). This might have resulted due to more pollen available to fertilize the flowers buds at optimum temperature regimes which led to more fruit set and thus formation of large quantity of seed with

**Table 2.** Effect of time of pollination and number of fruit pickings on seed biochemical parameters in chilli hybrid UARChH42 (JCH42)

| Treatments | EC (dS $cm^{-1}$) | | | DEA (OD value) | | | AAEA (mm) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2016 | 2017 | Pooled mean | 2016 | 2017 | Pooled mean | 2016 | 2017 | Pooled mean |
| Pollination time- T | | | | | | | | | |
| $T_1$= 9 to 10 am | 0.120 | 0.117 | 0.119 | 0.193 | 0.197 | 0.195 | 11.23 | 12.13 | 11.68 |
| $T_2$ = 10 to 11 am | 0.096 | 0.092 | 0.094 | 0.270 | 0.280 | 0.275 | 14.74 | 15.62 | 15.18 |
| $T_3$ = 11 to 12 pm | 0.132 | 0.129 | 0.131 | 0.173 | 0.173 | 0.173 | 10.79 | 11.18 | 10.99 |
| $T_4$ = 2 to 3 pm | 0.102 | 0.097 | 0.100 | 0.219 | 0.232 | 0.226 | 13.36 | 13.87 | 13.61 |
| $T_5$ = 3 to 4 pm | 0.138 | 0.134 | 0.136 | 0.155 | 0.158 | 0.156 | 9.36 | 9.63 | 9.49 |
| $T_6$ = 4 to 5 pm | 0.140 | 0.136 | 0.138 | 0.139 | 0.140 | 0.139 | 8.87 | 8.96 | 8.92 |
| CD (p=0.01) | 0.003 | 0.003 | 0.002 | 0.007 | 0.005 | 0.004 | 0.71 | 0.77 | 0.59 |
| Fruit picking- P | | | | | | | | | |
| $P_1$ = $1^{st}$ picking | 0.121 | 0.118 | 0.120 | 0.191 | 0.197 | 0.194 | 11.13 | 11.67 | 11.40 |
| $P_2$ = $2^{nd}$ picking | 0.116 | 0.112 | 0.114 | 0.200 | 0.203 | 0.202 | 12.74 | 12.99 | 12.86 |
| $P_3$ = $3^{rd}$ picking | 0.127 | 0.122 | 0.125 | 0.184 | 0.190 | 0.187 | 10.31 | 11.03 | 10.67 |
| CD (p=0.01) | 0.002 | 0.002 | 0.001 | 0.005 | 0.003 | 0.003 | 0.51 | 0.54 | 0.41 |
| T×P | | | | | | | | | |
| $T_1P_1$= 9 to 10 am + $1^{st}$ picking | 0.120 | 0.117 | 0.119 | 0.192 | 0.198 | 0.195 | 9.86 | 10.87 | 10.36 |
| $T_1P_2$= 9 to 10 am + $2^{nd}$ picking | 0.114 | 0.113 | 0.114 | 0.202 | 0.207 | 0.205 | 12.68 | 13.87 | 13.28 |
| $T_1P_3$= 9 to 10 am + $3^{rd}$ picking | 0.126 | 0.122 | 0.124 | 0.186 | 0.185 | 0.186 | 11.15 | 11.66 | 11.40 |
| $T_2P_{1=}$ 10 to 11 am + $1^{st}$ picking | 0.099 | 0.096 | 0.097 | 0.267 | 0.280 | 0.273 | 14.60 | 15.22 | 14.91 |
| $T_2P_2$= 10 to 11 am + $2^{nd}$ picking | 0.089 | 0.083 | 0.086 | 0.281 | 0.288 | 0.285 | 16.65 | 17.43 | 17.04 |
| $T_2P_3$= 10 to 11 am + $3^{rd}$ picking | 0.101 | 0.097 | 0.099 | 0.264 | 0.271 | 0.267 | 12.96 | 14.21 | 13.58 |
| $T_3P_1$=11 to 12 pm + $1^{st}$ picking | 0.129 | 0.124 | 0.129 | 0.172 | 0.175 | 0.173 | 10.16 | 10.82 | 10.49 |
| $T_3P_2$= 11 to 12 pm + $2^{nd}$ picking | 0.128 | 0.135 | 0.126 | 0.184 | 0.175 | 0.180 | 11.51 | 11.58 | 11.55 |
| $T_3P_3$= 11 to 12 pm + $3^{rd}$ picking | 0.140 | 0.135 | 0.138 | 0.162 | 0.170 | 0.166 | 10.70 | 11.14 | 10.92 |
| $T_4P_1$= 2 to 3 pm + $1^{st}$ picking | 0.106 | 0.100 | 0.103 | 0.220 | 0.231 | 0.225 | 14.70 | 14.89 | 14.79 |
| $T_4P_2$= 2 to 3 pm + $2^{nd}$ picking | 0.092 | 0.088 | 0.090 | 0.227 | 0.238 | 0.233 | 15.12 | 15.43 | 15.27 |
| $T_4P_3$= 2 to 3 pm + $3^{rd}$ picking | 0.109 | 0.103 | 0.106 | 0.210 | 0.229 | 0.220 | 10.27 | 11.28 | 10.78 |
| $T_5P_1$= 3 to 4 pm + $1^{st}$ picking | 0.138 | 0.134 | 0.136 | 0.153 | 0.153 | 0.153 | 8.97 | 9.93 | 9.45 |
| $T_5P_2$= 3 to 4 pm + $2^{nd}$ picking | 0.133 | 0.131 | 0.132 | 0.161 | 0.167 | 0.164 | 10.47 | 10.05 | 10.26 |
| $T_5P_3$= 3 to 4 pm + $3^{rd}$ picking | 0.142 | 0.136 | 0.139 | 0.151 | 0.153 | 0.152 | 8.64 | 8.90 | 8.77 |
| $T_6P_1$= 4 to 5 pm + $1^{st}$ picking | 0.136 | 0.128 | 0.134 | 0.140 | 0.142 | 0.141 | 8.48 | 8.30 | 8.39 |
| $T_6P_2$= 4 to 5 pm + $2^{nd}$ picking | 0.138 | 0.135 | 0.137 | 0.144 | 0.145 | 0.144 | 9.99 | 9.59 | 9.79 |
| $T_6P_3$= 4 to 5 pm + $3^{rd}$ picking | 0.145 | 0.141 | 0.143 | 0.132 | 0.132 | 0.132 | 8.15 | 8.98 | 8.57 |
| Mean | 0.121 | 0.118 | 0.119 | 0.192 | 0.197 | 0.194 | 11.392 | 11.896 | 11.644 |
| CD (p=0.01) | NS | 0.005 | 0.003 | NS | NS | NS | 1.24 | 1.33 | 1.02 |

EC= Electrical Conductivity, DEA= Dehydrogenase Enzyme Activity & AAEA= Alpha Amylase Enzyme Activity maximum seed quality. Similar results trend was observed by Kumar et al (2008) in tomato, Priya et al ( 2009) in chilli, , Sandra (2012) in bitter gourd and Kalyanrao et al (2015) in bottle gourd. Lowest seed quality parameters were observed in 4 to 5 pm crossing which could be due to light weight seed with depleted food reserves. The maximum dehydrogenase enzyme activity (0.27), α-amylase (15.18) and lowest electrical conductivity (0.094) were in T2 treatment (Table 2).

The picking of fruits which were developed from early crossed flower buds showed significant variation in seed quality parameters. Maximum germination (78.62), root length (14.29), shoot length (10.76), seedling vigour index (1988) and speed of germination (12.17) were observed in P2 (2nd picking i.e. crossing female flower in mid-October to mid-November, were harvested in December). These results are in conformity with findings of Rajsekaran (2004) in brinjal and Natesh et al (2005b) in chilli. The increase in all the seed quality parameters in the early fruits may due to better mobilization of reserve food materials, mobilized by the mother plant for the proper development of seeds with sound physiological quality. Lowest values were recorded in P3 (3rd picking i.e. crossing female flower in mid-November to mid-December and harvesting in January) due to complete utilization of applied fertilizers by the earlier set fruits and reduced mobilization of the food reserves from the source to sink as the age of plant is advanced. Second picking fruits also showed maximum seed biochemical observations (Table 2) viz., dehydrogenase enzyme activity (0.202), α-amylase activity (12.86) and less electrical conductivity (0.114) . The interaction of time of pollination and fruit picking also significantly affected some of the seed quality and biochemical parameters and best combination was 10 to 11 am pollination time for crossing female line and harvesting red ripe dried fruits at second picking.

## CONCLUSION

The 10 to 11 am pollination time for crossing female line and harvesting red ripe dried fruits at second picking (crossing female flower in mid-October to mid-November and harvesting fruits in December) induces positive results obtaining better seed quality of CGMS chilli hybrid UARChH42 (JCH42). Hence this treatment combination can be recommended for high quality seed production of hybrid chili in the H-K region chilli of Karnataka.

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# Toxicological Evaluation of Aromatic Hydrocarbons Using Toxi-Chromo Test and Mice Model

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***Abstract:*** This study aimed to evaluate the toxic effects of aromatic hydrocarbons using Toxi-chromotest and mice model. The methods employed for the toxicological evaluation involving physicochemical analysis of the marine wastewater, Toxi – chromo test using mutant *Escherichia coli*, and sub-chronic toxicity testing using mice model. The Nembe waterside wastewater sample contains higher fractions of physicochemical parameters than other sampled locations. Toxi – chromo testing revealed that the three sampling sites and aromatic hydrocarbons solutions were categorized in the order of toxicity: Onne wastewater > Nembe wastewater > Abonema wastewater and pyrene distilled water > anthracene distilled water > xylene distilled water with $EC_{100}$ of 0.28, 0.35, 0.40 and 0.22, 0.30 and 6.44 %, respectively. The mice model revealed no significant changes in the weight of tissues but significant differences were detected in the haematological parameters of the different doses of the aromatic hydrocarbons and their controls. These findings revealed ecological and animal toxicity potentials of these samples and the aromatic hydrocarbons and urgent attention is therefore needed to combat their hazardous consequences.

***Keywords*:** Acute Toxicity, Albino mice, Aromatic hydrocarbons, *E. coli*, Marine wastewater

The ecosystem of the Niger Delta in Nigeria is exposed to human-made changes and extremely vulnerable to growing environmental decline because of the discharge of petroleum pollutants (John and Okpokwasili 2012). Environmental contaminants such as hydrocarbons, heavy metals, and pesticides have been identified to have immediate toxicity impacts when discharged into the aquatic environment (Taiwo et al 2012). Aromatic hydrocarbons are environmental pollutants with toxic, mutagenic teratogenic, genotoxic, as well as carcinogenic characteristics and so their existence in the environment poses human health problems (Wanjohi et al 2015). Xylene, which is an associate of BTEX compounds, is part of aviation and gasoline fuels that could be cancerous and toxic to the central nervous system of most organisms (Hemalatha and Veeramanikandan 2011). The sixteen (16) PAHs priority list by USEPA implicated pyrene, anthracene and they make up most of the constituents of industrial wastes discharged into the aquatic and soil ecosystems. Evaluation of water or sediment samples using ecotoxicological assays should comprise a set of tests, with at least three or more tests. The model set of tests should be illustrative of all the plants and animals, and all trophic stages of the ecosystem of the study (Asker 2011). Several studies conducted emphasized the applications of Toxi-chromo test as well as an animal model as tools for the risk evaluation of contaminated sites and pollutants, both in water and on land. Asker (2011) reported that Toxi-chromo test can be applied in the environmental screening of surface and groundwater, industrial effluents, municipal discharges, etc. for the presence of toxic pollutants. Larsen (2013) reported diverse haematological effects that were observed in animals after exposure to benzo[*a*]pyrene. After oral administration (125 mg/kg body weight/day) for 13 days, aplastic anemia, pancytopenia, a severe reduction in peripheral blood leucocytes, and severe bone marrow depression with the almost destruction of pluripotent haematopoietic stem cells have been observed in female DBA/2 mice. The present study is one of the first studies carried out on the effects of marine wastewater on bacterial enzyme inhibition in the marine environment of Rivers State in Nigeria, as well as the toxic effects of representative aromatic hydrocarbons using mice model. This study was undertaken to determine the toxicological effects of aromatic hydrocarbons based on the inhibition of the enzyme *β* -galactosidase, in a mutant strain of *Escherichia coli* and changes in the weights of body and tissue as well as haematological parameters in Wistar albino mice.

## MATERIAL AND METHODS

**Sampling area description:** The studied areas were Abonema Wharf Water Front in Akuku-Toru Local

Government Area within Rivers West senatorial district (latitude 4°46'15.82"N to latitude 4°46'38.01"N and longitude 7°0'0.54"E to longitude 7°0'34.82"E), Nembe Water-Side in Port Harcourt Local Government Area within Rivers East senatorial district (latitude 4°45'8.72"N to latitude 4°45'26.42"N and longitude 7°1'11.37"E to longitude 7° 2'14.54"E) and Onne Light Flow Terminal Seaport located in Eleme Local Government Area of Rivers State within Rivers Southeast senatorial district (latitude 4°41'32.58"N to 4°41'58.18"N and longitude 7°9'26.34"E to 7°10'48.82"E). The sampling sites were selected because they are the major locations in the three senatorial districts exposed to human-induced pressures/activities resulting from urbanization, industrialization, and intensive navigation across the Rivers State marine environment.

**Sample collection and extraction:** Ten samples of each of the marine wastewater were collected randomly per point of the ten designated points from the three sampling sites. The samplings were done once in each of the three sampling sites in May - June 2017. The samples were pooled together, after which three composite /representative water samples were obtained and taken for analysis. The water samples were collected at the air-water interface by hand-dipping the 95 % ethanol - sanitized clean, dry, leak-proof, cylindrical-shaped 2 L plastic containers. The containers with lids slightly opened were rinsed with the samples thrice before aseptically collecting the samples. They were analyzed at Chukwuemeka Odumegwu University, Uli Campus, Nigeria. The marine water samples were prepared by filtering using a membrane filtration technique. The suspensions were filtered through membrane filters (d = 0.45 μm) to separate the suspended and dissolved solids in the samples before the physicochemical and toxicological analyses (Aruoja et al 2011, Uba 2019).

**Analytical chemicals and reagents:** The aromatic compounds namely xylene, anthracene, and pyrene used in this study were selected as revealed in the chemical characterization of the marine water samples and are representatives of simple, lower and higher molecular weights aromatic hydrocarbons. Xylene of analytical grade was from MERCK (PTY) Limited, South Africa (CAS NO: 1330 - 20 -7, $C_8H_{10}$, MW: 106.17 g/mol: MP: -34 °C, BP: 136 °C, VP: 8.29 at 25 °C). The test chemical is greater than or equal to 98.5 % pure (HPLC). Different concentrations (400, 1000, and 2000 mg/kg) of xylene were prepared using olive oil as a diluent for the animal study. Anthracene of analytical grade was from MERCK (PTY) Limited, South Africa (CAS: 129 - 00 - 0, $C_{14}H_{10}$, MW: 178.23 g/mol, MP: 213 - 216 $^0$C, BP: 342 $^0$C). The test chemical is greater than or equal to 96 % pure (HPLC). Different concentrations (100, 500, and 1000 mg/kg) of anthracene were prepared using olive oil as a diluent for the animal study. Pyrene of analytical grade was from Sigma Aldrich, United Kingdom (CAS: 129 – 00 - 0, $C_{16}H_{10}$, MW: 202.25 g/mol, MP: 145-148 °C (lit.), BP: 404 °C). The test chemical is greater than or equal to 98 % pure (HPLC). Different concentrations (50, 120, and 250 mg $kg^{-1}$) of pyrene were prepared using olive oil as a diluent for the animal study. Benzo (a) pyrene of analytical grade was from Sigma Aldrich, China (CAS: 50-32-8, $C_2OH_{12}$, MW: 252.31 g $mol^{-1}$, MP: 177-180°C (lit.), BP: 495°C (lit.)). The test chemical is greater than or equal to 96 % pure (HPLC). Different concentrations (20, 40, and 100 mg $kg^{-1}$) of benzo (a) pyrene were prepared using olive oil as a diluent for the animal study and use as standard PAH.

**Physicochemical analysis:** The basic parameters such as the pH, conductivity, temperature, total nitrogen, phosphorus, potassium, calcium, total dissolved solids (TDS), Total suspended solids (TSS), Total solids (TS), chemical oxygen demand (COD), dissolved oxygen (DO) and biological oxygen demand (BOD) were analyzed in the marine water samples. The heavy metal concentration was determined using an atomic absorption spectrophotometer with oxidizing air - acetylene flame (FS240AA - Agilent, USA). The total aromatic hydrocarbon (TAH) content was analyzed using the scientific gas chromatographic system with a flame ionization detector equipped with an on-column, automatic injector, mass spectroscopy (M530 buck, CA USA) (Uba 2019).

**Ecological Assay**

**Toxi-chromo test:** The toxicity of the samples was performed using Toxi–Chromo test kit according to the standard method of EBPI (2016) Toxi-chromo test procedure version 4.0 and as previously described by Uba (2018). Results of the analysis were determined by recording the complete *β* - galactosidase synthesis inhibition that results from the lowest sample concentration i.e. without color development and is expressed as the water' Effective Concentration 100 ($EC_{100}$) value expressed as a percentage.

**Sub-chronic Health Effects**

**Laboratory animals:** Adult Wistar albino mice (78 mice of 13.17 g - 30 g body weight, 12 weeks of age) obtained from Christian Farm beside General Hospital Mgbakwu, Awka North L.G.A. Anambra State Nigeria were used. The mice were housed in aluminum wire cages, 6 mice per aluminum wire cage, and maintained on a standard commercial diet. They had free access to water and food in a controlled room at 25 ± 3°C in a 12 h light: dark cycle, for one week (for acclimatization) before the start and throughout the experimental period (35 days). The mice were allowed *ad libitum* to grower feed of poultry (TOP VITAL FEEDS, Kano

State, Nigeria) and water. The animals were handled following the principle of laboratory animal care (Issa and El – Sherif 2015, Morcos et al 2015).

**Establishment of animal models:** The animal models were established as described by the standard method of the EU (2010). Briefly, twenty - four adult Wistar albino mice were used for preliminary experiments to determine suitable dosages for the aromatic hydrocarbons.

**Subchronic animal studies:** A total number of 78 adult Wistar albino mice at a 1:1 sex ratio was assigned to a control group and 4 treatment groups. The test chemicals (xylene, anthracene, pyrene, and benzo (a) pyrene (standard)) were dissolved in olive oil and 0.1 ml was administered orally. After one week of acclimatization, the animals were weighed and randomly divided into five groups (6 mice in group 1 and 18 mice each in groups 2, 3, 4, and 5). Groups 2, 3, 4 and 5 were subdivided into 3 subgroups (6 mice per subgroup) according to the dose concentrations (400, 1000 and 2000 mg xylene $kg^{-1}$; 100, 500 and 1000 mg anthracene $kg^{-1}$; 50, 125 and 250 mg pyrene $kg^{-1}$ and 20, 40 and 100 mg benzo (a) pyrene $kg^{-1}$). Group 1 was administered orally with 0.1 ml olive oil only and served as control while groups 2, 3, 4, and 5 were treated with xylene, anthracene, pyrene, and benzo (a) pyrene and administered with similar doses daily for 35 days (Issa and El-Sherif 2015, Morcos et al 2015, Wang and Xue 2015).

**Body and organ weight determination:** After the experimental period (35 days) sub-chronical exposure of the hydrocarbons treated mice, the weights of the animals were determined and the blood samples were collected in a vacuum blood collection tube (Agary) by cardiovascular aspiration puncture using agarine sterile insulin syringe (Wuxi-Yoshou, China) containing an anticoagulant (EDTA) (Morcos et al 2015). In the same vein, the tissues (stomachs, livers, kidneys, and lungs) were removed intact by dissecting the mice, weighed, and fixed in 10 % neutral buffered formalin (Okani et al 2013, Issa and El-Sherif 2015, Wang and Xue 2015).

**Haematological analysis:** By adopting the method of Morcos (2015), the haematological parameters used in this study includes white blood cell (WBC), red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV), platelets, neutrophils, lymphocytes, monocytes, eosinophils, and basophils. The blood haematological levels were analyzed at Comax International Laboratory Ogidi, Anambra State, Nigeria.

**Statistical analysis of data:** Two-tailed paired T-TEST and repeated measures one-way analysis of variance followed by post-Tukey's; multiple comparison tests were carried out on the mean ± S.D. Graph-Pad Prism statistical software version 7.00 was used.

## RESULTS AND DISCUSSION

**Physicochemical characterization:** The conductivity at 25°C (EC25) for Onne, Abonema, and Nembe waters was 6.99, 1.97, and 1.32 dS $cm^{-1}$, respectively (Table 1). This revealed that the Onne wastewater had greater content ions, carry more current, and saltier than other sampling locations and the conductivities values tend to give surrogate values of levels of salinities and total dissolved solids, (TDS). Onne water samples exhibited lower pH values (6.41) followed by Abonema (6.55) and Nembe (7.31) revealing that the marine water samples were found to be acidic and neutral. Nembe had the highest total nitrogen of 970.00 mg $l^{-1}$ whereas Abonema had the highest total phosphorus , potassium , and calcium content of 3.72 mg $l^{-1}$, 4.60 ppm, and 06.35 ppm for water samples, respectively. The sampled locations contained a high content of total nitrogen, total phosphorus, and exchangeable bases of potassium and calcium in the water samples, and the possible reasons for these occurrences may be due to pH and human activities observed along the study area which include agricultural land use and farming operation, anthropogenic activities and industrialization. Furthermore, Abonema water showed the highest COD (210.00 mg $l^{-1}$), DO (25.30 mg $l^{-1}$), BOD (106.60 mg $l^{-1}$), TDS (7.48 mg $l^{-1}$), TSS (0.15 mg $l^{-1}$) and TS (7.63 mg $l^{-1}$) followed by Nembe and Onne water samples. All the sampled locations COD and BOD were higher than WHO (2008) standards except TDS, TSS, and TS were below the maximum recommended limits. The possible reasons for these phenomenal could be due to the agricultural and physical practices in the sampled locations and similar observations were reported by Rana et al (2016) and Devi and Nagendran (2017) who observed the TDS, BOD, COD, DO, $Cl^-$ and $NO_3^-$ contents in surface water were attributed to agricultural land use, movement, and temperature. There were more TAH contents in Nembe water samples (21.3 ppb) than the other two marine water samples. The iron metal had the highest fractions in the Abonema water sample (3.27 ppm) than the samples from the Nembe and Onne sample. The order of concentration abundance of these metals in the marine water from the three locations is in the sequence: Fe > Zn > Hg > Ni > Cd > Co > Cr > Cu > Pb > As. Non-significant differences ($P > 0.05$) were detected in all the sampled parameters. All metals were found to be lower except iron, cadmium nickel, and mercury which were within the WHO (2008) maximum permissible recommended limits (MPL). Owamah (2013), reported that the enrichment factors for Cd, Cr, Cu, Fe, Ni, and Pb were very high for water and sediment samples in Niger Delta, Nigeria.

**Toxi – chromo testing:** The pyrene Onne water had 0.20 % while the positive control ($HgCl_2$) had 1.38 % of $EC_{100}$ (Table

2). There were significant differences among the treatment group of wastewater samples, aromatic hydrocarbons, and their controls. Toxicity ($EC_{100}$) was classified according to the percentage concentration of the sample required to produce an $EC_{100}$ effect. The three sampling sites were categorized in the order of toxicity: Onne wastewater > Nembe wastewater > Abonema wastewater with $EC_{100}$ values of 0.28, 0.35, and 0.40 %, respectively while the aromatic hydrocarbons were classified in the order of toxicity: pyrene distilled water > anthracene distilled water > xylene distilled water with $EC_{100}$ values of 0.22, 0.30 and 6.44 %, respectively. The possible reason could be due to the presence of heavy metals and other physicochemical substances detected in the wastewater. All the samples had their coefficient of variation (CV) to be less than 25 percent (< 25 %) thereby substantiating the biological validity criterion of the test which agreed with the guiding principle of EBPI (2016) that for Toxi-ChromoTest™ results to be considered valid. Uba (2019) demonstrated the potential of Toxi-ChromoTest™ to be sensitive for detecting bioavailable toxicants in the wastewater. The $EC_{50}$ results for bioassay of wastewater samples of Abonema, Nembe, and Onne sites of Niger Delta Nigeria containing polyaromatic hydrocarbons (PAHs) and heavy metals were classified as toxic -, very toxic - and toxic, respectively.

**Animal Toxicity**

**Determination of body and organ weight:** The xylene 1000 mg/kg exposed mice had the highest body weight of 27.87 g before exposure, and control exposed mice without hydrocarbon had the highest body weight of 26.44 g after exposure (Table 3). The, increase in the dose of xylene led to a decrease in weight of stomach and liver from 0.93-0.49 g, while kidney and lung increase in weight from 0.17-0.28 g, respectively (Fig. 1). The increase in the dose of anthracene led to a decrease and increase in weight of stomach and lung from 0.68-0.15 g while liver and kidney increase and

**Table 1.** Physicochemical features of the marine water samples of the three sampling locations (Mean ± standard error)

| Parameters | Water sampling locations | | |
|---|---|---|---|
| | Abonema | Nembe | Onne |
| Conductivity (dS /cm) at 25 °C | 1.97 ± 0.03 | 1.32 ± 0.13 | 6.99 ± 0.27 |
| pH | 6.55 ± 0.02 | 7.31 ± 0.16 | 6.41 ± 0.02 |
| TDS (mg $L^{-1}$) | 7.48 ± 0.01 | 6.56 ± 0.02 | 5.76 ± 0.62 |
| TSS (mg $L^{-1}$) | 0.15 ± 0.00 | 0.01 ± 0.00 | 0.07 ± 0.00 |
| TS (mg) | 7.63 ± 0.01 | 6.56 ± 0.00 | 5.83 ± 0.21 |
| COD (mg $L^{-1}$) | 210.00 ± 2.52 | 66.67 ± 31.41 | 80.00 ± 46.84 |
| DO (mg $L^{-1}$) | 22.50 ± 0.05 | 25.30 ± 0.08 | 19.30 ± 0.15 |
| BOD (mg $L^{-1}$) | 106.60 ± 65.56 | 46.00 ± 0.20 | 46.00 ± 0.20 |
| TN (mg $L^{-1}$) | 280.00 ± 0.01 | 970.00 ± 0.01 | 550.00 ± 0.03 |
| TP (mg $L^{-1}$) | 3.72 ± 0.02 | 2.16 ± 0.01 | 2.07 ± 0.02 |
| Potassium (ppm) | 4.64 ± 0.01 | 3.88 ± 0.01 | 4.28 ± 0.00 |
| Calcium (ppm) | 6.35 ± 0.01 | 5.78 ± 0.00 | 4.10 ± 0.00 |
| TAH content (ppb) | 14.60 ± 0.16 | 20.40 ± 0.34 | 16.10 ± 0.18 |
| Iron (Fe) (ppm) | 03.27± 0.25 | 1.11± 0.105 | 2.20± 0.20 |
| Cobalt (Co) (ppm) | 0.05± 0.02 | ND | ND |
| Copper (Cu) (ppm) | ND | ND | ND |
| Lead (Pb) (ppm) | ND | ND | ND |
| Cadmium (Cd) (ppm) | 0.01± 0.00 | 0.01± 0.00 | ND |
| Chromium (Cr) (ppm) | 0.02± 0.02 | ND | ND |
| Zinc (Zn) (ppm) | 0.06± 0.02 | 0.13± 0.00 | 0.10± 0.01 |
| Nickel (Ni) (ppm) | ND | 0.11± 0.02 | 0.05± 0.02 |
| Mercury (Hg) (ppm) | 0.13± 0.02 | 0.05± 0.02 | ND |
| Arsenic (As) (ppm) | ND | ND | ND |

ABW = Abonema water, NEW = Nembe water; ONW = Onne water, NA = Not available; ppb = part per billion; values are mean ± Standard deviation of triplicate determination; Detection limit: PAH = 0.0001 ug /g; xylene, anthracene and naphthalene = 0.002 µg /mL; ND = Not detected; Detection limit = Fe, Co,Pb, Cd, Cr, Ni and As = 0.0002 ppm; Cu and Zn = 0.0001 ppm; Hg = 0.0005 ppm decrease in weight from 0.12-0.31 g, respectively (Fig. 2). The increase in the dose of pyrene led to a decrease and increase in weight of stomach, liver, and kidney from 1.49-0.29 g while lung increase and decrease in weight from 0.15-0.20 g, respectively (Fig. 3) and finally increase in the dose of benzo (a) pyrene led to increase and decrease in weight of stomach, liver, and lung from 0.08-2.74 g while kidney increase in weight from 0.17-0.24 g, respectively (Fig. 4). There were no statistically significant differences ($P > 0.05$). Niaz et al (2015) reported a 16% reduction in body weight when the rat was exposed to 700 ppm dose of xylene for 7 days while Larsen (2013) reported that 120 mg benzo[*a*]pyrene/kg BW/day via the diet was developmentally toxic in mice.

