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# Comparison and Extraction of Watershed Using Arc Swat and Arc Hydro from High Resolution Digital Elevation Model

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**Abstract:** The watershed modelling is carried out using two different modelling modules for extraction of streams and associated watershed area. Arc swat tool from SWAT and Arc hydro tool are used for watershed modelling. High resolution Cartosat‑1 DEM with 10m resolution is used to delineate the accurate watershed. The study aims in modelling two different watersheds using two different modules with same dataset provided for modelling. Both modules provide different way of processing the DEM and extraction of watershed. The performance of both Arcswat and Archydro differs in watershed extraction. The Arcswat module allows to integrate information such as land use and soil type and optimal for small watershed studies. Arcswat automatically performs operations while Archydro provides manual options for extraction of watershed. Archydro tools are optimal for large watershed studies and easy for managing datasets for modelling. The study also includes three dimensional watershed analysis by deriving contours, TIN model and draping of stream networks over the terrain surface using satellite imagery as base layers.

**Keywords**: Hydrological analysis, Watershed modelling, Arc swat, Arc hydro, GIS

Water is one of the most significant natural resources which is the reason for life existence in this planet. Earth is called as blue planet because the existing water accounts for 70 per cent of the earth's surface. Hydrology is the science that explains about the occurrence, distribution, movement and properties of the water on the earth and their interaction with the environment. Modern hydrology includes modern mathematical models for study of difficult hydrological processes including advanced techniques such as remote sensing and GIS for estimating water resources, hydrological modelling, quality and quantity parameters, etc. Remote sensing technologies such as SAR and LIDAR replaced traditional surveying techniques (Merwade *et al*., 2008). In recent years, Geographical Information Systems (GIS) are being used to support hydrological models and water resources management more efficiently using high end technology such as microwave remote sensing, hyperspectral remote sensing methods and use of LIDAR, etc. Remote sensing and GIS software such as ArcGIS, Envi, Erdas are capable of handling large datasets and efficiently performing hydrological functions and modelling (Bunting *et al*., 2013). Image processing is also an important part of remote sensing in which spectral characteristics of the satellite sensor products are utilized for change detection, flood assessment, water index calculation, etc. GIS based modelling and decision support system is used in watershed management (Sun *et al*., 2016). Multiple data from various locations are integrated easily on condition of data frame under same spatial conditions. GIS is used to create complex database in which watershed characteristic is digitally represented for hydrologic modelling. GIS and remote sensing makes hydrologic analysis less time consuming and economic. Watershed is modelled from high resolution DEM using Arc hydro and Arcswat modelling tools. Both models are provided with similar input for modelling the watershed. The objective of the study is to extract watershed from high resolution digital elevation models by using two different modelling tools and comparing the models for suitable watershed analysis. The study illustrates the capabilities of Archydro and Arcswat for effective modelling of the watershed and different applications. Watershed study provides a powerful way of water conservation and management. Accurate and suitable models for different watersheds provide an efficient way of sustainability and management of watershed and its based ecosystem.

## MATERIAL AND METHODS

**Data Used:** Indian Remote Sensing (IRS) launched a sequence of satellite system for monitoring natural resource's especially water based features. Resourcesat, Cartosat used in the study, are among the satellite systems maintained by IRS. The data used in the study are :‑

Cartosat‑1 DEM (10m Resolution), Resourcesat 2 LISS IV (5.8m Spatial Resolution), Fused Resourcesat 2 and Cartosat‑1 data. Land‑Use and Land‑Cover map

**Study Area:** The study area covers the Tirupati and Western

side of Tirupati, Chittoor district, Andhra Pradesh, India. The area of the site is approximately 38.5 km$^2$. The study area covers 13˚ 41' 60" to 13˚ 20' 60" N latitude, 79˚ 6' 36" to 79˚ 27' 36" E longitude. Fig 1 illustrates the geographical location of the study area.

**Methodology:** The flow diagram illustrates sequence in Archydro watershed modelling. Using Cartosat–1 DEM, pre-processing of terrain is done by levelling the DEM. Usual operation such as filling DEM, creating flow direction and flow accumulation raster is done. Slope raster is created with use of raw DEM data (Oikonomidis et.al, 2015). Topographic parameters such as slope, aspect, and stream/drainage are derived from Archydro tools. Terrain morphology tools of Archydro provide options to calculate the drainage area characterization, drainage boundary definition. Drainage line, longest flow path and catchment grid can also be extracted by using Arc hydro tools. Arc swat tools, watershed modelling is derived using watershed delineator tool, in which automatic watershed delineation process is initiated. DEM data is required for delineating the watershed. Arcswat provides option to use DEM or predefined watershed and streams as input. DEM based method is applied in watershed delineation (Luo *et al*., 2011). Flow direction and accumulation raster are created. Streams and outlets are delineated and defined respectively from the provided data.

Outlet and inlet are defined and edited manually. Watershed outlet is defined after defining the outlets, creating watershed model. Further processing such as HRU analysis can be done using suitable data such as land use data, soil data and slope data.

**Hydrology tools and Software**: Surface water modelling is supported by ArcGIS Hydrology Toolset (Spatial analyst), ArcScene 3D analyst is used to extrapolate surface visualisation of the study area and Arcswat is used to delineate automated watershed. The main models used largely using GIS and remote sensing includes ArcSWAT and Archydro for stream delineation and for watershed estimation (Vimal et.al, 2012). By default, basic hydrological tools are present in ArcGIS and are used for basic operation such as filling the DEM, calculation of flow direction and accumulation. Integration of hydrologic models with spatial database enables to perform modelling more accurately (Kulkarni *et al*., 2013). Hydrological tool present in the software is based upon mathematical models and modern algorithms for accurate assessment of watershed. Spatial analysis has huge impact on hydrological operations (Khatami *et al*., 2014). Complex hydrological modelling tasks are simplified using GIS and remote sensing techniques.

**Arc hydro tool:** Arc hydro is also an extension module of ArcGIS used for creating, manipulating and representing
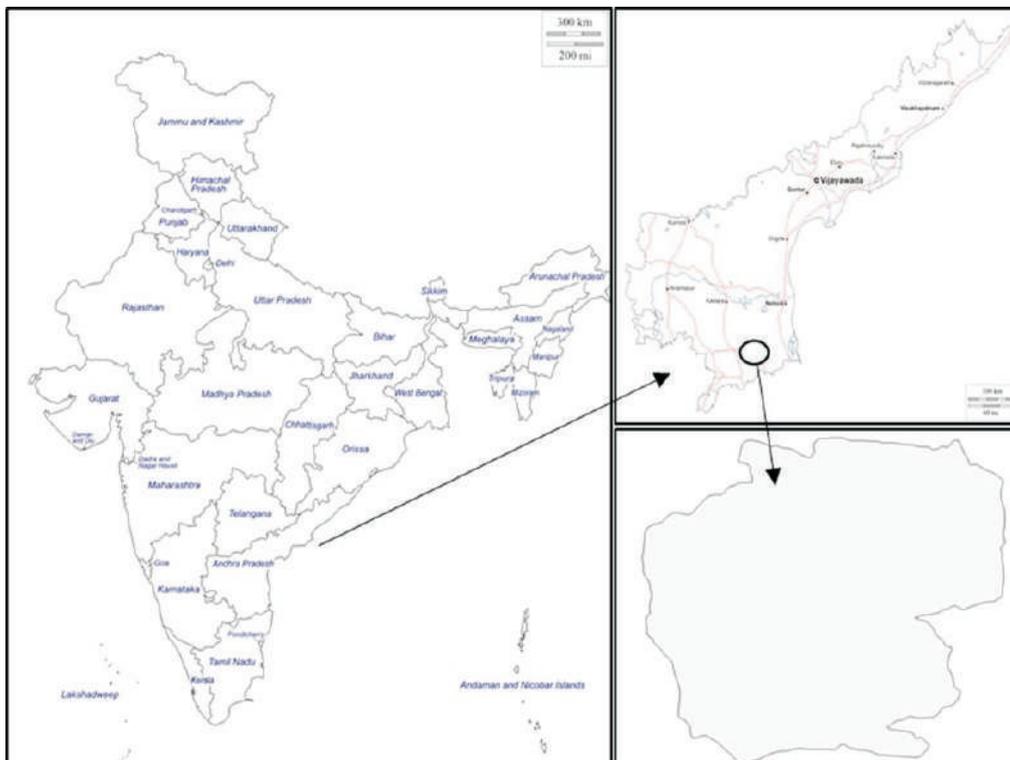


**Fig. 1.** Study area

hydrologic features to improve temporal and geospatial analysis (Khatami *et al.*, 2014). Arc hydro has an advantage when it comes for watershed modelling. It has the capability of modelling large watersheds accurately. Archydro provides number of operations and liberty in watershed modelling. Set of operations/tools are provided for creating and delineating watershed with use of DEM data. Complete profile for streams and drains are modelled using Archydro (Mantasb *et al.*, 2016). It also allows the GIS scripting users to enhance the process by modifying the functionality using python. The

Archydro tools are used to extract watershed through a set of operations. The voids in the DEM are filled and flow direction, flow accumulation raster's are generated using the slope information extracted from the digital elevation model. The stream is generated and stream order is defined to extract the basins and associated sub-basins in the watershed. In the study, watershed modelling is performed using Archydro tools associated with ArcGIS. The DEM is leveled and sinks are filled to avoid errors. Operations such as flow direction and flow accumulation are performed to delineate the
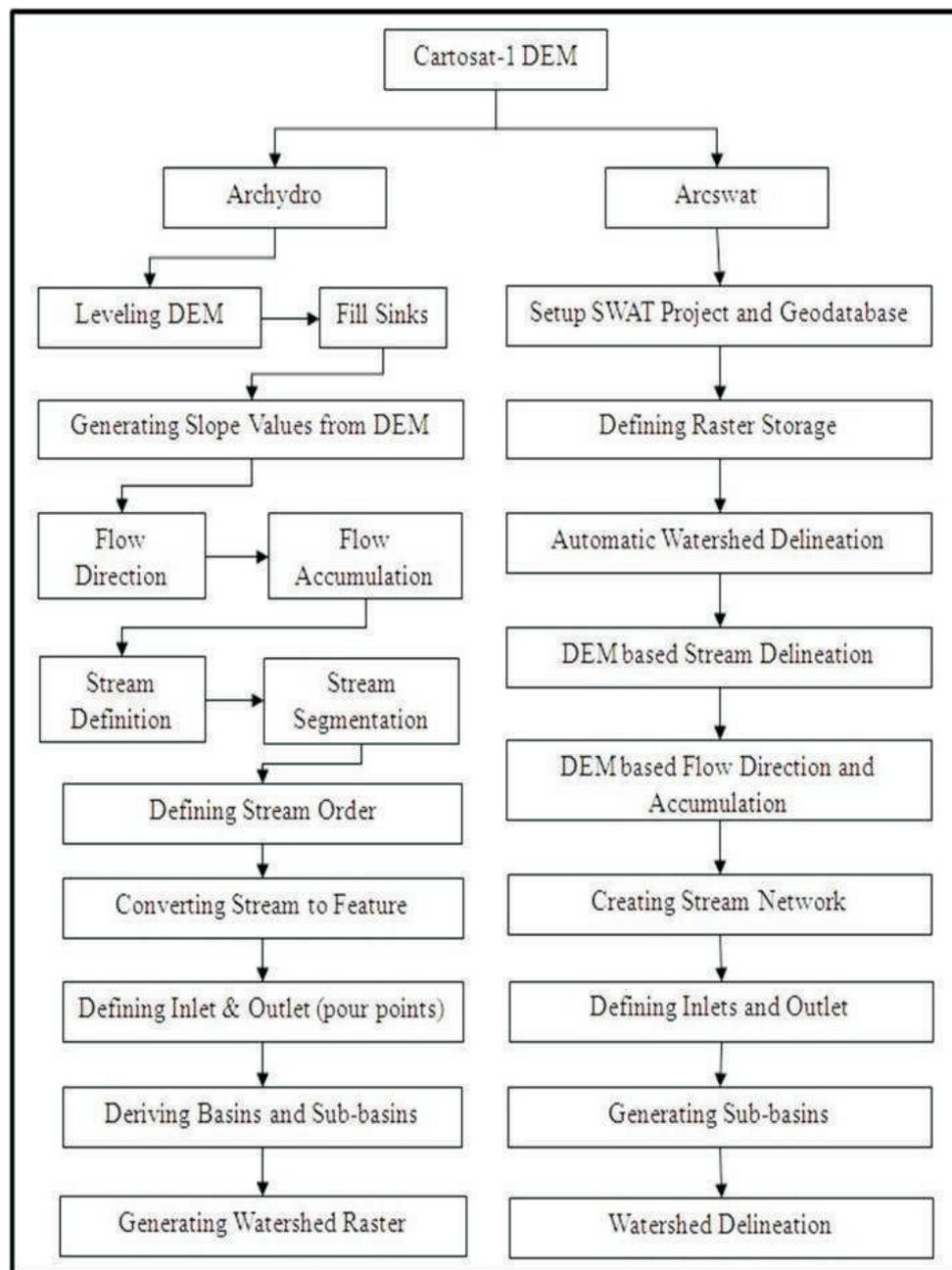


**Fig. 2.** Watershed methodology using Archydro and Arcswat

streams. Based on the streams, basins are generated and watershed is modelled. Figure 3 illustrates the delineated watershed using Arc hydro watershed model.

**Arcswat tool:** Soil and water assessment tool combine with GIS is used to delineate stream and watershed (Briak *et al*., 2016). Arcswat is an extension module of SWAT Soil and Water Assessment Tool used for hydrologic operations like watershed delineation, water quality and quantity, stream flow, etc. Arcswat provides options including physical parameters, deriving effective and accurate models. Automated processing of data is enabled in ArcSWAT that allows the user to feed in specific data for modelling. Land use land cover data, soil data and precipitation data are integrated into modeling by user of ArcSWAT. It has the capability of running simulations over a defined period of time.