**Haematological profile:** There were reductions in RBC (5.10 - 4.00 /L), Hb (14.40 - 12.10 g/dL), PCV (46.40 - 36.00 %), monocytes (10.40 - 6.00 %), basophils (2.0 - 0.00 %); decrease and increase in platelets and lymphocytes; increase and decrease in WBC, neutrophils and eosinophils as the dose/ concentration of xylene increased from 400-2000 mg kg$^{-1}$ (Table 4). There were reductions in RBC, Hb, PCV, and platelets; an increase in lymphocytes and basophils; a decrease and increase in WBC and neutrophils; increase and decrease in monocytes and eosinophils as the dose/ concentration of anthracene increase from 100 – 1000

**Table 2.** $EC_{100}$ toxic response of mutant *E. coli* to the aromatic hydrocarbons and wastewater samples from the three sampled locations ( Mean ± standard error)

| Sample | Toxicity $EC_{100}$ (%) |
|---|---|
| Positive control ($HgCl_2$) | 1.38 |
| Xylene + Distilled water | 6.44 |
| Xylene + Abonema water | 0.36 |
| Xylene + Nembe water | 3.10 |
| Xylene + Onne water | 0.75 |
| Anthracene + Distilled water | 0.30 |
| Anthracene + Abonema water | 0.33 |
| Anthracene + Nembe water | 0.44 |
| Anthracene + Onne water | 0.52 |
| Pyrene + Distilled water | 0.22 |
| Pyrene + Abonema water | 0.39 |
| Pyrene + Nembe water | 0.37 |
| Pyrene + Onne water | 0.20 |
| Abonema water | 0.40 |
| Nembe water | 0.35 |
| Onne water | 0.28 |

**Key:** Toxicity classification: (a) very toxic, if the sample concentration was < 12.5 %; (b) moderately toxic, if the sample concentration was > 12.5 % and < 50.0 %; and (c), non-toxic, if the sample concentration was > 50 %

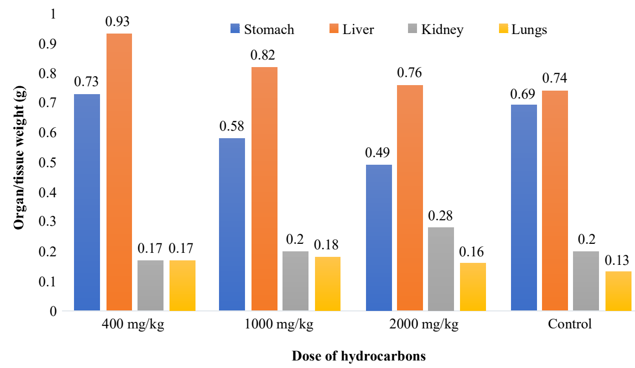


**Fig. 1.** Organ/tissue weight of xylene treated Wistar albino mice after 35 days of exposure

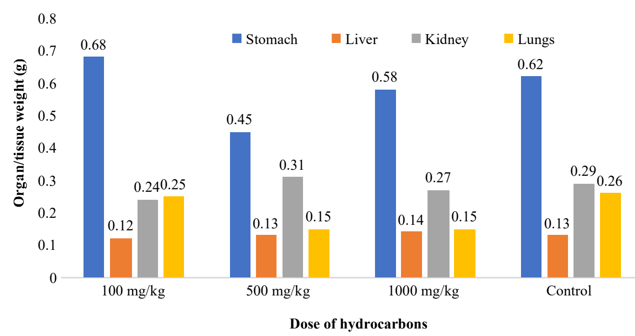


**Fig. 2.** Organ/tissue weight of anthracene treated Wistar albino mice after 35 days of exposure

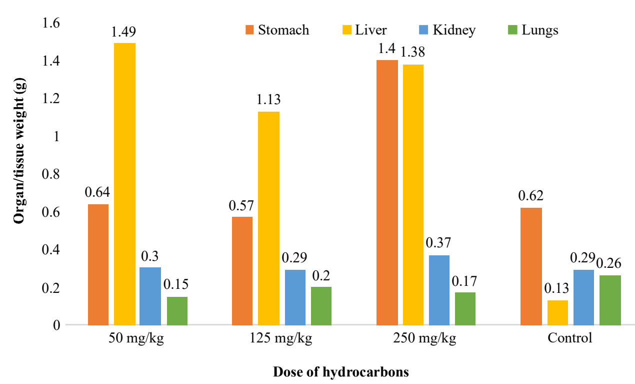


**Fig. 3.** Organ/tissue weight of pyrene treated Wistar albino mice after 35 days of exposure

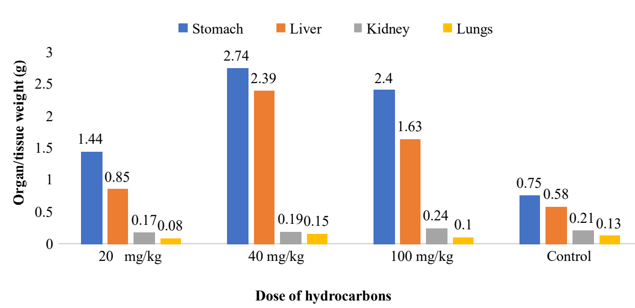


**Fig. 4.** Organ/tissue weight of benzo (a) pyrene treated Wistar albino mice after 35 days of exposure

mg $kg^{-1}$ (Table 5). There were reductions in RBC, Hb, PCV, and platelets; an increase in monocytes and basophils; decrease and increase in WBC, eosinophils, and lymphocytes; increase and decrease in neutrophils as the dose/ concentration of pyrene increased from 50-250 mg $kg^{-1}$ (Table 6). There were reductions in WBC, RBC, Hb, platelets, monocytes, eosinophils, and basophils; decrease and increase in neutrophils and PCV; increase and decrease in lymphocytes as the dose/ concentration of benzo (a) pyrene increased from 20-100 mg $kg^{-1}$ (Table 7). The control values were within the reference range except the treatment groups with either higher or lower values. There were extremely significant differences detected among the dose-effect of the treatment group of aromatic hydrocarbons and their controls but not significant among the haematological parameters ($P > 0.05$). The possible reasons for the variable changes in the haematological parameters of the aromatic hydrocarbon exposed mice could be due to apoptosis of red blood cell progenitors, pre - B cells, and B lymphocytes induced by the toxic effects of these aromatic hydrocarbons and their metabolites thereby leading to a detrimental effect on bone marrow haematopoiesis. These findings corroborate with the observation of Morcos et al (2015) that xylene induces apoptosis of the red blood cells.

The Onne wastewater had greater content ions, carry more current, and saltier than other sampling locations and the conductivities values tend to give surrogate values of levels of salinities and total dissolved solids, (TDS) (Table 1). The marine water samples were acidic and neutral. All the sampled locations showed high content of total nitrogen (TN), total phosphorus (TP), and exchangeable bases potassium and calcium in the water samples, and the possible reasons for these occurrences may be due to pH and human activities observed along the study area which includes agricultural land use and farming operation, anthropogenic activities and industrialization. In the same vein, all the sampled locations COD and BOD were higher, standards except TDS, TSS, and TS that were found below the maximum recommended limits. A significant elevation of water indices such as pH, BOD, nitrate, phosphate, and TSS was observed. It is well known that oxygen depletion in water bodies could cause fish death while an increase in BOD signifies a high load of organic matter. All metals except iron,

**Table 3.** Average body weight (g) of aromatic hydrocarbon treated Wistar Albino mice before and after 35 days of oral exposure

| Dose (mg $kg^{-1}$) | Before (g) | After (g) |
|---|---|---|
| Control | | |
| 0 | 27.11 ± 0.28 | 26.44 ± 0.91 |
| Xylene | | |
| 400 | 24.13 ± 1.11 | 23.26 ± 0.51 |
| 1000 | 27.87 ± 1.11 | 21.81 ± 0.56 |
| 2000 | 24.62 ± 0.57 | 22.93 ± 0.89 |
| Anthracene | | |
| 100 | 23.21 ± 0.94 | 21.12 ± 0.34 |
| 500 | 25.10 ± 0.65 | 24.01 ± 0.39 |
| 1000 | 26.40 ± 1.34 | 26.37 ± 1.71 |
| Pyrene | | |
| 50 | 23.12 ± 0.40 | 20.88 ± 0.37 |
| 125 | 23.30 ± 0.35 | 19.43 ± 0.04 |
| 250 | 23.30 ± 0.35 | 17.88 ± 0.09 |
| Benzo (a) pyrene | | |
| 20 | 20.20 ± 0.32 | 20.19 ± 0.40 |
| 40 | 21.50 ± 0.13 | 20.77 ± 0.21 |
| 100 | 23.78±0.18 | 20.00 ± 0.33 |

Values are mean of three actual values ± standard error

**Table 4.** Effect of xylene on haematological parameters in Wistar Albino mice at different doses after 35 days of exposure

| Parameters | Control (0 mg $kg^{-1}$) | 400 mg $kg^{-1}$ | 1000 mg $kg^{-1}$ | 2000 mg $kg^{-1}$ |
|---|---|---|---|---|
| WBC (L) | 5.80 ± 0.50 | 4.60± 0.13 | 5.00 ± 0.13 | 2.30 ± 0.13 |
| RBC (L) | 3.30 ± 0.60 | 5.10 ± 0.13 | 4.20 ± 0.13 | 4.00 ± 0.13 |
| Hb (g/dL) | 9.90 ± 0.70 | 14.40 ± 0.13 | 12.00 ± 0.16 | 12.10 ± 0.16 |
| PCV (%) | 30.00 ± 0.20 | 46.40 ± 0.16 | 37.80 ± 0.08 | 36.00 ± 0.17 |
| Platelets (/L) | 185.00 ± 0.30 | 421.00 ± 0.16 | 198.00 ± 0.13 | 401.00 ± 0.16 |
| Neutrophils (%) | 23.00 ± 0.70 | 19.90 ± 0.17 | 24.10 ± 0.14 | 17.20 ± 0.16 |
| Lymphocytes (%) | 60.00 ± 0.50 | 61.70 ± 0.16 | 51.30 ± 0.14 | 71.60 ± 0.13 |
| Monocytes (%) | 12.00 ± 0.40 | 10.40 ± 0.16 | 8.20 ± 0.16 | 6.00 ± 0.16 |
| Eosinophil (%) | 5.00 ± 0.70 | 6.00 ± 0.17 | 16.40 ± 0.16 | 5.20 ± 0.13 |
| Basophils (%) | 0.00 ± 0.00 | 2.00 ± 0.81 | 0.00 ± 0.00 | 0.00 ± 0.00 |

Values are mean of three actual values ± standard error
KEY: WBC = White blood count; RBC = Red blood count; Hb = Haemoglobin; PCV = Packed cell volume

**Table 5.** Effect of anthracene on haematological parameters in Wistar Albino mice at different doses after 35 days of exposure

| Parameters | Control (0 mg $kg^{-1}$) | 100 (mg $kg^{-1}$) | 500 (mg $kg^{-1}$) | 1000 (mg $kg^{-1}$) |
|---|---|---|---|---|
| WBC (L) | 5.80 ± 0.50 | 9.50 ± 0.13 | 3.80 ± 0.13 | 5.60 ± 0.13 |
| RBC (L) | 3.30 ± 0.60 | 7.80 ± 0.13 | 3.90 ± 0.13 | 3.60 ± 0.13 |
| Hb (g $dL^{-1}$) | 9.90 ± 0.70 | 14.60 ± 0.13 | 11.00 ± 0.16 | 11.00 ± 0.16 |
| PCV (%) | 30.00 ± 0.20 | 46.90 ± 0.16 | 35.20 ± 0.08 | 33.00 ± 0.17 |
| Platelets (L) | 185.00 ± 0.30 | 739.00 ± 0.16 | 264.00 ± 0.13 | 185.00 ± 0.16 |
| Neutrophils (%) | 23.00 ± 0.70 | 58.00 ± 0.17 | 19.30 ± 0.14 | 24.00 ± 0.16 |
| Lymphocytes (%) | 60.00 ± 0.50 | 32.00 ± 0.16 | 56.30 ± 0.14 | 58.00 ± 0.13 |
| Monocytes (%) | 12.00 ± 0.40 | 7.00 ± 0.16 | 8.20 ± 0.16 | 6.00 ± 0.16 |
| Eosinophil (%) | 5.00 ± 0.70 | 3.00 ± 0.17 | 15.40 ± 0.16 | 11.00 ± 0.13 |
| Basophils (%) | 0.00 ± 0.00 | 1.00 ± 0.81 | 1.00 ± 0.81 | 1.00 ± 0.13 |

Values are mean of three actual values ± standard error
KEY: WBC = White blood count; RBC = Red blood count; Hb = Haemoglobin; PCV = Packed cell volume

Table 6. Effect of pyrene on haematological parameters in Wistar Albino mice at different doses after 35 days of exposure

| Parameters | Control (0 mg $kg^{-1}$) | 100 (mg $kg^{-1}$) | 500 (mg $kg^{-1}$) | 1000 (mg $kg^{-1}$) |
|---|---|---|---|---|
| WBC (L) | 5.80 ± 0.50 | 5.30 ± 0.40 | 2.30 ± 0.40 | 2.90 ± 0.50 |
| RBC (L) | 3.30 ± 0.60 | 5.50 ± 0.50 | 4.60 ± 0.50 | 4.60 ± 0.50 |
| Hb (g $dL^{-1}$) | 9.90 ± 0.70 | 16.50 ± 0.50 | 13.90 ± 0.50 | 13.80 ± 0.60 |
| PCV (%) | 30.00 ± 0.20 | 51.00 ± 0.60 | 42.00 ± 0.40 | 42.60 ± 0.70 |
| Platelets (%) | 185.00± 0.30 | 88.50 ± 0.50 | 53.30 ± 0.50 | 13.50 ± 0.60 |
| Neutrophils (%) | 23.00 ± 0.70 | 30.00 ± 0.20 | 40.00 ± 0.50 | 30.00 ± 0.20 |
| Lymphocytes (%) | 60.00 ± 0.50 | 68.60 ± 0.50 | 54.20 ± 0.60 | 60.00 ± 0.50 |
| Monocytes (%) | 12.00 ± 0.40 | 1.00 ± 0.40 | 3.80 ± 0.80 | 5.80 ± 0.50 |
| Eosinophils (%) | 5.00 ± 0.70 | 0.40 ± 0.10 | 2.00 ± 0.60 | 3.00 ± 0.80 |
| Basophils (%) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 1.00 ± 0.40 |

Values are mean of three actual values ± standard error
KEY: WBC = White blood count; RBC = Red blood count; Hb = Haemoglobin; PCV = Packed cell volume

**Table 7.** Effect of benzo (a) pyrene on haematological parameters in Wistar Albino mice at different doses after 35 days of exposure

| Parameters | Control (0 mg $kg^{-1}$) | 100 (mg $kg^{-1}$) | 500 (mg $kg^{-1}$) | 1000 (mg $kg^{-1}$) |
|---|---|---|---|---|
| WBC (L) | 5.80 ± 0.50 | 5.40 ± 0.17 | 4.70 ± 0.18 | 4.70 ± 0.08 |
| RBC (L) | 3.30 ± 0.60 | 4.70 ± 0.21 | 4.00 ± 0.21 | 4.00 ± 0.35 |
| Hb (g $dL^{-1}$) | 9.90 ± 0.70 | 14.20 ± 0.13 | 12.10 ± 0.26 | 12.10 ± 0.13 |
| PCV (%) | 30.00 ± 0.20 | 42.00 ± 0.17 | 33.90 ± 0.14 | 36.40 ± 0.17 |
| Platelets (/L) | 185.00 ± 0.30 | 526.00 ± 0.16 | 416.00 ± 0.17 | 218.00 ± 0.13 |
| Neutrophils (%) | 23.00 ± 0.70 | 40.00 ± 0.13 | 25.00 ± 0.16 | 30.00 ± 0.17 |
| Lymphocytes (%) | 60.00 ± 0.50 | 48.00 ± 0.13 | 67.00 ± 0.17 | 65.10 ± 0.17 |
| Monocytes (%) | 12.00 ± 0.40 | 8.00 ± 0.17 | 5.00 ± 0.17 | 3.00 ± 0.21 |
| Eosinophils (%) | 5.00 ± 0.70 | 3.00 ± 0.16 | 3.00 ± 0.08 | 1.00 ± 0.13 |
| Basophils (%) | 0.00 ± 0.00 | 1.00 ± 0.22 | 0.00 ± 0.00 | 0.00 ± 0.00 |

Values are mean of three actual values ± standard error
KEY: WBC = White blood count; RBC = Red blood count; Hb = Haemoglobin; PCV = Packed cell volume

cadmium nickel, and mercury were lower and within for wastewater samples in comparison to the Federal Ministry of Environment, water standards for aquatic life fresh (FWA) Nigeria, maximum permissible recommended limits (MPL). Owamah (2013) reported that the enrichment factors for Cd, Cr, Cu, Fe, Ni, and Pb were very high for water and sediment samples in Niger Delta, Nigeria.

Toxicity (EC100) was classified according to the percentage concentration of sample required to produce an EC100 effect: (a) very toxic, if the sample concentration was <12.5%; (b) moderately toxic, if the sample concentration was >12.5% and <50.0%; and (c), non-toxic, if the sample concentration was >50%. The three sampling sites were categorized in the order of toxicity: Onne wastewater > Nembe wastewater > Abonema wastewater with EC100 values of 0.28%, 0.35, and 0.40%, respectively while the aromatic hydrocarbons were classified in the order of toxicity: pyrene distilled water > anthracene distilled water > xylene distilled water with EC100 values of 0.22%, 0.30% and 6.44%, respectively. The possible reason could be due to the presence of heavy metals and other physicochemical substances detected in the wastewater. All the samples had their coefficient of variation (CV) to be less than 25 per cent (<25%) thereby substantiating the biological validity criterion of the test which agreed with the guiding principle of EBPI (2016) that for Toxi-ChromoTestTM results to be considered valid, the CV between the absorbance values of negative controls and sample replicates <25%. Previous research has demonstrated the Toxi-ChromoPad_solid phase test to be sensitive for detecting bioavailable toxicants in sediments. Besides, based on the EC50 results for bioassay, Ballymacoda, Douglas, and the East Wall site estuarine sediments of Irish Coast containing polyaromatic hydrocarbons (PAHs) and heavy metals were ranked as non-, moderately- and very toxic, respectively.

The 1000 mg $kg^{-1}$ had the highest body weight before exposure and control without hydrocarbon had the highest body weight after exposure while the results in Fig. 1- 4 revealed that there were increase and decrease in the mean weight of the tissues (lung, stomach, liver, and kidney). Xylene leads to reduced fetus weight and especially develops offspring size with skeletal deformities. So, it was concluded that xylene in huge amount leads to a foetus and parental toxic effects.

The control values were within the reference range but higher or lower than the treatment groups. The reasons for the variable changes in the haematological parameters of the hydrocarbon treated mice could be due to apoptosis of red blood cell progenitors, pre - B cells, and B lymphocytes induced by toxic effects of these aromatic hydrocarbons and their metabolites thereby leading to a detrimental effect on bone marrow haematopoiesis. These findings corroborate with the observation of Morcos et al (2015) that xylene induces apoptosis of the red blood cells.

## CONCLUSION

Nembe waterside wastewater sample is more polluted than other sampled locations. The toxic response to mutant *Escherichia coli* to aromatic hydrocarbon is in the order: pyrene > anthracene > xylene. The response to wastewater is in the order: Onne water > Abonema water > Nembe water. Higher toxicity effects were observed when the wastewater and the representative aromatic hydrocarbon samples were in combined preparations than in single preparations. The results signify the toxicity potentials of the wastewater and the representative aromatic hydrocarbon samples to the aquatic environments. There were reductions in the tissue weights (lung, stomach, liver, and kidney) of the mice and Also, varying changes in hematological parameters of the Wistar albino mice were observed at different doses of the aromatic hydrocarbons analyzed during the 35 days study period. The results also signify the animal toxicity potentials of the representative aromatic hydrocarbon samples when discharged indiscriminately. The findings of this study could be employed in the toxicological risk assessment of these aromatic hydrocarbons in the Rivers State marine environment.

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# Identification and Antimicrobial Susceptibility Profiles of *Salmonella* spp. Isolated from Chicken Flocks and their Feed and Water in Karbala, Iraq

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***Abstract***: The objective of study was determining the most prevalent *Salmonella* spp. and their antimicrobial susceptibility in broilers and laying chickens and their feed and drinking water in five chicken farms in Karbala, Iraq over the period from August to October 2020. A total of 289 samples, including 217 cloaca swabs, 46 water and 26 feed samples were collected. *Salmonella* spp. was identified firstly by routine diagnostic methods, followed by applying the API 20E kit, the Vitek2 system, and serology. There was significant differences in *Salmonella* prevalence among different types of samples, mainly cloaca swabs reported a high isolation rate (21.7%). In contrast, feed samples were completely free of contamination. The highest rate of isolation was in September on the $4^{th}$ to $6^{th}$ weeks of age. The presence of different *Salmonella* types in the collected samples excludes the possibility of outbreak occurrence among these farms. However, many isolates were diagnosed as *S. paratyphi* B. The tested isolates were 100% resistant to Ampicillin, Amikacin, Gentamicin, and Ciprofloxacin. By contrast, they were susceptible to Ceftazidime, Cefepime, and Ertapenem. The study provides an insight into the distribution and antimicrobial resistance of *Salmonella* spp. circulating in several poultry farms in Karbala, Iraq.

***Keywords***: *Salmonella* spp, Broiler chicken, Layer chicken, Identification, Serotyping

*Salmonella* spp. is a Gram-negative intracellular enteric bacteria that is important to public health (Li et al 2018) and is a major zoonotic pathogen that causes illness in both humans and animals, adding to being the most common bacterial foodborne illness in both developed and developing countries (Wibisono et al 2020). Typhoidal and non-typhoidal salmonellosis are caused by *Salmonella*, and millions of these cases occur annually, resulting in substantial economic losses and even human death (Luvsansharav et al 2020). The majority of cases of non-typhoidal *Salmonella* (NTS) illness are linked to infected animal-based foods, notably poultry meat and, in some circumstances, vegetables (Pandey and Goud 2021). *Salmonella* infections are important in the poultry production industry as well as in humans. Salmonellosis in poultry is an important disease that seriously hinders the development of the poultry industry (Sarker et al 2021). It causes reduced growth and fatality in chickens (Jazi 2019). Every year, *Salmonella* infection causes not only reduced performance of poultry production and even death, but also contaminate the human food chain, resulting in significant economic losses in the poultry sector as well as posing a threat to public health (Sylejmani et al 2016). Despite that chickens are typically considered as asymptomatic carriers who shed the germs in their feces and are significant reservoirs of bacteria. Infected birds can serve as a vehicle for disease transmission (Sylejmani et al 2016). Infection may be contracted through both direct and indirect contact with animals. Indirect transmission might result due to contact with contaminated things around poultry farms or with the environment around (Wibisono et al 2020). Chicks can become infected with *Salmonella* spp. by vertical transmission from infected parents or horizontal transmission from hatcheries, cloacal infection, or transfer through feed and equipment (Al Mamun et al 2017). Antimicrobial resistance to *Salmonella* has been a serious public health problem across the world (Sarker et al 2021). Resistance to antimicrobials by pathogenic bacteria is a global public health problem, particularly in developing countries (Akbar and Anal 2015, Rahman et al 2018). The primary determinants for the occurrence of multidrug-resistant pathogenic bacteria are the outcomes of unwise use of antimicrobial drugs to reduce bacterial infection or as a growth booster in poultry production (Akbar and Anal 2015, Rahman et al 2016). Therefore, the management of *Salmonella* infections with standard treatments is extremely challenging because of the occurrence of multidrug-resistant *Salmonella* isolates (Nair et al 2018).

Finally, control measures, such as those contained in National Control Plans, have been undertaken in the EU to minimize the prevalence of salmonellosis and other foodborne illnesses in poultry production due to the involvement of poultry in the spread of *Salmonella* spp. (Sibanda et al 2018). Despite numerous preventative and control methods, such as drug and vaccine use as well as eradication campaigns, *Salmonella* infection is still one of the most serious concerns globally. From what was mentioned above, *Salmonella* continues to cause major economic losses in many countries and consumes a significant amount of resources in other countries for testing and control efforts. In Iraq, this bacterium still poses a significant threat to poultry flocks. Therefore, the present study aimed at investigating the possible sources of *Salmonella* occurrence in five chicken farms in Karbala, Iraq, either in chicken themselves or their feed and water.

## MATERIAL AND METHODS

**Specimens' collection:** A total of 289 samples were collected from two different locations in the holy Karbala governorate, Iraq, including: Al-Husseinia and Al-Zubeilia during the period from August till November 2020. *Salmonella* spp. was isolated from three different types of samples, including: cloaca cotton swabs, water and feed (Table 1). All samples were treated aseptically, in which 1 gm of chicken feed sample was individually inoculated in a test tube containing 9 ml of peptone water and incubated at 37°C for approximately 18-24 hr. Cotton swabs were inoculated into 10 ml of peptone water, water samples taken from the same fields were centrifuged and 1 ml of the sediment was moved to another test tube containing 10 ml of tetrathionate broth (TTB) and incubated at 37°C for 18-24 hr.

**Bacterial isolation and identification:** For bacterial isolation, 1 ml of peptone water medium already inoculated with a sample was transferred to 10 ml of tetrathionate broth (TTB), which inhibited the growth of all bacteria except for *Salmonella*, and then the medium was incubated at 37°C for 24 hr. Later, a loopful from the cultured enriched broth was streaked onto plates of MacConkey, SS, XLD, and Brilliant green agars, and incubated at 37ºC for 24 hr. Salmonella-Shigella (SS) agar, was used as a moderately selective and differential medium for the isolation, cultivation and differentiation of *Salmonella* spp. MacConkey's agar was used for the isolation of Gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting bacteria (Jaffer 2013). Moreover, other *Salmonella* spp. were identified and differentiated by using HiCrome Salmonella agar.

On the basis of colony features, staining properties, and routine biochemical tests, organisms were isolated and initially identified. Afterwards, the bacterial growth was purified on Brian hart infusion agar to be used the next day for inoculating the commercial kits. Analytical profile index 20 for *Enterobacteriaceae* (API 20 E) kit was used for the detection of *Salmonella* spp and this was followed by serotype identification by serological tests. The serological diagnosis was done at The Central Health Laboratories, Baghdad, Iraq, by the use of slip stacking assay with a standard polyvalent antigen of the O and H antigen groups (phase I and II). In addition, the Vitek2® system was used for confirmation of some isolates.

**Identification with the API 20E system:** The identification of the bacterial isolates by the API 20E system was done according to the procedure stated by the manufacture (BioMerieux). Twenty standard biochemical tests were determined by this system. To do this test, the bacterial suspension was prepared from the well-isolated colonies of the suspected isolates by using the API suspension medium, and the turbidity was adjusted to 0.5 McFarland tube ($1x10^8$ CFU/ml). By using a sterile Pasteur pipette, the bacterial

**Table 1.** Types, numbers and locations of samples from which *Salmonella* spp. were isolated

| Location | Chicken age $day^{-1}$ | No. samples | Type of samples | Specimen | Farm |
|---|---|---|---|---|---|
| Al-Husseinia | 12-45 | 41 | Cloaca cotton swabs | Broiler chicken | A |
| | | 6 | Water | | |
| | | 6 | Feed | | |
| Al-Husseinia | 13-45 | 44 | Cloacal cotton swabs | Broiler chicken | B |
| | | 10 | Water | | |
| | | 6 | Feed | | |
| Al-Husseinia | 14-45 | 44 | Cloacal cotton swabs | Broiler chicken | C |
| | | 10 | Water | | |
| | | 5 | Feed | | |
| Al-Zubeilia | 12-47 | 44 | Cloacal cotton swabs | Layers chicken | D |
| | | 10 | Water | | |
| | | 5 | Feed | | |
| Al-Zubeilia | 13-47 | 44 | Cloacal cotton swabs | Layers chicken | E |
| | | 10 | Water | | |
| | | 4 | Feed | | |

suspension was transferred to the 20 micro tubes and inoculated according to the manufacturer's instructions. Then, after incubation at 37°C for 24 hr, the isolates were identified by matching the numerical coding of the API system.

**Vitek2 diagnostic method (BCL identification card):** Some isolates suspected to be *Salmonella* were identified by the automated Vitek2 system with its identification card at Imam Al-Hijjah Hospital, located in Karbala, Iraq. The 64-well card contained 43 colorimetric substrates for the phenotypic identification of bacterial species. For detecting of the bacterial identity using Vitek2, the isolate was plated onto XLD agar and incubated overnight at 37°C. The next day, a suspension of the organism was prepared in saline (0.45-0.50% NaCl) inside a polystyrene tube to a density equivalent to a McFarland tube number 0.5. The density was determined using a Vitek2 DensiChek spectrophotometer. Subsequently, the tube and the card were inserted into the Vitek2 cassette, and the card was auto-inoculated within the Vitek2 instrument via a vacuum-release method. The wells of the card were optically scanned and read each 15 min, with a total incubation time of approximately 8 hours.

**Antimicrobial susceptibility test:** Using the Vitek2 system, the antimicrobial resistance of 11 of the *Salmonella* isolates was examined against 21 antimicrobial agents according to the manufacturer's instructions. To determine the microbial resistance, the purified isolates were streaked onto nutrient broth and incubated overnight at 36°C. The antimicrobial agents included: Ampicillin, Cofotaxime, Ceftazidime, Cefepime, Ertapenem, Amikacin, Gentamicin, Ceftriaxone, Amoxicillin/Clavulanic acid, Piperacillin/Tazobactam, Imipenem, Meropenem, Ciprofloxacin, Cefazolin, Cefoxitin, Levofloxacin, Tigecycline, Fostomycin, Norfloxacin, Nitrofurantoin and Trimethoprim/Sulfamethoxazole.

**Statistical analysis:** A paleontological statistics software package for education and data analysis (PAST3) version 3.09 was used to analyze the data of this study. The findings were evaluated by using chi-square analysis, in addition, the probability P-value was estimated, in which values equal to or less than 0.05 were indicated as significant differences.

## RESULTS AND DISCUSSION

**Detection of *Salmonella* spp.**

**Routine bacteriological diagnosis:** Figure 1 and 2 show detection of suspected *Salmonella* spp. on many different bacteriological media. In addition, biochemical tests were also indicative of this bacterium (Fig. 3).

**Diagnosis by Api 20E:** The Api 20E system identified the suspected isolates as *S. enterica* (Fig. 4). By comparing the API 20E system with the conventional biochemical tests, the first one was able to identify *Salmonella* isolates at rates of 84%, while the traditional tests showed identification rate of 76% (Ahmed and Khudor 2019).

**Diagnosis using the Vitek2 system:** Thirteen isolates of suspected *Salmonella* collected randomly from the five farms were confirmed by the Vitek2 system as *S. enterica* subsp. *enterica*. The diagnosis probabilities ranged from 97% to 99% (Table 1).

**Serotyping of *Salmonella* isolates:** Serology was able to confirm some of the isolates to the serotype level as *S. enterica* sub sp. *enterica* serotype Arizonae. *Salmonella* spp. may be present in 65% of individuals in a flock. Serotypes colonizing the gastrointestinal tract of poultry are variable, depending on the geographic location and the time of the year (Nidaullah et al 2017). *Salmonella* serovars are distributed differently in poultry across countries and areas. In Jordan, for example, *S. enteritidis* and *S. typhimurium* caused poultry infection rates of approximately 8% and 13%, respectively (Nisafi and Abdelaziz 2006).

Similarly, in Egypt, the most frequent serotypes isolated from retail stores and broiler chickens were *S. enteritidis* and *S. typhimurium* (Elkenany et al 2019). By contrast, Singh et al (2013) isolated *S. typhimurium* at a prevalence rate of 4.4% from cloaca samples of layer chickens. Moreover, a Japanese study found only *S. infantis*, *S. manhattan*, and *S. schwarzengrund* in broiler chicken cecal samples (Duc et al 2019). In comparison with a study performed in China, both *S. typhimurium* and *S. enteritidis* were the most common serotypes that constituted 15.3% and 69.8%, respectively (Zhu et al 2017). Despite serotyping is most widely used phenotypic method, it fails to provide correct information because of the complex serotyping scheme and lacking of comparison among various laboratories, thereby limiting its application to the reference laboratories only (Parmley et al 2013).

**Incidence of *Salmonella* in different sources of samples:** In this study, out of 289 collected samples, *Salmonella* spp. were isolated from 61 (21.1%) of the samples. In comparison with another study performed in Basra, Iraq Al-Abadi and Al-Mayah (2011) obtained 34 *Salmonella* spp. isolates from 370 samples with a prevalence rate of 9.2%. These isolation rates were higher than the rate of 5% assumed by the National *Salmonella* Control Program in 2004. Many other studies were performed in other countries, for example, the study of Choi et al (2014) Korea, in which *Salmonella* spp. were isolated from 195 out of 1214 (16.1%) samples collected from various stages of the integrated broiler production firm, such as broiler mother farms, broiler farms, broiler trucks, slaughterhouses, and retail chicken meat. There were significant differences in *Salmonella* spp. isolation rates among different types of samples. In the present study, water was highly contaminated with *Salmonella* spp. with an isolation rate of 30.4%, followed by cloaca swabs (21.7%), compared with the feed samples where no contamination was reported at all. Concerning water contamination with *Salmonella* spp., these microorganisms have an important characteristic, which is their ability to grow and multiply outside the bodies of living host organisms, this increases the chance of their survival in comparison with other organisms (Winfield and Groisman 2003). These microorganisms have been identified in approximately 29% of water samples collected from South India (Patel et al 2020). Poultry drinking water was found to be contaminated with *Salmonella* at an isolation rate of roughly 17.2% (Islam et al 2014), while the rate of contamination was 28.6% in the study of Al Mamun et al (2017). By contrast, other studies performed in Argentina and Algeria, for instance, found that the drinking water was either negative for *Salmonella* or carry very low rate of 2.18%, respectively (Soria et al 2017, Djeffal et al 2018).

In contrast to this study, cloaca swabs collected from chickens in South Africa showed very low *Salmonella* incidence rate of 3.2% (Mathole et al 2017). However, Karim et al (2017) isolated *Salmonella* spp. from 46% of cloaca samples. This result was also consistent with other studies (Akond et al 2012, Rahman et al 2018). Interestingly, Paul et al (2017) obtained extremely high prevalence (80%) in

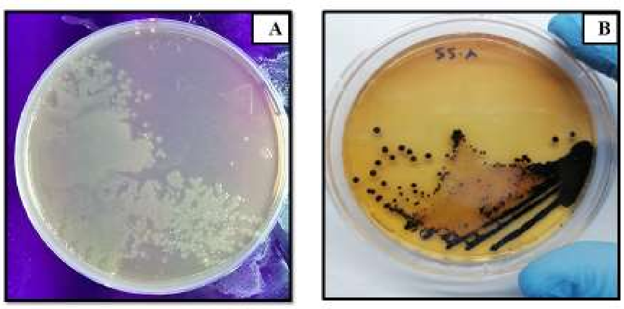


**Fig. 1.** Lactose non-fermenter bacteria suspected to be *Salmonella* spp. grown on MacConkey's agar (A), and SS agar with $H_2S$ production (B)

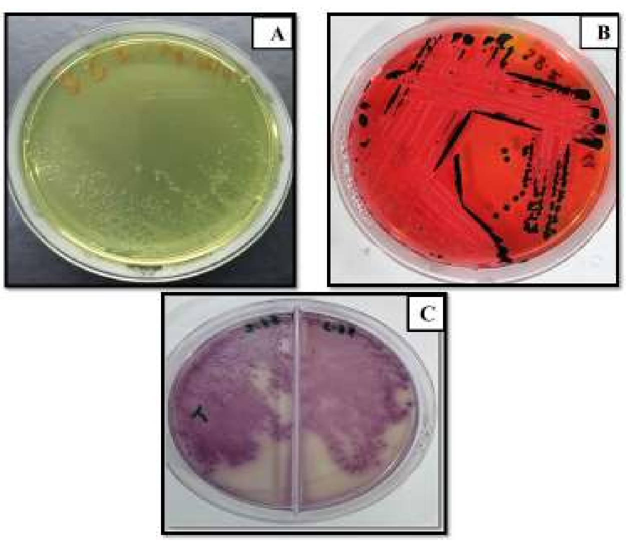


**Fig. 2.** Bacteria suspected to be *Salmonella* spp. grown on Brilliant green agar (A), XLD agar (B), and HiCrome Salmonella agar (C)

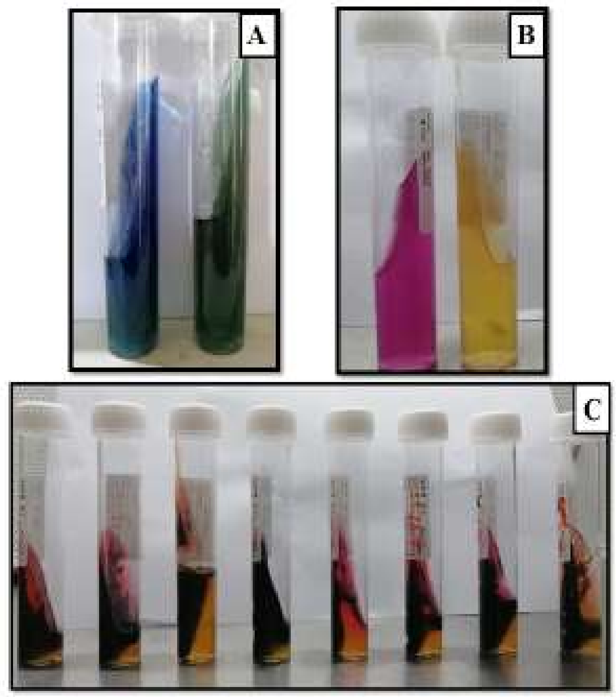


**Fig. 3.** Examples of biochemical tests inoculated with suspected *Salmonella* spp. A- Simmon's citrate agar: positive reaction (left tube). B- Urease test: negative result (right tube). C- Series of samples suspected to be *Salmonella* spp. inoculated into TSI agar

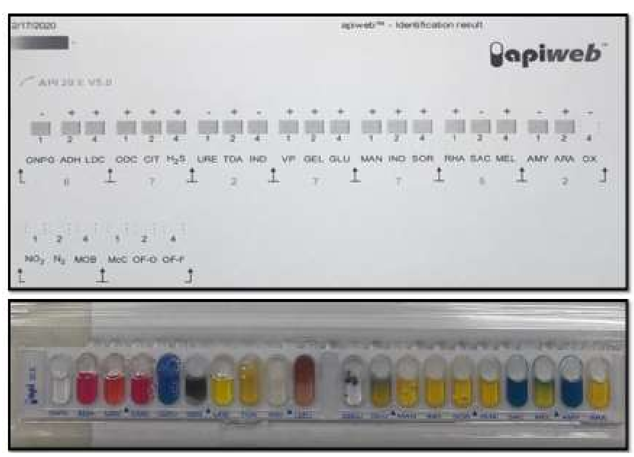


**Fig. 4.** Api 20E strip shows an example of a sample diagnosed as *S. enterica*

cloaca swabs. Sodagari et al (2020) reported *Salmonella* prevalence of 35% in the environment of 26 commercial layer farming flocks in Western Australia, and the greatest *Salmonella* recovery rates were seen in pooled fecal samples (54.5%). Similarly, another study conducted in China reported an isolation rate of 30% from different samples, approximately 48% of which were isolated from broiler cecal contents (Zhu et al 2017).

With respect to *Salmonella* spp. isolation from poultry feed, while Djeffal et al (2018) in this study found that all of the tested samples (n= 160) were Salmonella free, Kingsbury et al (2019) in New Zealand, found only one (3%) of 33 examined feed samples was positive for *Salmonella*. Despite the essential use of protein sources and feed additives in chicken ration for improving growth and performance, they have been implicated as reservoirs for a variety of *Salmonella* serovars (Almrsomi et al 2021). Contamination of feed components, such as animal protein sources, or the use of contaminated trucks for delivering feed, might have a big role in *Salmonella* outbreaks on farms (Hulaj et al 2016). The current study conflicts with that of Maqsood (2012), who stated that the major source of *Salmonella* infection in chicken was contaminated feed. Despite the fact that feed is generally recognized as a possible source of contamination, disease outbreaks linked to feed are extremely rare, and serotypes recovered from feed do not match those isolated from sick chicken flocks (Eguale 2018). Poultry feed pellets have been demonstrated to be effective to decrease the occurrence of *Salmonella* contamination (Boltz 2019).

**Prevalence of *Salmonella* in broiler and layer chicken farms:** *Salmonella* spp. is one of the diseases that affect broiler and layer hens at various ages and seasons. Among

**Table 3.** Isolation rates of *Salmonella* spp. from the collected samples

| Source | No. samples | No. positive samples | Isolation (%) |
|---|---|---|---|
| Cloaca swabs | 217 | 47 | 21.7 |
| Water | 46 | 14 | 30.4 |
| Feed | 26 | 0 | 0 |
| Total | 289 | 61 | 21.1 |
| Statistics | Chi square ($X^2$) = 6.96<br>Degree of freedom = 2<br>P < 0.05 | | |

**Table 4**. Prevalence of *Salmonella* spp. in broiler and layer farms

| Loction | Breed | No. samples | No. positive samples | Incidence (%) |
|---|---|---|---|---|
| AL-Husseinia | Broiler | 172 | 46 | 26.7 |
| Al-Zubailia | Layer | 117 | 15 | 12.8 |
| Total | - | 289 | 61 | 21.1 |
| Chi square ($X^2$) = 5.417 | | | | |

**Table 2.** Identification of *Salmonella* spp. by the Vitek2 system

| Farm code | Type of sample | Bacterial species | Probability | ID message confidence level |
|---|---|---|---|---|
| A | W | *S. enterica* subsp *enterica*<br>*S. paratyphi* B | 99% | Excellent identification |
| | C | *S. enterica* subsp *enterica*<br>*S. paratyphi* B | 99% | Excellent identification |
| B | W | *S. enterica* subsp *enterica* | 97% | Excellent identification |
| | C | *S. enterica* subsp *enterica*<br>*S. enteritidis*<br>*S. paratyphi* B<br>*S. paratyphi* C | 99% | Excellent identification |
| C | W | *S. paratyphi* C<br>*S. enteritidis* | 99% | Excellent identification |
| | C | *S . enterica* subsp *enterica*<br>*S. paratyphi* B | 99% | Excellent identification |
| D | W | *S. enterica* subsp *enterica*<br>*S. paratyphi* B | 99% | Excellent identification |
| | C | *S. enterica* subsp *enterica*<br>*S. paratyphi* B | 99% | Excellent identification |
| E | W | *S. enteritidis*<br>*S. paratyphi* B | 99% | Excellent identification |
| | C | *S. enterica* subsp *enterica* | 99% | Excellent identification |

A, B and C: broiler farm; D and E: layer farm; W: water sample; C: cloaca swab

172 samples taken from broiler farms located in Al-Husseinia, 46 (26.7%) *Salmonella* spp. was isolated. However, lower than this rate (15 isolates, 12.8%) of *Salmonella* contamination was identified out of 117 samples collected from layer farms of Al-Zubeilia region (Table 3). Thus, this study shows that the infection rate is higher in broiler than laying chicken.

**Effects of age and month on *Salmonella* infections in broiler and layer birds:** Chicks in age of 30-37 days were more susceptible to infection (32.8%) followed by the age group of 21-27 days (30.8%). However, birds at the age of 45 days were less susceptible (10.5%). In case of layer birds, the age of 47 days witnessed the highest infection rate of 30.8% (Table 4). Similarly, a significant increment in infection rates occurred on day 35 (Marin and Lainez 2009). The last authors also observed that during the first 3 weeks of rearing, detection of these bacteria in feces increased. Furthermore, Djeffal et al (2018) found more *Salmonella* contamination occurred in the samples collected at the age of 3 weeks than those taken at the end of the production period. High infection rates of *Salmonella* at that age was due to the elimination of antimicrobial use as growth stimulants in poultry diets especially antimicrobials are known to alter bacterial flora in the chicken intestine (Al-Taie 2009). Conversely, other studies stated that the highest excretion of *Salmonella* occurred nearly 14 days of rearing due to immature immune system. As a result, detection of *Salmonella* decreased and persisted uncommon until the day of slaughter (Van Immerseel et al 2004). The least isolation rate occurred on day 45 in broiler chicken. Marin and Lainez (2009) mentioned that slight decrease in the infection rates was at the end of rearing. However, layer birds showed the opposite situation in the current study, in which high isolation rate was observed on day 47 (Table 4). Van Immerseel et al (2004) observed that the young chicks infected with *Salmonella* continue excretion of these bacteria for at least 18 weeks of rearing. Additionally, young birds, regardless of their age at *Salmonella* exposure, would persist in infection till 10 or 12 weeks, after the age of slaughtering broilers (Beal et al 2004).

The higher infection rates occurred in September than August or October in broiler flocks (Table 4). Generally, *Salmonella* infection is more common in the summer. The optimum conditions for *Salmonella* growth are warmer weather and unrefrigerated foods (CDC 2020). The salmonellae in chicken samples collected from China were more common during spring and summer than in autumn and winter (Li et al 2020). Regalado-Pineda et al (2020) in Mexico reported a significant high prevalence of these microorganisms during the spring, summer, autumn and winter. These findings emphasize the importance of health threats of *Salmonella* that need to be tackled urgently.

**Antimicrobial susceptibility test:** Multi-drug resistance (MDR) in *Salmonella* spp. is a growing worry across the world, especially in the developing nations where numerous antimicrobials are used indiscriminately at chicken farms to increase the production (Seo et al 2019). Antibiotic resistance can be developed as a result of the prophylactic use of several antimicrobials in chicken feed (Rajagopal and Mini 2013). The improper use of chemotherapeutic agents and growth promoters in poultry farms resulted in the emergence of MDR in *Salmonellae* (Magdy et al 2020). Against 21 antimicrobials tested in this study, *Salmonella* isolates collected from different farms showed MDR. Importantly, all the 11 isolates were 100% resistant to 4 antimicrobials, including: Ampicillin, Amikacin, Gentamicin, and Ciprofloxacin (Table 5).

In case of Ampicillin, other studies also documented *Salmonella* resistance to this antimicrobial agent, and the resistance increased significantly (98.4%) in 2019 compared with the 2017 report (87.8%) indicating that the farms have

**Table 5.** Effects of age and month on *Salmonella* infections in broiler and layer chicken

| Breed | Age/days | No. examined samples | No. positive (%) | August | September | October |
|---|---|---|---|---|---|---|
| Broiler | 12-14 | 18 | 4 (22.2) | 1 | 3 | 0 |
| | 21-27 | 26 | 8 (30.8) | 0 | 8 | 0 |
| | 30-37 | 67 | 22 (32.8) | 0 | 15 | 7 |
| | 45 | 19 | 2 (10.5) | 0 | 0 | 2 |
| Layer | 12-13 | 16 | - | 0 | 0 | 0 |
| | 20 | 20 | 5 (25) | 0 | 5 | 0 |
| | 35 | 20 | - | 0 | 0 | 0 |
| | 43 | 18 | 2 (11.1) | 0 | 0 | 2 |
| | 47 | 13 | 4 (30.8) | 0 | 0 | 4 |
| Total | | 217 | 47 (21.7) | 1 | 31 | 15 |

**Table 6.** Susceptibility tests for *Salmonella* isolates to different antimicrobials

| Antimicrobial | 1 AW | 2 AC | 3 BW | 4 BC | 5 CW | 6 CC | 7 DW | 8 DC | 9 EW | 10 EC | 11 CC |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ampicillin | R | R | R | R | R | R | R | R | R | R | R |
| /Amoxicillin Clavulanic acid | I | I | I | I | R | I | R | I | I | I | S |
| /Piperacillin Tazobactam | S | S | S | S | I | S | S | I | S | S | S |
| Cofotaxime | S | S | S | S | S | S | S | S | I | I | S |
| Ceftazidime | S | S | S | S | S | S | S | S | S | S | S |
| Cefepime | S | S | S | S | S | S | S | S | S | S | S |
| Ertapenem | S | S | S | S | S | S | S | S | S | S | S |
| Imipenem | S | S | S | S | S | S | S | S | R* | S | S |
| Meropenem | S | S | S | S | S | S | S | S | I | I | S |
| Amikacin | R | R | R* | R* | R | R | R | R | R* | R* | R* |
| Gentamicin | R | R | R | R | R | R | R | R | R* | R* | R* |
| Ciprofloxacin | R | R | R | R | R | R | R | R | R | R | R |
| Cefazolin | / | / | / | / | / | / | / | / | R* | R* | / |
| Cefoxitin | / | / | / | / | / | / | / | / | R* | R* | / |
| Ceftriaxone | / | / | / | / | / | / | / | / | S | S | / |
| Levofloxacine | / | / | / | / | / | / | / | / | R | R | / |
| Tigecycline | / | / | / | / | / | / | / | / | S | S | / |
| Norfloxacin | R | R | R | R | R | R | R | R | S | I | R |
| Fostomycin | S | S | S | S | S | S | S | S | I | I | S |
| Nitrofurantoin | I | S | I | I | S | S | S | I | I | S | S |
| Trimethoprim/Sulfamethoxazole | R | R | R | R | R | R | R | R | S | S | S |

A, B, C, D & E refer to the five farms; the 2[nd] C refers to cloaca sample; W refers to water sample. The numbers refer to: 1- *S. paratyphi* B, 2- *S.* paratyphi B, 3- *S. enterica* subsp *enterica*, 4- *S. enteritidis* or *S. paratyphi* B or C, 5- *S. paratyphi* C or *S. enteritidis*, 6- *S. paratyphi* B, 7- *S. paratyphi* B, 8- *S. paratyphi* B, 9- *S. enteritidis* or *S. paratyphi* B, 10- *S. enterica* subsp *enterica*, 11- *S. paratyphi* B; *: AES (Advanced Expert System) modified; R: Resistant, S: Susceptible; I: Intermediate with MIC within ±1 doubling dilution

had more applications of Ampicillin (Zhu et al 2017). A study conducted in Egypt reported that 95% of the isolates were resistant to Penicillin, 85% to Norfloxacin, and 75% to Gentamicin (Magdy et al 2020). In comparison with a previous study, all *Salmonella* isolates were resistant to Ampicillin, Kanamycin, Cefotaxime, Erythromycin, Streptomycin, Neomycin, Novobiocin and Spectinomycin (Shah and Korejo 2012). The isolates of the present study were 100% resistant to each of Gentamicin and Ciprofloxacin. *Salmonella* strains were highly sensitive to these antimicrobials in the research performed by Bahnass et al (2015) and Baran et al (2019)..

In comparison with the study of Yu et al (2021), partial simililarity was noticed, particularly, the sensitivity of the isolates to Imipenem; however, that study reported susceptibility of the isolates to each of Amikacin and Amoxicillin/Clavulanic acid, which contradict the data of this study. Here, Amikacin showed 100% resistance as mentioned above, whereas intermediate susceptibility was exerted by the combination Amoxicillin/Clavulanic acid towards 8 isolates, with 2 isolates were resistant to the same combination. Moreover, in the current study, 9 isolates were resistant to Norfloxacin (approximately 82% resistance, Table 5). This result is lower than that reported in China by Zhu et al (2017) where *Salmonella* isolates were found to be 99.5% resistant to Norfloxacin, respectively. This resistance could be due to the overuse of Norfloxacin in chicken farms in China compared to Iraq. Regarding Trimethoprim/ Sulfamethoxazole used in the present study, this mixture did not inhibit the growth of 8 isolates (72.7% resistance). On the other hand, all of the isolates were susceptible to Ceftazidime, Cefepime, and Ertapenem. Furthermore, 10, 9, 9, and 6 isolates were inhibited by Imipenem, Meropenem, Fostomycin, and Nitrofurantoin (Table 5). Thus, controlling the use of growth promoters and antimicrobial drugs in animals is critical to prevent the development of resistant strains.

## CONCLUSIONS

Different *Salmonella* spp. were isolated from cloaca and water samples, while all chicken feed samples were completely free of *Salmonella* contamination in five farms in Karbala province. The most prevalent *Salmonella* spp. isolated was *S*. *paratyphi* B according to Vitek2. *S*. *enteritidis* and *S*. *diarizonae* were identified in some samples. Identification of *Salmonella* spp. by Api 20E, serotyping, or by Vitek2 showed inconsistent and rather inaccurate results, particularly, at the serotype level. Therefore, these tests may be inefficient and inappropriate for *Salmonella* detection in general. The emergence of different types of *Salmonella* contaminating water samples collected from the five chicken farms excludes the possibility of the epidemic spread among these farms, especially they are located in different regions within the same governorate. A significant degree of antimicrobial profile similarity was exhibited by *Salmonella enterica* serotypes. All *Salmonella* spp. isolated were 100% resistant to four antimicrobials, including: Ampicillin, Amikacin, Gentamicin, and Ciprofloxacin. By contrast, all of the isolates were susceptible to Ceftazidime, Cefepime, and Ertapenem. Therefore, extensive study need to be done to explore the prevalent *Salmonella* serotypes circulating in different chicken farms throughout the Iraqi governorates.

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# Evaluation of the Efficacy of Asparagus (*Asparagus officinalis* L.) Root Powder and Aqueous Extract on the Physiological and Immunological Performance of Broilers

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***Abstract*:** The 315 one -day old broiler chicks (Ross- 308) were randomly assigned to seven treatments with three replication. The first treatment was fed a basal diet (control). Second, third, and fourth treatments were fed basal diet supplemented with the Asparagus(*Asparagus officinalis* L.) root powder (ARP) at 5, 10, 15 (g kg$^{-1}$), while aqueous extract of ARP was administrated to drinking water at 5, 10, 15 (ml l$^{-1}$) in fifth, sixth, and seventh treatments respectively. There was significant improvement in number of RBC, WBC (ARP extract at 5, 10, and 15 ml l$^{-1}$), hemoglobin, PCV compared to control, and ARP (5 g kg$^{-1}$). Serum total protein, globulin, and albumin were improved significantly in comparison with control, ARP (5 and 10 g kg$^{-1}$), while a significant decrease was observed in serum glucose, cholesterol, and triglyceride compared to control. Phagocytic activity, phagocytic index, immune response, and value of antibodies titer against Newcastle and Gumboro disease were significantly better than control. In comparison with control, feeding Asparagus improved lymphocyte and decreased heterophils and H/L ratio. The study concluded that the best results were achieved at level 15 (g kg$^{-1}$) of the ARP and level 15 ml l$^{-1}$ of its aqueous extract in improving the physiological and immune performance of broilers.

***Keywords*:** Broilers, *Asparagus officinalis*, Blood parameters, Immune traits

*Asparagus officinalis* L. is a perennial vegetable, which was classified under the family *Liliacea* in the past, while at present it comes under the *Asparagaceae* family (Joanna et al 2019). *Asparagus officinalis* L. is a perennial herb with various bioactivities and has been widely used as medicine and food since ancient times, and it originated from the eastern Mediterranean and Asia Minor and has been cultivated for more than 2000 years (Guo et al 2020). *A. officinalis* rich in bioactive compounds including antioxidants and the most important characteristic of asparagus is a vegetable that offers multiple health benefits due to containing flavonoids, alkaloids, phenols, saponins, tannins, which possess strong antioxidant properties (Minh et al 2019), which is a precursor to many pharmacologically active stimulants. Roots and leaves are important parts to use medicinally. Besides that, a recent study suggested that asparagus may constitute a good source of natural antioxidants to be used in our diet, as well by industries for functional food preparations (Joanna et al 2019). The *A. racemosus* (Shatavari) is used to enhance the body's resistance against infections and improve the immune system, and it is widely used for the treatment of various ailments as it contains many different phytochemicals (Singh et al 2018). Asparagus is also, contains various phytochemical compounds such as polysaccharides, polyphenols, anthocyanins, and saponins, which exhibit anticancer, antitumor, antioxidant, immunomodulatory, hypoglycemic, antihypertensive, and antiepileptic effects through *in vitro*, and *in vivo* experiments (Guo et al 2020). The root powder of *A. racemosus* is used as an herbal feed supplement in poultry feed, Shatavari augments the appetite and stimulates the liver (Shukla et al 2018). Shatavari possess anabolic properties viz., growth promotion, increase the production potential in broilers by using herbs possessing therapeutic potential (Gaikwad et al 2018). Several studies have been undertaken to assess the influence of dietary supplementation of *Asparagus racemosus* (Shatavari) root powder in broilers, to improve body weight, feeding efficiency, conformation traits and carcass yield (Chikwae t al 2018), immunity (Dahale et al 2014), removed cold stress and improves immune status of broiler chicks(Kant et al 2014). Due to the rarely of studies that deal with Asparagus (*Asparagus officinalis* L.) as a feed additive in poultry. The study was designed to investigate the effect of *A. officinalis* L. root powder, and its aqueous extract as dietary supplementation on some haemato-serobiochemical parameters, and the immunologic status of broiler chickens.

## MATERIAL AND METHODS

**Animals and experimental diets:** The present study was conducted for 35 days from Dec 16, 2020. Three hundred and fifteen (315) one-day-old mixed-sex broilers (Ross 308) were used on a completely randomized design (CRD) in seven treatment groups each having 45 chicks and each group was further divided into three replicates containing 15 chicks each. The chicks in the control group (T1) were given no supplement, whereas in treatments T2, T3 and T4, chicks were supplemented with Asparagus (*A. officinalis*) root powder (ARP), at 5, 10, 15 (g $kg^{-1}$) of feed, respectively. The aqueous extract of ARP was administrated to drinking water at levels 5, 10, 15 (ml $l^{-1}$) in T5, T6, and T7 respectively. The feeding program consisted of a starter diet, which was fed until day 21, and a grower diet, which was fed from day 22 until 35 and was based on yellow corn, wheat and soybean meal as shown in Table 1. The diets were formulated to meet the nutrient requirements of the broiler (commercial recommendation). The chicks had free access to feed and water throughout the experiment period.

**Collection of plant material:** The roots of the Asparagus plant were collected from the Eastern Island of Teeb, East of Maysan Governorate. The roots were cleaned from the dust, washed with tap water, cut into small pieces, dried in the shade, and ground into powder. The fine powders were stored in black plastic bags at room temperature (25°C) until the extraction process was performed.