**Watershed:** The use of high resolution data results in better topographic information derived from it (Cook *et al*., 2009). The study uses Cartosat‒1 generated DEM for watershed extraction. Streams are generally formed in watershed due to saturated surface and precipitation resulting in surface runoff (Jin *et al*., 2015). The streams are generated using flow direction and flow accumulation raster using the surface elevation changes derived from the DEM. The inlet and outlet of the watershed is defined after stream is generated. Watershed is generated with relevance to the defined inlet
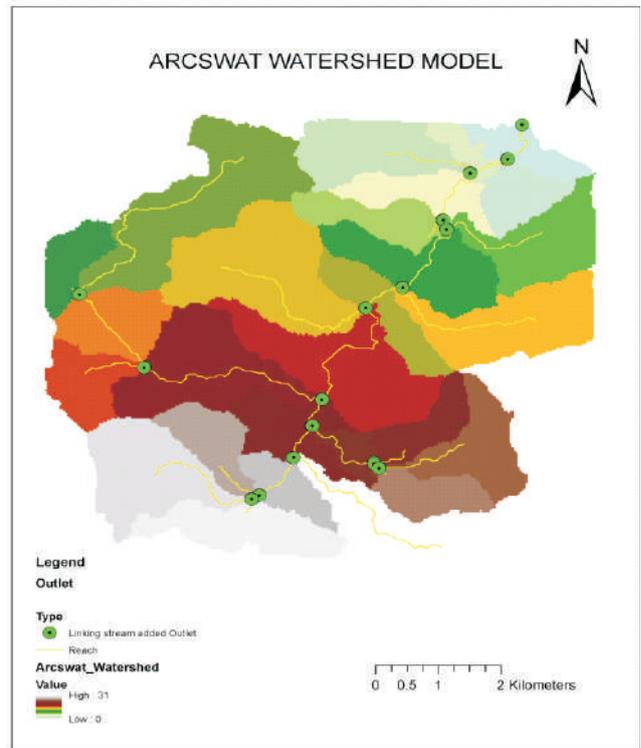


**Fig. 4.** Arcswat watershed model
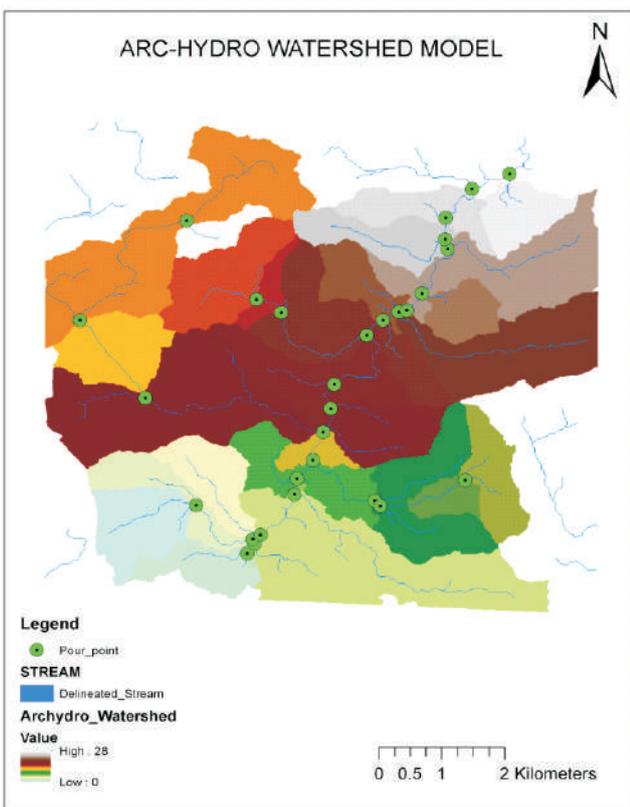
and outlets. Delineation of watershed has application such as monitoring surface water features, forecasting floods, groundwater studies, etc (Sharma *et al*., 2014). Watershed characteristics such as drainage area, catchment, watershed slope, elevation can be derived with use of GIS (Ames *et al*., 2008).

**Land-use and Land-cover:** Remote sensing products such as IRS Resourcesat 2 LISS IV, Fused Resourcesat 2 and Cartosat 1 are used to identify the land use and land cover pattern. Such land use and land cover information provides a detailed report of regions that are more prone to flood, drought, etc. Physical, biological, economic and social factors affect the land use considerably (Onate‒Valdivieso *et al*., 2010). Reclassification of existing land use classes into desired groups results in better information on estimation of any disaster occurrences and damage assessment. Temporal analysis can be carried out for detecting the change in land use and land cover and to shows the status of surface water availability/change in an area and better management of resources (Subbu Lakshmi and Yarrakula, 2016, Butt *et al*., 2015). Figure 5 shows different LULC classification such as Agriculture lands, Barren rocks, Built‒up/urban regions, lakes and ponds, crop land, forest areas, mining and industrial areas, river and sandy regions.

**Contour, DEM and TIN models:** Contour are continuous



**Fig. 3.** Archydro watershed model

lines that starts from a point and ends at the same point and continuous over a similar level of elevation over the terrain. Figure 9 illustrates the contour, DEM and TIN map. Digital elevation model (DEM) is a model for 3D representation of an area/ terrain. It is a simple, equally distributed elevation grid, generated by interpolation of surface height by using satellite or remotely sensed products. In the present study, Cartosat 1 generated DEM is used for modelling. DEM derived from LIDAR and high resolution sensors provide high quality DEM and advantageous over other DEMs (Burke *et al.*, 2016). Triangular Irregular Network model (TIN) is a simple alternative for DEM. A raster created by connecting the vertices of points generated from DEM forming 3D surface morphology of the terrain. TIN is based upon delaunay triangulation, majorly used for analysis of surface slope and aspect. High resolution DEM provides dense network of TIN.

**Table 1.** IRS remotely sensed data products

| Satellite | Sensor | Launch date |
|---|---|---|
| IRS-P6/Resourcesat-1 | LISS-4, LISS-3, AWIFS-A, AWIFS-B | 2003 |
| Cartosat-1 | PAN FORE, PAN-AFT | 2005 |
| Cartosat-2 | PAN | 2007 |
| Cartosat-2A | PAN | 2008 |
| Oceansat-2 | OCM, SCAT, ROSA | 2009 |
| Cartosat-2B | Panchromatic camera (PAN) | 2010 |
| Resourcesat-2 | LISS-3, LISS-4, AWIFS | 2011 |

*Source:* Indian Space Research Organization (ISRO)



**Fig. 6.** TIN model



**Fig. 5.** Land use and Land cover map



**Fig. 7.** Surface elevation profile of different DEMs

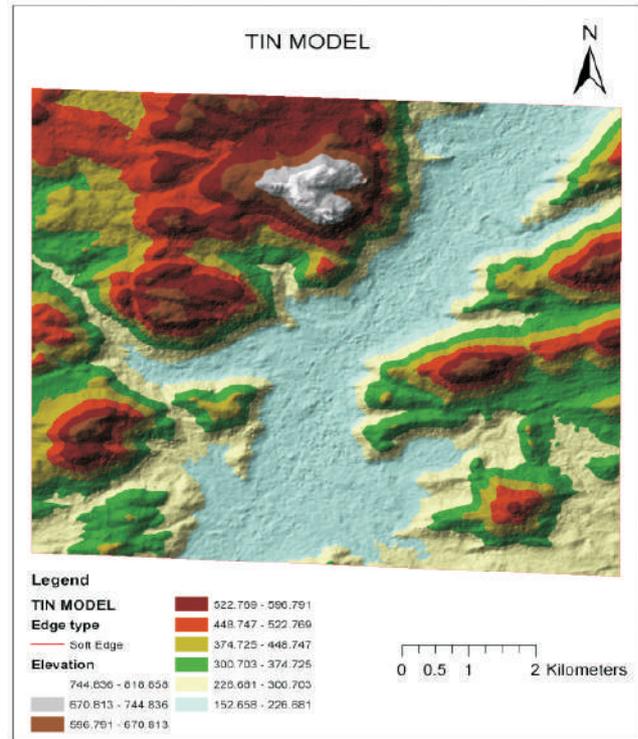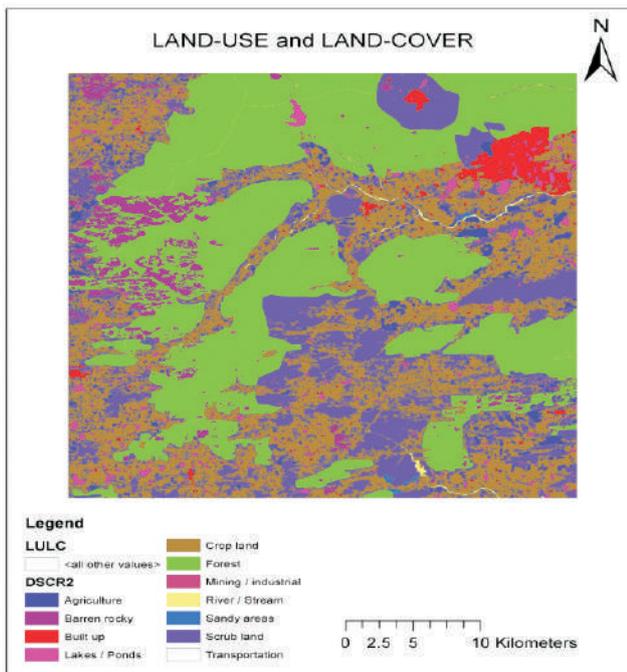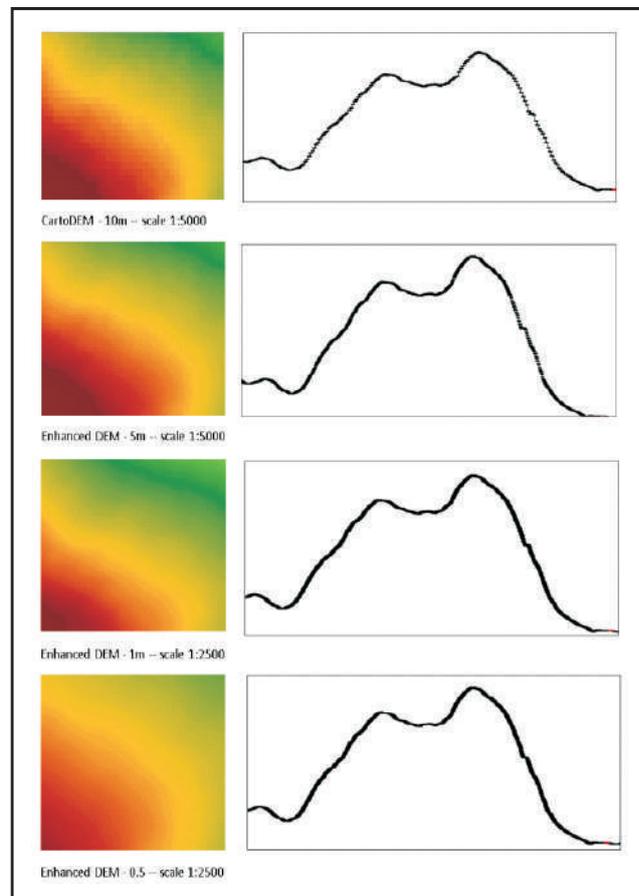**Table 2.** Basic operations of watershed delineation

| Operation | Definition |
|---|---|
| Fill | Fills sinks in a surface raster to remove small imperfections in the data |
| Flow Direction | Creates a raster of flow direction from each cell to its steepest down slope neighbor |
| Flow Accumulation | Creates a raster of accumulated flow into each cell |
| Basin | Creates a raster delineating all drainage basins |
| Stream | (Map algebra, Raster conversion) Delineates stream from DEM |
| Stream Link | Assigns unique values to sections of a raster linear network between intersections |
| Stream Segmentation | Segments the stream to order into several classes based on the grid code. |
| Stream Order | Assigns a numeric order to segments of a raster representing branches of a linear network. |
| Snap Pour Point | Snaps pour points to the cell of highest flow accumulation within a specified distance |
| Watershed | Determines the contributing area above a set of cells in a raster |

*Source*−Arc map Tool help, Hydro tools help

**Table 3.** Data processing for watershed modelling

| Input | Output |
|---|---|
| DEM | Filled DEM |
| Filled DEM | Flow direction raster |
| Flow direction raster | Flow accumulation raster |
| Flow direction raster | Basin layer |
| Flow accumulation | Stream layer |
| Stream raster, Flow direction | Stream link layer |
| Stream layer | Segmented stream layer |
| Segmented stream layer | Stream order layer (Grid code) |
| Stream points, Flow accumulation | Pour point layer |
| Pour point layer, Flow direction | Watershed |



a) Contour Map

b) Digital Elevation Model

c) TIN Model

**Fig. 8.** Contour map, DEM model and TIN model

Such TIN models are used for both hydrologic and hydrodynamic river flow simulations (Subbu Lakshmi and Yarrakula, 2017).

**DEM enhancement and comparison of DEM surface elevation profile:** Watershed modeling and hydrological analysis, the key input is digital elevation model (Singh *et al*., 2014). The resolution of DEM is high, the accuracy is also high. Data sets such as SRTM and ASTER are some of the freely available DEMs and these are acquired from USGS. To ensure the quality of the watershed, Cartosat−1 generated DEM with 10m resolution is used for precise modelling. To derive accurate surface elevation profile, the DEM is enhanced or rescaled/resampled to several higher resolutions. Comparing the DEMs, the surface elevation profile shows the difference in the arrangement of points. Fine resolution DEM provide more number of points. Table 2 explains various basic hydrological modelling operations that are available in hydrology tools in ArcMap as default. Such operations are performed using appropriate input data. On providing the suitable data, the tool is used to derive hydrologic outputs such as flow direction raster, flow accumulation raster, delineate streams, etc.