**Table 1**. Ingredients and nutrient composition of broiler starter and grower diets

| Ingredient (%) | Starter diet 1-21 days | Grower diet 22-35 days |
|---|---|---|
| Yellow maize | 56.20 | 61.00 |
| Wheat | 04.00 | 04.00 |
| Soybean meal (48%) | 32.00 | 26.50 |
| Vegetable oil | 1.50 | 2.50 |
| [1]Broiler protein concentrates (40%) | 5.00 | 5.00 |
| Limestone | 0.80 | 0.50 |
| Premix | 0.25 | 0.25 |
| Common salt | 0.25 | 0.25 |
| Total | 100 | 100 |
| Calculated composition[2] | | |
| Metabolizable energy (Kcal $Kg^{-1}$) | 3023 | 3137 |
| Crude protein (%) | 22.70 | 20.47 |
| Crude fat (%) | 2.77 | 2.89 |
| Crude fiber (%) | 2.36 | 2.22 |
| Calcium (%) | 0.61 | 0.49 |
| Phosphorus available (%) | 0.28 | 0.24 |
| Lysine (%) | 1.21 | 1.15 |
| Methionine + Cysteine (%) | 0.88 | 0.81 |
| Calorie: protein ratio | 133.17 | 153.24 |

[1]Broiler protein concentrate (Brocorn-5 special W), Exported by (Wafi B.V., Alblasserdam – Holland),supplied per kilogram of feed: Crude protein 40%, 2017 kcal/kg M.E, 5% fat, 2.20% crude fiber, 7.10% moisture, 28.30% ash, 4.20% calcium, 2.65% total phosphorus, 3.85% lysine, 3.70% methionine, 4.12% methionine+ cysteine, 0.42% tryptophan, 1.70% threonine, 2.50% sodium, 4.20% chloride, 200 mg/kg copper, 1.600 mg/kg manganese, 2.000 mg/kg zinc, 2.000 mg/kg iron, 20.00 mg/kg iodine, 5.00 mg/kg selenium. [2]Was calculated according to the chemical composition of feedstuff contained in NRC (1994)

**Preparing of *Asparagus officinalis* root aqueous extracts:** The aqueous extract was prepared according to the method described by Sharma et al (2012). Five grams (5g) of dry fine powder of Asparagus root was extracted with distilled water (500 ml), and then the solution mix well to obtain a homogeneous mixture then leave for 4 hours in a horizontal shaker at medium speed, after which the sample is left to stabilize for an hour and then filter the mixture using filter paper (Whatman No.1). After that, the mixture was evaporated by using the drying oven at a temperature of 37°C for a period of seven days, after which the concentrated extract obtained in a thick viscous form, scrape the product, and store in the refrigerator for future use.

**Haematological parameters and serum biochemistry:** At 35 days old, blood samples were taken from the brachial vein from three birds of an average weight selected from each treatment (chick/replicate) randomly. Blood samples were used for a fresh blood count. The hematological analysis includes red blood cell (RBC) count, white blood cells (WBC) count, hemoglobin (Hb), packed cell volume (PCV). Blood smears were also performed to count lymphocytes (L) and heterophils (H), which made it possible to determine the H: L ratio. For serum biochemical indices, a blood sample was drawn and allowed to stand for an hour at room temperature (18°C) to serum collection. Serum was separated by centrifugation at a speed of 3000 rpm for 15 minutes and then stored at −20°C for further analysis. Serum total protein (TP) and albumin were analyzed by a colorimetric method using commercial kits (Spinreact, Spain). Sera globulin was calculated by subtraction from TP. Blood serum glucose, cholesterol, and triglyceride were calculated by using special kits (Biolabo AS, France).

**Relative weight of lymphoid organs and immunoglobins:** At the end of the experimental (35 days) period, three birds of an average weight selected from each treatment (chick/replicate) randomly. After the birds were manually eviscerated and dressed. Lymphoid organs (spleen and bursa of Fabricius) were carefully removed, weighed and expressed as a percentage of the live weights. Thereafter, the bursa index was calculated. Immunoglobulin tests were performed by means of the ELISA test (Enzyme-Linked Immune Sorbian Assay) to determine the concentration of immunoglobulin's IgM, IgA, and IgG. Phagocytosis and Phagocytosis index was measured according Erhard et al (1992). The determination of the Titer for serum antibody directed against the Newcastle disease and infectious bursal disease (IBD) (Gumboro) HI test (Hemoagglutination Inhibition Test) according to Enzyme-Linked Immuno Sorbent Assay (ELISA) was used to determine the titer of antibodies.

**Statistical analysis:** Data were analyzed as a completely randomized design by using SPSS program software (2015).

## RESULTS AND DISCUSSION

**Hematological and biochemical parameters:** The hematological parameters of red blood cell (RBC), white blood cell (WBC in T5, T6 and T7),) hemoglobin (Hb), and pack cell volume (PCV), were significantly improved in broilers fed with Asparagus root powder diet compared to the control and T2 (Table 2). The level of 15 ml $l^{-1}$ of *A. officinalis* extracts was achieved the highest values in these parameters. This positive effect of these parameters may be attributed to the vital function of the bioactive compounds in the root of the *Asparagus* plant that offers multiple health benefits owing to containing flavonoids, phenolic, alkaloids, saponins, and tannins compounds, which own strong antioxidant properties (Minh et al 2019), hence higher values indicate a greater potential for these function and a better state of birds health, which reflects positively on increasing hematological attributes. Kant et al (2014) reported a significant improvement in RBC count, Hb, and PCV with respect to Shatavari and vitamin E treated groups than the control group of broilers. On other hand, Shukla et al (2018), showed an improvement in the health, physiological, immunological traits, and haematobiochemistry characteristics of the blood after supplement *Asparagus* root powder to a broiler diet. As well, in a study on rats, Chaudhary et al (2016) noted that methanol extract from *A. racemosus* root powder led to a significant improvement in RBC count and hemoglobin concentration compared to the control, when anemic rats were treated.

The biochemical changes observed in the study included significant improvements (with 15 g $kg^{-1}$ and ARP extract groups) in total protein, globulin, and albumin concentrations, as compared to control, T2 and T3, while glucose, cholesterol, and triglycerides levels were significantly (decreased by the dietary treatments of broilers (Table 3). Improvements in total protein, globulin, and albumin in birds fed ARP and its aqueous extract may be due to asparagus root powder that supports the immune system of birds (Rekhate et al 2010, Kant et al 2014), which was positively reflected in the improvement of these serum parameters. The reduction in the levels of serum total glucose when the chicks supplemented with ARP may be related to the main active constituents of the roots of Asparagus (steroidal saponins and sapogenins), which have antidiabetic properties. Mathews et al (2006) illustrated that aqueous extract of *A. adscendens* have antidiabetic potentials as it stimulated both the secretion and action of insulin as well as inhibiting starch digestion in the clonal pancreatic β cell line. Additionally, Visavadiya and Narasimhacharya (2007) observed that the phytosterol and saponin contents of *A. racemosus* root besides polyphenols, flavonoids, and ascorbic acid could be responsible for increased fecal sterol excretion and decreased cholesterol levels in the hyperlipidemic rats. Bhardwaj et al (2009) observed a significant increase in serum total protein due to the inclusion of ARP in broiler diets.In accordance with the present results, Yadav et al (2018) concluded that supplementation of the diet with Shatavari (*A. racemosus)* meal at 0.5% and above reduced plasma cholesterol in coloured chicken. Similar findings were reported by Ashwini et al (2019), that roots of *A. racemosus* of broiler diets decreased serum cholesterol.

### Immunological Parameters

Supplementation of Asparagus root powder in treatment

**Table 2.** Hematological analysis of broilers under different levels of *A. officinalis* root powder and its aqueous extract at 35 days of age

| Treatments | RBC ($10^6$/mm$^3$) | WBC ($10^3$/mm$^3$) | Hb (g 100 ml$^{-1}$) | PCV (%) |
|---|---|---|---|---|
| T1 | $3.62^{d}$ ±0.09 | $21.68^{d}$±0.17 | $9.66^{d}$ ±0.26 | $28.99^{d}$±0.79 |
| T2 | $3.84^{cd}$ ±0.07 | $22.32^{cd}$ ±0.03 | $10.18^{cd}$ ±0.16 | $30.55^{cd}$ ±0.49 |
| T3 | $3.98^{bc}$±0.01 | $22.38^{cd}$ ±0.08 | $10.62^{bc}$ ±0.04 | $31.87^{bc}$ ±0.13 |
| T4 | $4.03^{ab}$±0.09 | $22.65^{bcd}$±0.09 | $10.75^{abc}$±0.25 | $32.25^{abc}$ ±0.76 |
| T5 | $3.95^{bc}$±0.08 | $23.12^{abc}$±1.07 | $10.53^{bc}$ ±0.23 | $31.61^{bc}$ ±0.69 |
| T6 | $4.16^{ab}$±0.10 | $23.92^{ab}$±0.44 | $11.09^{ab}$ ±0.27 | $33.27^{ab}$ ±0.81 |
| T7 | $4.24^{a}$±0.02 | $24.14^{a}$±0.26 | $11.31^{a}$ ±0.06 | $33.94^{a}$ ±0.19 |

*Values in the same column with no common superscript differ significantly (p≤0.05). ** T1 - control; T2, T3 and T4 - Asparagusroot powder at 5, 10 and 15 (g $kg^{-1}$) in basal diet; T5, T6 and T7 Asparagus aqueous extract - at 5, 10 and 15 (ml $l^{-1}$) in drinking water, respectively groups caused significant improvement in phagocytosis, phagocytosis index, and immunoglobulin's levels (IgM, IgA, and IgG) as compared to control (Table 4). The improvement in the immune response of broiler chicks may be attributed to saponins (active principle) properties. Saponins have been ascribed to a number of pharmacological actions, such as immunomodulatory potential via cytokine interplay, and have tremendous cytotoxic (Sun et al 2009). Shaha and Bellankimath (2017) reported that the tuberous roots of this *A. racemosus* plant are widely applied in pharmaceutical preparations as well on the biotechnology scale, in the preparation of various herbal preparations for they possess a distinct potential and a defense system. Singh et al (2018), Shatavari can enhance the body's resistance against infections and improve the immune system. Wang et al (2020) concluded that the pectic-like polysaccharides from white asparagus (*A. officinalis*) skin can be potentially used as an immunomodulatory agent in functional foods. Kumari et al (2012) concluded that the extract from the *A. racemosus*, could significantly increase the humoral and cell-mediated immune responses and had immuno-modulatory effects of the treated birds.

Experimental groups showed higher antibody titers against Newcastle (ND), and IBD (Gumboro) disease as compared to the control group (Table 5). The maximum was observed in group T7 (3246.00), and (2738.33) for ND and IBD disease respectively, which was administered with 15 (ml $l^{-1}$) *A. officinalis* root extract drinking water. T1 showed the lowest value reached (2652.67) and (1893) for both diseases respectively. The improvement in titer value against ND and IBD disease for supplementary treatments may be due to the herbs like Asparagus contain different a variety of active components that affect the properties of cell-mediated

**Table 5**. Effect of treatments on serum titers of Newcastle and Gumboro disease in broiler chickens at 35 days of age

| Treatment | Newcastle disease | Gumboro disease |
|---|---|---|
| T1 | 2652.67$^{e}$ ±50.53 | 1893$^{f}$ ±2.30 |
| T2 | 3066.00$^{d}$ ±4.72 | 2247.33$^{e}$ ±5.54 |
| T3 | 3077.67$^{bc}$ ±2.02 | 2518.33$^{d}$ ±3.48 |
| T4 | 3084.33$^{c}$ ±2.90 | 2583 00$^{c}$ ±4.72 |
| T5 | 3078.00$^{bc}$ ±2.08 | 2555.67$^{bc}$ ±3.93 |
| T6 | 3125.67$^{b}$ ±4.66 | 2681.67$^{b}$ ±5.20 |
| T7 | 3246.00$^{a}$ ±9.50 | 2738.33$^{a}$ ±18.35 |

See Table 2 for details

**Table 3**. Biochemical parameters in broilers supplemented to *A. officinalis* root powder and its aqueous extract at 35 days of age

| Treatments | Total protein (g 100ml$^{-1}$) | Albumin (g 100ml$^{-1}$) | Globulin (g 100ml$^{-1}$) | Glucose (mg 100ml$^{-1}$) | Cholesterol (mg 100ml$^{-1}$) | Triglycerides (mg 100ml$^{-1}$) |
|---|---|---|---|---|---|---|
| T1 | 4.20$^{d}$±0.03 | 1.77$^{c}$±0.06 | 2.42$^{d}$±0.02 | 254.28$^{a}$ ±1.00 | 168.68$^{a}$±1.85 | 95.14$^{a}$±0.91 |
| T2 | 4.44$^{d}$±0.04 | 1.80$^{c}$±0.08 | 2.62$^{cd}$±0.11 | 245.76$^{b}$ ±1.43 | 161.4$^{b}$ ±0.71 | 85.03$^{b}$ ±0.22 |
| T3 | 4.51$^{cd}$±0.20 | 1.84$^{c}$±0.04 | 2.67$^{bcd}$±0.03 | 239.61$^{bc}$±1.14 | 156.29$^{cd}$±1.18 | 82.85$^{c}$ ±0.74 |
| T4 | 5.28$^{b}$±0.01 | 2.19$^{b}$±0.01 | 3.09$^{ab}$±0.16 | 231.90$^{cd}$ ±4.81 | 156.12$^{cd}$ ±1.28 | 77.27$^{d}$ ±0.54 |
| T5 | 4.77$^{c}$±0.17 | 1.84$^{c}$±0.07 | 2.93$^{abC}$±0.13 | 239.75$^{bc}$±3.93 | 158.44$^{bc}$ ±0.67 | 79.23$^{d}$ ±1.01 |
| T6 | 5.52$^{ab}$±0.05 | 2.24$^{b}$±0.04 | 3.27$^{a}$±0.29 | 225.67$^{de}$ ±2.11 | 153.9$^{de}$ ±0.44 | 75.00$^{e}$ ±0.33 |
| T7 | 5.75$^{a}$±0.03 | 2.51$^{a}$±0.03 | 3.24$^{a}$±0.16 | 222.10$^{e}$ ±1.77 | 152.10$^{e}$±0.35 | 73.89$^{e}$ ±0.63 |

See Table 2 for details

**Table 4.** Effect of *A. officinalis* root powder and its aqueous extract on phagocytosis and immune response of broiler chickens

| Treatment | Phagocytosis (%) | Phagocytic index | Immune response | | |
|---|---|---|---|---|---|
| | | | IgG (mg ml$^{-1}$) | IgA (mg ml$^{-1}$) | IgM (mg ml$^{-1}$) |
| T1 | 42.00$^{f}$ ±0.57 | 49.83$^{d}$ ±1.27 | 1.95$^{e}$ ±0.02 | 2.26$^{d}$ ±0.01 | 2.61$^{f}$ ±0.03 |
| T2 | 44.67$^{e}$ ±1.45 | 53.28$^{bc}$±1.22 | 2.08$^{d}$ ±0.03 | 2.68$^{bc}$±0.01 | 3.36$^{e}$ ±0.04 |
| T3 | 46.33$^{d}$ ±1.45 | 53.71$^{bc}$±1.39 | 2.19$^{c}$ ±0.01 | 2.75$^{c}$ ±0.03 | 4.44$^{c}$ ±0.02 |
| T4 | 50.33$^{c}$ ±2.84 | 62.59$^{ab}$±1.21 | 2.31$^{ab}$±0.01 | 3.42$^{b}$±0.03 | 4.49$^{b}$±0.01 |
| T5 | 47.33$^{d}$ ±2.33 | 58.72$^{c}$ ±3.20 | 2.17$^{bc}$±0.02 | 3.33$^{ab}$±0.03 | 3.50$^{d}$ ±0.03 |
| T6 | 52.33$^{b}$ ±1.45 | 63.54$^{b}$ ±0.96 | 2.35$^{b}$±0.02 | 3.44$^{b}$±0.01 | 4.30$^{c}$ ±0.04 |
| T7 | 55.01$^{a}$ ±1.66 | 67.62$^{a}$ ±0.49 | 2.81$^{a}$±0.03 | 3.82$^{a}$±0.01 | 4.58$^{a}$±0.01 |

See Table 2 for details

**Table 6**. Effect of treatments on the relative weight of lymphoid organs, lymphocytes, and heterophils cell in broiler chicken at 35 days of age

| Treatment | Spleen (%) | Bursa of fabricius (%) | Bursa Index | lymphocytes (%) | Heterophils (%) | H/L |
|---|---|---|---|---|---|---|
| T1 | 0.15±0.008 | $0.09^{a}$ ±0.003 | $1.00^{a}$±0.000 | $66.08^{c}$ ±0.86 | $25.24^{a}$ ±0.41 | $0.382^{a}$ ±0.0097 |
| T2 | 0.16 ±0.008 | $0.09^{a}$ ±0.003 | $1.02^{a}$±0.040 | $67.23^{bc}$ ±1.59 | $23.18^{b}$ ±0.48 | $0.346^{b}$±0.0155 |
| T3 | 0.15±0.005 | $0.09^{ab}$ ±0.004 | $0.98^{a}$±0.047 | $71.36^{a}$ ±0.52 | $22.23^{bc}$ ±0.62 | $0.311^{c}$±0.0064 |
| T4 | 0.15±0.005 | $0.08^{bc}$ ±0.002 | $0.86^{b}$±0.032 | $71.49^{a}$ ±2.12 | $22.89^{bc}$ ±0.91 | $0.320^{bc}$±0.0108 |
| T5 | 0.18±0.006 | $0.07^{c}$ ±0.001 | $0.79^{b}$±0.012 | $72.36^{a}$ ±0.57 | $23.13^{b}$ ±0.52 | $0.319^{bc}$±0.0075 |
| T6 | 0.16 ±0.013 | $0.07^{c}$ ±0.006 | $0.80^{b}$±0.068 | $70.56^{ab}$ ±1.39 | $21.39^{c}$ ±0.30 | $0.303^{c}$±0.0061 |
| T7 | 0.17 ±0.003 | $0.06^{d}$ ±0.001 | $0.66^{c}$±0.016 | $71.83^{a}$ ±1.26 | $21.83^{bc}$ ±0.27 | $0.304^{c}$±0.0097 |

See Table 2 for details

immunity, such as lipopolysaccharides, polymers, saponins. Saponins-based adjuvants have the ability to stimulate the cell-mediated immune system, as well as to enhancing antibody production and it has the advantage that only a low dose is required for auxiliary activity (Rajput et al 2007). Kumari et al (2012) observed that the use of *Asparagus racemosus* extract in broilers feed has significant positive effects in both humoral and cell mediated immune responses of the birds which were evident by increased antibody titer after HI test after they study the immunomodulatory effects of *A. racemosus* extract against Newcastle disease in one week old normal and immunocompromised broiler chicks. Patil et al (2012) reported that the activating action is attributed to the isoprinosine content of the asparagus root plant, which has been shown to increase cytokine production, increase active T cells and induce T-cell surface markers on protein cells, besides increase lymphocyte proliferation. The findings in the current study are supported by Tekade et al (2008) who recorded higher antibody production than normal due to more stimuli to the immune system in broilers treated with *A. racemosus* alone as well as in different combinations with *Sida cordifolia* was and *Levamisole* starting from $28^{th}$ day of age for 2 weeks. Similar observations were reported by Yadav et al (2018), that dietary supplementation of Shatavari root meal @ 0.5% improves immunity in coloured chicken (*Chabro*).

No significant differences were observed in the relative weight of the spleen among treatment groups (Table 6). However, there was a significant difference in the bursa of Fabricius (%), bursa index, lymphocytes (L), heterophils (H) populations and H/L ratio. ARP and ARP extract significantly decreased bursa relative weight and bursa index (except in T2 and T3), heterophils, and H/L ratio, whereas the lymphocytes population was increased significantly as compared to control. The *Asparagus racemosus* plant has immunostimulating properties (Sharma and Sharma 2013), also modulates the action of the immune system (Shaha and Bellankimath 2017). Mishra et al (2017) indicated that extracts and formulations prepared from *A. racemosus* aids in increases in white cell counts, absolute neutrophil counts, haemagglutinating, and contribute to increasing the phagocyte activity of infected laboratory mice, thus eliminating the negative effects and preserving on the titer of antibody to blood in mice, also showed that asparagus is an immune stimulant, along with *Withania somnifera*, *Tinospora cordifolia* and *Picrorhiza kurroa*, significantly suppresses the chemotactic activity and production of interleukin-1 and TNF-α by macrophages.

## CONCLUSION

The supplementation of 15 g $kg^{-1}$ of *A. officinalis* root powder and (15 ml $l^{-1}$) of aqueous extract to drinking water, improve the heamatobiochemical parameters, phagocytosis, immunoglobulin's (IgM, IgA, IgG), and antibody production against ND and IBD, and improve lymphocyte, while it reduced heterophils and H/L ratio of broiler chicks.

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# Effect of Adding Phytase to Low Protein Diets on Some Productive Traits of Quail

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***Abstract:*** This study was conducted in to observe the effect of adding phytase enzyme to low protein diets on some productive traits of quail. The 360 one-day-old" quail chicks were used, bred collectively during the first week and given a standard diet containing 24% protein and at the beginning of second week chicks were randomly distributed to 6 treatments. The treatments were control and second treatments included standard diet of 24% protein with the addition of phytase enzyme, third and fourth treatments were 22% low in protein with the addition of phytase enzyme for fourth treatment. The fifth and sixth treatments were with 21% lower in protein with addition of phytase enzyme for sixth treatment. Percentage by adding phytase enzyme to additional treatments was 500 g/ton. The results of showed a significant superiority at fifth and sixth week of first treatment, control compared with rest of the treatments in mean live body weight and weight gain. There wax significant decrease for third treatment compared to first, second, fifth and sixth treatments at fifth week, while in sixth week that there was a significant superiority for the third treatment compared to second, fourth and sixth treatments. There was a significant improvement in average feed conversion for a fifth and sixth week, as well as a total conversion factor for the first treatment (control) compared with rest of the treatments.

***Keywords:*** Phytase, Low protein diets, Quails

The poultry industry is one of the main pillars in developed countries to provide food to consumers with high value, such as meat and eggs. It is one of the main sources of animal protein in the human diet. The Food and Agriculture Organization of the World (FAO) observed that the real challenge is to produce enough food to meet needs of society while maintaining and enhancing the natural resource base in proportion to a growing population. Therefore, poultry industry had an importance of providing a source of protein in form of meat and eggs, as well as impact of this on polluting environment. Man is not only one who causes environmental pollution, but birds contribute to this through intensive production in poultry factories, which is a major cause of the deterioration of environmental life in major industrialized countries, which is reflected in pollution of land, air and water with chemicals, parasites, viruses and minerals, especially phosphorous associated with undigested and exogenous phytic acid with poultry glaucoma (Cowieson et al 2004, Musapuor et al 2005). The researchers were interested in studying poultry nutrition as it is one of most important administrative factors that the breeder must take into account in order to obtain birds with normal growth and high production efficiency. In addition, feeding costs approximately 60-70% of total production costs (Cowieson et al 2004).Quail is a small, dual-purpose migratory bird, adaptable to environmental conditions, easy to handle, and has many advantages such as rapid growth rate, early sexual maturity, short incubation period for eggs, high egg production, as well as low feeding requirements compared to other species (El-Tarabany 2015). The largest proportion of feedstock raw materials is in form of grains or cakes as a source of carbohydrates and protein, but may be responsible for for presence of different amounts of anti-nutritional factors (ANF's) (Farhat et al 2012). The cause of nutritional disturbances for birds is low rates of digestion and absorption of nutrients, as well as a decrease in growth and a deterioration inefficiency of food conversion. Among these factors is the phytic acid complex, which binds to main stock of organic phosphorous by 70% to form non-absorbable compounds (Tahir et al 2012) and is also associated with amino acids (Ravindran et al 2006), some mineral elements such as Ca, Mg, Fe, and Zn (Kaya et al 2009) and starch and protein (Angel et al 2005, Liu et al 2007). In addition, high levels of phytic acid in plants lower representative energy availability and reduce ability to process and digest phosphorous in ileum (Ravindran et al 2006). The phytic acid has a role in inhibiting the activity of some enzymes inside the body such as pepsin, trypsin and alpha-amylase and are difficult to digest in poultry due to and most of the phosphorus is excreted in glaucoma and this leads to a problem of excess phosphate in soil in areas where poultry production is concentrated and causes environmental pollution (Selle and

Ravindran 2007). Therefore, some feed additives were introduced to reach the best production as they effectively treated some nutritional problems of poultry, and these additives increased utilization of basic nutritional compounds such as proteins, fats, carbohydrates, vitamins and minerals. The addition of enzymes, including phytase enzyme, had an effective effect on improving performance of domestic birds (Applegate and Angel 2005). The main objective of sturdy was to observe the effect of adding phytase enzyme to low protein diets on some productive traits of quail.

## MATERIAL AND METHODS

This study was carried out at University of Mosul. At first fertilized eggs from animal production department research field were approved in experiment and entered hatchery on October 14, 2020. After hatching, 360 one-day-old chicks were used. A sample of the chicks was weighed to find out the initial weight of chicks. The experiment lasted for six weeks from October 31, 2020. The chicks were raised collectively during 1st week of age in a semi-open hall, after initial weight of the chicks was calculated and were placed in a large iron cage on which all washing and sterilization operations were carried out and after drying it was spread with sawdust 3-5 cm thick. Feed and water were provided freely throughout the study period by means of plastic trays and water by inverted septic tanks. The distance of 2.5 cm from the circumference of circular feeder to each bird and 3 cm from circular feeder to each bird until the end of the experiment was maintained. The temperature ranged between 37-38°C and lighting was continuous for the first days for 24 hours and then 23 hours until the end of the experiment. The hall was provided with electricity and oil heaters and heaters distributed in hall and inside cages to monitor temperature. Air vacuums were also adopted in hall to ensure good ventilation. After the chicks reached second week, the chicks were distributed collectively to treatments and replicates after weighing each replicate separately while ensuring homogeneity of replicate weights ± 25 g. The replicates were distributed inside a large wooden cage consisting of three floors, each floor containing four cages with dimensions 50 * 50 * 50 cm in length, width and height, respectively and each room had a special door equipped with a 1 kg feeder and an inverted plastic water manifold suspended in the center of cage with a capacity of 850 ml.

The average live body weight, average weight gain, average amount of feed consumed and average food conversion factor for each the bird was estimated at end of each week.

Average live body weight (gm bird$^{-1}$) = Total bird weights (gm) / total birds counts per replicate (Ibrahim 1987).

Average weekly weight gain (gm bird$^{-1}$) = total live body weight at the end of the week (gm) - total live body weight at the beginning of the week (gm) / number of birds.

Average amount of feed consumed (gm bird$^{-1}$ week$^{-1}$) = Amount of feed provided during the week - Amount of remaining feed at the end of week/Number of birds( Alzubaidi1986).

Average weekly of feed conversion efficiency = Amount of feed consumed during the week / weekly weight gain rate.

The Statistical analysis of the data was carried out according to using of Completely Randomized Design (CRD) by applying the ready-made SAS program (2003) and comparison between the means of the studied traits was carried out by using Duncan's test (1955) to determine the significance level at probability level of 0.05.

The treatments were distributed as follows:

T1 = Control (24% CP.), without phytase enzyme.

T2 = Control (24% CP.), adding with phytase enzyme.

T3 = Ration with crude protein (22% CP.), without phytase enzyme.

T4 = Ration with depression of crude protein (22% CP.), adding with phytase enzyme.

T5 = Ration with depression of crude protein (21% CP.), without phytase enzyme.

T6 = Ration with depression of crude protein (21% CP.), adding with phytase enzyme.

## RESULTS AND DISCUSSION

There were significant differences in live body weight of birds in the different treatments during growth period (Table 2). In the second week, there was a significant decrease for treatment T5, (23.58 gm) compared with treatments T1, T4 and T6. In the third and fourth weeks there were no significant differences between treatment and during fifth week were significantly superior to treatments T2, T4 and T6, At end of sixth week, The addition of phytase enzyme during this period (growth period) to feed and led to activation of phytase enzyme and thus body was provided with a protein source that can be used in building muscular skeleton body weight gain.his result agreed with (Silversides and Hruby 2009).

The effect of adding phytase enzyme to low-protein diets indicated no significant differences between treatments for the second, third and fourth weeks (Table 3). During the fifth week, significant superiority was observed in T1 amounted (37.68 gm) as compared to T4 and (25.05, 29.11 gm). In the sixth week, control (T1-30.93 gm)) was significantly superior to T2, T5 and T6 (24.00, 24.03 and 20.23 gm, respectively). There was between control ,T3 and T4 treatments. This may be due to increased level of phosphorous and availability, as well as the protein's association with phytic acid. Phytase works to release phosphorous and other minerals such as calcium, magnesium, manganese, iron and zinc from phytate present in components of poultry ration, especially yellow corn, which leads to an increase in readiness of these elements to perform their action and increase their absorption and thus benefit from them, as well as liberating carbohydrates and fat from phytate complexes, raising energy level and increasing utilization of inositol by animal body after hydrolysis of phytic acid by enzyme phytase (Kies et al 2001, Wu et al 2006).