**3D Surface generation and Visualization**: ArcScene is used to create 3D analysis of DEM data. In the present study, digital elevation model is enhanced to several levels like contour lines, TIN model, DEM data (Fig. 8), slope map (Fig. 9) and streams are draped into a series of layers. The layers show the 3−Dimentional view of the study area.

The generated stream from watershed model is draped over the layer to show the stream flow. Terrain analysis is important for hydrological modelling. 3D spatial analyst tool is used for performing spatial operations in the data (Khatami *et al*., 2014). GIS and remote sensing allows a user to estimate the parameters needed for modelling, that results in simulation near to real world.

**RESULTS AND DISCUSSION**

Archydro and Arcswat are used for hydrologic modelling

d) Slope Map with Streams

e) Satelite Image draped over the terrain
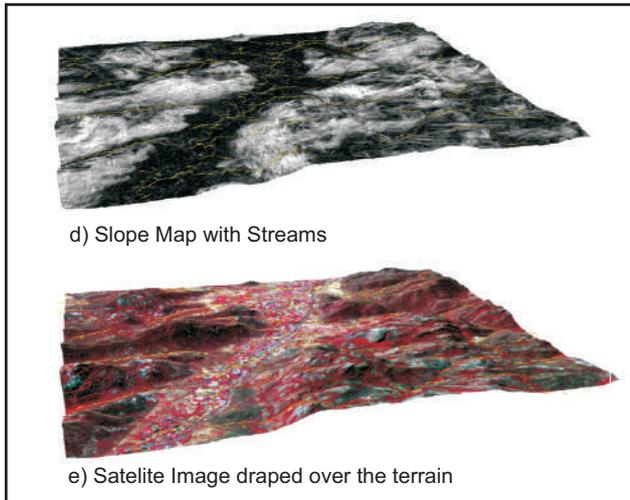
**Fig. 9.** Slope map and Resourcesat LISS IV overlaid on DEM

(watershed) using different types of set of processes the modelling outputs are obtained. Models are resultant of similar input data. Land use and land cover map is created using the database provided on the number classes that are available on the study area. Using DEM and contour map, TIN model is created. The comparison between two watershed models are performed and results showed that Arc Hydro shows good performance when operation are carried out for a large watershed and Arcswat has flexibility for including more parameters in modelling. Both watershed models are compared and both models provide different end applications.

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# Economic Growth and CO$_2$ Emissions in India: An Environmental Kuznets Curve Approach

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*Abstract:* The present paper was attempted to examine the existence of EKC in India's context. By using the time series data from 1978-2015 for the variables CO$_2$ emission per capita, real GDP per capita, Energy consumption per capita and FDI inflows collected from World Bank Development Indicators and World Energy Statistics database. To test co-integration or long run relationship between variables, ARDL (Auto-Regressive Distributive Lag) bound test was employed and for causality VECM (Vector Error Correction Model) Granger causality test was used. Finally to examine the existence of EKC hypothesis, OLS (Ordinary Least Square) regression was employed. The results indicate the existence of long run relationship between CO$_2$ emission per capita, GDP per capita, FDI inflows and energy consumption per capita. The causality result of VECM granger causality shows the bi-directional causality between CO$_2$ emission per capita and FDI, CO$_2$ emission per capita and energy consumption. But, found unidirectional granger causality running from GDP per capita to CO$_2$ emission per capita. From regression result can conclude that the EKC hypothesis i.e. inverted U-shaped curve does not exist between GDP per capita and CO$_2$ emission per capita in India's context. It may be cubic shape curve or N-shape curve.

*Keywords*: Economic growth, Environmental degradation, ARDL, VECM, Environmental Kuznets Curve, India

Increased economic growth leads threat to environmental deterioration and global warming. The major increase in Greenhouse Gas (GHG) emissions leads to global warming and climate change (Saboori *et al.,* 2012). The Inter-governmental Panel on Climate Change reported that Greenhouse gas emissions vary over a wide range in 21$^{st}$ century and it is depending on both socio-economic development and climate policy. The Continued emission of greenhouse gases will cause further warming and long-lasting changes in all components of the climate system, increasing the likelihood of severe, pervasive and irreversible impacts for people and ecosystems (IPCC, 2014). The anthropogenic emissions of greenhouse gases are highest in history and large fraction of anthropogenic climate change resulting from CO$_2$ emissions (IPCC, 2014). Carbon dioxide (CO$_2$) is the main source of Greenhouse gas (GHG) emissions changing day by day. Cumulative emissions of CO2 largely determine global mean surface warming by the late 21$^{st}$ century and beyond (IPCC, 2014). Now days, carbon emissions has great importance in the debate on environmental protection and sustainable development. However, from the seventies, when a major concern on the environment's deterioration took place at international level, some economists argued that enhancement in per capita income could eventually reduce the level of environment degradation. Such measures may lead to a negative impact on environment. Hence, depending upon the long term relationship between carbon emissions, energy consumption, foreign direct investment and economic growth for different countries may resort different strategies to fight against environmental degradation. In long run relationship between carbon emissions, energy consumption foreign direct investment and economic growth by research focus on the relationship between economic growth and environmental pollutants can be explored (Ghosh, 2010). These underlying ideas were enthusiastically accepted when an early set of papers (Grossman and Krueger, 1991; Shafik and Bandyopadhyay, 1992; Panayotou, 1993) provided the first formal evidence about an inverted U-shape relationship between per capita income and environmental degradation following, therefore, a Kuznets type curve. Nowadays, we can appeal to many studies that show the existence of an environmental Kuznets curve (EKC) for several countries by using different sample periods, econometric modeling and empirical methods. In this context the present study was examined the existence of EKC hypothesis in India's context using dependent variable as CO$_2$ emission per capita and independent variables as real GDP per capita, Energy consumption per capita and FDI inflows.

## MATERIAL AND METHODS

For the study time series data from 1978–2015 on real GDP per in capita (in constant 2010 US\$), CO$_2$ emission per capita (in metric tons), FDI net inflows (BoP, current US\$) and Energy consumption (kg of oil equivalent per capita) were collected from the World Bank development indicators and World Energy Statistics database. To test the co-integration or long run relationship and causal relationship between these variables were found in two steps: first examining the co-integration or long run relationship between these two variables by using ARDL (Auto Regressive Distributed Lag) bound test method and secondly, testing the causal relationship between variables using Granger causality test through VECM (Vector Error Correction Method). Time series data has problem of non-stationary and it may results the spurious regression. To make data stationary, Augmented Dickey Fuller (ADF) test was employed. After this, to examine co-integration or long-run relationship between the variables real GDP per in capita, CO$_2$ emission per capita, FDI net inflows and Energy consumption per capita, the ARDL (Auto-Regressive Distributive Lag Model) bound test of co-integration was employed, which was introduced by Pesaran and Pesaran (1997), Pesaran and Smith (1998), Pesaran and Shin (1999) and Pesaran *et al*. (2001). ARDL is based on general to specific modelling technique.

ARDL framework of the model as follows:

$$Inc_t = \beta_o + \sum_{i=1}^{p} \delta_i \Delta InC_{t-i} + \sum_{i=1}^{p} \theta_i \Delta InY_{t-i} + \sum_{i=1}^{p} \omega_i \Delta InY^2_{t-i}$$
$$+ \sum_{i=1}^{p} \delta_i \Delta InFD_{t-i} + \alpha_1 InC_{t-1}$$
$$+ \alpha_2 InY_{t-1} + \alpha_3 InY^2_{t-1} + \alpha_4 InFD_{t-1} + U_t$$

Where,

$\beta_o$ Drift component; C= CO2 emission per capita; Y= Real per capita income; $Y^2$ = Square of real per capita income; FD: Foreign direct investment; $U_t$ =Error term

In ARDL bound test, first step was estimate equation (1) by using OLS (Ordinary Least Square) estimation at different lag lengths. Here, null hypothesis is no long run relationship or no co-integration between variables, $H_o$. $\alpha_1 = \alpha_2 = \alpha_3 = \alpha_4 = 0$ as against alternative hypothesis there is long run relationship or co-integration $H_A$. $\alpha_1$ $\alpha_2$ $\alpha_3$ $\alpha_4$ 0. To check the long run relationship between variable F-test was performed. The test value of F-statistics was compared with pesaran and pesaran (1997) and pesaran *et al*. (2001) critical values of F-statistics. Two sets of critical value such as I(0) and I(1) which means lower bound and upper bound critical values. Conclusion can be made based on if the computed F-statistics is higher than upper critical value the null hypothesis of no long run relationship or no co-integration is rejected, it means variables has co-integration or long run relationship. If computed F-statistics below the lower bound critical value the null hypothesis of no long run relationship or no co-integration cannot be rejected, it means variables are not co-integrated or has no long run relationship. If it is lies between upper bound and lower bound critical value the result is inconclusive. Once co-integration or long run relationship test was confirmed, then there would be expectation of causal relationship between variables at least in one direction. To find causal direction between variables Vector Error Correction (VEC) Granger causality test was used. Granger causality test is easy to find the causal direction between two variables X and Y).

## RESULTS AND DISCUSSION

Based on the ADF test, all variables are non-stationary at level, but after taking second difference, it would become stationary. Thus, it was concluded that these variables are stationary of order two i.e. I (2). Once data was confirms with stationary, the co-integration or long run relationship was tested by using ARDL (Auto Regressive Distributive Lag) model. The results of lag length structure were presented in the figure 1. In this study maximum lag length structure p=4, 1, 2, 2, 4 have been selected. The estimated F-statistics was found to be 29.6743, when CO$_2$ emission per capita was dependent variable (Table 2). The computed F-statistics was found to be more than upper bound critical value at 1, 2.5, 5 and 10 per cent level of significance. This confrms that there is existence of strong long run relationship between the variables in the model.

After establishment of long run relationship there would be expectation of causal relationship between variables at least in one direction. The bi-directional causality between CO$_2$ emission per capita and FDI inflows, which means when FDI inflows increases the CO$_2$ emission per capita increases and vice versa (Table 3). The bi-directional causality between CO$_2$ emission per capita and energy consumption was

**Table 1.** Results of unit root test

| Level (Constant and Trend) | | 2$^{nd}$ Difference (constant and no trend) | |
|---|---|---|---|
| Variable | ADF (t-statistics) | Variable | ADF (t-statistics) |
| CO$_2$ | 1.0104 | CO$_2$ | –4.5089 |
| GDP | 2.1545 | GDP | –5.8233 |
| Square of GDP | 5.3476 | Square of GDP | –6.6136 |
| FDI | 2.9069 | FDI | –5.6049 |
| Energy use | –3.1750 | Energy use | –7.2657 |

observed and it can also interpreted as when energy consumption per capita increases the $CO_2$ emission per capita increases and vice versa. But, found unidirectional granger causality running from GDP per capita to $CO_2$ emission per capita i.e. when economic growth takes place in the country leads to economic degradation.

Finally, the $CO_2$ emission per capita was regressed on explanatory variables real GDP per capita, Energy consumption per capita and FDI inflows. The results indicate that all the coefficients are significant at 1 percent level (Table 4). Expected sign for the GDP per capita coefficient was positive as suggested by Selden and Song (1994) and Kaufmann et al. (1998). It shows that the early stage of economic growth in the country leads to increase in the $CO_2$

emission. Coefficient of squared of GDP per capita was negative as suggested by Selden and Song (1994) and Kaufmann et al. (1998) and coefficient of cubic of GDP per capita was positive. From this can conclude that there is no existence of EKC i.e. inverted U-shaped curve in the Indian context. It may be cubic curve or N-shaped curve.

## CONCLUSIONS

In this paper examined long run and causal relationship between $CO_2$ emission and GDP per capita based on data collected from World Bank Development Indicators and World Energy Statistics database from 1978-2015. The long run relationship or co-integration test was conducted by using ARDL approach developed by Pesaran et al. (2001)

**Table 2.** ARDL bound test results

| Equation | F-statistics | |
|---|---|---|
| $F_{CO_2}$(CO$_2$ | GDP, SGDP, FDI, ENE) | 5.6743 | |
| Critical value bounds | | |
| Significant | I 0 bound | I 1 bound |
| 10 % | 2.2 | 3.09 |
| 5 % | 2.56 | 3.49 |
| 2.5 % | 2.88 | 3.87 |
| 1 % | 3.29 | 4.37 |

**Table 4.** Environmental Kuznets Curve regression

| Variable | Coefficient | Standard error | t-value | Prob. |
|---|---|---|---|---|
| Constant | −1.11039 | 0.0851 | −13.0443 | 0.0000 |
| GDP per capita | 0.0028 | 0.0002 | 10.5638 | 0.0000 |
| Square of GDP per capita | $-2.94*10^{-6}$ | $2.60*10^{-7}$ | −11.3015 | 0.0000 |
| Cubic of GDP per capita | $9.52*10^{-10}$ | $8.18*10^{-11}$ | 11.6341 | 0.0000 |
| FDI | $3.66*10^{-10}$ | $8.23*10^{-13}$ | 4.4443 | 0.0001 |
| ENE | 0.0027 | 0.0003 | 7.7779 | 0.0000 |