The adding phytase enzyme to low protein diets on amount of feed consumed varied significantly (Table 4). In the first week the breeding was collective and average consumption of bird was 16.5 g. There were no significant differences between treatments in each of second, third and fourth weeks while at the end of fifth week treatments T1 , T2, T5 and T6 were significantly superior (85.14, 85.18, 81.86 and 84.65 gm) over T3. In the sixth week, it was confirmed that there was a significant superiority of treatment T3 in feed consumption (125.35 gm) over treatments T2, T4 and T6

**Table 1.** Rations components and chemical composition of diet

| Constituents | First diet protein (24%) | Second Diet Protein (22%) | Third Diet Protein (21%) |
|---|---|---|---|
| Yellow corn | 55 | 59 | 62.5 |
| Soyabean meal (48%) | 40 | 35.5 | 32.5 |
| Sunflower oil | 3.5 | 4 | 3.5 |
| Limestone | 0.8 | 0.8 | 0.8 |
| Dicalcium phosphate | 0.2 | 0.2 | 0.2 |
| Table salt | 0.25 | 0.25 | 0.25 |
| Premix | 0.25 | 0.25 | 0.25 |
| Calculated chemical composition (NRC 1994) | | | |
| Moisture (%). | 12.025 | 12.0475 | 12.148 |
| Crude protein (%). | 24 | 22 | 21 |
| Ether extract (%). | 6.0125 | 6.6125 | 6.4064 |
| Crude fibers (%). | 4.2625 | 4.165 | 4.12375 |
| Ashe (%). | 4.2285 | 3.9797 | 3.8189 |
| Gross energy ($K_{cal}$). | 3156.565 | 3229.06 | 3237.433 |

**Table 2.** Effect of adding phytase to low-protein diets on average live body weight from 2 - 6 weeks (Mean ± standard error)

| Treatments | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|---|---|---|---|---|---|
| T1 | 24.47 ± 0.02a | 38.94 ± 0.32 | 62.93 ± 5.25 | 100.61 ± 7.62a | 131.55 ± 9.60a |
| T2 | 23.96 ± 0.34ab | 36.28 ± 0.35 | 53.68 ± 1.87 | 83.93 ± 2.33b | 107.9 ± 4.18b |
| T3 | 24.24 ± 0.25ab | 37.35 ± 1.27 | 56.70 ± 2.47 | 88.61 ± 4.33ab | 104.53 ± 3.16ab |
| T4 | 24.39 ± 0.21a | 36.78 ± 0.85 | 53.56 ± 2.27 | 78.61 ± 3.26b | 114.11 ± 3.61ab |
| T5 | 23.58 ± 0.11b | 38.05 ± 0.27 | 54.04 ± 1.38 | 88.66 ± 6.80ab | 112.70 ± 8.27b |
| T6 | 24.41 ± 0.15a | 36.74 ± 1.48 | 54.08 ± 0.98 | 83.20 ± 2.23b | 103.43 ± 1.21b |

*Averages with different letters vertically represent the presence of significant differences below level of probability ($p \leq 0.05$)

**Table 3.** Effect of adding phytase to low-protein diets on average weight gain of (Mean ± standard error).

| Treatments | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|---|---|---|---|---|---|
| T1 | 10.28 ± 0.13 | 14.46 ± 0.29 | 23.99± 4.98 | 37.68 ± 2.44a | 30.93 ±2.99a |
| T2 | 10.23 ± 0.07 | 12.32 ± 0.54 | 17.39 ± 1.83 | 30.25± 2.92ab | 24.00 ± 1.85b |
| T3 | 10.36 ± 0.11 | 13.10 ± 1.05 | 19.34 ± 2.14 | 31.91 ± 1.93ab | 15.91 ± 2.82ab |
| T4 | 10.10 ± 0.08 | 12.39 ± 0.89 | 16.78 ± 1.89 | 25.05 ± 1.03b | 26.50 ± 0.41ab |
| T5 | 10.08 ± 0.00 | 14.46 ± 0.36 | 15.99 ± 1.65 | 30.13 ± 4.20ab | 24.03 ± 1.72b |
| T6 | 10.30 ± 0.09 | 12.33 ± 1.36 | 17.34 ± 2.10 | 29.11 ± 1.30b | 20.23 ± 1.02b |

*Averages with different letters vertically represent the presence of significant differences below level of probability ($p \leq 0.05$)

**Table 4.** Effect of adding phytase to low-protein diets on average feed consumption. (Mean ± standard error)

| Treatments | Week 2 | Week3 | Week4 | Week 5 | Week6 | Total feed consumption |
|---|---|---|---|---|---|---|
| T1 | 35.38±0.41 | 46.85±2.81 | 52.16±3.94 | 85.14±0.65a | 121.60±3.88ab | 357.64±9.67 |
| T2 | 35.86±0.52 | 44.26±1.28 | 49.23±0.25 | 85.18±0.61a | 118.41±1.95b | 349.46±3.57 |
| T3 | 35.30±0.25 | 44.11±1.44 | 49.18±1.07 | 81.05±0.96b | 125.35±0.52a | 351.50±2.38 |
| T4 | 35.71±0.42 | 43.26±0.64 | 49.934.21 | 81.95±1.63ab | 118.51±0.82b | 345.88±6.04 |
| T5 | 34.93±0.04 | 44.00±1.15 | 51.56±1.23 | 81.86±2.50a | 119.03±2.01ab | 347.90±2.28 |
| T6 | 35.05±0.07 | 43.28±1.70 | 48.33±0.78 | 84.65±0.39a | 114.95±0.48b | 342.76±3.08 |

*Averages with different letters vertically represent the presence of significant differences below level of probability ($p \leq 0.05$)

**Table 5.** Effect of adding phytase enzyme to low-protein diets on average feed conversion factor (Mean ± standard error)

| Treatments | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 | Total conversion factor |
|---|---|---|---|---|---|---|
| T1 | 3.44±0.08 | 3.24±0.25 | 2.28±0.28 | 2.27±0.13b | 3.98±0.29b | 17.71±0.62b |
| T2 | 3.50±0.07 | 3.60±0.15 | 2.89±0.32 | 2.88±0.31ab | 4.94±0.44ab | 20.50±0.62ab |
| T3 | 3.41±0.06 | 3.35±0.26 | 2.59±0.23 | 2.55±0.14ab | 7.87±0.54a | 19.46±0.41ab |
| T4 | 3.53±0.07 | 3.53±0.29 | 3.01±0.24 | 3.27±0.06a | 4.47±0.05ab | 20.50±0.22ab |
| T5 | 3.46±0.00 | 3.04±0.12 | 2.60±0.30 | 2.94±2.35ab | 4.99±0.33ab | 22.48±1.69ab |
| T6 | 3.39±0.03 | 3.56±0.23 | 2.89±0.42 | 2.92±0.13ab | 5.71±0.27ab | 20.98±0.12ab |

*Averages with different letters vertically represent the presence of significant differences below level of probability ($p \leq 0.05$)

(118.41, 118.51 and 114.95 gm, respectively). This may be due to a discrepancy in rate of feed consumption between treatments as normal ration that meets the necessary needs of calcium and phosphorous for bird, while addition of phytase enzyme reduced calcium and phosphorus in proportions based on the percentage of addition of phytase enzyme. The insignificant increase, symmetry, or decrease of these treatments has positive results compared to the control treatment as phytase enzyme works to release unavailable phosphorous stored in plant sources in form of phytate phosphorous in grains and oilseeds such as wheat and corn, as well as dissociating it with many mineral elements in the form of phytate. The adding a mixture of enzymes from the first day increases its effectiveness with the age of birds and this leads to the expansion of digestive canal and an increase in the amount of feed consumed (Cowieson and Adeola 2005). The decrease in feed consumption in some phytase supplementation treatments indicates that consumed feed satisfies birds' energy and protein needs available in ration, thus reducing amount of feed consumed (Liu et al 2008). The total feed consumption during these weeks indicated no significant differences between the treatments.

The adding phytase enzyme to low protein diets on average feed conversion factor showed no significant differences in all treatments in the second, third and fourth weeks. During the fifth week that there was a significant superiority of control of the treatment (T1) which amounted to 2.27 gm compared to treatment of T4, while no significant differences were observed between the aforementioned treatment and rest of treatments. At age of six weeks, significant superiority of the treatment T1 was (3.98 g) was observed compared with T6. The total conversion factor in control treatment was 17.71 gm.

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# Molecular Study of Cryptosporidiosis in Dogs in the Central Region of Iraq

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***Abstract***: *Cryptosporidium* spp are important zoonotic protozoan parasites that infect more than 280 species of vertebrate animals and can cause acute or chronic diarrhea and even death. For the purpose of determining the *Cryptosporidium* species that infect dogs, this study was designed. One hundred dogs fecal samples were collected from both sexes and different age groups, in Babylon and Al-Qadisiyah provinces / central of Iraq, from November 2020 to Jun 2021. Each sample was subject to nested PCR assay method using two sets of primers to amplify small subunit ribosomal RNA gene, followed by sequencing and phylogenetic analysis to determine the *Cryptosporidium* species. The prevalent rate of infection with *Cryptosporidium* spp was 12%, where the females (14.81%) and youngers (25%) recorded the highest rate. The sequencing and phylogenetic assay identified two *Cryptosporidum* species *C. parvum* and *C. canis*. *Cryptosporidium* in these two referred species are an endemic protozoan in study area, the sex and age have no significant effect on infection occurs.

***Keywords***: *Cryptosporidium* species, Dogs, Molecular study, *C. parvum* and *C. canis*

*Cryptosporidium* parasite required one host to complete its direct life cycle (monoxenous life cycle), approximately two days, with prepatent period of 3-10 days according to many factors as age, sex, host immune, species virulence and infection site (Bouzid et al 2013, AL-Mudhafar et al 2020). Cryptosporidiosis remains a clinically significant opportunistic in immune-compromised hosts causing potentially life-threatening diarrhea (Chalmers 2013). Epidemiological studies have publicized that the most important ways of transmission are water born, human-animal and dog to dog contact (Zahedi and Ryan 2020). Prevalence of *Cryptosporidium* infection is correlated with a variety of parameters, such as sampling, age, season, country and interaction with domestic animals. Immune-compromised hosts are more susceptible than healthy hosts (Aldeyarbi et al 2016). Chalmers et al (2013) confirmed that the symptoms of cryptosporidiosis are not pathognomonic; laboratory verification is required to confirm the diagnosis. This is usually done by the detection (presence or absence) of oocytes in stool samples by a microscopic examination of smears stained with tinctorial stain (usually acid fast, such as modified Ziehl-Neelsen stain), fluorescent stain (such as auramine O), or immune-fluorescent stain. In last decade polymerase chain reaction (PCR) technique is used as a confirmative or protozoan species determination (Chalmers et al 2013, Mahmoud et al 2019). *Cryptosporidium* spp coccidian parasite is an untreated parasite in dogs (Fayer R 2008, Aldeyarbi et al 2016, Bustani and Baiee 2021).

To prevent the distribution and decrease the infection (Natapol and Supawadee 2018, Baiee et al 2020) indicated the necessity of treating raw water taken from rivers through series of processes, including coagulation, flocculation, sedimentation, and filtration. Also boiling the water is the most reliable way to decontaminate drinking water that may be contaminated by *Cryptosporidium* (Alibraheemi et al 2021). The study was conducted for incidence of cryptosporidiosis with related risk factors in dogs, and to determine the *Cryptosporidium* species.

## MATERIAL AND METHODS

This study was conducted during November 2020 to June 2021 in Babylon and Al-Qadisiyah provinces located in central Iraq, between latitude 31.97 and 32.50˚N, and longitude 44.58 and 45.83ºE. About 5 grams of fecal samples were collected from 100 stray and housed dogs (46 males and 54 females) with different ages groups included less than 2 years, 2-4 years and more than 4 years. These samples were kept in sterile disposable plastic containers on which the required information of each dog were fixed, and then transported in cold conditions to the laboratory of parasitology in College of Veterinary Medicine / University of Al-Qadisiyah for the laboratory examination. DNA was extracted from each fecal samples by using Presto™ Stool DNA Extraction Kit (Geneaid/Taiwan) according to manufacturer's instructions. The purity of extracted genomic DNAs were checked by using Nanodrop spectrophotometer

(THERMO / USA), at 260 /280 nm absorbance, then the produced DNAs were stored at -20ºC.

The nested PCR technique was performed for detection *Cryptosporidium* spp. based on heat shock protein 70 (hsp70) gene according to method described by (Silvia et al 2013). Two runs of amplification were done by used two different sets of ssRNA primers (Bioneer /Korea) and Go taq Green PCR Master (Promega/USA) that which contain the PCR reaction requirements (Taq DNA polymerase, dNTPs (dATP, dCTP, dGTP, dTTP), Tris-HCl pH 9.0, KCl, $MgCl_2$, Stabilizer and Tracking dye). The first run constructed of 50µl total volume consist of: 5µl DNA template, 2µl (20pmol) of both foreword (AGACGGTAGGGTATTGGCCT) and reverse (TCTGATCGTCTTCGATCCCCT) primers, 12.5µL Master mix and 28.5µL deionized sterile distilled water (Biolab / UK), The amplification was performed in an automated thermocycler (Mltigene/China) by following the program: Initial denaturation at 94 ºC for 5 min followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 58ºC for 30 sec and elongation at 72°C for 2 min, then final extension at 72°C for 5 min and holding at 4°C. The expected PCR product was 664bp where electrophoresed through a 1.5% agarose gel. The second run consisted of the same components as the first one, except for the use of 3µL of the first run product as a DNA template, 2µl (20pmol) of an interior primers (foreword CGGGTAACGGGGAATTAGGG and reverse TCCTTGGCAAATGCTTTCGC) and 30.5 µL deionized sterile distilled water. The amplification conditions as same as the first round except annealing at 60ºC. The yield PCR product expected to be 580bp.

To determine the *Cryptosporidium* species, the DNA sequencing assay was performed for the purest positive nested PCR products, used the AB sequencing system )Bioneer/Korea). Then the phylogenetic analysis was aligned on NCBI-blast Alignment identification and Unweight Pair Group Method with Arithmetic Mean tree (UPGMA tree).

## RESULTS AND DISCUSSION

*Cryptosporidium*, the causative agent of cryptosporidiosis, is an ubiquitous protozoan parasite, it causes gastrointestinal disease in a wide variety of vertebrate hosts and human, Several *Cryptosporidium* species are known to be zoonotic with animals as major reservoirs (Feng et al 2018, Al-Dhalimy et al 2021). Twelve percent (12%) out of 100 examined dogs fecal samples indicated a positive result according to nested PCR technique when amplified the 580 bp product, which confirms the infection with *Cryptosporidium* spp (Fig. 1). The current study which depend upon molecular assay revealed that the obtained results were less than what was stated in molecular

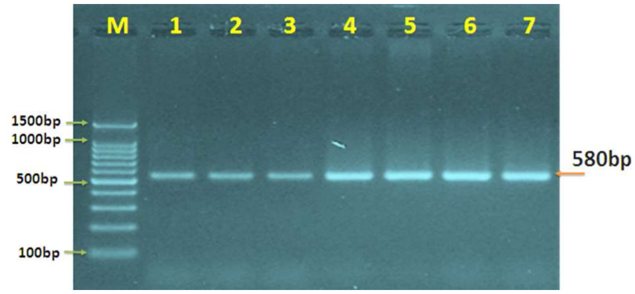


**Fig. 1.** Agarose gel electrophoresis image that showed the nested PCR product analysis of small subunit ribosomal RNA gene in *Cryptosporidium* spp. M: DNA marker ladder (1500-100bp), lanes 1-7 positive samples (580bp)

study of Faraj (2019) Baghdad city, where it was proven that the infection rate is 28.6%. Several microscopic studies were conducted in different governorates in Iraq, most of them included stray dogs and a few of them cared for guard dogs, the incidence rates of which were 8.9%, 16.5%, 20.8%, and 6.5% in Mosul, Saladin, Baghdad and Kirkuk respectively (Mahmoud 2015, Hadi and Faraj 2016, Hassan and Barazinji 2018, Zeny et al 2020). The differences in infection rates in our study and other studies may be due to the difference in the dogs' living conditions, the laboratory tests used and the surrounding environmental conditions. The observation on effect of certain risk factors including sex and age, the results revealed that the females are appeared with higher rate (14.81%,) compared to males (8.69%), but without significant differences. While Young dogs within age group less than two years are showed in highest rate (25%, of cryptosporidiosis infection, while the lowest rate (9.43%) was in dogs with age between 2-4 years, (Table 1).

These results are in accordance with that demonstrated by Mundim et al (2007). However, the findings of many recent reports applied PCR assay detected that *Cryptosporidium* infection in dogs having no significant differences between the positive male and female dogs for *Cryptosporidium* infection (Tavalla et al.2017, Alves et al 2018, Hussein et al 2021). The reasons for these results may be as mentioned in the microscopic study. Alves et al (2018) showed that there no significant differences between different age groups positive for *Cryptosporidium* infection. Other studies demonstrated that *Cryptosporidium* infection in animals is most frequent in young or immunosuppressed animals because of several factors factors (Corza, and Tangtrongsup 2010, URiggio et al 2013, Jian et al 2014). However, age-related infection rates followed the known pattern of negative correlation with increasing age.

The DNA sequencing and phylogenetic analysis was carried out for only seven positive samples, which were with high DNA purity, whereby the results revealed that there are two *Cryptosporidium* species, which are C. parvum (57.2%,) and C. canis (42.8%,). The identity of C. parvum and C. canis with their respective species that which previously recorded in GenBank which were under accession numbers

**Table 1.** Incidence of dogs cryptosporidiosis according to the sex and age

| Sex | Examined samples | Positive samples | |
|---|---|---|---|
| | | No. | % |
| Male | 46 | 4 | 8.69 |
| Female | 54 | 8 | 14.81 |
| Total | 100 | 12 | 12 |
| X2 | | 0.881 | |
| P value | | 0.348* | |
| Age | | | |
| < 2 | 8 | 2 | 25 |
| 2-4 | 53 | 5 | 9.43 |
| >4 | 39 | 5 | 12.82 |
| Total | 100 | 12 | 12 |
| X2 | | 1.63 | |
| P value | | 0.441* | |

* No significant differences (P>0.05)

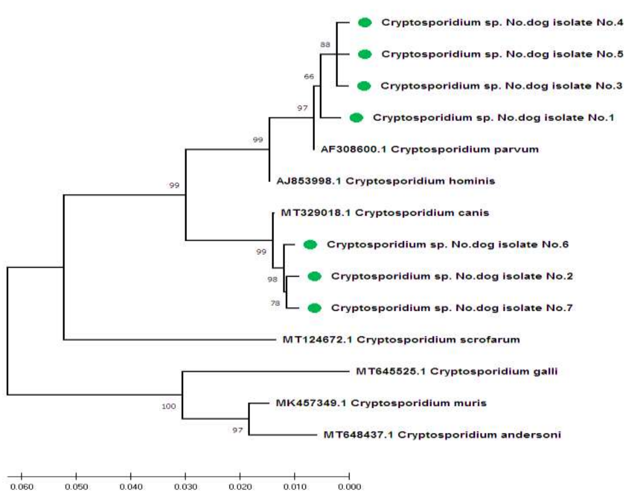


**Fig. 2.** Phylogenetic tree analysis based on small subunit ribosomal RNA gene partial sequence in local *Cryptosporidium* sp. dogs isolates that used for genetic species identification. The phylogenetic tree was constructed using Maximum Likelihood method and Tamura-Nei model tree method) in (MEGA X version).The local *Cryptosporidium* sp. dogs isolate (No. 1-No. 3, No, 4 and No. 5) were showed genetic closed related to NCBI-BLAST *Cryptosporidium* parvum (AF308600.1). The local *Cryptosporidium* sp. (No. 2, No. 6, and No. 7) were showed genetic closed related to NCBI-BLAST *Cryptosporidium* canis (MT329018.1) at total genetic changes (0.060-0.010%)

AF308600.1 in C. parvum and MT329018.1 in C. canis. The nucleotide sequences of ssrRNA gene of *Cryptosporidium* isolates were submitted in the GenBank and were given an accession numbers MZ377133, MZ377134, MZ377135, MZ377136, MZ377137, MZ377138 and MZ377139 (Fig. 2).

The C. parvum isolates in current study are closed identity to American isolate (AF308600.1) which recorded by Blancqab et al (1997) and Barudin et al (2019) while the C. canis isolates are homology to Iraqi isolate (MT329018.1) recently reported by AL-Yasary and Faraj (2021) Only two studies were conducted previous to our study concerning in the determine the *Cryptosporidium* species, whereby the first one confirmed presence of *Cryptosporidium* parvum in Baghdad Faraj (2019), and the second study was done in Karbala city and emphasized two species, *C. parvum* and C. canis AL-Yasary and Faraj (2021). *Cryptosporidium parvum* has been reported globally from dogs and cats; but at lower rates than C. canis in dogs and C. felis in cats (Alves et al 2018).

## CONCLUSION

The *Cryptosporidium parvum* and *C. canis* are an endemic protozoans in Babylon and Al-Qadisiyah provices in central Iraq, and the risk factors of sex and age have no significant effect on infection rate.

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# First Record of Occurrence of Indian Tree Shrew (*Anathana elliotí*) in Gautam Buddha Wildlife Sanctuary, Gaya, Bihar (India)

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***Abstract***: A study was conducted on the sloth bear distribution along with avifaunal species diversity in Gaya forest division (GFD) at Gautam Buddha Wildlife Sanctuary (GBWLS), (Bihar). During the course of field study, Indian tree shrew (*Anathana ellioti*) was first time evidently recorded in GBWLS Bihar with photographs taken at Barachatti forest range (Madgadba sub-beat). In addition to this, its occurrence was also visually seen and reported at Sankhba sub-beat, Barachatti forest range, GFD. Thus, the occurrence of Indian tree shrew has extended the distributional pattern of this new species in Bihar.

***Keywords****:* Tree shrew Gaya forest division, Gautam Buddha wildlife Sanctuary, Barachatti Forest range, Bihar

Gautam Buddha Wildlife Sanctuary (GBWLS) situated in Gaya district of Bihar (138.4 km2) and Hazaribagh, Chatra district of Jharkhand (121.14 km2) between 24.54º N and 85.56º E with a total area of 259 $km^2$ (Fig. 1). The sanctuary covers the portion of the lower Gangetic plains characterised by moist deciduous forests and Chota Nagpur dry deciduous forest ecoregions. This sanctuary is rich in floral and faunal diversity with more than 100 species of plants and 75 species of birds. This sanctuary also exists with a favourable habitat for some of the rare, endangered and threatened species like Sloth bear and Egyptian vulture. However, this sanctuary is constantly facing the biodiversity and habitat loss with a rapid rate due to anthropogenic activities. One of the most detrimental effects has been due to activities of the extremist group, balu mafias, poppy dealers, etc. Hence, an urgent action plan to conserve and manage the sanctuary is the top priority concern. During the study of sloth bear distribution and avifaunal diversity in GBWS (Fig. 2), surprisingly, Indian Tree Shrew was first time evidently seen in this sanctuary. The Indian tree shrew (*Anathana ellioti*) also known as Madras tree shrew is a small mammal belongs to scandentia order of family Tupaiidae. It is endemic species to southern peninsular India. They are native to the Southeast Asia and endemic to the Indo-Burma Biodiversity Hotspot (Majumder and Agarwal 2015) and are basically seen in the deciduous and evergreen primary and secondary forest, more commonly in the karst, associated natural scrub vegetation from sea level to upper montane areas. The species is widely distributed in North-Eastern and central India. The Indian tree shrew has been reported from Assam, Meghalaya, Manipur, Mizoram, Nagaland, Sikkim and Tripura states of India (Majumder and Agarwal 2015). Indian Tree Shrew has the tail coloured the same as its back, the colour as photographed below being reddish-brown and the feet and hind legs are buff or ochraceous. It has olive-brown or greyish-brown coat and a buff or orange venter (Fig. 3). They have got a long bushy tail which is equivalent to their body length. The length of their body (head and body) is 16.5 to 19.5 cm. Tree Shrews are omnivores and have a symbiotic relationship with the pitcher plants on which it perches on. Shrews lick nectar from the bottom of the leaf and defecate inside the tube from which the plants derive the nutrients. In wild their food comprises insects, seeds and fruits. They are diurnal comes under the Least Concern (LC) category in the IUCN Red List (Han et al 2008) and is enlisted in Appendix II of cites (UNEP-WCMC 2001). It is a Schedule II animal of the Indian Wildlife (Protection) Act, 1972 amended up to 2008. The Indian shrew plays a significant role in the ecosystems by its seed dispersal activity.

## MATERIAL AND METHODS

During the field study on the Sloth bear distribution and avifaunal species diversity from the period of September to October 2020 in Gaya forest division at GBWS, surprisingly Indian tree shrew was first time sighted by our study team. For the field study we generally preferred early morning and afternoon. At first, it was visually seen but because of its confusing morphology of strip less squirrel or a young mongoose cannot be confirmed and assured for its presence in this region. Survey of avifaunal study was conducted by following Line Transect Method using Garmin etrex 10x GPS and Nikon P900 camera in the study area (Fig. 2). during this study its occurrence was first time sighted with a photographic evidence record at the Barachatti Forest Range (24° 30' 59.99"N and 85° 00' 60.00"E) of the GBWLS, Gaya, Bihar on the 8 September, 2020 at around 17.00 hours by our team member Ahbar Alam and Mohammad Maaz. This species is recorded for the first time in Bihar. The photographs taken was further also authentically identified by the report of Patel et al (2019) and recognised as Indian Tree Shrew.

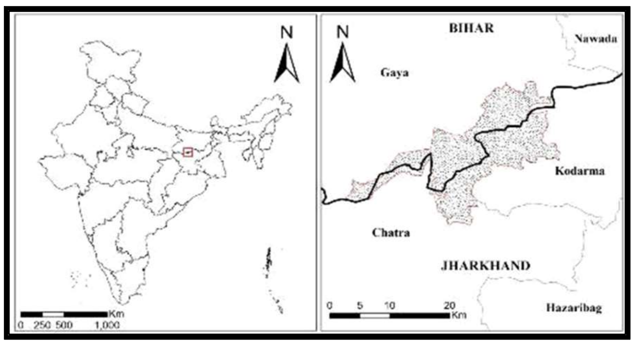


**Fig. 1.** Gautam Buddha Wildlife Sanctuary

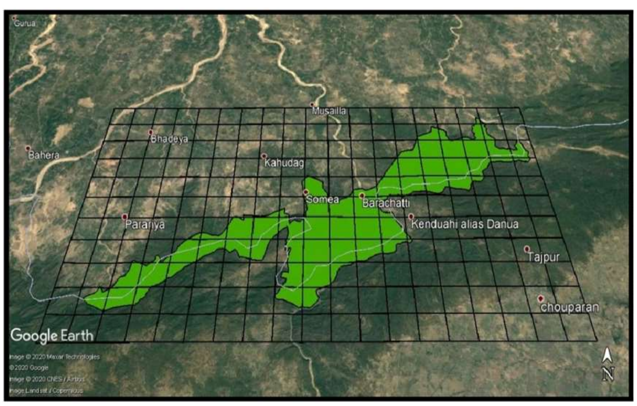


**Fig. 2.** Study area for the survey (ref: Google earth)

**Fig. 3.** Indian Tree Shrew at Gautam Buddha Wildlife Sanctuary

## RESULTS AND DISCUSSION

During the course of field study, maximum 6 sighting reports of Indian Tree shrew were recorded in various location at Barachatti forest range (Madgadba sub-beat). In addition to this, its occurrence was also visually seen and reported at Sankhba sub-beat, Barachatti forest range, GFD in Bihar (Table 1). Several earlier reports have also recorded the presence of Indian Tree Shrew across all the different states of India including, Kerala, Tamil Nadu, Maharashtra (Shrivastava et al 2013), Andhra Pradesh (Srinivasulu and Srinivasulu 2011), Madhya Pradesh (Ashish and Talmale 2013) and West Bengal (Sanya et al 2012, Anweshan et al 2019). Moreover, the spotting of another species of tree shrew i.e., the Northern tree shrew was earlier recorded in Bhimbandh Wildlife Sanctuary, Munger, Bihar in 2014 (Khan et al 2016). This record marks the first spotting with photographic evidence of this Indian Tree shrew species in Bihar. Thus, the occurrence of Indian tree shrew from Bihar has added a new location that extended the distributional pattern of this new species in the country.

The anthropogenic activities like agriculture, poaching, illegal mining, human encroachment for settlement, grazing, etc. and some natural activities like drought, forest fire, irregular monsoon, etc. has not only disturbed the habitat of this species but also increased the pressure on their survival. The effective management plan and a better action plan is needed to save the species of this forest area as the whole ecosystem is dependent on this and once this species becomes extinct the climate change will accelerate and the whole mankind will get vulnerable. Small mammals have significant influences on vegetation and soils, exerts predatory pressure on the insects and other animals and also provide food for other predators. Tree shrews also plays a role in seed dispersion (Karthikeyan 2003) Hence, they are important for the wildlife and forest to function in a smoother way. This first sighting record of occurrence of Indian tree shrew from Gaya forest division, Bihar identified the new location of this species which has expanded their distribution pattern in the country.

**Table 1.** Sighting details of Indian tree shrew at Gautam Buddha Wildlife Sanctuary, Gaya

| Date | GPS location | Time |
|---|---|---|
| 08/09/2020 | 24°28'35.85"N and 85° 9'23.22"E | 5:00 PM |
| 08/09/2020 | 24°28'40.33"N and 85° 9'44.99"E | 6:51 AM |
| 12/09/2020 | 24°28'22.81"N and 85° 9'5.17"E | 8:37 AM |
| 23/09/20 | 24°28'37.79"N and 85°10'4.66"E | 3:47 PM |
| 03/10/20 | 24°26'43.26"N and 85° 8'47.38"E | 6:01 PM |
| 15/10/20 | 24°26'25.50"N and 85° 8'52.94"E | 7:12 AM |

## CONCLUSION

The tree shrew as an important part of ecosystem, plays a significant role in maintaining ecological balance. This species acts as a seed- disperser which is vital for forest ecosystem. The major threat issues involved in decline of the population of this species is potentially due to their habitat loss as a consequence of deforestation, agriculture expansion and hunting by the local residing people. Conservation efforts for this species should be highly recommended with priority for their protection by considering under the Indian Wildlife Protection act schedule.

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# Decision Making of Maize Growing Farmers in Punjab State

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***Abstract*:** The study entitled socio-economic characteristics and decision making statements of maize growers in Punjab state with the objectives to study socio-economic characteristics and decision making of maize growers. Five districts from five agro-climatic zones namely Gurdaspur, Rupnagar, Jalandhar, Firozpur and Muktsar were selected, four blocks from each district and further one village from each block was selected randomly. From each village, ten farmers having experience of fifteen year in maize cultivation were selected randomly. Thus, a total of 200 farmers were selected for this study. The data was collected through personal interview method through structured interview schedule. Statistical tools like frequency and percentage, rank and correlation were used to interpret the data. More than half of the respondents were in age group of 30-46 years and half of the respondents used zero tillage sowing method for maize sowing. All the respondents adopted conventional type of farming. More than ¾ of the respondents had their own tractors and farming done on independent decision. Age and farming experience, age and type of farming, operational land holding and source of irrigation, farming experience and practice followed as compared to other farmers.

***Keywords***: Socio-economics maize, Decision making statements, Punjab state

Climate change is one of the greatest challenges being faced by global community in 21st century. Air temperature near the earth surface rose by 0.74°C from 1906 to 2005 and it is estimated to increase as much as 6.4°C on average during the 21st century (Anonymous 2007a). Rainfall trends showed increase trends in different districts of Haryana, for Kaithal significant increase was observed with z-value of 2.59 (Shaloo et.al 2016). Climate change refers to any change in climate overtime, either due to natural variability or as a result of human activity. The changes occur due to variation in different climatic parameters such as cloud cover, precipitation, temperature and increase in greenhouse gases (GHG's) emission through human activities. It is a change in the mean and variability of its properties that persist for an extended period, typically decades or longer (Anonymous 2007b). Agriculture is the one of sensitive areas upon which society depends for the food, feed and fiber that enables sustainable livelihoods and is one of the sectors that are most vulnerable to climate change. In agriculture, climate can reasonably be considered as a resource and climate change can lead to changes in agricultural productivity of a region (Darwin 2004). In Punjab the major crops of kharif season are rice and maize. In 2019, the area under maize crop was recorded as 1.59 lakh hectare. The farmer is a critical decision maker if agricultural lands are to be effectively managed to adapt to changing climate conditions. There are many evidence of climate variability in Punjab that affect maize production. Relatively small climate changes can cause large water resource problems in many areas in Punjab state. In Punjab, very few studies have been conducted on climate change. The present study was designed to understand the socio-economic characteristics and decision-making statements of respondents of perception of maize growers towards climate change in Punjab state.

## MATERIAL AND METHODS

The study was conducted in Punjab and on the basis of five agro-climatic zones, one district from each zone, four blocks from each district and further one village from each block was selected randomly. From each village, ten farmers having experience of fifteen year in maize cultivation were selected randomly. Thus, a total of 200 farmers were selected for this study. The data was collected through personal interview method through structured interview schedule. Statistical tools like frequency and percentage, rank and correlation were used to interpret the data in meaningful way.

## RESULTS AND DISCUSSION

**Demographic profile:** The demographic of socio personal characteristics is given in Table 1. Social factors like age, educational level and operational land holding greatly influence interest and understanding of respondents towards new innovations in Agriculture (Asrani and Kaushik, 2008). Most of the farmers ranged from 20-40 years of age group and also reported that climate change was greatly influenced by socio economic characteristics (Mustapha, 2012). About 83.33 per cent of the total respondents was educated upto matric level and above. More than 54 per cent family members were educated up to senior secondary and graduation level (Nikhil and Brij Bala 2021).

The 65.50 per cent of the farmer practiced zero tillage sowing method of maize, 19.50 per cent practiced trench sowing method of sowing while only 15.00 per cent of the farmer had practiced bed/ridge sowing method of maize. The distribution of respondents according to type of farming shows that 100 per cent of the farmer adopted conventional farming. Only few farmers (6.50 per cent) had organic by default and Only 4 per cent farmer had certified organic farming. This is due to the fact that farmers were less aware towards organic and the process of organic certification is complex for them. For main source of irrigation, 78.00 per cent had bore-well (submersible pumps) as their source of irrigation while 51.50 per cent have river/canal as a source of irrigation. About 38.50 per cent of the farmers had open-well (monoblock pumps) as a source of irrigation. No farmer is dependent on only rain and tank as their source of irrigation. Majority (88.50%) of the farmers had their own tractor. Only 11.50 per cent farmers used the leased tractors and cultivating with animals/bullocks and working with hands in not done by the farmers. The 81.00 per cent of the respondents take decision regarding farming themselves and 13.50 per cent involve other family members whereas only 5.50 per cent of the respondents take collective decision regarding farming. Most of the respondent themselves involved in work(91 per cent), 64.50 per cent hired labour on daily wages, 58.50 per cent farmers had permanent labour whereas 37.50 per cent of respondents involve other family member in the work on farm. The 99.00 per cent use their own experience for making farming decision and 52.50 per cent took advice from pesticides dealer, followed by media releases, extension personnel's, family and friends and other farmers/ farmer's club/ society/organization and *arthia's*. The farmers used methods like farm literature,

**Table 1.** Distribution of respondents according to socio personal characteristics

| Socio-personal characteristics | Category | f | Per cent | Rank |
|---|---|---|---|---|
| Age (Years) | 30-46 | 127 | 63.50 | I |
| | 47-63 | 58 | 29.00 | II |
| | 64-80 | 15 | 7.50 | III |
| Family type | Nuclear | 83 | 41.50 | II |
| | Joint | 117 | 58.50 | I |
| Family size | Small (<4 members) | 70 | 35.00 | II |
| | Medium (5-8) | 114 | 57.00 | I |
| | Large (> 8 members) | 16 | 8.00 | III |
| Marital status | Married | 197 | 98.50 | I |
| | Unmarried | 3 | 1.50 | II |
| Educational qualification | Illiterate | 16 | 8.00 | V |
| | Primary school | 28 | 14.00 | IV |
| | Middle | 34 | 17.00 | III |
| | Matriculation | 45 | 22.50 | II |
| | Senior secondary | 57 | 28.50 | I |
| | Graduates | 14 | 7.00 | VI |
| | Postgraduates | 6 | 3.00 | VII |
| Operational land holding (In acres) | Marginal (<2.5) | 21 | 10.50 | IV |
| | Small (2.5-5) | 32 | 16.00 | III |
| | Semi-Medium (5-10) | 81 | 40.50 | I |
| | Medium (10-25) | 58 | 29.00 | II |
| | Large (>25) | 8 | 4.00 | V |
| Farming experience (Years) | 15-26 | 153 | 76.50 | I |
| | 27-38 | 36 | 18.00 | II |
| | 39-50 | 11 | 5.50 | III |

**Table 2.** Distribution of respondents according to technologies adopted for sowing by farmers

| Technology adopted | f* | Per cent | Rank |
|---|---|---|---|
| Trench sowing | 39 | 19.50 | II |
| Bed/Ridge sowing | 30 | 15.00 | III |
| Zero tillage sowing | 131 | 65.50 | I |
| Distribution of respondent according to adoption of type of farming | | | |
| Type of farming | f* | Per cent | Rank |
| Conventional | 200 | 100.00 | I |
| Organic by default | 13 | 6.50 | II |
| Certified organic | 4 | 2.00 | III |
| * Multiple responses | | | |
| Distribution of respondent according to main source of irrigation | | | |
| Source of irrigation | f* | Per cent | Rank |
| River\Canal | 103 | 51.50 | II |
| Tank | 0 | 0.00 | IV |
| Bore-well (Submersible pumps) | 156 | 78.00 | I |
| Open-well (Monoblock pumps) | 77 | 38.50 | III |
| Only Rain | 0 | 0.00 | IV |
| * Multiple responses | | | |
| Distribution of respondents according to mode of cultivation | | | |
| Own tractor | 177 | 88.50 | I |
| Leased tractor | 23 | 11.50 | II |
| Bullocks/Animal power/Working with hand tools | 0 | 00.00 | III |
| Distribution of respondents according to decision taken for farm operation | | | |
| Farmer himself | 162 | 81.00 | I |
| Farmer's wife | 0 | 0.00 | IV |
| Other family members | 11 | 5.50 | III |
| It's a collective decision | 27 | 13.50 | II |
| Distribution of respondents according to work done on farm | | | |
| Farmer himself | 183 | 91.50 | I |
| Farmer's wife | 0 | 0.00 | V |
| Other family members | 75 | 37.50 | IV |
| Permanent labour | 117 | 58.50 | III |
| Daily wages labour | 129 | 64.50 | II |
| * Multiple responses | | | |
| Distribution of respondents according to advice taken for making a farming decision | | | |
| Own experience | 198 | 99 | I |
| Family and Friends | 43 | 21.50 | V |
| Farmer's wife | 0 | 0.00 | VIII |
| *Sarpanch* of the village | 5 | 2.50 | VII |
| Other farmers/farmers' club/society/organization | 39 | 19.50 | VI |
| Village priest | 0 | 0.00 | VIII |
| Extension personnel | 54 | 27.00 | IV |
| Media releases (TV, radio, newspaper) | 86 | 43.00 | III |
| Pesticide dealer | 105 | 52.50 | II |
| Others (*Arthia's* etc.) | 39 | 19.50 | VI |

**Cont...**

**Table 2.** Distribution of respondents according to technologies adopted for sowing by farmers

| Technology adopted | f* | Per cent | Rank |
|---|---|---|---|
| * Multiple responses | | | |
| Distribution of respondents according to practice followed are compared to other farmers | | | |
| Like to do experiments and try new things | 71 | 35.50 | II |
| Follow neighbouring farmers | 114 | 57.00 | I |
| Continue to follow own traditional practices | 15 | 7.50 | III |
| Distribution of respondents according to commercial or subsistence farming | | | |
| Commercial | 200 | 100.00 | I |
| Subsistence farming | 0 | 0.00 | III |
| Kind of both | 191 | 95.50 | II |
| * Multiple responses | | | |
| Distribution of respondents according to labour hired for farm operation | | | |
| Never | 0 | 0.00 | IV |
| Frequently, as needed | 182 | 91 | I |
| Hired at least one permanent worker | 162 | 81 | II |
| Only during sowing and harvesting period | 16 | 8.00 | III |
| * Multiple responses | | | |
| Distribution of respondents according to perceived land holding | | | |
| An average farm in the area | 109 | 54.50 | I |
| A relatively small farm | 68 | 34 | II |
| A relatively big farm | 23 | 11.50 | III |

**Table 3.** Coefficient of correlation between various aspects

| Aspects | 'r' value |
|---|---|
| Age and farming experience | 0.9748 |
| Age and technology adopted | -0.7402 |
| Age and type of farming | 0.9394 |
| Operational land holding and source of irrigation | 0.7327 |
| Operational land holding and work done on farm | -0.3020 |
| Farming experience and practice followed as compared to other farmers | 0.2389 |
| Farming experience and practice followed (commercial or subsistence or both) | 0.3873 |

newspaper and other farmers to enhance knowledge and take decisions (Gill et al 2018). The university literature, extension personnel, KVK's and farm magazines worked as a good source of dissemination of valuable information regarding agricultural innovations and new varieties developed in the field of agriculture (Sharma et al 2018). All the respondents did commercial farming. No one was interested in practicing subsistence farming. Majority (91 per cent) of the respondents frequently needed the labour. More than three fourth (81 per cent) of the respondents hired labour permanently while only 8.00 per cent respondents needed the labour during sowing and harvesting period. The 54.50 per cent of the respondents perceived that their farm was an average size farm and 34.00 per considered small farms. The decision of farmers mostly based on their income, access to irrigation, access to credit facility and landholding size (Kisauzi 2012).

The correlation between age and farming experience was positive. The age and farming experience were positively correlated, and the farming experience increase with the increase in the age and vice-versa (Table 3). Age and type of farming, operational land holding and source of irrigation, farming experience and practice, farming experience and practice followed (commercial or subsistence or both) shows positive correlation. Age and technology adopted operational land holding and work done on farm show negative correlation, where other factor decreases with increase in first factor and vice-versa.

## CONCLUSION

The maize growers in Punjab state have experience of 15 to 26 years and only few have large land holdings. Most of the farmers used zero tillage method for sowing of maize and all the respondents doing conventional type of farming. Most of the respondents used river/canal and bore-well as source of irrigation and no one is dependent on rainfall. More than ¾ of the respondents have independent decision. Most of the farmer himself working in the field and also prefer permanent labour as well as daily wage labour. Age and farming experience, age and type of farming, operational land holding and source of irrigation, farming experience and practice followed as compared to other farmers, farming experience and practice followed (commercial or subsistence or both) shows positive correlation.

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# Identification of Suitable Sites for Water Harvesting Structures in Wadi Al-Meleh Using GIS

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***Abstract*:** Water harvesting can be effectively used to harness unused surface runoff and increase groundwater feeding. This research identifies suited locations for rainwater harvesting designs in the watershed of Wadi Al-Meleh, which is located in the northern part of Iraq. Thematic maps were created for slope, stream order, land use–land cover, runoff depth, and types of soil using ArcGIS program. Suitable locations for the selected water harvesting structures were identified by overlaying these thematic maps according to the required criteria. Check dams and farm ponds can be constructed in a wide region of the study area. Subsurface dyke structures can be constructed in a limited region at the south west of the basin. The study provides a quick and socioeconomically beneficial solution for the planning and construction of rainwater harvesting designs within the drainage basin.

***Keywords:*** GIS, Overlay map, Rainwater harvesting, Locations property analysis

Water resources are an essential component of life. After the first UN-sponsored international conference on human environment in Stockholm, Sweden, in 1972, water gained global focus (Al Sulaiman 2016). This focus has intensified with the rising consequences of climate change on water cycle (Ammar et al. 2016). The consequences of this influence in the climate are evident in the Arab region through shrinkage of agricultural land (Karami 2019). In particular, Iraq suffers from a water crisis caused by climate change and this is exacerbated by the absence of successful management (Al-Khafaji 2018). This study analyzes the possible locations of water harvesting structures in the northern part of the semi-arid region of Wadi Al-Meleh where water supply is scarce because of drought (Boryczko et al 2021). Water harvesting structures in this region can support farmers and increase agricultural land and remove the complete dependence on rain for crop production (Velasco-Muñoz et al 2019). A hot semi-arid climate covers most of the basin area, which is characterized by very hot summers and mild or warm winters, while the rest of the basin is characterized by warm summers and wet winters (Ameen et al., 2018). Therefore, the valley receives heavy rainfall within a short period each year. Consequently, most of the agricultural activities in the basin suffer due to a water deficit and the success of crops is at high risk. Furthermore, this climate and the present land use and land cover pattern result to high surface runoff and loss in soil cover (Lopes et al 2019) causing a decrease in agricultural land, damage to crops, and an increase in the frequency of flooding through the Tigris River in the city of Mosul, which lies south of the valley. This research includes the preparation of the appropriate thematic maps of Wadi Al-Meleh for the identification of suitable locations for rainwater harvesting designs applying remote sensing and (GIS) techniques.

## MATERIALS AND METHODS

**Location of study area:** The Wadi Al-Meleh basin is placed in North-West Iraq within 43°09´35"–42°53´36" E and 36°45´35"–36°27´35" N (Fig. 1). Tectonically, the basin cuts across the anticlinal structure of Qand, which is approximately 33 km north of Mosul within the Butma–Mosul belt of the Low Folded Zone (Husain 2018). The headwaters begin at the north in the mountains of Alqosh, which is the southern part of the structure, and to Qand. The valley extends further south and reaches the Tigris River at the village of Gededa Al-Malla.

**Data accumulation:** The Digital Elevation Model from Aster (gained by http://earthexplorer.usgs.gov) was used for the analysis. Rainfall data were collected from the Mosul meteorological station, which is located close to the basin and also from the World Weather website. Satellite imagery acquired through the Sentinel-2 satellite were also utilized. The satellite images that were chosen had a cloud cover of ≤ 4.54% and were selected from the U.S. Geological Survey (USGS) database (http://glovis.usgs.gov/). The soil types of this watershed were identified by Buringh.

**Slope map:** Hydrological surface parameters such as runoff, recharge, and water movement are controlled by the slope of


**Fig. 1**. Location map of study area

the watershed. Digital elevation model from the shuttle radar topography mission (SRTM) dataset with 30-m resolution was used to create the slope map of the study area. Spatial analyst tools were used to generate the slope map using the surface option.

**Stream network map:** The stream network map was generated using DEM through the "Hydrology" option of the "Spatial Analyst Tools." The DEM raster georeferenced and then conveyed to the coordinate system type projected prior to use. A set of steps including determination of flow direction and flow accumulation led to the generation of the stream network. The Strahler classification of stream order was used to delineate the stream network.

**Land use-Land cover map:** The land use and land cover map takes an important aspect in identifying suitable rainwater harvesting structures near the agricultural lands (Maina and Raude, 2016). Sentinel-2 satellite images were taken to produce the map of land use–land cover. A supervised classification technique was applied to determine the land use and land cover classes in the area of interest. This classification procedure is highly accurate especially when it is applied with the likelihood method algorithm (Marapareddy et al 2017).

**Soil map:** The soil map is essential for position choosing for rainwater harvesting design (Rasul et al 2019) as soil permeability influences the infiltration rate through the soil column (Naseef and Thomas 2016). The soil types of this catchment area were specified by Buringh.

**Runoff map:** Runoff estimation of a watershed is required for the design of storm water management facilities (Towsif Khan et al 2020). Surface runoff is affected by the watershed characteristics. Such as shape of the basin, size, slope, soil classes, land use-land cover, specification of precipitation and drainage area pattern (Park et al 2021). The Soil Conservation Service–Curve Number (SCS–CN) was utilized to measuring the amount of Meleh watershed's surface runoff. The SCS–CN approach was established depend on the equation of water balance using two of theoretical assumption (Satheeshkumar et al 2017). The first hypothesis states about that the proportion of the actual amount of direct runoff to the maximum probable runoff is equal to the proportion of the quantity of actual infiltration to the quantity of the potential maximum retention. The second assumption relates that the volume of initial abstraction is a portion of the potential maximum retention. The SCS–CN method uses the following empirical equation to estimate the direct runoff in a watershed (Thankachan et al 2020).

$$Q = (P-I_a)^2 / (P-I_a+S) \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(1)}$$

Where Q is the direct runoff (mm), Ia is the initial abstraction (mm), P the precipitation depth (mm) and S is the potential maximum retention (mm).The initial abstraction can be assumed as a function of S (Thankachan et al 2020), as follows:

$$I_a = 0.2\,S \quad \ldots\ldots\ldots\ldots \text{(2)}$$

The potential maximum retention after runoff starts can

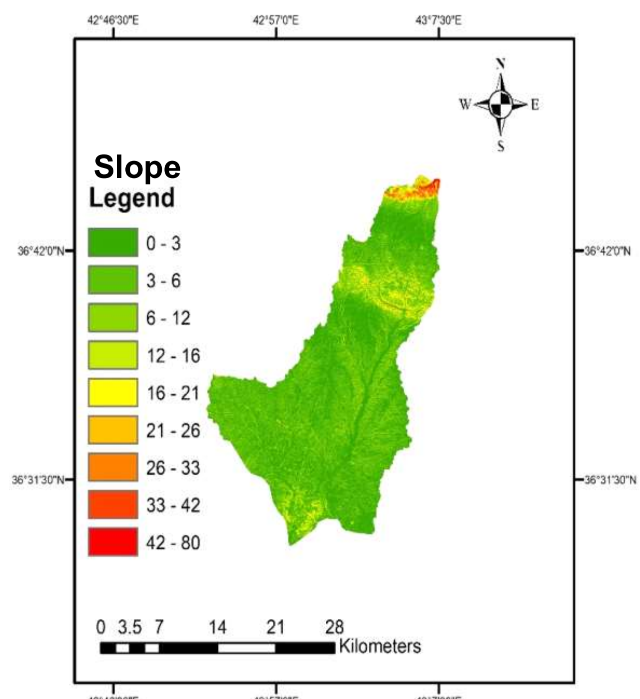


**Fig. 2.** Slope map of study area

be calculated in value terms of the curve number (CN) using the following equation:

S = 25400/CN-254…………….... (3)

Where CN is a dimensionless parameter with ranging from 0 to 100, S indicates the maximum volume of water that can enter the drainage basin by interception, surface depression storage, infiltration, and other hydrological losses. The curve number values is a function of the soil infiltration capacity with respect to earth covering type and antecedent soil moisture situation. The soil based on SCS are divided in to 4 soil hydrological groups (Table 1)

**Selection of suitable locations for rainwater harvesting designs:** Rainwater-harvesting designs are selected depend on a set of criteria. The current study included the suggestion each of check dam, farm pond and subsurface dyke as rainwater harvesting structures. A check dam is a widely utilized rainwater harvesting structure. A check dam is a small dike constructed across a drain water ditch or other drainage water features to reduce the velocity of flow (Huzita et al 2020). Where, a farm pond is a feature of a land filled with water that is smaller than a lake. The water collected in it is used for livestock and irrigation. While, a subsurface dyke is constructed, and it is established on impermeable bedrock to intercept groundwater flow of a seasonal water system from flowing into sandy drainage (Ramasesha et al 2020). Table 2 shows the criteria for the selection of rainwater harvesting structures of study area.

## RESULTS AND DISCUSSION

**Slope map:** The slope analysis shows that most of the basin has a very gentle to gentle slope of less than 6° (Fig. 2) and a very steep slope was limited to the northern part of the basin.

**Stream network map:** The result of stream order delineating by DEM of study area can see at (Fig. 3). According to Strahler classification, highest order stream is found to be 6th order for the area of interest.

**Land use–Land cover map:** The results of applied supervised classification technique over the area of study can bee see in (Fig. 4). The classified land use and land cover zones of the study area are listed in Table 3. The agricultural land class covered the maximum ratio of land use / land cover classes with more than 44%.

**Soil map:** Five classes of soils are present in Wadi Al-Meleh (Fig. 5). Deep brown soil phase type of soil covers most of the study area (Table 4). The Brown soil deep phase is represents the most common type in the study area. This type of soil has moderate permeability with high to moderate runoff

**Runoff map:** The SCS–CN method showed that the runoff depth map of Wadi Al-Meleh was arrived at with 456 mm of annual rainfall (as measured at the Mosul meteorological

**Table 2**. Guidelines for selection the rainwater harvesting structures in the study area

| Type of structure | Slope | Runoff | Soil permeability | Drainage |
|---|---|---|---|---|
| Check dam | Gentle Slope | High/Moderate | Low | 1–4 |
| Farm pond | 5 < | High/Moderate | Low | 1 |
| Subsurface dyke | 3 < | Low | High | > 4 |

**Table 3**. Classes of land use/land cover zones in Wadi Al-Meleh

| Classes | Area (Km$^2$) | Area (%) |
|---|---|---|
| Vegetation land | 85.88 | 26.27 |
| Agriculture land | 144.98 | 44.34 |
| Rangeland | 87.36 | 26.74 |
| Outcrops | 6.54 | 2.00 |
| Urban | 1.82 | 0.55 |
| Seasonal lakes | 0.012 | 0.0036 |
| Total | 326.9 | 99.90 |

**Table 4**. Hydrological soil classes present in the study area and their properties

| Soil classes | Permeability | Runoff |
|---|---|---|
| Rough broken and stony land | Slow to moderate | High/Moderate |
| Brown soils, medium, deeply eroded phase | Moderate | Moderate/ High |
| Brown soils, medium, over gypsum | High to moderate | Moderate/ Low |
| Brown soils deep phase | Moderate | High/Moderate |
| Lithosolic soils in sand stone and gypsum | High | Low |

**Table 1.** Soil groups and runoff potential description

| Soil groups | Runoff potential-infiltration | Type |
|---|---|---|
| A | Low runoff - high infiltration rate | Sand, Loamy sand, and sandy loam |
| B | Moderate runoff- moderate infiltration | Silty loam and loam |
| C | High/moderate runoff -slow infiltration rates | Sand clay loam |
| D | High runoff potential - very low infiltration | Clay loam, silty clay loam, sandy clay, silty clay, and clay |

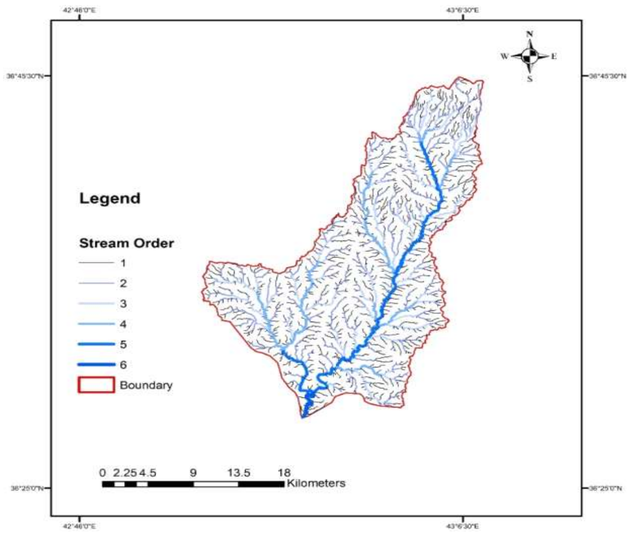


**Fig. 3.** Stream order map of the study area

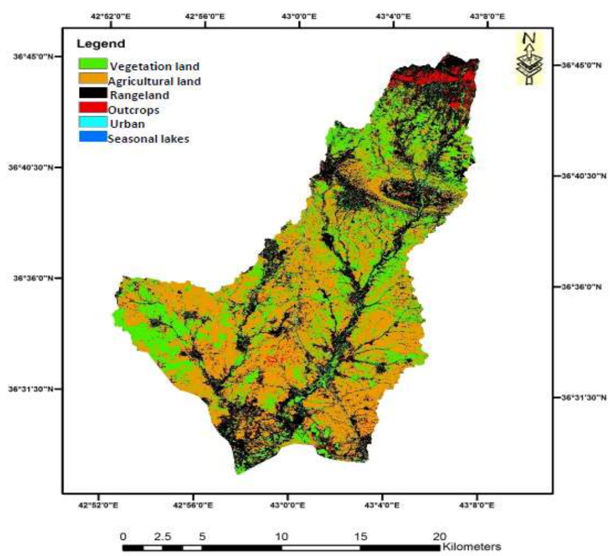


**Fig. 4.** Land use-land cover map of Wadi Al-Meleh

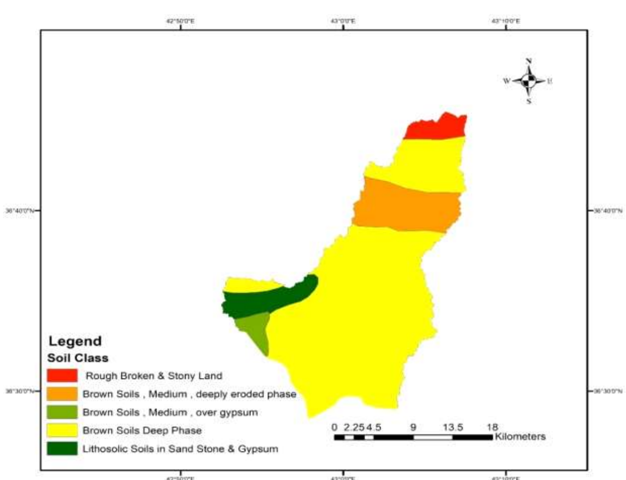


**Fig. 5.** Soil map of Wadi Al-Meleh

station). The annual runoff depth values ranged between 347 mm to 406 mm (Fig. 6).

**Selection of suitable locations for rainwater harvesting designs:** The selected locations for the three chosen water harvesting structures were arrived at by overlaying slope, stream order, land use–land cover, soil types, and runoff depth maps using ArcGIS software. The suitable sites for the suggested check dam, pond, and subsurface dyke water harvesting structures are shown in Figure 7.