**Table 3.** VEC Granger causality/Block exogenity wald tests

| Dependent variable | Null hypothesis | Chi-square | Probability | Status |
|---|---|---|---|---|
| $CO_2$ | SGDP does not cause $CO_2$ | 10.6142** | 0.0140 | Reject |
| | GDP does not cause $CO_2$ | 7.5027** | 0.0575 | Reject |
| | ENE does not cause $CO_2$ | 20.9091*** | 0.0001 | Reject |
| | FDI does not cause $CO_2$ | 20.7814*** | 0.0001 | Reject |
| FDI | SGDP does not cause FDI | 15.5619*** | 0.0014 | Reject |
| | GDP does not cause FDI | 7.6598** | 0.0536 | Reject |
| | ENE does not cause FDI | 34.7390*** | 0.0000 | Reject |
| | $CO_2$ does not cause FDI | 17.2318*** | 0.0006 | Reject |
| ENE | SGDP does not cause ENE | 6.2801* | 0.0987 | Reject |
| | GDP does not cause ENE | 2.9883 | 0.3934 | Accept |
| | FDI does not cause ENE | 15.2427*** | 0.0016 | Reject |
| | $CO_2$ does not cause ENE | 9.5745** | 0.0226 | Reject |
| GDP | SGDP does not cause GDP | 0.4922 | 0.9206 | Accept |
| | ENE does not cause GDP | 2.2327 | 0.5255 | Accept |
| | FDI does not cause GDP | 1.3169 | 0.7251 | Accept |
| | $CO_2$ does not cause GDP | 0.5605 | 0.9054 | Accept |
| SGDP | GDP does not cause SGDP | 1.2891 | 0.7317 | Accept |
| | GDP does not cause SGDP | 2.1418 | 0.5435 | Accept |
| | ENE does not cause SGDP | 2.6543 | 0.4480 | Accept |
| | $CO_2$ does not cause SGDP | 0.6003 | 0.8964 | Accept |

*Note:* *, **, *** represents 10%, 5% and 1% level of significance, respectively
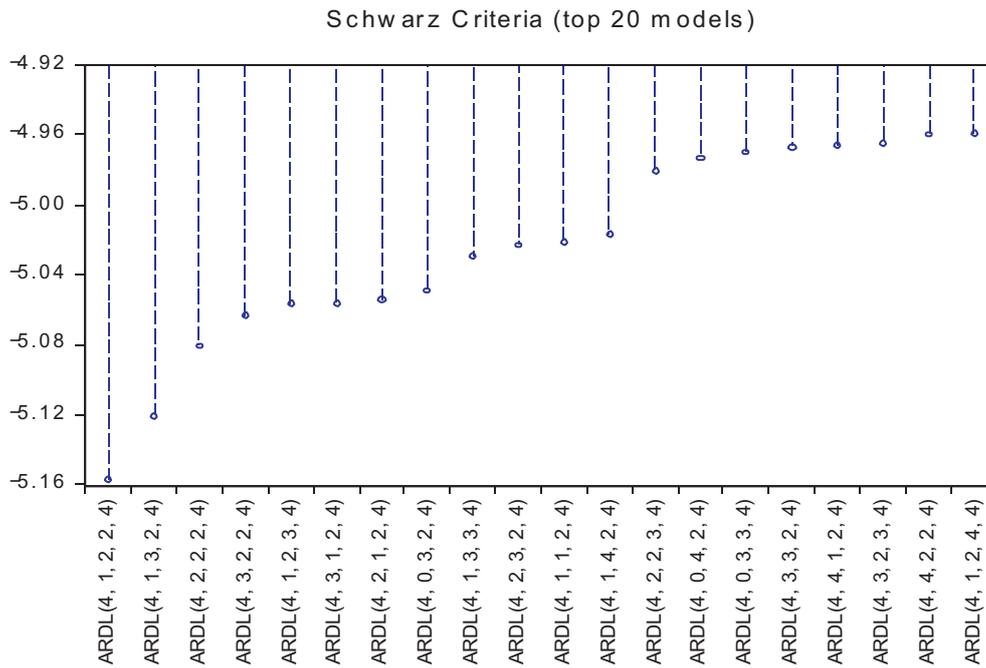
Schwarz Criteria (top 20 models)



**Fig. 1.** Schwarz criteria for model selection

and causality by using granger causality through VECM. Study establishes long run relationship or co-integration between $CO_2$ emission, GDP per capita, energy consumption and FDI. Found bidirectional granger causality between CO2 emission per capita and FDI, energy use. But GDP per capita granger cause CO2 emission per capita. The EKC hypothesis i.e. inverted U-shaped curve does not exist between GDP per capita and $CO_2$ emission per capita in India's context. It may be cubic shape curve or N-shape curve.

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# Watershed Prioritization Based on Morphometric Parameters Using Remote Sensing and Geographical Information System

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*Abstract:* In this study, remote sensing (RS) and geographical information system (GIS) were used to compute the morphometric parameters of thirteen sub-watersheds of Gagas watershed of Ramganga River basin situated in Almora district of Uttarakhand, India. The morphometric parameters used for this purpose are based on the linear/aerial aspects of watershed, namely bifurcation ratio, drainage density, stream frequency, texture ratio and mean length of overland flow; along with shape parameters such as form factor, circularity ratio, compactness coefficient and elongation ratio. After analysis of morphometric parameters, compound rank is calculated for each sub-watershed and fixed their priority rank based on compound rank. The sub-watershed (SW3) that has the lowest compound parameter value as 5.56 is likely to be subjected to maximum soil erosion, hence, highest priority rank one was given to this sub-watershed for implementing appropriate soil and water conservation measures for its development. Morphological parameters-based prioritization is good for field investigation carried out during the field work.

*Keywords*: Remote sensing, GIS, Gagas watershed, Morphometric analysis

Our land is subjected to soil disintegration and degradation primarily due to rain and wind activity, which gradually change waterway morphology, supply sedimentation and reduction in storage capacity of reservoirs, poor crop yields etc. Remote sensing and GIS can be utilized as a part of soil erosion appraisal and watershed prioritization studies. In recent time, various studies on morphometric parameters based prioritization of sub-watersheds utilizing remote sensing and GIS such as Srinivasa *et al.* (2004) utilized remotely detecting and GIS in morphometric parameters examination of sub-watersheds of Pawagada zone, Tumkur region, Karnataka. Chopra *el al.* (2005) completed morphometric examination of Bhagra-Phungotri and Hara Maja sub-watersheds of Gurdaspur region, Panjab. Nookaratnam *et al.* (2005) prioritized small scale watersheds based on the sediment yield index (SYI) and morphometric parameters examined using remote sensing and GIS. Khan *et al.* (20l1) utilized remote sensing and GIS for watershed prioritization in the Guhiya bowl, India. Gajbhiye *et al.* (2013) used RS and GIS for morphometric analysis and prioritization of 14 sub-watersheds of Manot River catchment, is a tributary of the Narmada River, India. Gajbhiye *et al.* (2015) utilized remote sensing and GIS for morphometric analysis and prioritization of the sub-watersheds of Shakkar River Catchment, Narsinghpur district in Madhya Pradesh State, India.

In the present study, morphometric analysis was done

using remote sensing and GIS for prioritization of thirteen sub-watersheds of Gagas watershed lying in Almora district of Uttarakhand, India.

## MATERIAL AND METHODS

**Study area:** The Gagas watershed, lies in the middle and outer range of Himalaya located between 79˚20'36" to 79˚33'15" E longitudes and from 29˚51'55" to 29˚35'49" N latitudes (Fig. 1) with an area of 511.19 km$^2$ on Ramganga River basin in the South-Western Almora region of
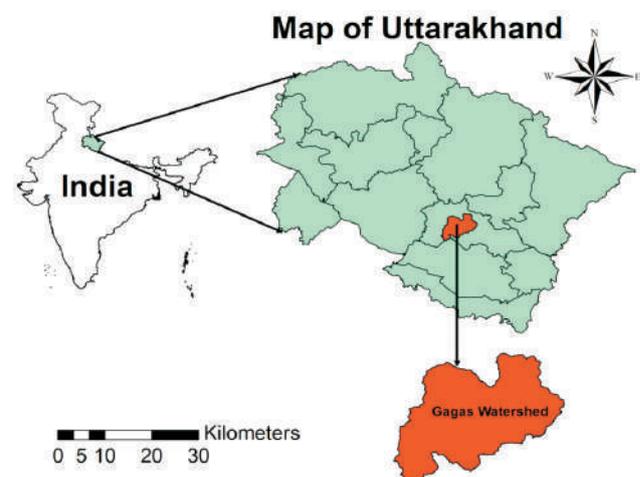


**Fig. 1.** Location map of Gagas watershed

Uttarakhand, India. The altitude of the region varies from 2742m to 774m above the mean sea level. The mean yearly rainfall fluctuates from 903 to 1281mm, with a mean of 1067mm. Roughly, over 80% of the yearly rainfall in the region occurs from the South-West monsoon, which begins in the third week of June and can lasts till mid-October.

**Data and methodology:** Survey of India (SOI) topographic maps (No. 53-O/5, 53-O/6, 53-O/9, 53-O/10) at 1:50000 scales were used to outline the natural drainage; and ASTER data, with 30m x 30m spatial resolution, were used for DEM generation (Fig. 1).

The parameters computed in the present study using ArcGIS 10.2 include area, perimeter, stream order, stream length and stream number, which were obtained from the digitized coverage of the drainage network map. However, linear/aerial aspects of the sub-watersheds such as bifurcation ratio ($R_b$), drainage density ($D_d$), stream frequency ($F_s$), texture ratio ($R_t$), mean length of overland flow ($L_{om}$), and the shape parameters, such as form factor ($F_f$), circularity ratio ($R_c$), compactness coefficient ($C_c$) and elongation ratio ($R_e$) were calculated by the standard formulae (Table 1).

Prioritization of sub-watersheds was done on the basis of morphometric parameters were assessed according to the linear/aerial and shape parameters, because linear/aerial parameters are directly related to the gross soil erosion from the sub-watersheds, the highest value of each parameter was given highest priority rank starting from one and so on with decreasing value of the parameter. The shape

parameters have inverse relationship to the gross soil erosion from the sub-watersheds, hence the parameters with the lowest value was given highest priority of one and so on with increasing value of the parameter (Nookaratnam, 2005; Gajbhiye *et al*., 2013). Final priority ranking was made on the basis of the compound (average) rank of each sub-watersheds such that the lowest value of compound rank for a sub-watershed was given the highest priority rank one and so on for all the sub-watersheds of Gagas watershed.

## RESULTS AND DISCUSSION

The stream order analysis and drainage network map of the Gagas watershed was prepared using the spatial analyst tools of ArcGIS 10.2 software (Fig. 2). The study area was divided into thirteen sub-watersheds (Fig. 3). Gagas watershed shows a dendritic drainage pattern. Table 2 shows the highest bifurcation ratio is 4.563 for sub-watershed SW6; highest circularity ratio is 0.498 for sub-watershed SW9; and highest elongation ratio of 0.456 is for sub-watershed SW7, which indicates a possibility of less soil erosion form these sub-watersheds. The form factor values for all sub-watersheds are in the range of 0.076 to 0.164 (Table 3).

All the sub-watersheds were given ranks on the basis of their linear/aerial and shape parameter values (Table 4). Finally, the compound rank of all the sub-watersheds was evaluated on the basis of these parameters and prioritization ranking was done as given in Table 4. Sub-watershed SW3 with the minimum compound rank of 5.56 is assigned the

**Table 1.** Various formula used for computation of morphometric parameters

| Morphologic parameters | Formula | References |
|---|---|---|
| Stream order ($u$) | Hierarchical rank | Strahler (1964) |
| Mean stream length ($L_{sm}$) | $L_{sm} = L_t/N_u$ where, $L_u$ = total length of stream of order $u$, $N_u$ = total number of stream segments of order $u$ | Strahler (1964) |
| Basin length ($L_b$) | $L_b = 1.312 * A^{0.568}$ where, A = area of the basin (km$^2$) | Nookaratnam *et. al*. (2005) |
| Bifurcation ratio ($R_b$) | $R_b = N_t/N_{u+1}$ where, $N_{u+1}$ number of stream segments of $(u+1)^{th}$ order | Schumn (1956) |
| Drainage density ($D_d$) | $D_d = (L_u)/A$ where, $(L_u)$ = total stream length of all orders (km) | Horton (1945) |
| Mean length of overland flow ($L_{om}$) | $L_{om} = ½ * D_d$ | |
| Stream frequency ($F_s$) | $F_s = (N_u)/A$ | Horton (1945) |
| Texture ratio ($R_t$) | $R_t = (N_u)/P$ | Horton (1945) |
| Form factor ($F_f$) | $F_f = A/Square\ of\ Lb$ | Horton (1945) |
| Elongation ratio ($R_e$) | $R_e = 1.128 A^{0.5}/L_b$ | Schumn (1956) |
| Circularity ratio ($R_c$) | $R_c = 12.57 A/P^2$ | Miller (1953) |
| Compactness coefficient ($C_c$) | $C_c = 0.2821 P/A^{0.5}$ | Horton (1945) |

**Table 2.** Sub-watershed wise physiographic parameters of Gagas watershed

| Sub-watershed code | Number of streams for the order ($u$) | | | | Total number of streams | Total length of streams (km) | Drainage area (km²) | Length of basin (km) | Perimeter of basin (km) |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | | | | |
| SW 1 | 17 | 5 | 1 | 0 | 23 | 46.77 | 59.36 | 19.62 | 39.76 |
| SW 2 | 16 | 2 | 0 | 1 | 19 | 41.49 | 44.28 | 20.48 | 41.49 |
| SW 3 | 22 | 4 | 1 | 0 | 27 | 50.72 | 61.65 | 21.28 | 42.78 |
| SW 4 | 16 | 4 | 1 | 0 | 21 | 33.38 | 41.73 | 17.33 | 35.7 |
| SW 5 | 3 | 1 | 0 | 1 | 5 | 7.92 | 9.26 | 8.34 | 16.44 |
| SW 6 | 19 | 5 | 1 | 0 | 25 | 45.71 | 51.76 | 18.45 | 42.14 |
| SW 7 | 3 | 0 | 0 | 1 | 4 | 12.78 | 12.02 | 8.56 | 18.03 |
| SW 8 | 1 | 3 | 1 | 0 | 5 | 26.23 | 27.63 | 19.05 | 39.05 |
| SW 9 | 1 | 0 | 0 | 1 | 2 | 4.67 | 4.34 | 5.32 | 10.46 |
| SW 10 | 7 | 6 | 1 | 1 | 15 | 37.91 | 41.95 | 16.7 | 32.97 |
| SW 11 | 9 | 3 | 1 | 0 | 13 | 25.89 | 26.34 | 14.93 | 31.43 |
| SW 12 | 9 | 8 | 2 | 1 | 20 | 69.94 | 83.89 | 25.91 | 52.64 |
| SW 13 | 18 | 3 | 1 | 0 | 22 | 45.6 | 47.4 | 17.48 | 34.91 |