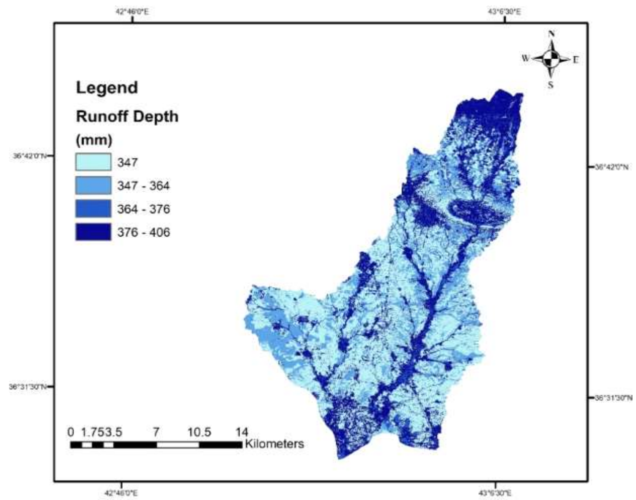


**Fig. 6.** Runoff depth map

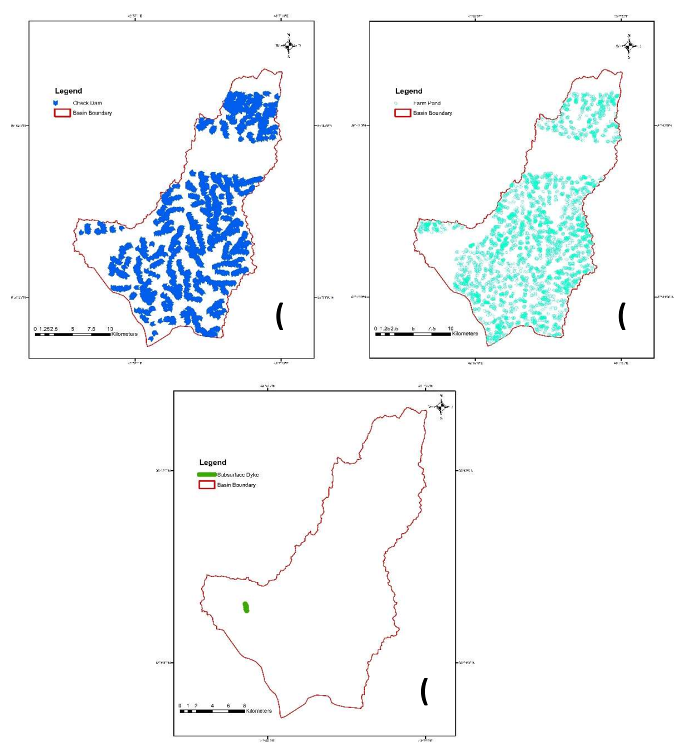


**Fig. 7.** Applicable locations for proposed rainwater harvesting designs: (A) check dam; (B) farm pond; and (C) subsurface dyke

The farm pond and check dam can be successfully installed in many parts of the Wadi Al-Meleh basin, mainly at North and South-East of the watershed. However, the construction of subsurface dyke is suitable only in a small area confined to the south west of the study area wherein the soil properties and hydrological system of drainage are conducive.

## CONCLUSIONS

This study successfully identified applicable sites within Wadi Al-Meleh basin for the construction of rainwater harvesting designs like ponds, check dams, and subsurface dykes by applying techniques of GIS with remote sensing data. A composite overlay map was also created using the thematic slope, stream order, earth cover type, soil group, and runoff depth in Wadi Al-Meleh watershed covering an area of 327 km2. Economic resources can be best utilized by selecting the optimum type and location of the water harvesting structure for construction, and the study provided a basis for achieving this. The most useful and widely installable water harvesting structures in this water shed are farm ponds and check dams. However, there are some limitation should be noted and further work suggestions. First, analysis the rainfall and evaporation data at the selection structures locations. Second, infiltration rate of soil with holding capacity and depth of soil will give more reliability for selected sites.

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# Prevalence of Monogenean Parasites in Some Freshwater Fishes of Jammu Region, India

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***Abstract*:** The present study was conducted to investigate the occurrence and seasonal incidence of monogenean parasites in the freshwater fishes of Jammu region. A total of 654 fishes comprising *Labeo rohita, Puntius sophore*, *Tor sp*. and *Puntius ticto* were collected and examined for monogenean parasites from March 2018 to February 2019. Three monogenean ectoparasites belonging to group Platyhelminthes recovered from the gills of infected fishes. *Dactylogyrus bucinus* and *Diplozoon guptai* were identified to the species level while *Gyrodactylus* species identified to the generic level only. Among the monogenean parasites recovered *Dactylogyrus bucinus* showed highest rate of prevalence of 47.78% and 43.20% in *Puntius ticto* and *Tor sp.* respectively, whereas *Gyrodactylus sp.* showed lowest prevalence of 04.28% in *Puntius sophore*. The seasonal prevalence for monogenean infestation was the maximum in summer season, followed by spring, autumn and winter among infected host. Among, the fish hosts, Cyprinidae was found to be most suitable host for monogeneans and dactylogyridae was the most diverse family.

***Keywords*:** Parasites, Platyhelminthes, *Dactylogyrus, Gyrodactylus, Diplozoon,* Prevalence

In the past two decades the fish culture in India has transformed into a main commercial activity due to increase demand for fish as a source of protein. But the intensification and expansion of fish culture is facing severe threats from pathogens as they are the prime cause for chronic mortalities and poor growth thus, affecting the yield and marketability of fishes. Environmental pollution of aquatic ecosystem may either increase or decrease the pathogens burden in aquatic organisms mainly the fish host (Sures 2008, Blanar et al 2009) Application of therapeutic measures to get rid of these pathogens requires a sound base of parasitic taxonomy. Among all the vertebrates, fish is the most parasitized and the importance of parasitic infection on fish production has largely remained an issue of concern to fish farming industries. Monogeneans are the ubiquitous and abundant group of parasites in the aquatic environment and predominantly ectoparasitic on skin and gills of fish (Ivona 2004). Monogeneans are hermaphroditic worms and have a direct life cycle without involving intermediate host and due to their life strategies and adaptations to parasitic life they can be regarded as very successful parasites (Valigurova et al 2011). Direct development with high fecundity generally results in heavy infection with these parasites in host fishes (Shameem 2007). These parasites were found both in wild and captive fishes and caused excessive mortalities as they are attached to the skin and gills of host fish with their equipped haptor armature and feed upon mucus and other host tissues. In the natural environment, monogeneans are often in equilibrium with their hosts, and can cause serious morbidity and mortality during fish breeding resulting in significant economical losses. Monogenean diversity from Indian region is still in infancy only low percentage of these parasites that are presently known (Chaudhary et al 2013).The present study, aims to identify monogenean parasites and also to find out parasitic prevalence, mean intensity and abundance in the freshwater fishes of Jammu region.

## MATERIAL AND METHODS

Fishes were caught using drag nets by local fisherman and were brought alive to the laboratory, dissected and examined for the parasites. In total of 654 fishes, specimens belonging to five species were examined, including *Labeo rohita, Puntius ticto*, *Tor sp.*, and *Puntius sophore.* Gill arches were cut out through the edges, placed in water and examined individually. Monogeneans were picked up with a micropipette and placed on a micro slide in a drop of water, washed with 0.7-0.9% saline solution, fixed in Aceto- Formal-Alcohol (AFA) or hot alcohol. After fixation the parasites were washed with water to remove excessive fixative then dehydrated, stained and mounted in canada balsam. Parasites recovered were identified using standard keys (Tripathi 1957, Pandey and Aggarwal 2008).

**Statistical analysis:** The prevalence and intensity of the parasite were computed following the formulae proposed by Margolis et al (1982).

$$\text{Prevalence} = \frac{\text{No. of hosts infected}}{\text{Total no. of hosts examined}} \times 100$$

$$\text{Mean Intensity} = \frac{\text{No. of parasites recovered}}{\text{Total no. of infected hosts}}$$

$$\text{Relative density} = \frac{\text{No. of parasites recovered}}{\text{Total no. of hosts examined}}$$

## RESULTS AND DISCUSSION

***Dactylogyrus bucinus:*** Small sized worm measuring 0.312-0.428×0.013-0.035mm; anterior region bears two pairs of eye spots and four pairs of cephalic glands; Pharynx muscular spherical structure measuring 0.020-0.024mm; Haptor is distinct from body proper and measures 0.032-0.068×0.033-0.85mm; Armature of haptor comprised of a pair of anchor, a dorsal bar and seven pairs of marginal hooks; Each anchor is winged measuring 0.018-0.037mm and having well developed strong shaft, base and recurved tip; shaft length 0.026-0.032mm; inner root length 0.007-0.010mm; outer root length 0.002-0.003mm; Dorsal bar length 0.008-0.012×0.001-0.002mm; marginal hooks seven pairs, $5^{th}$ pair large 0.015-0.026mm in total length; $3^{rd}$ pair smaller in size; copulatory tube thin, coiled measuring 0.005-0.009mm in length; accessory piece 0.012-0.017mm in length; vaginal tube sclerotized measuring 0.003-004mm. Host:*Puntius ticto, Tor sp.* Site of infection: Gills (Fig. 1 & 2)

Genus *Dactylogyrus* was established by Diesing (1850) to describe *D.auriculatus* from the fishes, *Cyprinus carpio, Phoxinus phoxinus* and *Abramis brama* as the type species. In India, Price (1938) first recorded the genus *Dactylogyrus* to describe a new species *D. moorthyi*, subsequently, various workers added new and already described species. The present fluke infesting gill filaments of *Puntius sophore* and *Tor sp.* agrees with *D. bucinus* in morphomertic characters such as shape of bar, haptor and having seven pair of marginal hooks. Therefore the present monogenean fluke is identified and reported here as *D. bucinus* Gussev (1976). *Dactylogyrus bucinus* was first described by Gussev (1976) as parasitizing the gills of *Barbus dorsalis* (now known as *Puntius dorsalis*) collected from Bhawani sagar reservoir near Coimbatore, India. Ahmed (2016) reported this species from the host fish *Crossocelius latius* of Poonch district, J&K.*Puntius ticto* and *Tor sp.* are new host record for *D.bucinus* from Jammu region.

***Gyrodactylus* sp.:** Body small, elongated, dorso-ventrally flattened, measuring 0.347-0.412 mm in length and 0.048-0.085mm in width at mid body; Cephalic lobes are well developed; Eyes absent; Head organs elongated extends up to pharynx; Pharyngeal bulb well developed elongated measuring 0.23-0.028mm; haptor with one pairs of anchors supported by a dorsal and a ventral bar and 16 marginal hooklets; haptor sub-ovate measuring 0.058-0.090×0.041-0.073 mm; ovary pretesticular. Host:*Puntius sophore* Site of infection:Gills and scales (Fig. 3). Bakke et al (2002) and Bommakanti (2016) noted the host specificity dynamics and observations of gills on gyrodactylid. *Gyrodactylus sp.* is being recorded for the first time from the gills of *Puntius sophore* from Jammu division.

***Diplozoon guptai* Ahmad & Chishti, 1997:** Body 1.78-2.85 mm in length; Fore body 0.90-1.65×0.32-0.50 mm; Hind body 0.62-1.18×0.16-0.24 mm; Anterior extremity of fore body has terminal mouth surrounded by a muscular sucker; Pre-pharynx 0.03-0.04 mm; muscular oval pharynx 0.05-0.06×0.04-0.05 mm; Oesophagus short; Intestinal caecum give off many lateral diverticula, extend in hind body up to clamps; Anterior sucker 0.03-0.04×0.04-0.05 mm; Hind body includes reproductive organs and haptors; Haptor with four pairs of transversely oval clamps; Clamps with 7 rods (1 median, 2 posterior, 2 middle and 2 anterior); Testis single, spherical 0.06-0.07×0.06mm; Ovary pre-testicular; Vitelline follicle extensive, scattered in whole fore body.Host: *Labeo rohita,* Site of infection: Gills (Fig. 4)

The specimen of Monogenea collected from gills of *Labeo rohita* when subjected for detailed morphological and comparative studies was identified as *Diplozoon guptai* Ahmad and Chishti (1997). However, some differences recorded were insufficient to create a new species. Therefore, present work deals with the redescription of *D. guptai.* Ahmad and Chishti (1997) reported this species from *Schizothorax Niger, Schizothorax esocinus, Labeo sp.* and *Carassus carassus* from Kashmir. Ahmed (2016) has also

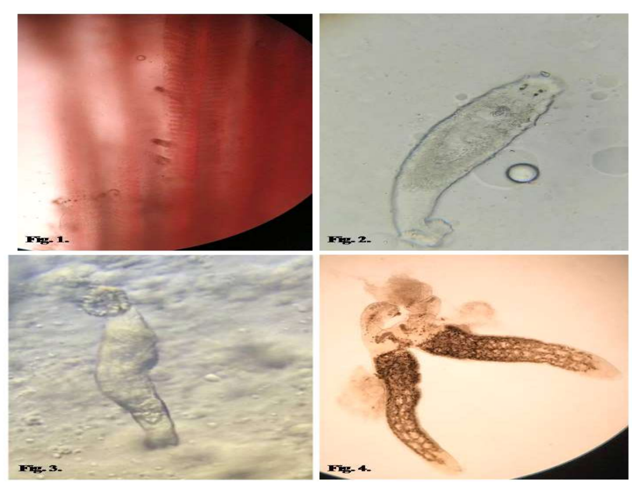


**Fig. 1.** Showing *Dactylogyrus bucinus* attached to gill filament of *Puntius ticto*; **Fig. 2.** *Dactylogyrus bucinus*; **Fig. 3.** *Gyrodactylus* sp.; **Fig. 4.** *Diplozoon guptai*

reported *D. guptai* from the gills of *Schizothorax richardsonii* from Jammu province of district Poonch. The present species is recorded from *Labeo rohita*, new host record from Jammu region.

**Seasonal occurrence of parasites:** A total of 205 fishes were infested out of 654 fishes examined. From the infested host 399 monogeneans were recovered. The total prevalence was 31.34%, mean intensity was 1.94 and relative density was 0.62. The lowest prevalence of *Gyrodactylus sp.* was in *Puntius sophore* being 03.58%, the mean intensity was 2.20 and abundance was 0.07, whereas the highest prevalence of *Dactylogyrus bucinus* was in *Puntius ticto* being 47.78%, mean intensity 2.35 and abundance value of 1.12 (Table 1).

The highest seasonal prevalence of monogenean parasites was characteristically evident in summer followed by spring then autumn and winter (Table 2). The higher infection of *Dactylogyrus bucinus* was observed during summer (62.50%) followed by spring (57.58%) than autumn (43.48%) and lowest observed during winter (23.33%) in *Puntius ticto*. Similarly the prevalence of *Gyrodactylus sp.* and *Diplozoon guptai* was highest during summer followed by spring than autumn and with no infection during winters. Mean intensity and relative density of monogeneans infection are represented in Table 2.

The seasonal intensity of the monogenean in general was highest in summer and spring. The prevalence of monogenean species *viz. Dactylogyrus bucinus, Gyrodactylus sp.* and *Diplozoon guptai* decreased in autumn and winter whereas was at peak in summer followed by spring. Osman (2005) and Noor El Deen (2007) also observed the highest prevalence during summer. Similarly

**Table 1.** Showing prevalence, mean intensity and relative density values of monogenean parasites recovered from host fishes

| Host Fish | Parasites | No. of fishes examined | No. of fishes infected | No. of parasites recovered | Prevalence (%) | Mean intensity | Relative density/ Abundance |
|---|---|---|---|---|---|---|---|
| *Puntius ticto* | *Dactylogyrus bucinus* | 157 | 75 | 176 | 47.78 | 2.35 | 1.12 |
| *Tor* sp. | *Dactylogyrus bucinus* | 162 | 70 | 135 | 43.20 | 1.92 | 0.83 |
| *Puntius sophore* | *Gyrodactylus* sp. | 140 | 06 | 12 | 04.28 | 2.00 | 0.07 |
| *Labeo rohita* | *Diplozoon guptai* | 195 | 54 | 76 | 27.69 | 1.40 | 0.38 |

**Table 2.** Showing seasonal infection prevalence (%), mean intensity and relative density values of monogenean parasites in host fishes

| Seasons | Host fish | Parasites | No. of host fish examined | No. of host fish infected | No. of parasites recovered | Prevalence (%) | Mean intensity | Relative density/ Abundance |
|---|---|---|---|---|---|---|---|---|
| Spring | *Puntius ticto* | *Dactylogyrus bucinus* | 33 | 19 | 46 | 57.58 | 2.42 | 1.40 |
| | *Tor sp.* | *Dactylogyrus bucinus* | 41 | 22 | 43 | 53.65 | 1.95 | 1.05 |
| | *Puntius sophore* | *Gyrodactylus sp.* | 30 | 02 | 03 | 06.67 | 1.50 | 0.10 |
| | *Labeo rohita* | *Diplozoon guptai* | 49 | 12 | 20 | 24.49 | 1.66 | 0.40 |
| Summer | *Puntius ticto* | *Dactylogyrus bucinus* | 48 | 30 | 79 | 62.50 | 2.63 | 1.64 |
| | *Tor sp.* | *Dactylogyrus bucinus* | 44 | 25 | 54 | 56.81 | 2.16 | 1.22 |
| | *Puntius sophore* | *Gyrodactylus sp.* | 40 | 03 | 07 | 07.50 | 2.33 | 0.17 |
| | *Labeo rohita* | *Diplozoon guptai* | 57 | 19 | 28 | 33.33 | 1.47 | 0.49 |
| Autumn | *Puntius ticto* | *Dactylogyrus bucinus* | 46 | 20 | 40 | 43.48 | 2.00 | 0.87 |
| | *Tor sp.* | *Dactylogyrus bucinus* | 50 | 18 | 30 | 36.00 | 1.67 | 0.60 |
| | *Puntius sophore* | *Gyrodactylus sp.* | 41 | 01 | 02 | 02.44 | 2.00 | 0.05 |
| | *Labeo rohita* | *Diplozoon guptai* | 54 | 20 | 25 | 37.04 | 1.25 | 0.46 |
| Winter | *Puntius ticto* | *Dactylogyrus bucinus* | 30 | 07 | 16 | 23.33 | 2.29 | 0.53 |
| | *Tor sp.* | *Dactylogyrus bucinus* | 27 | 05 | 09 | 18.52 | 1.80 | 0.33 |
| | *Puntius sophore* | *Gyrodactylus sp.* | 29 | 00 | 00 | 0 | 0 | 0 |
| | *Labeo rohita* | *Diplozoon guptai* | 35 | 03 | 03 | 08.58 | 1.00 | 0.08 |

Dar et al (2015) reported that the prevalence and intensity are higher in the summer and lower in winter season. Khanum et al (2011) observed that the higher infection was in rainy season and lower in winter season. On the contrary Ramudu et al (2018) reported the highest prevalence of *Dactylogyrus* sp. and *Gyrodactylus* sp. during winter (December to February) and lowest during summer season (June to August). These differences in the prevalence of monogenean parasites may be attributed to the difference in environmental conditions and type of fish examined. The family Dactylogyridae of class monogenea exhibit highest abundance due to competitive skill for successfully establishing on their respective host.

## CONCLUSION

The three species of monogeneans were recovered from four species of freshwater fishes of Jammu. Among the monogeneans, *Dactylogyrus bucinus* and *Gyrodactylus sp.* are Monopisthocotyleans and *Diplozoon guptai* is Polyopisthocotyleans. Highest prevalence of infection was shown by *Dactylogyrus bucinus* and lowest by *Gyrodactylus sp.* The prevalence of all the parasites recovered was highest during summer season probably due to the fact that this season favors their reproduction as well as the continuity of generation due to frequent availability of their intermediate hosts. During autumn and winter season the prevalence was lowest. This decrease in frequency during autumn and winter may be due to environment conditions which are also not in much favour of their intermediate hosts. The life cycle and reproduction of monogeneans is retarded or completely stopped in cold seasons.

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# Effect of Pre-sowing Seed Treatments and Time of Sowing on *Terminalia chebula* Retz. in the Nursery

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***Abstract***: This study included three pre-sowing treatments (control, seeds soaking in sulphuric acid and mechanical breaking of seed coat) at different sowing times (March, June and July) for seed germination and seedling growth of *T. chebula*. The highest rate of germination (40.83%) was observed in mechanical breaking of seed coat and the lowest (18.33%) was found in control. The growth parameters like shoot length, root length, number of leaves, total dry biomass, and root-shoot ratio recorded optimum and substantially higher in mechanical breaking as compared to others. Considering the practicality of the nursery growing technique of the species, mechanical breaking and seed soaking in sulphuric acid for few minutes revealed the best pre-treatment choice obtained in this study for large-scale plantation programmes.

***Keywords***: Germination, Seedling, Fruit, Biomass, Nursery, Parameters

*Terminalia chebula* is one of the multipurpose and medicinal tree species in India, Sri Lanka, Myanmar, Thailand and China. It occurs in Indian forests and agroforestry systems that stretch from sub-temperate to tropical regions (Singh 1982). The species' fruits are used locally in many medicines and are an essential component of 'Triphala' (a medicinal stew) and are used widely in other Ayurvedic medicines to treat heart pain, flatulence, dyspepsia, liver and spleen diseases, asthma and constipation. It is estimated that production in India is 100,000 tons, 20 per cent of which is exported to neighboring nations, Europe and the USA. During winters when other green fodder is very limited, the tree is lopped for leaves to feed the livestock. The leaves contain 1.73 per cent nitrogen (equivalent to 10.80% protein content) and 2.75 per cent calcium (Singh 1982). Large-sized fruits get higher prices in domestic and international markets and thus fruit size is an important feature. Limiting factors for its adoption in agroforestry systems are poor germination capacity, lack of natural regeneration and knowledge regarding its propagation. The species' juvenile period is also longer, as maximum fruit production occurs at about 20 years (Rana and Sood 2012, Chander and Chauhan 2014).

The population of this important medicinal tree species is dwindling continuously due to immense population pressure, market demand, seizure, and inefficient use of forest resources. Therefore, the government, non-governmental institutions, private organizations and individual efforts have recently taken several initiatives for planting and managing species of medicinal plants along with other timber and tree species through the effective participation of people across the country. Planting system' success chiefly relies on seed germination and seedling development in the nursery. The nursery practice should be sufficiently potent to yield ample amounts of quality seedlings within a suitable lapse of time.

## MATERIAL AND METHODS

The study was conducted in the Khaggal farm nursery of the College of Horticulture and Forestry, Neri, Hamirpur situated at the intersection of latitude 31º39′59″N and longitude 76º29′57″E. The climate of the study site is sub-tropical with cold winters. According to the Köppen-Geiger climate classification, Hamirpur climate is known to be Cwa. In summers, the temperature rises to a high of 40°C and in winters, to a drop of 3-7°C with an annual average of 21.6°C. During the monsoon season (July-August) an average annual precipitation of 400-600 mm occurs. The experiment was performed in two years *viz*., 2018 and 2019. Before starting of the experiment, the seeds were collected from Nahan (HP) for the study. Three treatments were given to the seeds for improvement of germination was $T_1$: control, $T_2$: Dipping in sulphuric acid (95 per cent) for 5-10 min and $T_3$: Mechanical breaking of hard seed coat. $S_1$: March, $S_2$: June and $S_3$: July were selected for different time of sowing to examine the seed germination capacity. There were 20 seeds in each treatment, and 3 replications were included for analysis. Treatment impact was analyzed using two-way ANOVA. The fields were designed to make it loose and friable by ploughing and raking the soil up to 4-5 inches deep. In the months of March, June and July, the pre-treated seeds were sown. Light irrigation was given in the beds only after seed had been sown. Timely weeding was also done on the nursery beds. The following germination and growth parameters were recorded to observe the success of germination of harar seedlings in the nursery.

**Germination percentage and germination energy:** Germination percentage was calculated by counting the number of seeds germinated out of total number of seeds sown and expressed in percentage. The numbers of seeds germinating daily in each treatment were recorded from the date of sowing until the completion of germination to compute germination energy.

**Number of days taken to complete germination:** The dates of commencement and completion of germination were recorded to calculate number of days taken to complete germination.

**Shoot length and root length (cm):** The shoot and root lengths of seedling were measured from the collar region to the growing tip while root length was measured from the collar region to the tip of taproot, using measuring scale and expressed in centimeters. In case of coiled root, a thread was run along the root and thread length was measured to determine the root length.

**Number of leaves per plant:** Number of leaves per plant were counted by averaging total number of leaves counted in five randomly selected plants.

**Total dry biomass of seedling (gm):** Total dry weight per plant was recorded for the biomass estimation. The seedlings were dried at 80°C for 48 hours for recording dry weight. The dry weight of the seedlings was taken using electronic balance.

**Root/shoot ratio:** The root/shoot ratio was worked out on dry weight basis by dividing the weight of dry root by the weight of dry shoot of respective plant.

## RESULTS AND DISCUSSION

**Germination per cent and germination energy:** During the year 2018, the highest germination (36.67%) was shown in pre-treatment $T_3$ followed by $T_2$ (28.33%). The maximum germination (30.00%) was found in sowing time $S_2$ followed by $S_3$ (29.44%). In treatment combination, the highest germination per cent were recorded in $T_3S_2$ (Table 1). In 2019, pre-treatment $T_3$ gave maximum germination per cent (36.11%). With regard to sowing time, the highest germination (33.33%) was registered in $S_3$ which is statistically at par with $S_2$ (29.44%). With respect to factor combination (pre-sowing seed treatment and sowing time), $T_3S_3$ showed maximum germination (45.00%). The highest germination per cent was registered in $T_3$ (36.39%), whereas the maximum germination was recorded in $S_3$ (31.39%) when the data was pooled for two years. In treatment combinations, the highest germination per cent was registered in $T_3S_3$ (40.83%) statistically followed by $T_3S_2$ (40.00%).

**Plate 1.** Initial seedling growth of scarified harar seeds in July (A) and after 45 days (B)

**Table 1.** Germination per centduring the year 2018 (2019)

| Time of sowing | $T_1$ | $T_2$ | $T_3$ | Mean |
|---|---|---|---|---|
| $S_1$ | 30.00 (11.67) | 20.00 (23.33) | 31.67 (25.00) | 27.22 (20.00) |
| $S_2$ | 15.00 (21.67) | 33.33 (28.33) | 41.67 (38.33) | 30.00 (29.44) |
| $S_3$ | 20.00 (23.33) | 31.67 (31.67) | 36.67 (45.00) | 29.44 (33.33) |
| Mean | 21.67 (18.89) | 28.33 (27.78) | 36.67 (36.11) | |
| CD (p=0.05) | T= 6.20 (4.66), S= 10.75 (4.66) and T×S= NS (NS) | | | |
| Pooled data (2018 and 2019) | | | | |
| | $T_1$ | $T_2$ | $T_3$ | Mean |
| $S_1$ | 20.83 | 21.67 | 28.33 | 23.61 |
| $S_2$ | 18.33 | 30.83 | 40.00 | 29.72 |
| $S_3$ | 21.67 | 31.67 | 40.83 | 31.39 |
| Mean | 20.28 | 28.06 | 36.39 | |
| CD (p=0.05) | T= 3.43, S= 3.43 and T×S= 5.94 | | | |

$T_1$= Control, $T_2$= Sulphuric acid @ 95% concentration, $T_3$= Mechanical breaking of seed coat, $S_1$= March, $S_2$= June, $S_3$= July and NS= Non-significant

The maximum germination energy (17.33) was observed in pre-sowing treatment, $T_3$ statistically at par with $T_2$ (14.44) and $T_1$ (11.11). The highest germination energy (14.89) was found in sowing time $S_3$. In treatment combination, the maximum germination energy (18.33) was shown in $T_3S_3$ in 2018 (Table 2). During the year 2019, pre-treatment $T_3$ showed highest germination energy (19.33) and with respect to sowing time, the maximum germination energy (18.56) was recorded in $S_2$ which was statistically at par with $S_3$ (16.11). In treatment combinations, $T_3S_2$ showed highest germination energy (23.67). The maximum germination energy was registered in $T_3$ (7.33) when the data was pooled over the years. In sowing time, the highest germination energy was recorded in $S_3$ (6.39) statistically at par with $S_2$ (6.17). In factor combination (pre-sowing seed treatment and sowing time), the maximum germination energy was recorded in $T_3S_3$ (8.17).

Tigabu and Oden (2001) recorded maximum germination percent of all treatments of *Albizia grandibracteata* in scarified seeds and seeds treated with sulphuric acid. Likewise, it was found by Rashid *et al*., fruits of *T. chebula*, soaked in water for 48 hours with 10 per cent sulphuric acid subsequent treatment for 20 minutes, showed up to 70 per cent germination. Sub-treatments of $T_1$ which attained the highest germination capacity within few days of sowing had lower germination energy and have better chances of survival compared with those which had lower germination capacity (Willan 1987).

**Days taken to complete germination:** During the year 2018, the least days taken to complete germination (29.11) was observed in pre-sowing treatment $T_2$ (Table 3). With regard to sowing time, the minimum days taken to complete germination (28.11) was observed in sowing time $S_2$. With respect to factor combinations, the lowest number of days taken to complete germination (28.00) was shown by $T_1S_2$ and $T_3S_2$. In 2019, pre-sowing treatment $T_1$ showed minimum number of days taken to complete germination (28.11). In time of sowing, the least days taken to complete germination (26.11) were registered in $S_3$. With respect to treatment combination, the minimum number of days taken to complete germination (25.67) was shown in $T_1S_3$. In pooled data, the lowest number of days taken to complete germination was recorded in $T_1$ (28.83). With regard to sowing time, the minimum number of days taken to complete germination was recorded in $S_3$ (27.44). The minimum days taken to complete germination were shown by $T_1S_3$ (27.00). A high germinative capacity is indicative of high vigour and high field emergence compared with a low germinative capacity seen in delayed germination. Treatments with lower germinative capacity have lower competitive ability than early emerging one (Pourhadian and Khajehpour 2010).