**Table 3.** Linear/aerial and shape parameters of different sub-watersheds of Gagas watershed

| Sub-watershed code | Linear/aerial parameters | | | | | Shape parameters | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $R_b$ | $D_d$ | $F_s$ | $R_t$ | $L_{om}$ | $F_f$ | $R_c$ | $C_c$ | $R_e$ |
| SW 1 | 4.397 | 0.7873 | 0.387 | 0.578 | 0.634 | 0.154 | 0.471 | 1.444 | 0.442 |
| SW 2 | 3.175 | 0.936 | 0.429 | 0.457 | 0.533 | 0.105 | 0.323 | 1.745 | 0.366 |
| SW 3 | 4.448 | 0.822 | 0.437 | 0.631 | 0.607 | 0.136 | 0.423 | 1.525 | 0.416 |
| SW 4 | 4.000 | 0.799 | 0.503 | 0.588 | 0.625 | 0.138 | 0.411 | 1.547 | 0.420 |
| SW 5 | 1.442 | 0.855 | 0.539 | 0.304 | 0.584 | 0.133 | 0.430 | 1.512 | 0.411 |
| SW 6 | 4.563 | 0.884 | 0.482 | 0.593 | 0.566 | 0.152 | 0.366 | 1.640 | 0.439 |
| SW 7 | 3.000 | 1.063 | 0.332 | 0.221 | 0.4702 | 0.164 | 0.464 | 1.456 | 0.456 |
| SW 8 | 1.442 | 0.949 | 0.180 | 0.128 | 0.526 | 0.076 | 0.227 | 2.080 | 0.311 |
| SW 9 | 1.000 | 1.076 | 0.460 | 0.191 | 0.464 | 0.153 | 0.498 | 1.405 | 0.441 |
| SW 10 | 1.864 | 0.903 | 0.357 | 0.454 | 0.553 | 0.150 | 0.485 | 1.425 | 0.437 |
| SW 11 | 3.000 | 0.982 | 0.493 | 0.413 | 0.508 | 0.118 | 0.335 | 1.714 | 0.387 |
| SW 12 | 2.289 | 0.833 | 0.238 | 0.379 | 0.599 | 0.124 | 0.380 | 1.609 | 0.398 |
| SW 13 | 3.780 | 0.962 | 0.464 | 0.630 | 0.519 | 0.155 | 0.488 | 1.419 | 0.444 |

**Table 4.** Ranking of sub-watersheds on the basis of linear/aerial and shape parameters

| Sub-watershed code | Linear/aerial parameters | | | | | Shape parameters | | | | Compound rank value | Final priority rank | Final priority category |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R_b$ | $D_d$ | $F_s$ | $R_t$ | $L_{om}$ | $F_f$ | $R_c$ | $C_c$ | $R_e$ | | | |
| SW 1 | 3 | 13 | 9 | 5 | 1 | 11 | 10 | 4 | 11 | 7.44 | 9 | Medium |
| SW 2 | 6 | 6 | 8 | 6 | 8 | 2 | 2 | 12 | 2 | 5.78 | 2.5 | Very high |
| SW 3 | 2 | 11 | 7 | 1 | 3 | 6 | 7 | 7 | 6 | 5.56 | 1 | Very high |
| SW 4 | 4 | 12 | 2 | 4 | 2 | 7 | 6 | 8 | 7 | 5.78 | 2.5 | Very high |
| SW 5 | 11 | 9 | 1 | 10 | 5 | 5 | 8 | 6 | 5 | 6.67 | 6 | High |
| SW 6 | 1 | 8 | 4 | 3 | 6 | 9 | 4 | 10 | 9 | 6.00 | 5 | Very High |
| SW 7 | 7.5 | 2 | 11 | 11 | 12 | 13 | 9 | 5 | 13 | 9.28 | 13 | Very Low |
| SW 8 | 12 | 13 | 13 | 13 | 9 | 1 | 1 | 13 | 1 | 8.44 | 12 | Low |
| SW 9 | 13 | 1 | 6 | 12 | 13 | 10 | 9 | 1 | 10 | 8.33 | 11 | Low |
| SW 10 | 10 | 7 | 10 | 7 | 7 | 8 | 11 | 3 | 8 | 7.89 | 10 | Medium |
| SW 11 | 7.5 | 3 | 3 | 8 | 11 | 3 | 3 | 11 | 3 | 5.83 | 4 | Very High |
| SW 12 | 9 | 10 | 12 | 9 | 4 | 4 | 5 | 9 | 4 | 7.33 | 8 | Medium |
| SW 13 | 5 | 4 | 5 | 2 | 10 | 12 | 12 | 2 | 12 | 7.11 | 7 | Medium |

**Fig. 2.** Drainage network map and sub-watersheds of Gagas watershed
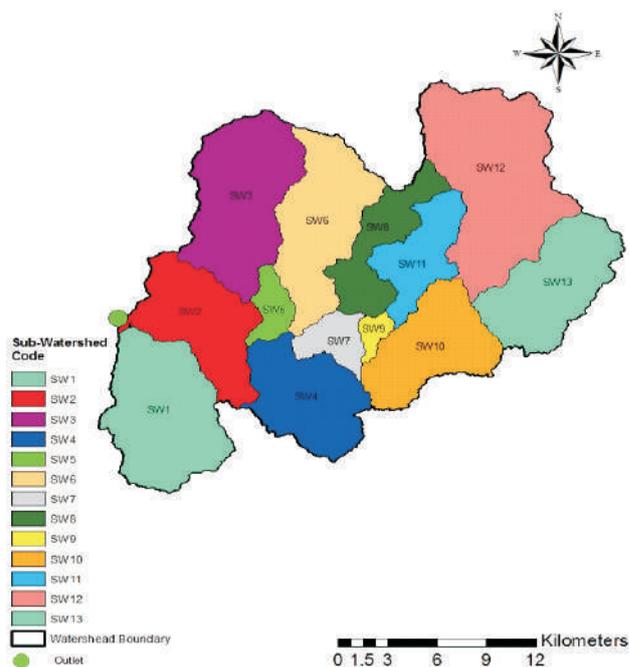


**Fig. 3.** Sub-watersheds code map of the Gagas watershed
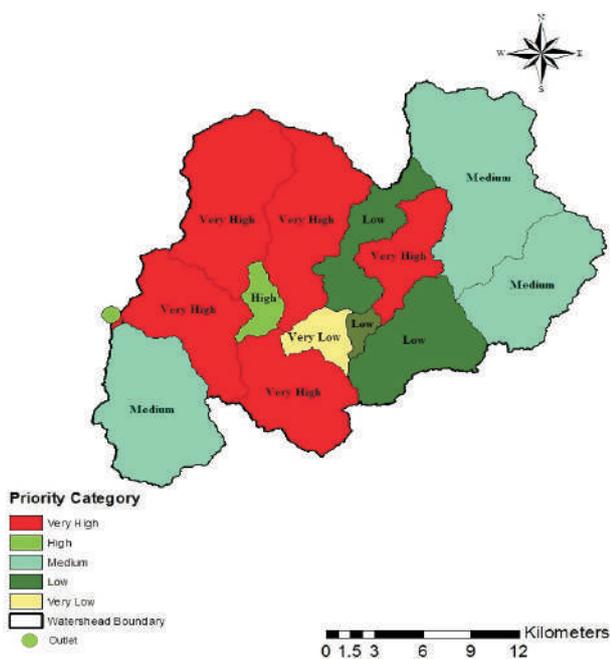


**Fig. 4.** Priority map showing priority rank category of sub-watersheds of Gagas watershed

highest priority rank one, followed by sub-watershed SW2 and SW4 with compound rank value of 5.78, and so on. The highest priority indicates greater risk of soil erosion from the watershed which requires better and earliest soil and water conservation measures for better development and

management. The final prioritization map of the study area indicating all sub-watersheds with different colors is shown in Fig. 4.

**CONCLUSION**

Present study makes use of remote sensing and GIS techniques for morphometric analysis and prioritization of the sub-watersheds in Gagas watershed of Ramganga River basin in Uttarakhand state of India. The morphometric attributes of various sub-watersheds demonstrate their relative qualities for hydrologic reaction of the Gagas watershed. Consequences of morphometric investigation demonstrate that sub-watersheds SW3, SW2 and SW4 were highly prone to soil erosion, and should be taken up first followed by others as per their ranks for the execution of proper soil and water conservation and management techniques for soil erosion control and safeguard the land from further erosion. This technique is must be followed by all the agencies involved with the task of planning, development and management of watershed programs in the country.

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# Assessing Decline in Open Water Area of Vellayani Fresh Water Lake Ecosystem: A Remote Sensing Approach

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**Abstract**: Vellayani lake, is a sole freshwater lake of Thiruvananthapuram, is facing several anthropogenic stresses such as reclamation, encroachment, sand mining and pollution. Consequently, the quality and quantity of water in the lake had declined considerably, which affected many ecosystem services provided by the lake. The extent of degradation of the lake using remote sensing techniques is attempted here. For mapping the changes in the geographical extent of the water body, Landsat imagery of February 1973, March 1992, September 2001 and IRS imagery of March 2011 were used. The area transformation statistics indicated that the area of water body which was 558.93 ha during 1973 was reduced to 243.39 ha in 2011. The study revealed that the drastic reduction in the area of the lake is due to irrational human activities like unsustainable exploitation of the ecosystem services due to demographic pressures and urbanization.

**Keywords**: Fresh water lake, Anthropogenic stress, Remote sensing, Mapping, Urbanisation, Unsustainable exploitation

Kerala, the green state of the country, is blessed with 44 rivers, back water lagoons, lakes, beaches, manmade reservoirs and water logged land which accounts for nearly 4.13% of geographical area (ISRO, 2011). Still the state faces acute water shortage during the summer months owing to non availability of freshwater. Access to clean freshwater ecosystems plays a crucial role in sustaining life. In Thiruvananthapuram, the capital city of Kerala the total wetland area is 5942 ha with Vellayani lake as major fresh water source. But owing to rapid development activities and indiscriminate use of the lake, it is subjected to acute pressure. The lake is facing several anthropogenic stresses like reclamation, encroachment, sand mining and pollution.

During 1950s for facilitating paddy cultivation major portion of the lake was drained yearly. Thus the fragile ecosystem was subjected to acute manmade stresses which caused temporal and spatial variability in the lake system. Owing to crop loss and changes in natural ecosystem, in 1992, Government of Kerala discontinued dewatering the lake and after that the lake is kept as a drinking water source. But, even after discontinuing paddy cultivation, the anthropogenic pressures due to development activities and indiscriminate use continued by ways of encroachment, constructions, sand mining and pollution. In the present scenario of acute water scarcity due to degradation of water sources, a study on the extend of degradation of the lake assumes prime importance. So this study attempts to analyze the temporal and spatial variation of Vellayani lake

system using Geographical Information System (GIS).Geographical Information Systems provides accurate information for decision makers of any natural resource conservation programme (Dirim *et al*, 2009). Remote sensing technique provides fast, accurate and economic method for extraction of wetlands in an area(Kavyashree and Ramesh, 2016). Hence the present study attempts a spatio − temporal analysis of Vellayani lake in order to understand the extend of Vellayani lake degradation.

## MATERIAL AND METHODS

Lying on the South East of the Thiruvananthapuram city, Vellayanilake (Plate 1) is one of the three major fresh water lakes of Kerala. The lake is an elongated water body, allinged in a North South direction. It lies between 8˚24'09" − 8˚26'30" N Latitudes; 76˚59'08"−76˚59'47" E Longitudes and is situated 11km south of Thiruvananthapuram city and 7 km from Kovalam. Lying hardly about 3 kms away from sea, the lake is surrounded in all sides by gently sloping hillocks. The whole terrain lies 29 m above MSL, and the lake bed is 0.1 to 1.5 m below the MSL. The lake stretches from Venniyoor − Muttakadu region in the South to reservoir in the North and from Poonkulam in the West, to Kakkamoola in the East. No base line inventory about the lake was available. So satellite maps were used as a guide for study.

**Satellite images used:** Remote sensing of wetlands using satellite data dates back to 1972 with the launch of Landsat−1 MSS (Deka *et al.*, 2011). For mapping the changes in the

area of the water body LANDSAT imagery of February 1973, March 1992, September 2001 and IRS imagery of March 2011 were used. Field investigations were also done for cross checking.   The cadastral maps of the villages surrounding the Vellayani Lake were scanned, geo referenced, mosaiced and digitized in Arc GIS Desktop software. The satellite images of 1973, 1992 and 2001 were downloaded from the Global Land Cover Facility (GLCF). GLCF provides remotely sensed satellite data and products with a focus for promoting research on environmental systems. The IRS‑P6 image for 2011 was accessed from Bhuvan, the Indian Geo‑portal managed by ISRO (Table 1).

These images were subjected to linear contrast enhancement for improving tonal variation. Each satellite image was properly geo referenced to fit exactly with cadastral map. For each satellite image, a corresponding vector layer was created and the lake boundary was digitized accurately in Arc GIS desktop environment. After digitization, geometrical area of the lake was calculated for each layer, in projected co‑ordinate system in order to derive the area in metric system. After deriving this, a new column was created in the attribute table, and the area was recalculated to hectares, using field calculator.

### RESULTS AND DISCUSSION

The study revealed that the lake was subjected to wide shrinkage in geographical extend from 1973 (Table 2, Plate 2).The satellite imageries indicated that the area of water body during 1973 was 558.93 ha which showed a sharp decline further. Since the image was taken during February, there is every probability that the lake was dewatered for paddy cultivation for raising the summer crop. So the actual area of water body might be much more than this. Landsat 5 satellite image indicated that the coverage of water body has been drastically reduced to 267.89 ha by 1992.The reduction in area by about 50 per cent by 1992 shows that reclamation of the lake for paddy cultivation had resulted in all round destruction in the water spread area of the lake.  In a study on Loktak and Pumlenlake of Manipur, Singh and Moirangleima (2009) reported similar shrinkage in area to a tune of 50 per cent from 1989 to 2002 in open water area due to human pressure. After the cessation of paddy cultivation in 1992 the further loss of area was meagre. The latest available data on area of water body of the lake using Indian Remote Sensing Satellite‑P6 is 243.39 ha. The reduction in water spread from 2001 to 2011 was mainly due to the slow process of urbanization occurring in the surrounding areas which resulted in encroachment and reclamation of the lake. Soumyashree and Ramachandra (2011) also reported that urban sprawl was the major cause for the environmental

degradation and wetland loss.

The public property status of the lake and improper delineation of the lake boundary enhanced illegal encroachment. The periphery of the lake was planted with perennials like coconut with the selfish motive of conversion of wetland. The aesthetic and recreational value of the lake attracted many people to acquire land near the lake to construct water front houses. To facilitate transportation across the lake and to have connectivity to

**Table 1.** Satellite imageries used for study

| Satellite | Sensor | Date of Acquisition |
| --- | --- | --- |
| Landsat 1 | MSS | 09‑02‑1973 |
| Landsat 5 | TM | 11‑03‑1992 |
| Landsat 7 | ETM+ | 04‑09‑2001 |
| IRS‑P6 | LISS III | 02‑03‑2011 |

**Table 2.** Temporal variation in lake area

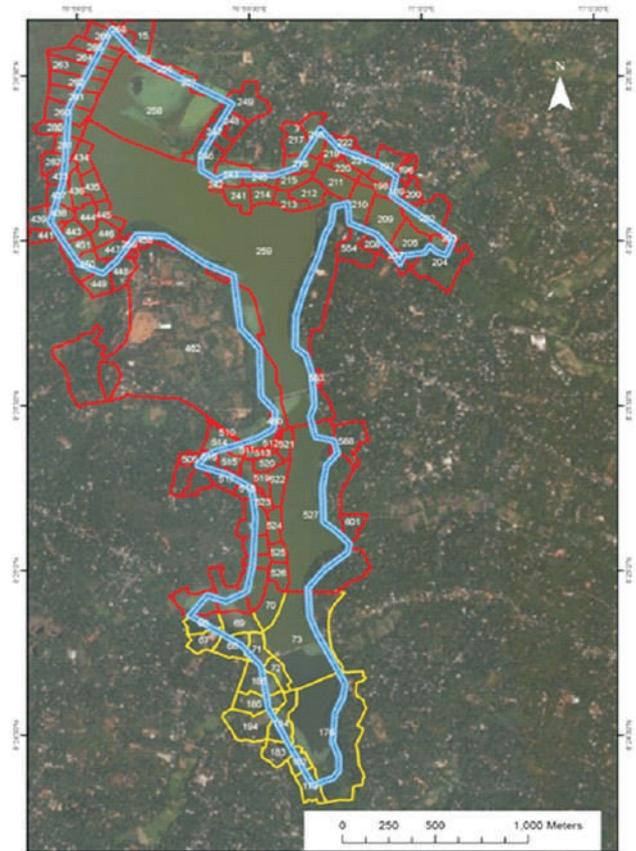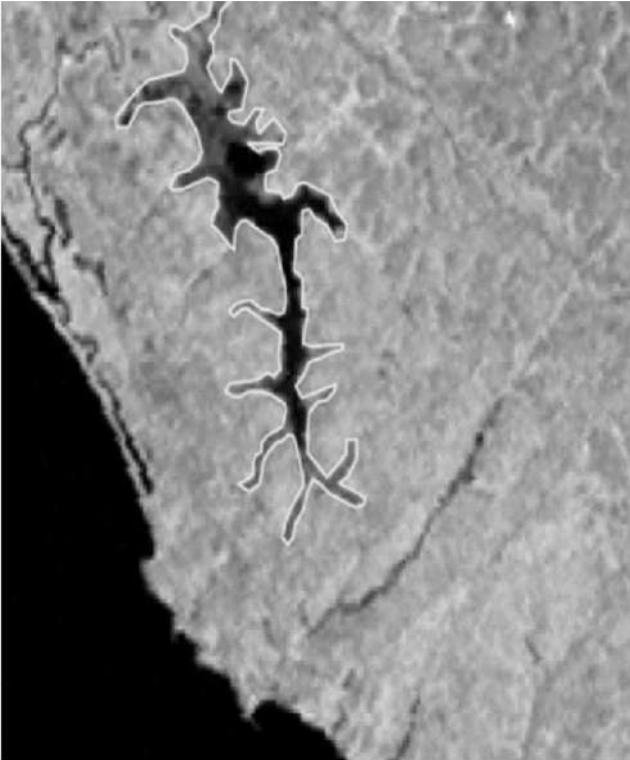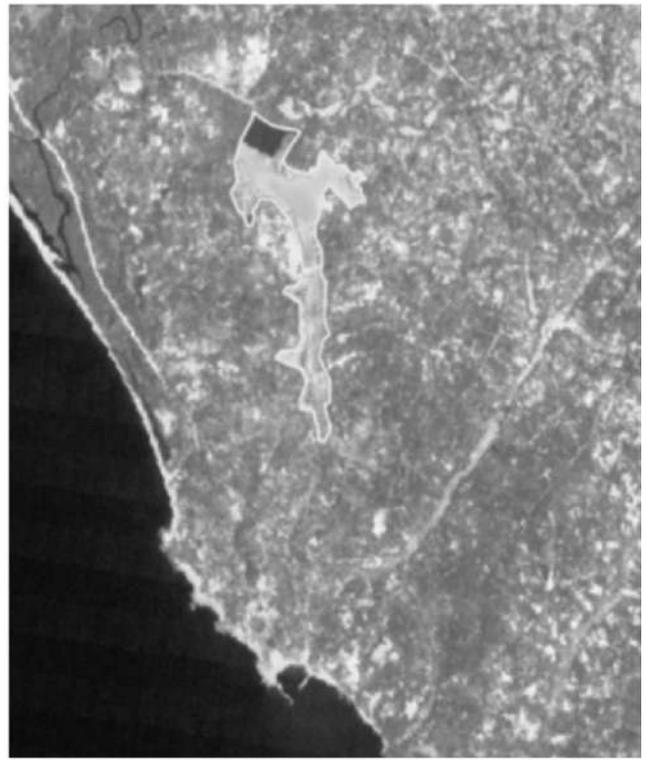| Year | Area of water body (ha) | % variation |
| --- | --- | --- |
| 1973 | 558.93 | − |
| 1992 | 267.89 | 52.07 |
| 2001 | 267.14 | 0.28 |
| 2011 | 243.39 | 8.89 |



**Plate 1.** Vellayanilake

Vellayani Lake in 1973
(Satellite Image–Landsat 1, MSS, 09-02-1973)

Vellayani Lake in 1992
(Satellite Image–Landsat 5, TM, 11-03-1992)

Vellayani Lake in 2001
(Satellite Image–Landsat 7, ETM+, 04-09-2001)

Vellayani Lake in 2011
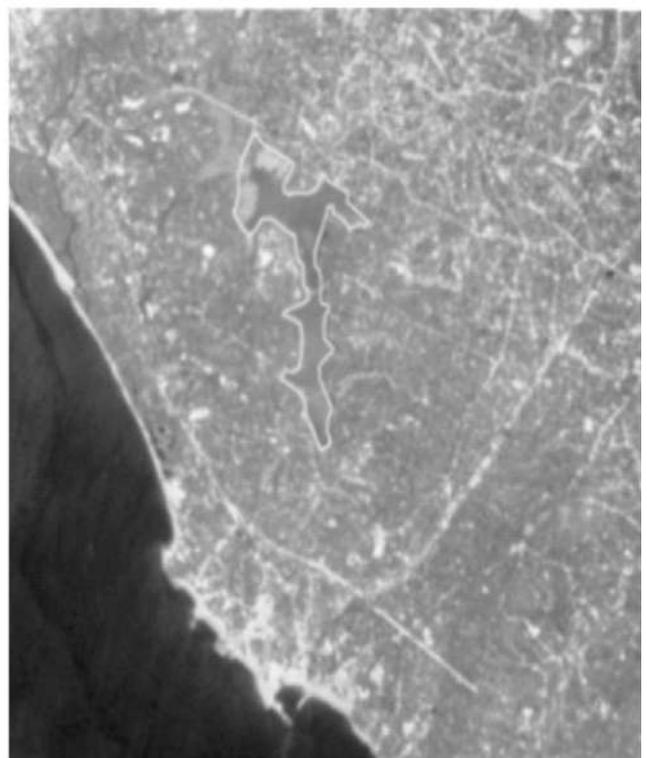(Satellite Image–IRS-P6 LISS III, 02-03-2011)



**Plate 2.** Temporal variation in lake area

Thiruvanathapuram city two bund roads were constructed across the lake *viz*. Kakkamoola bund road and Vavvamoola bund road. These bund roads even though enhanced the transportation facilities of the people, fragmented the lake and hindered free flow of water across and also resulted in the decline in open water area of the lake. The lake is mainly rainfed. The water from surrounding area was carried through small inlets or streams to the lake. Urbanisation of surrounding area led to the depletion of these inlets and reduced the quantity of water carried to the lake. Many small streams identified in Landsat 1 imagery was missing in satellite images of the consequent years. The field visits confirmed that many small streams leading towards the lake were depleted partly or completely. These inlets carrying water to the lake was also polluted with domestic waste from the houses which in turn polluted the lake. It can be inferred that the drastic reduction in the area of the lake is mainly due to irrational human activities consequent upon gradual transition from rural to urban set up. The increasing demographic pressure and the public property status of the lake system had resulted in the large scale destruction of the ecosystem. The lack of awareness on the significance of this valuable wetland ecosystem system among the public could be another reason for the exploitation and sharp decline in the area with degradation of ecological environment. Alarming levels of human induced environmental degradation of fresh water lakes and subsequent reduction in area was also reported by Rao *et al*. (2004) and Reddy (2014) through digital processing of the IRS–1D LISS–III image of Kolleru lake. This indicates that the degradation of wetlands happening all over the country is also visible in Vellayani lake system.

## CONCLUSION

The study using Remote sensing techniques indicated that there is considerable reduction in the area of Vellayani fresh water lake. If not properly managed through institutional interventions, death of the invaluable services provided by the lake cannot be replaced by any means. It is important to keep a balance between growth of urbanization and level of exploitation of natural resource. The alarming level of degradation of lake as revealed by the study demands sustainable strategies for conserving the lake.

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# Strategic Ecological Assessment of the State of the Transboundary Catchment Basin of the Dnieper River under Extensive Agricultural Load

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**Abstract**: The most promising is a practical evidence-based implementation of basin principle of nature, which involves the use of an integrated approach to land-and water conservation measures on the basis of the systematic research. The basis of the research objectives is entrusted with the implementation of Strategic Environmental Assessment (SEA) of space-time transformation of landscape of the eco-systems of the Dnieper river catchment area on the basis of river basin approach. Strategic environmental assessment and spatial clustering was carried out on six indicators of agrogenic transformation of structural and functional state of landscape ecosystems of the Dnieper river basin: forest cover, plowing, squares slopes more 1°, squares slopes of southern exposure, the area cultivated slopes, eroded areas of arable land. As a result, SEA and geo-modeling determined that more than 50% of the Dnieper basin has high agrogenic transformation of the structural and functional status of the landscape ecosystems. The main criterion for the destabilization of agricultural landscapes is a high level of tillage. In this case the most effective tool for improving the ecological balance of the territory of the trans-boundary basin study is the reduction of arable land for other land or environmental fund 470 sub-basin boundaries with a total area of ~ 346.3 thousand km$^2$. Rational land use in accordance with the strategic environmental assessment should take into account the characteristics and features of the landscape, promote the protection and reproduction of soil, vegetation and other natural components in a single agreement between the countries of trans-boundary basin.

**Keywords**: Strategic environmental assessment, Agricultural development, Forest area, Erosion potential, River basins, Dnieper river, Geo-modeling, GIS-technologies

In recent years, the need and importance of operations of the strategic level, aimed at regulating and ensuring environmental sustainability of geo-hydro system of trans-boundary basins are growing. To ensure a comprehensive approach to the environmental assessment and management of trans-boundary basins the environmental assessment methods have been set that require constant improvement and amendments by the principles adapted to policies, plans and programs of neighboring countries, primarily in the areas of trans-boundary basins. In European practice, evaluation of policies, programs and development plans and ensuring environmental sustainability units Directive 2001/42 /EU "On Strategic Environmental Assessment" is used. Strategic Environmental Assessment (SEA) is the main instrument for integrating environmental considerations (including health) in the development of plans and programs in ensuring the environmental sustainability of trans-boundary basins. Directive 2001/42/EU defines the obligations of the government (or those of private organizations that provide public services) to identify and assess potentially significant environmental effects of the

proposed plans and programs (not politicians), including cross-border cooperation, view to easing or avoiding a potentially significant negative impact on the environment prior to approval of such a plan or program. SEA promotes sustainable development through the promotion of environmental issues in economic and social development and the integration of green economy objectives and sustainable consumption and production in the strategic decision making. The issue of the need, development, and methodological development of the direction and feasibility of using strategic environmental assessment was studied earlier (Palekhov, 2005; Khlobystov, 2005; Chernykhivska, 2011).