## Growth Parameters

**Shoot length and root length:** During the year 2018, the highest shoot length (7.68cm) was observed in pre-sowing treatment $T_3$ (Table 4). In sowing time, the maximum shoot length (8.68cm) was found in $S_1$ statistically at par with $S_3$ and $S_2$. With regard to treatment combinations (pre-sowing seed treatment and sowing time), maximum shoot length (9.30cm) was shown in $T_3S_1$. In 2019, pre-treatment $T_2$ showed highest shoot length (5.59cm). With regard to sowing time, the maximum shoot length (5.74cm) was registered in $S_3$ statistically followed by $S_2$. In factor combination, $T_2S_3$ showed maximum shoot length (6.07cm). In pooled data of both years, the highest shoot length was registered in $T_3$ (6.58cm). With respect to sowing time, the maximum shoot length was recorded in $S_1$ (6.81cm). With regard to treatment

**Table 2.** Germination energy during the year 2018 (2019)

| Time of sowing | $T_1$ | $T_2$ | $T_3$ | Mean |
|---|---|---|---|---|
| $S_1$ | 14.00 (4.33) | 10.00 (10.33) | 17.67 (12.67) | 13.89 (9.11) |
| $S_2$ | 9.00 (13.00) | 17.33 (19.00) | 16.00 (23.67) | 14.11 (18.56) |
| $S_3$ | 10.33 (1033) | 16.00 (16.33) | 18.33 (21.67) | 14.89 (16.11) |
| Mean | 11.11 (9.22) | 14.44 (15.22) | 17.33 (19.33) | |
| CD (p=0.05) | T= 4.70 (2.62), S=NS (2.62) and T×S= NS (NS) | | | |
| Pooled data (2018 and 2019) | | | | |
| | $T_1$ | $T_2$ | $T_3$ | Mean |
| $S_1$ | 4.33 | 4.33 | 5.83 | 4.83 |
| $S_2$ | 4.00 | 6.50 | 8.00 | 6.17 |
| $S_3$ | 4.50 | 6.50 | 8.17 | 6.39 |
| Mean | 4.28 | 5.78 | 7.33 | |
| CD (p=0.05) | T= 0.70, S= 0.70 and T×S= 1.21 | | | |

See Table 1 for details

combination, the highest shoot length was shown by $T_3S_1$ (7.00cm).

In 2018, pre-sowing treatment $T_2$, registered the maximum root length (7.34cm). In sowing time, the highest root length (9.24cm) was found in $S_1$ (Table 5). With regard to factor combination, maximum root length (9.67cm) was shown in $T_2S_1$. During 2019, the highest root length was recorded in $T_2$ (6.21cm) statistically followed by $T_3$ and $T_1$. With regard to sowing time, the maximum root length (5.91cm) was registered in $S_3$. In treatment combinations, $T_2S_3$ showed maximum root length (6.80cm). In pooled data, the highest root length was registered in $T_2$ (6.81cm). In sowing time, the maximum root length was recorded in $S_1$ (7.49cm). In treatment combinations, the highest root length was shown in $T_2S_1$(7.77 cm).

Our results were supported by Olatunji et al (2012), they found the maximum shoot length value of 5.92 cm was recorded highest among seedlings that were produced from seeds treated with Sulphuric acid for 5 min. of *Acacia auriculiformis*. Dhupper (2013) compared the root length of three tree species viz., *Acacia nilotica*, *Albizia lebbeck* and *Prosopis cineraria.* The maximum root length was observed in *Prosopis cineraria* (48 cm) in hot water treatment for 30 min and lowest were in *Albizia lebbeck* (25 cm).

**Number of leaves per seedling:** During the year 2018, pre-sowing treatments $T_1$ and $T_2$ showed the maximum number of leaves per seedling (6.22). The highest number of leaves per seedling (7.33) was recorded in sowing time $S_1$ which was statistically at par with $S_3$ (Table 6). In treatment combination, the maximum number of leaves per seedling (7.67) was

**Table 3.** Days taken to complete germination during the year 2018 (2019)

| Time of sowing | $T_1$ | $T_2$ | $T_3$ | Mean |
|---|---|---|---|---|
| $S_1$ | 32.33 (30.17) | 30.00 (29.33) | 31.00 (29.33) | 31.11 (28.89) |
| $S_2$ | 28.00 (30.67) | 28.33 (30.00) | 28.00 (30.00) | 28.11 (30.22) |
| $S_3$ | 28.33 (25.67) | 29.00 (26.67) | 29.00 (26.00) | 28.78 (26.11) |
| Mean | 29.56 (28.11) | 29.11 (28.67) | 29.33 (28.44) | |
| CD (p=0.05) | T= NS (NS), S= 1.15(1.58) and T×S= NS (NS) | | | |
| Pooled data (2018 and 2019) | | | | |
| | $T_1$ | $T_2$ | $T_3$ | Mean |
| $S_1$ | 30.17 | 29.67 | 30.17 | 30.00 |
| $S_2$ | 29.33 | 29.17 | 29.00 | 29.17 |
| $S_3$ | 27.00 | 27.83 | 27.50 | 27.44 |
| Mean | 28.83 | 28.89 | 28.89 | |
| CD (p=0.05) | T= NS, S= 0.86 and T×S= NS | | | |

See Table 1 for details

**Table 4.** Shoot length during the year 2018 (2019)

| Time of sowing | $T_1$ | $T_2$ | $T_3$ | Mean |
|---|---|---|---|---|
| $S_1$ | 8.80 (5.07) | 7.93 (5.07) | 9.30 (4.67) | 8.68 (4.93) |
| $S_2$ | 6.27 (5.00) | 5.53 (5.63) | 6.90 (5.87) | 6.23 (5.50) |
| $S_3$ | 6.60 (5.40) | 7.20 (6.07) | 6.83 (5.77) | 6.88 (5.74) |
| Mean | 7.22 (5.16) | 6.89 (5.59) | 7.68 (5.43) | |
| CD (p=0.05) | T= NS, S= 1.83 and T×S= NS | | | |
| Pooled data (2018 and 2019) | | | | |
| | $T_1$ | $T_2$ | $T_3$ | Mean |
| $S_1$ | 6.93 | 6.50 | 7.00 | 6.81 |
| $S_2$ | 5.67 | 5.60 | 6.40 | 5.89 |
| $S_3$ | 6.00 | 6.63 | 6.33 | 6.32 |
| Mean | 6.20 | 6.24 | 6.58 | |
| CD (p=0.05) | T= NS, S= NS and T×S= NS | | | |

See Table 1 for details

shown in $T_1S_1$. During the year 2019, pre-treatment $T_3$ showed highest number of leaves per seedling (3.44). With regard to sowing time, the maximum number of leaves per seedling (3.78) was registered in $S_3$. With respect to treatment combination, $T_2S_3$ had maximum number of leaves per seedling (4.33). In pooled data of both years, the maximum number of leaves per seedling was registered in $T_3$ (4.89). In sowing time, the highest number of leaves per seedling was recorded in $S_3$ (5.33) statistically at par with $S_1$. In factor combination (pre-sowing seed treatment and sowing time), the maximum number of leaves per seedling was found in $T_2S_3$ (6.00). Dhupper (2013) observed the comparison between the three species *vis*., *Acacia nilotica*, *Albizia lebbeck* and *Prosopis cineraria* and showed that the maximum number of leaves produced in *Acacia nilotica* when seeds were treated with hot water (15 min).

**Total dry biomass of seedling:** In 2018, the maximum dry biomass of seedling (1.83gm) was observed in pre-sowing treatment $T_2$. In sowing time, the highest dry biomass of seedling (2.18gm) was found in $S_1$ statistically followed by $S_3$. With respect to factor combination, maximum dry biomass of seedling (2.37gm) was registered in $T_1S_1$ (Table 7). During the year 2019, pre-treatment $T_2$ showed highest dry biomass of seedling (1.44gm). With regard to sowing time, the maximum dry biomass of seedling (1.68gm) was registered in $S_2$ which was statistically at par with $S_3$. In treatment combinations, $T_2S_2$ recorded the maximum dry biomass of seedling (1.83gm). In pooled data of both years, the highest root length was registered in $T_2$ (1.64gm). In sowing time, the maximum root length was recorded in $S_3$ (1.72gm). In treatment combinations, the maximum dry biomass of seedling was registered in $T_3S_3$ (1.82gm).

**Table 5.** Root length during the year 2018 (2019)

| Time of sowing | $T_1$ | $T_2$ | $T_3$ | Mean |
|---|---|---|---|---|
| $S_1$ | 8.83 (5.67) | 9.67 (5.77) | 9.23 (5.57) | 9.24 (5.67) |
| $S_2$ | 4.73 (4.73) | 4.73 (6.07) | 5.70 (5.73) | 5.06 (5.51) |
| $S_3$ | 4.90 (5.27) | 7.63 (6.80) | 6.53 (5.67) | 6.36 (5.91) |
| Mean | 6.16 (5.22) | 7.34 (6.21) | 7.16 (5.66) | |
| CD (p=0.05) | T= NS (0.68), S= 2.19 (NS)and T×S= NS (NS) | | | |
| Pooled data (2018 and 2019) | | | | |
| | $T_1$ | $T_2$ | $T_3$ | Mean |
| $S_1$ | 7.27 | 7.77 | 7.43 | 7.49 |
| $S_2$ | 4.77 | 5.43 | 5.73 | 5.31 |
| $S_3$ | 5.10 | 7.23 | 6.13 | 6.16 |
| Mean | 5.71 | 6.81 | 6.43 | |
| CD (p=0.05) | T= NS, S= 1.01 and T×S= NS | | | |

See Table 1 for details

**Table 6.** Number of leaves per seedling during the year 2018 (2019)

| Time of sowing | $T_1$ | $T_2$ | $T_3$ | Mean |
|---|---|---|---|---|
| $S_1$ | 7.67 (2.33) | 7.00 (2.00) | 7.33 (2.33) | 7.33(2.22) |
| $S_2$ | 4.67 (2.67) | 4.67 (2.33) | 4.33 (4.33) | 4.56 (3.11) |
| $S_3$ | 6.33 (3.33) | 7.00 (4.33) | 6.00 (3.67) | 6.44 (3.78) |
| Mean | 6.22 (2.78) | 6.22 (2.89) | 5.89 (3.44) | |
| CD (p=0.05) | T= NS (0.50), S= 1.27 (0.50) and T×S= NS (0.86) | | | |
| Pooled data (2018 and 2019) | | | | |
| | $T_1$ | $T_2$ | $T_3$ | Mean |
| $S_1$ | 5.33 | 4.67 | 5.00 | 5.00 |
| $S_2$ | 4.00 | 3.67 | 4.67 | 4.11 |
| $S_3$ | 5.00 | 6.00 | 5.00 | 5.33 |
| Mean | 4.78 | 4.78 | 4.89 | |
| CD (p=0.05) | T= NS, S= 0.71 and T×S= NS | | | |

See Table 1 for details

**Table 7.** Total dry biomass of seedling during the year 2018 (2019)

| Time of sowing | $T_1$ | $T_2$ | $T_3$ | Mean |
|---|---|---|---|---|
| $S_1$ | 2.37(0.93) | 2.13(1.17) | 2.03(0.87) | 2.18(0.99) |
| $S_2$ | 1.27 (1.62) | 1.17(1.83) | 1.37(1.60) | 1.27(1.68) |
| $S_3$ | 1.67 (1.50) | 2.20(1.32) | 1.93(1.70) | 1.93(1.51) |
| Mean | 1.77(13.5) | 1.83(1.44) | 1.78(1.39) | |
| CD (p=0.05) | T= NS (NS), S= 0.44(0.21) and T×S= NS(NS) | | | |
| Pooled data (2018 and 2019) | | | | |
| | $T_1$ | $T_2$ | $T_3$ | Mean |
| $S_1$ | 1.65 | 1.65 | 1.45 | 1.58 |
| $S_2$ | 1.44 | 1.50 | 1.49 | 1.48 |
| $S_3$ | 1.59 | 1.76 | 1.82 | 1.72 |
| Mean | 1.56 | 1.64 | 1.59 | |
| CD (p=0.05) | T= NS, S= NS and T×S= NS | | | |

**Table 8.** Root-Shoot ratio during the year 2018 (2019)

| Time of sowing | $T_1$ | $T_2$ | $T_3$ | Mean |
|---|---|---|---|---|
| $S_1$ | 1.00 (1.13) | 1.21(1.14) | 0.97 (1.22) | 1.06 (1.16) |
| $S_2$ | 0.86 (0.95) | 0.85 (1.07) | 0.83 (0.98) | 0.85 (1.00) |
| $S_3$ | 0.73 (0.97) | 1.10 (1.12) | 0.96 (0.98) | 0.93 (1.03) |
| Mean | 0.86 (1.02) | 1.05 (1.11) | 0.92 (1.06) | |
| CD (p=0.05) | T= NS (NS), S= 0.16 (0.13)and T×S= NS (NS) | | | |
| Pooled data (2018 and 2019) | | | | |
| | $T_1$ | $T_2$ | $T_3$ | Mean |
| $S_1$ | 1.07 | 1.18 | 1.10 | 1.11 |
| $S_2$ | 0.91 | 0.96 | 0.91 | 0.93 |
| $S_3$ | 0.85 | 1.11 | 0.97 | 0.98 |
| Mean | 0.94 | 1.08 | 0.99 | |
| CD (p=0.05) | T= 0.09, S= 0.09 and T×S= NS | | | |

See Table 1 for details

Amira et al (2011) conducted a study on the effects of Sulphuric acid and hot water pre-treatments on seed germination and seedling growth of *Cassia fistula L.* and resulted that the maximum dry weight per plant (3.46, 3.70 and 3.38 gm/plant) was recorded with hot water treatments, in the 1st season with significant differences between them.

**Root-Shoot ratio:** During the year 2018, the maximum root-shoot ratio (1.05) was observed in pre-sowing treatment $T_2$ (Table 8). In sowing time, the highest root-shoot ratio (1.06) was found in $S_1$ which was statistically followed by $S_3$ and $S_2$. With regard to factor combination (pre-sowing seed treatment and time of sowing), the maximum root-shoot ratio (1.21) was shown in $T_2S_1$. In 2019, pre-treatment $T_2$ showed highest root-shoot ratio (1.11). With regard to sowing time, the maximum root-shoot ratio (1.16) was registered in $S_1$ statistically at par with $S_3$ and $S_2$. In treatment combinations, $T_3S_1$ had the maximum root-shoot ratio (1.22). In pooled data, the highest root-shoot ratio was registered in $T_2$ (1.08) that was statistically followed by $T_3$ and $T_2$. In sowing time, the maximum root-shoot ratio was recorded in $S_1$ (1.11). In treatment combinations, the maximum root-shoot ratio was registered in $T_2S_1$ (1.18). Paliwal and Kannan (2000) analyzed the morphological and nutritional characteristics of four MPTs. Seedlings of *Adenanthera pavonina* had a higher root-shoot ratio than the seedlings of other species in his study.

## CONCLUSIONS

The presence of hard endocarp of *T. chebula* takes a long time to germinate hence nursery establishment is a time-consuming and less productive phase in the prospective large-scale planting programs for the crop. Softening and mechanical breaking of seed coat is then requisite to allow the embryo to get out quickly and safely.

The significant thing about prescribing a pre-sowing treatment is that growing ample seedlings with minimal expense and labour should suit to the farmers. It is assumed that this research has a wider potential to be utilized in the field as it uses easy mechanical breaking of seed coat and soaking in sulphuric acid for a few minutes, resulting in high germination percentage (40.83%), high germination energy (8.17) and low germination time (27 days). Furthermore, these pre-treatments culminated in a higher shoot length (7.00 cm), root length (7.77 cm), number of leaves per seedling (6.00), total dry biomass (1.82 g), and root-shoot ratio (1.18).

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# Determinants of Transfer of Agricultural Techniques to Farmers by Agricultural Extension Workers in Nineveh Governorate, Iraq

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***Abstract*:** This research aims to identify the constraints in transfer of agricultural technologies in and correlate with independent variables (age, position, academic achievement academic specialization guidance service, in-work train in delivering agricultural technologies, and participation in delivering agricultural technologies). The random sample was chosen with 25% of the total 70 employees. The he obstacles of delivering agricultural technologies in Nineveh governorate from agricultural workers view is moderate and the results explained also that the first problem of delivering agricultural technologies was low use many agricultural technologies because of lack of educational activities of agricultural guidance among farmers. The age, academic achievement and total guidance effect in defining problems and transfer of technologies to farmers.

***Keywords***: Agricultural techniques, Agricultural extension, Framers, Iraq

Agriculture plays a major role in Arabic world life as an income source and is the main source of providing food and raw materials for industries related to agricultural products (Al-Zaidi and Ashoak 2016). Agriculture represents an investment of fragile natural resources and failure to invest these resources will decline their natural features and value. The agriculture constitutes a vital part of economic activity of any country thus developing it is a key factor in developing any country's economy (Al-Shaabraki 2017, Dawood and Luma Monther 2020). Agricultural development can happen through developing precise agricultural technology with adoption of agricultural innovations and technology by farmers. In order to achieve agricultural development the farmers must be educated about modern technologies that are economically useful (Al-Sharrif 2013).Agricultural and human development is pillars of comprehensive development in both developed and developing countries. Human resources that comprehends and apply modern agricultural technologies efficiently and skillfully is the basic element introducing and development modern technologies (AL-Salhi 2016). This process needs transfer, demonstration and monitoring the acceptability of technology (Abdull 2015). Process of technological change is a dynamic response of economic and social circumstances.Technological breakthrough has always been the path of agricultural development (Ali and Mohammed 2017). The economic and technological shifts in agriculture in Iraq recently necessitates developing new means in production and enhance field experiences of farmers particularly in increasing production and managing resources (Ali 2016). Some assume that farmers knowledge is primitive or unscientific or hurdles development and therefore need based research and guidance must change their prejudice for development (Zakaria et al 2013). Naturally farmers are considered about economical aspect and not give any priority to the management of environment or judicious use of natural resources in the adoption of new technology in developing countries and face numerous problems due to various factors which also include the cost of adoption of new technology and risk factor (Mardini and Tony 2015). So agricultural guidance must address this issue. (Altalb 2017, Alhafidh 2019). The communication levels between research and guidance institutes lead in consequently for dominance of random opinions (Ngonga 2016). The more difference of opinions between those two institutes, the more rejection to adopt modern agricultural technologies. Results showed wide time gap between discovering modern technologies and adoption (Mardini and Tony 2015). The present study aimed at to understand the obstacles of introducing agricultural technologies in agricultural divisions in Nineveh and find correlation between obstacles of delivering agricultural technologies and the independent variables in this study.

## MATERIAL AND METHODS

The research was conducted at Nineveh Governorate, small random sample was chosen which constitute 25% of total number 70 employees. The data was collected by survey, includes all independent variables of agricultural extension employees (age, position, academic level, academic specialization, time spent in agricultural extension, in transfer of agricultural technologies, participation in transfer of agricultural technologies). The second part with 31 items/questions represents obstacles of transfer agricultural technologies in Nineveh governorate. Third part included suggestion to develop delivering agricultural technologies in Nineveh governorate.Dependent variables obstacles of transfer agricultural technologies and delivering agricultural technologies were based on five alternative replies , abundantly available (5), available (4), moderately available (3), few (2)and un available (1). By collecting answers on all items by respondents will get the total degree of extension workers and this number represents value of total obstacles which facing transfer of agricultural technologies from perspective of agricultural workers in Nineveh governorate.

## RESULTS AND DISCUSSION

**Obstacles of agricultural technologies transfer:** The highest value of extension workers answers about the obstacles was 137) and the least (46) with an average of 8o. Workers were divided to three categories regarding to the obstacles of transfer agricultural technologies in the agricultural branches in Nineveh governorate in general (Table 1). The highest rate of extension workers answers about the obstacles of agriculture technologies transfer was in middle category (77-107) scoring 54.30%. The high and low category (108-137), (46-76) scored 16%, for each one, meaning that obstacles of delivering agricultural technologies to farmers in Nineveh governorate from the point of view of agricultural workers are moderate due to the circumstances that agricultural sector an passed, through the region in particular and Iraq in general

**Ranking of obstacles in transfer of agricultural technologies according the average:** The first three obstacles (problems) of agricultural technologies transfer according to the answer of extension workers was low level of farmers adoption in the use many agricultural technologies because the weakness of agricultural extension activities, failure to provide some of input (such as machinery, seeds and fertilizers) at the time that farmers need it, which affects in negatively transfer of technology. The absence of coordination between scientific research institutes and the of absence of a clear formula for coordination between scientific research bodies and the directorate of Agriculture and non-availability of some agricultural process inputs like machines ,seeds, fertilizers. These obstacles are the most tangible obstacles in delivering agricultural technologies. The use of methods for the transfer of agriculture technologies is not suitable for rural of farmers due to the use of old concepts and methods

**Correlation between obstacles of delivering agricultural technologies and independent variables of research**

**Age:** The highest age of extension workers was 62 years and lowest was 23. Researches were categorized in to three divisions according to age of workers and its relation to obstacles of delivering agricultural technologies (Table 3). The maximum numbers of researchers fall in low category of 23-35 years (63), There is negative l correlation between obstacles of agricultural technologies transfer and age. Simple correlation conjunction factor of Pearson (-0.50) was significant indicating that employees age is related to obstacles of delivering agricultural technologies. The younger the employee is the more competitive he is in determining obstacles of transfer agricultural technologies to farmers

**Academic achievement:** The highest researches were grduates (45.7%) followed by secondary gradates. The master holders were 15.7%. The Spearman rank correlation between obstacles of delivering agricultural technologies academic achievement was significant (0.278) indicating that academic achievement and obstacles of delivering agricultural technologies related. The higher academic level of researches has more ability to determine these obstacles (Table 4).

**Position of extension workers:** Thee highest number was in region center (55.70%) followed by governorate center and district center. The Spearman rank correlation factor between obstacles of delivering agricultural technologies and position was 0.030 which shows that position doesn't affect deliverance of agricultural technologies (Table 5).

**Academic specialization** The highest rate was of workers was of non-specialized in agricultural guidance (78.60). There was no correlation between obstacles delivering agricultural technologies and academic specialization. Spearman rank correlation factors (0.027) indicate that academic specialization isn't related to obstacles of delivering agricultural technologies (Table 6).

**Table 1.** Respondents to the categories regarding to obstacles of agricultural technologies transfer

| Categories of extension workers | Number of researchers in sample | Per cent |
|---|---|---|
| Low (46 -76) | 16 | 22.85 |
| Medium (77 - 107) | 38 | 54.30 |
| High (108 – 137) | 16 | 22.85 |
| Total | 70 | 100 |

Correlation coefficient: -0.50

**Table 2.** Categorizing obstacles of transfer of agricultural technologies

| Average of obstacles | Questions | Rank | Rank of according to average |
|---|---|---|---|
| 4.23 | The low level of farmers efficiency in the use many agricultural technologies because the weakness of agricultural extension activities. | 17 | 1 |
| 4.10 | Failure to provide some of input of the agricultural process (such as machinery. seeds and fertilizers... Etc.) at the time that farmers need it. which affects in negatively to the transfer of technology. | 2 | 2 |
| 3.65 | The absence of a clear formula for coordination between scientific research institutes and the Directorate of absence of a clear formula for coordination between scientific research bodies and the Directorate of Agriculture in the province Agriculture in the province. | 18 | 3 |
| 3.41 | Attention to the crop production and without attention in the human aspects in some times. | 31 | 4 |
| 3.42 | There is no long strategic plan for agricultural extension in the field of agricultural | 5 | 5 |
| 3.34 | Most of articles specializing in the transfer of agricultural technologies have not actually been applied in a similar environment to that of the targeted o rural areas. | 26 | 6 |
| 3.29 | Weak the funding and financial support to the scientific research movement and agricultural sector in the governorate. | 8 | 7 |
| 3.25 | There is no a responsible institute. either the university or the agricultural administration. is concerned with evaluating the transfer of modern of agricultural technologies. | 16 | 8 |
| 3.22 | There is a gap between research which aimed to developing of agricultural technologies and applying the results of that field research. | 21 | 9 |
| 3.19 | Poor the follow-up of agricultural researchers for the results of new agriculture technologies which have been developed and adopted it by farmers. | 30 | 10 |
| 3.10 | The small number of workers in agricultural extension in the Ninawa Governorate compared with the number of farmers | 10 | 11 |
| 3.09 | The irregular distribution of agricultural guides in the Governorate. which is not commensurate with the rural areas in the research region. | 29 | 12 |
| 3.08 | There is no plan to transfer communicate the problems of farmers in the agriculture to the scientific research institutes and vice versa. | 7 | 13 |
| 3.07 | They don't depend on participatory approach in the adoption with farmers in the planning and implementation of the programs for the transfer of agricultural technologies | 27 | 14 |
| 3.06 | Most agricultural extension workers work in tasks not agricultural extension which leads to some losing their extension identity. | 28 | 15 |
| 3.05 | Lack of confidence for some researchers on the importance of role of agricultural extension | 6 | 16 |
| 3.04 | Lack of research institutes at the region level suitable for the work in agricultural research | 2 | 17 |
| 3.03 | The high prices of some agricultural technologies that agricultural extension is trying to spread between farmers. which affects in negative side in the process of transfer these technologies | 15 | 18 |
| 3.02 | Poor participation the university teachers in planning for most of the extension activities in the province | 9 | 19 |
| 3.01 | Lack of communication between farmers and agricultural extension on the one side and agricultural programs on the other side | 22 | 20 |
| 3 | Not give the required attention to the local leaders as an important link in the transfer and dissemination of modern of agricultural technologies | 14 | 21 |
| 2.98 | Weakness in the preparation and management of extension programs for the transfer of agricultural technologies to their region area | 24 | 22 |
| 2.95 | E lack of coordination between the Faculty of Agriculture and forestry and the agricultural sectors especially in the identifying the problems suffered by the Ninawa province. which effects on the transfer of agricultural techniques | 3 | 23 |
| 2.94 | absence of specialization for the most of extension workers in the agricultural extension in the Ninawa Governorate | 11 | 24 |
| 2.93 | Lack of benefit from agricultural research results in scientific research bodies by agriculture departments | 5 | 25 |
| 2.91 | The lack of agricultural television broadcasting programs that are interested transfer and spreading agricultural extension aspects and publishing agricultural technologies | 20 | 26 |
| 2.85 | The lack of acceptance of the percentage of farmers for modern ideas and methods in agriculture hinders the process of transportation | | 27 |
| 2.84 | Some of the teaching staff went to conduct research for scientific promotion purposes and not for the purpose of producing new agricultural technologies | 13 | 28 |
| 2.83 | There is no match with some of new technologies that farmers have already been adopted | 25 | 29 |
| 2.76 | Farmers' distrust with the agricultural extension system | 23 | 30 |
| 2.38 | Use of methods for the transfer of agriculture technologies is not suitable for the reality of the rural of farmers | 19 | 31 |

**Time spent in agricultural extension:** Thee highest number of year in guidance service agricultural employees in Nineveh administrate of agriculture was 16 years while the least was 1. Researches were categorized into three types according to years of guidance service (Table 7). The highest rate was in low category 1-6 reaching 55.70% and the lowest was 7.15% in high category 13-18. The correlation between obstacles delivering agricultural technologies and years of was significant (0.312) indicating relationship between years of guidance service and obstacles of delivering agricultural technologies.

**Training on the transfer of agricultural technologies:** Extension workers were ranked according to their training in the transfer of agricultural technologies. The highest number in training courses was 10 while the lowest was 2 (Table 8). The highest number researchers who trainees in directorate of agriculture in Nineveh was in category 5-7 (50%) and minimum in 8-10. There was no correlation between obstacles of delivering agricultural technologies and in-term training. Spearman rank correlation factor (1.000) indicates that in-term training to deliver agricultural technologies is not related to obstacles of delivering agricultural technologies

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## CONCLUSIONS

Lack of efficient farmers who can use agricultural technologies affects negatively transfer of technologies. Lack of coordination between academic agriculture and administrate of agriculture centers in the Nineveh Governorate and age, academic achievement, rank and total years in office matter of scientific personnel affect the transfer of technology.

**Table 3.** Categorizing of researchers according to their age and its relationship with obstacles

| Categories of age for extension workers (year) | Number | Percent | Correlation |
|---|---|---|---|
| Low (23-35) | 44 | 63 | 0.50- |
| Medium (36-48) | 17 | 24 | |
| High (49-62) | 9 | 13 | |

**Table 5.** Effect of position and relationship with obstacles of transfer of agricultural technologies

| Categories | Number | Percent | Correlation |
|---|---|---|---|
| District center | 6 | 8.60 | 0.030 |
| Region centre | 39 | 55.70 | |
| Governorate center | 25 | 35.70 | |

**Table 7.** Categorizing respondents according to the time spent in agricultural extension and relationship with obstacles of transfer of agricultural technologies

| Categories of time spent in agricultural extension | Number | Percent | Correlation |
|---|---|---|---|
| Low (1-6) | 39 | 55.70 | 0.312** |
| Medium (7-12) | 26 | 37.15 | |
| High (13-18) | 5 | 7.15 | |
| Total | 70 | 100 | |

**Table 8.** Categorizing of extension workers according to their training in the transfer of agricultural technologies and relationship with obstacles of agricultural technologies transfer

| Categories of interns in transfer of agricultural technologies | Number | Percent | Correlation |
|---|---|---|---|
| Low (2-4) | 25 | 35.70 | 1.000 |
| Medium (5-7) | 35 | 50 | |
| High (8-10) | 10 | 14.30 | |

**Table 4.** Categorizing workers of extension according to academic achievement and its relation with obstacles

| Categories of academic achievement for extension workers | Number | Per cent | Correlation |
|---|---|---|---|
| Secondary graduate agriculture | 16 | 22.90 | 0.278 |
| Agriculture institution | 11 | 15.70 | |
| Gradate holders | 32 | 45.70 | |
| Master | 11 | 15.70 | |

**Table 6.** Effect of categories according to academic specialization and relation to obstacles delivering agricultural technologies

| Categories of academic specialization for workers | Number | % | rs (value of correlation) |
|---|---|---|---|
| Specialized in agricultural extension | 15 | 21.40 | 0.027 |
| Non specialized in agricultural extension | 55 | 78.60 | |
| Total Number | 70 | 100 | |

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