The Dnieper river is one of the largest rivers of Europe, the swimming pool which is 511 thousand km$^2$ and is located within three states (Fig. 1) the Russia (19.8% of the total area of the pool), Belarus (22.9%), and Ukraine (57.3%). Cross-border Dnieper basin is a diversified complex that has a high natural and socio-economic value to the three neighboring countries, but the environmental crisis, caused by the construction on the Dnieper cascade reservoirs that
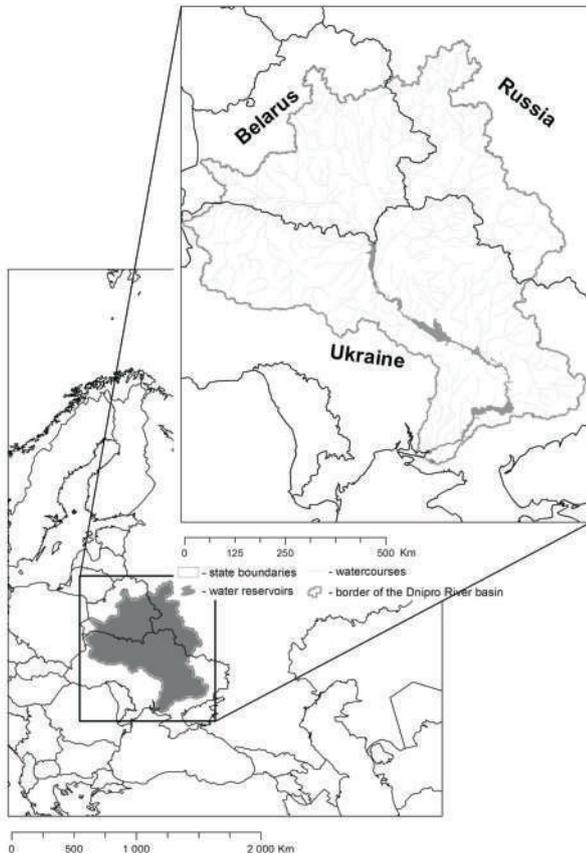
**Fig. 1.** Geographical location of the Dnieper basin

completely changed the dynamics of flow, extensive system management, large-scale land reclamation, construction within the basin of the industrial complex, vast amounts of water for industrial and agricultural production, dumping large amounts of contaminated water, etc. (Romanenko *at al.*, 2000; Lisetskii *at al.*, 2013; Pichura *at al.*, 2015), led to a significant deterioration in the functioning of integrity of geo–hydro-eco-system of the trans-boundary basin, and quality of drinking water and as a result, led to deteriorating the health of people. Because of historical patterns of development of the Ukrainian part of the Dnieper river basin in its territory is more than 60% of total industrial production in Ukraine. The area of arable land trans-boundary basin is 283 thousand km$^2$, or 55.4% of the total area of the basin, of which 206 thousand km$^2$ (72.8%) are in Ukraine. About half the state's population resides in the territory of the basin and two thirds drink water from the Dnieper. Therefore, taking into account the conditions of high anthropogenic load for the rational management of natural resources, the search for optimal interaction between management, man and nature is necessary, i.e. the formation of balanced relations between the exploitation geo-hydro-ecosystem their guard and purposeful transformation (Lisetskii *et al.*, 2015).

Taking into account the socio-economic situation of cross-border the environmental situation in the Dnieper basin is constantly unstable (Lisetskii *et al.*, 2014). Therefore, to solve the environmental problems of trans-boundary River, the most promising is a practical evidence-based implementation of basin principle of nature, which involves the use of an integrated approach to land-and water conservation measures on the basis of the systematic research. The main stage in the territorial organization of trans-boundary basin environmental management is to determine the spatial and temporal patterns of processes of degradation of the complex as a whole and determine specific relationships to research chain "environmental sustainability or breach of landscapes-the quality of surface water –human health" conditions for sustainable operation of the natural geo-hydro-ecosystem Dnieper basin, meeting the needs of the population and production of natural resources with minimal disturbances of geo-environment of natural integrity and improving the nation's health. Geo-planning of the territory of trans-boundary river basin should be based on assessment of agro-genic transformation of structural and functional state of landscape ecosystems as the main violator of integrity geo-hydro-ecosystem and environmental problems of the Dnieper river basin.

The basis of the research objectives is entrusted with the implementation of strategic environmental assessment of space-time transformation of landscape of the ecosystems of the Dnieper river catchment area on the basis of river basin approach. The advantages of using the basin approach for environmental research and the organization of environmental management indicates the clarity and simplicity of selection borders; hierarchical structure that allows you to jump to different territorial levels of government; organization unidirectional flows of matter, energy and information; geo-system relationship that enables to carry out all types of environmental monitoring; restriction of soil and vegetation, settlement system and environmental management to individual basin structures; localization anthropogenic sources of pollution along watercourses watershed.

**MATERIAL AND METHODS**

To separate annual sub-basin of the Dnieper river into groups depending on the order of the main channel, the approach of Stralera-Filosofova was used (Simonov *at al.*, 2004; Pichura, 2016). The ability to study geomorphological features of the load on the Dnieper basin and all components of the landscape in relation to the characteristics of water flow parameters allows the basin organization area at watersheds erosion forms IV procedure, which provides for the

identification of landscape heterogeneity territory.

Agricultural development and distribution of forest area in the basin of the Dnieper river is determined based on decoding of the data of the land remote sensing (LRS) using correctly calibrated series of MODIS satellite images (geometric distinction ~ 230×230 m) as of April 23 and August 13, 2015. Thematic image interpretation procedures conducted on the basis of dimensionless index NDVI– normalized differential vegetation index. The official statistics were used for the purpose of comparison and correction of the results.

To investigate the erosion potential of landscapes different-order catchment of river sub-basin by morphometric characteristics of the terrain (part of slopes is more than 1°, Part of the southern slopes, the proportion of cultivated slopes) using the working module Surface of Spatial Analyst Tools and Zonal Statistics of Spatial Analyst Tools.

Earlier a systematic approach was presented, using cluster analysis, multi-functional GIS-instruments and LRS data to analyze anthropogenic load capacity and display of water-erosion processes in agricultural landscapes in the territory of the regional geo-eco-system. Reproduced in part

approach used for grouping different-order watershed sub-basin of the Dnieper river for agricultural development and environmental condition. Spatial clustering was carried out on six indicators to assess agrogenic transformation of structural and functional state of landscape ecosystems of Dnieper river basin: forest cover, plowing, squares slopes more 1°, squares slopes of southern exposure, the area cultivated slopes, eroded areas of arable land. For this, Grouping Analysis of Spatial Statistics Tool was used. For processing, GIS analysis and mathematical modeling spatial heterogeneity of distribution of the studied parameters used licensed software ArcGIS 10.1, MathWorks MATLAB 7.9 R2009b and STATISTICA Advanced + QC for Windows v.10 Ru.

## RESULTS AND DISCUSSION

As a result of spatial hydrological gemodelling, almost half the length of all the erosion forms of the Dnieper river basin is forms of 1st order, and 90% of the total length is 1-4 orders of magnitude. For all the basin of the Dnieper (S = 511 thousand km$^2$) identified 776 sub-basins (Fig. 2) ranging from 1.9 to 22,680.2 km$^2$ IV-IX orders (Table 1). As a result, the comparative analysis of the structure of space slopes of
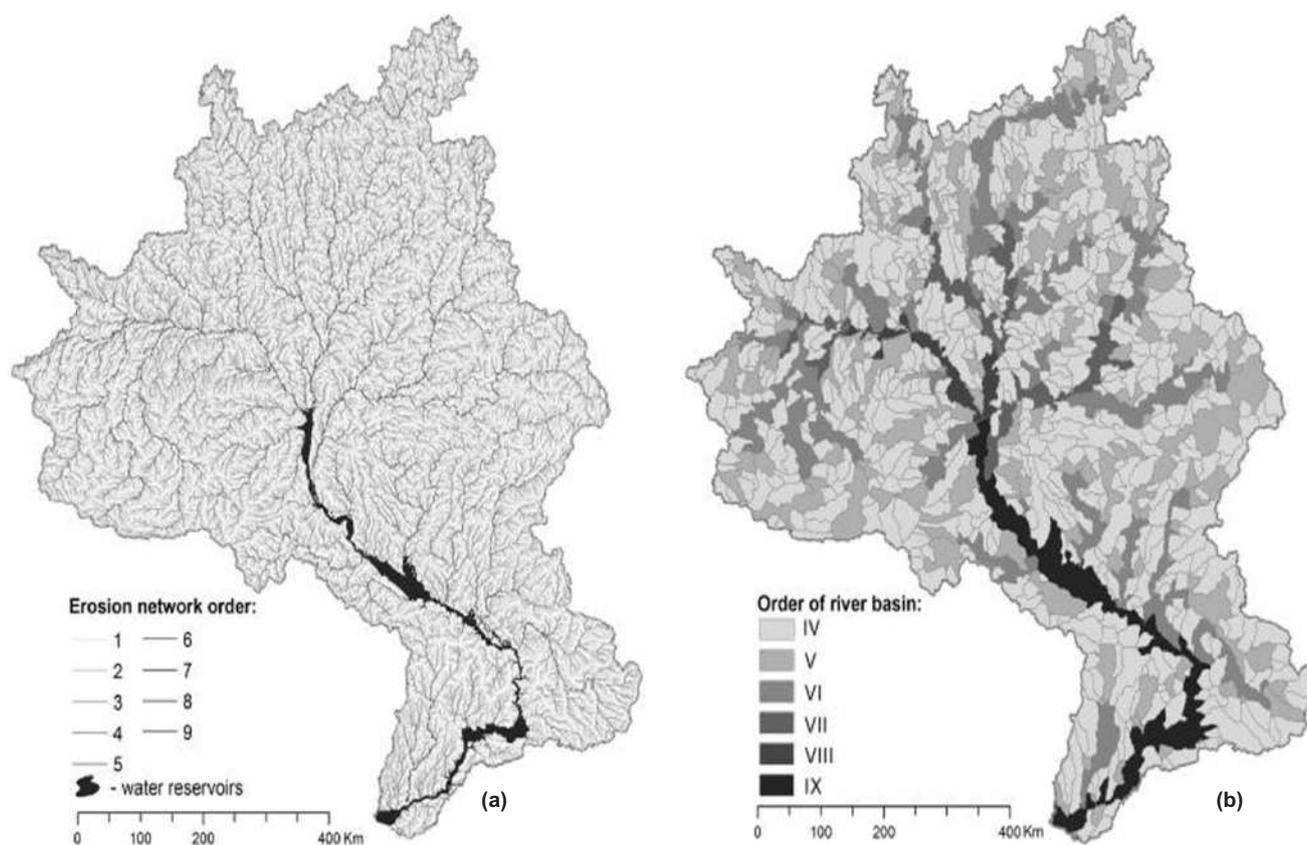


**Fig. 2.** Spatial organization of the internal structure of the Dnieper river basin: a. ordinal structure valley and ravine-beam network; b. basin structure

different orders in the Dnieper basin and that of modal pool found that the top link of the mainstream of the Dnieper basin VII −VIII orders decreased in 2 times, and IX order increased almost 3 times in accordance with the modal form. In the upper levels, slight increase by 1.2 times was observed. The length of the passed way and the time spent on the movement of water and sediment from the slopes in bed is associated with the area of the river basin. Relevant deviations from the modal type is anomalous figure basin planar structure, which allows to analyze the increase or decrease in value accumulation potential local loose sediments in line with the Dnieper. The analysis of the catchment area of different-order Dnieper basin showed that the area is drained by thalwegs from first through the fourth order of 58.4%, fifth and sixth order–33%, from the seventh to ninth–8.6%, so the main supply bed sediments carried by the top and middle managers (91.4%) and local power drifts lower level bed of the Dnieper is 1.8% (Pichura, 2016). The main power source are snow water in the upper reaches, they constitute about 50%, ground–27% and rain−more than 23% (Maksimovich *et al.*, 1985).

Woods, as part of the natural areas of the Dnieper basin perform a number of important and unique ecological and economic functions. They affect the water exchange and the state of aquatic ecosystems, prevent soil erosion, prevent from the formation of gullies and landslides, as well as landscapes and preserve the fertility of grants and so on. Degree river sub-basin watershed forest preserves the natural ecological balance of the entire trans-boundary basin, which significantly disrupted human activities. Land resources of the Dnieper river basin are characterized by a high level of development of business. When taking into account, the degree of tillage and forest to assess the consequences of the anthropogenic violation of the territory (Lisetskii *et al.*, 2016) transfer from medium to strong degree of transformation of land resources is associated with a share of more than 40% of arable land and forest cover at least 20%. The smallest loss of river flow observed in the weighted

average slopes to 2.5°, when plowing watershed is 40–60%, natural grasslands – 33–53% forest cover and shelter–7% (Lopyrev *at al.*, 1999; Buryak, 2015).

Forest resources are very unevenly distributed throughout the basin of the Dnieper River. Forests are mainly concentrated in the upper part of the basin and are underrepresented in its lower part, dominated by a relatively small area of artificial afforestation and forest shelter belts. The total forest area is approximately 175.4 km$^2$, including Belarus–48.6%, Russian Federation–31.6% and Ukraine−17.1%. Forest cover of trans-boundary Dnieper basin for individual catchment river sub-basin ranges from 0% to 95% (Fig. 3a). Most of sub-basin (~ 97%) of forestation> 20% are located in the boundaries of the upper reaches.

Land resources of the Dnieper river basin are characterized by a high level of development of economic. Almost three-fifths of the basin completely lost its natural landscape as a result of intensive economic use. Lopyrev *at al.* (1999) proposed to assess the sustainability of agricultural landscapes on parity "arable land / natural lands". On average for gently hilly terrain ratio "arable land / natural lands" defines the following types of state agricultural landscapes: 70:30–destruction, 60:40–not stable, 50:50−boundary stable, 40:60–minimally stable, 35:65–average stable, 30:70–resistant, 25:75–coverings, 0:25: 100–75−ecological balance with steady increase soil fertility. Based on this classification, the average value of ecosystem sustainability landscape Dnieper river catchment is estimated as unstable. Plow areas of trans-boundary Dnieper river basin in separate sub-basin catchment is distributed evenly (Fig. 3b) in the direction of the Dnieper river from north to south, it increases on average by 75% and from east to west by 60%. The ratio of arable and forest cover gives an idea of the system of strategic environmental assessment of the degree of environmental sustainability as each sub-basin and the entire basin.

The results of modeling indicate a high degree of environmental vulnerability and displays strong degraded

**Table 1.** Distribution of river sub-basins within the research area by order

| Order | Total, units | Total area, km$^2$ | Relative importance to the total area, % | $\overline{S}$, km$^2$ | Relative importance of the total area in the modal river basin, % | Deviation from the value , +/− |
|---|---|---|---|---|---|---|
| IV | 607 | 298379.8 | 58.4 | 492.5 | 50.0 | +8.4 |
| V | 131 | 104036.7 | 20.4 | 795.6 | 26.0 | –5.6 |
| VI | 30 | 64555.0 | 12.6 | 2155.8 | 12.5 | +0.1 |
| VII | 5 | 15391.1 | 3.0 | 3083.8 | 6.5 | –3.5 |
| VIII | 2 | 5957.2 | 1.2 | 2984.1 | 3.5 | –2.3 |
| IX | 1 | 22680.2 | 4.4 | 22680.2 | 1.5 | +2.9 |
| Total | 776 | 511000 | 100 | – | 100 | – |

land resources processes more than 70% of the trans‐boundary basin, leading to a significant disruption of hydro‐eco‐system of the Dnieper river.

The most valuable in the economic terms on which degraded water and erosion have the least impact on agricultural landscapes are upland areas. In the areas of slopes, significant signs of erosion processes are primarily caused by anthropogenic factor. Analysis of the situation of erosion suggests that it appears since the of slopes 1° (Oliinyk *et al.*, 2014). Spatial analysis of the morphometric characteristics of relief determined that slopes share of the boundaries of the Dnieper river basin (Fig. 4a) average increases from west to east by 2.7 times, in the direction of the river (north→ south) distribution is uneven with sinusoidal changes.

A part of the area of slopes of the southern exposure $P_{sc}$ is an additional criterion and displays a tendency to exercise pools erosion during snowmelt. $P_{sc}$ criterion for each basin was calculated based on the digital elevation model (DEM) division raster displays, sampling azimuth values ranging from 135 to 225˚. It is assumed if the $P_{sc}$ > 25%, the basin topography is characterized by high erosion potential during

snowmelt (Lisetskiy *et al.*, 2012; Buryak, 2015). A part of $P_{sc}$ is less than 20% (Fig. 4b) with 183 (23.9%) sub‐basin with a total area of 104.4 thousand $km^2$ ~ (20.4%) in the range of 20‐25% with 472 (61.6%) sub‐basin area ~ 341.6 thousand $km^2$ ~ (66.9%), increased erosion potential in snowmelt are 121 sub‐basin watershed with a total area of ~ 65.0 thousand $km^2$ (12.7 %).

In the cultivated slopes of the trans‐boundary basin, the modern water‐erosion processes occur mostly in the form of planar flushing. Natural factors of development and intensity of flow are features of topography (slope length and slope exposition), weather conditions (amount and intensity of rain and snowmelt rate), erosion and other soil properties. Plow slopes of the Dnieper river basin in some areas of sub‐basins reaches 64% (Fig. 5a), thus 596 (77.8%) of the sub‐basin watershed with the total area of 383.6 thousand $km^2$ (75.2%) have less than 20% of cultivated slopes; 20 to 50% of cultivated slopes‐174 (34.0%) with the area of 125.1 thousand $km^2$ (24.5%), more than 50% of the slopes are cultivated 6.0 (0.8%) with an area of 2.3 thousand. $km^2$ (0.5%). Plow slopes significantly increased (11 times) from west to east trans‐boundary basin, and this trend is observed
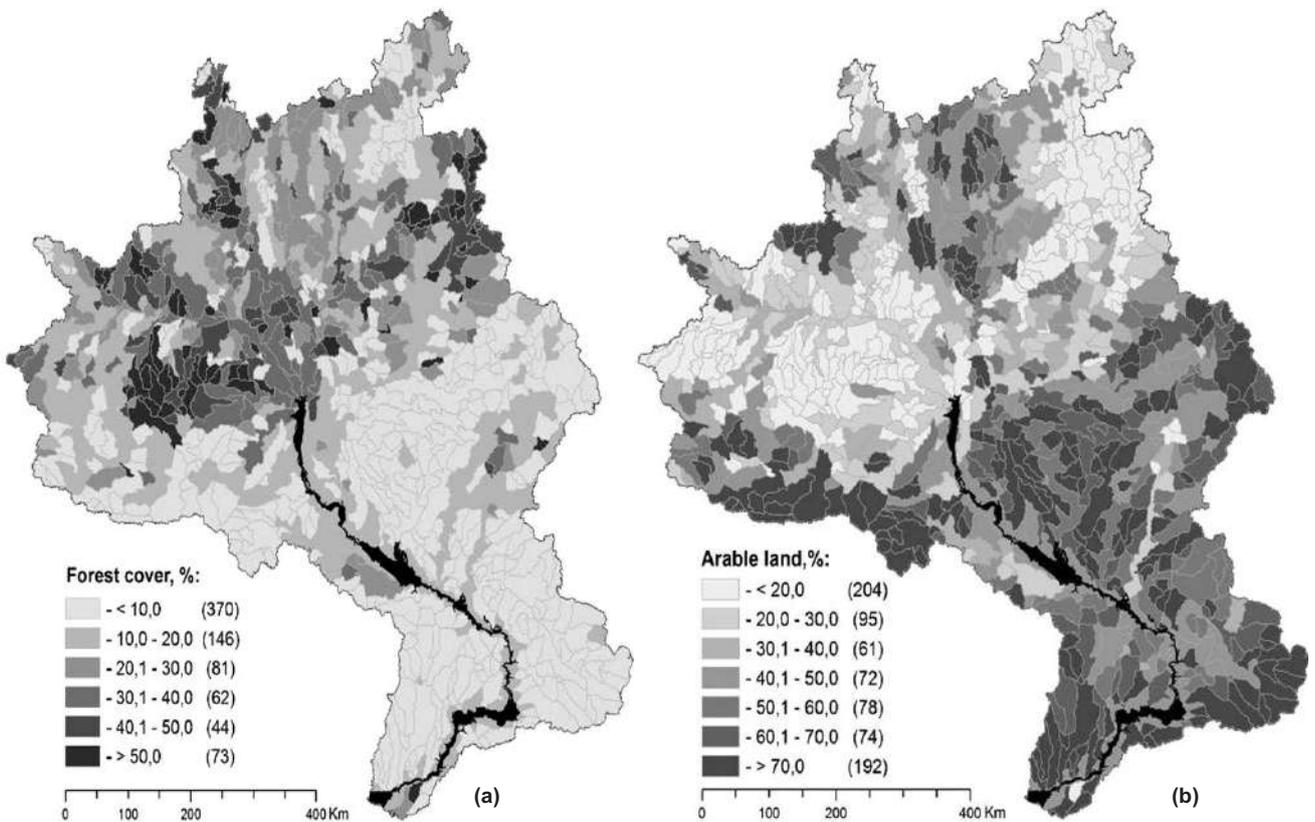


**Fig. 3.** Spatial distribution characteristics of forest area (a, %) and agricultural development landscape  (b, %) Dnieper river basin

from its source to the delta of the Dnieper, in the direction of plowing slopes landscape sub-basin increased by an average of 4-5 times.

As a result of excessive agricultural development of landscape of Dnieper river basin, including high tillage, extensive use of productive land, become widespread processes of soil degradation, characterized by erosion index. In the medium and strongly washed-off lands of opportunity soil infiltration is reduced to 30% and increases washing-off 1.5-2.0 times, which leads to a significant increase in accumulation processes in rivers of products erosive destruction of soil with agrochemicals mio-genes, heavy metals, including radionuclides, which significantly impair the quality of river water, cause eutrophication of water bodies, sedimentation ponds, reservoirs, valleys of small rivers that cause extinction of many of them in the borders of the Dnieper river basin. According to the historical and geographical research and decryption of satellite images it has been found that over the past 200 years, the total length of small rivers (100 km) has decreased by almost 40% with an average speed degradation of streams almost 15 km year^{-1} (Lisetskii *et al.*, 2015).

Erodibility of soils in the Dnieper basin varies of boundaries (Fig. 5b) of 0.13% (absent) to 74.5% (catastrophic): 436 (~ 56.2%) catchment of river basins with a total area ~ 260.2 thousand km$^2$ (almost 51% of the total area of the Dnieper basin) have weak or non-existent area of eroded lands; a significant degree of soil erosion with 149 (~ 19.2%) sub-basin with a total area of ~ 117.5 thousand km$^2$ (~ 23.0%); from severe to catastrophic erosion of soil with 191 (~ 24.6%) sub-basin with a total area of ~ 133.3 thousand km$^2$ (~ 26.0%). It is estimated that on average soils slightly eroded and shortfall crop is 10-20% (the average eroded – 40-60% and strongly eroded – up to 80%). Summary results of the evaluation indicators of the degree of transformation agrogenic structural and functional status of landscape ecosystems of the Dnieper river basin are shown in Table 2.

As a result of geo-modeling (spatial grouping or clustering) typing was made (Fig. 6) of watershed sub-basin by six indicators of the agrogenic transformation of structural and functional state of the landscape ecosystems of the Dnieper river basin. There are three groups (clusters), where defining classification signs were forestry, erodibility and tillage (including slopes) soil (Table 3), this section is consistent with
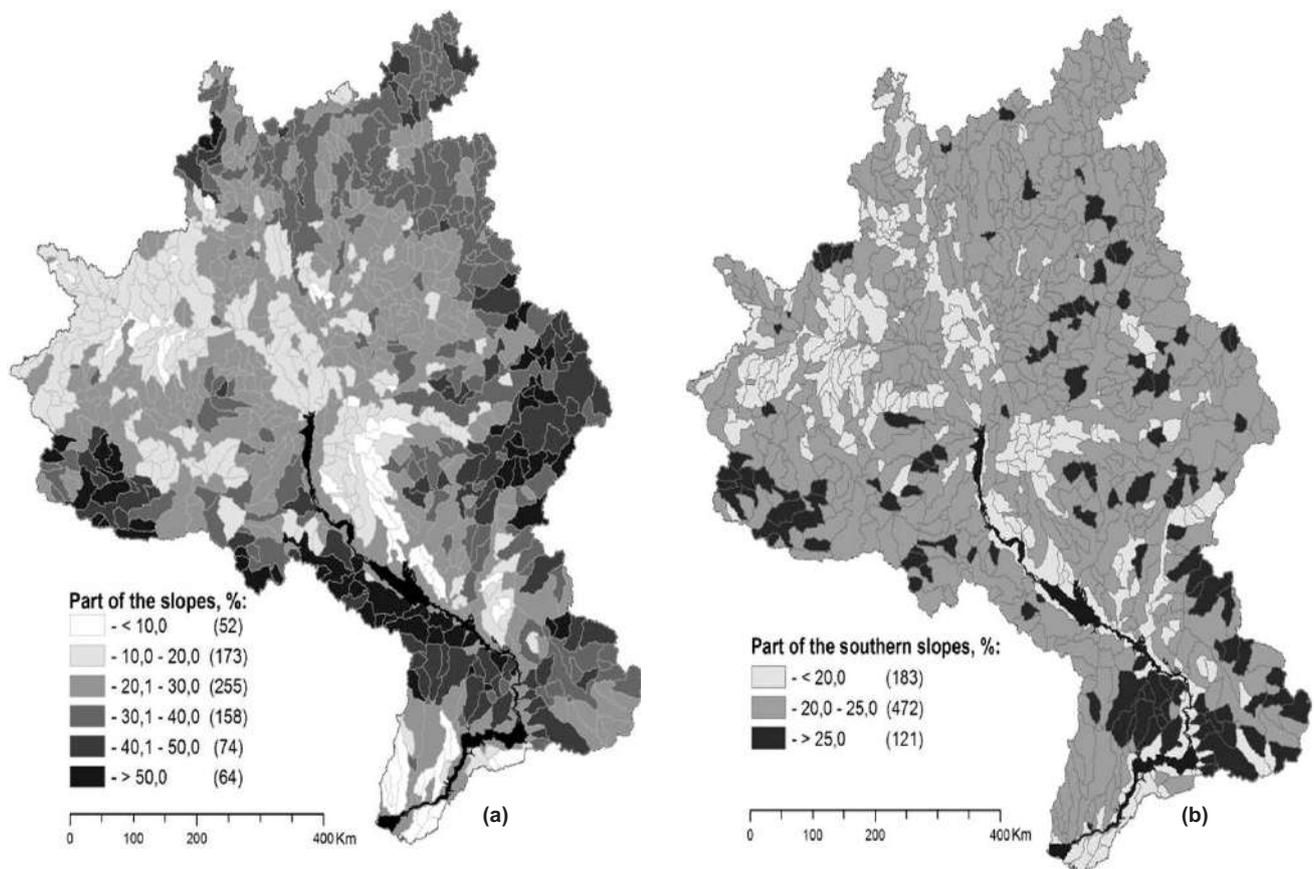


**Fig. 4.** Characteristics of morphometric parameters of slopes within the boundaries of different-order watershed sub-basin of the river Dnieper: a. part of the slopes more 1° %; b. part of the southern slopes (%)

*Vitalii Ivanovich Pichura et al*

the physical and geographic zone trans-boundary of Dnieper river basin and the degree of anthropogenic load, sub-basin of Group I confined to the forest zone, II and III groups to steppe and steppe zones. Tillage and soil erosion gives an idea of the results of large-scale long agrogenic load and modern structural and functional status of landscape ecosystems of Dnieper river basin.

Group I (landscape and ecologically stable or slightly broken) −sub-basin of absent or weak soil erodibility and low agrogenic transformation, where the proportion of arable land is on average about 19%, including on the slopes of 5%, average forest cover of about 30%. This group is 95% gone
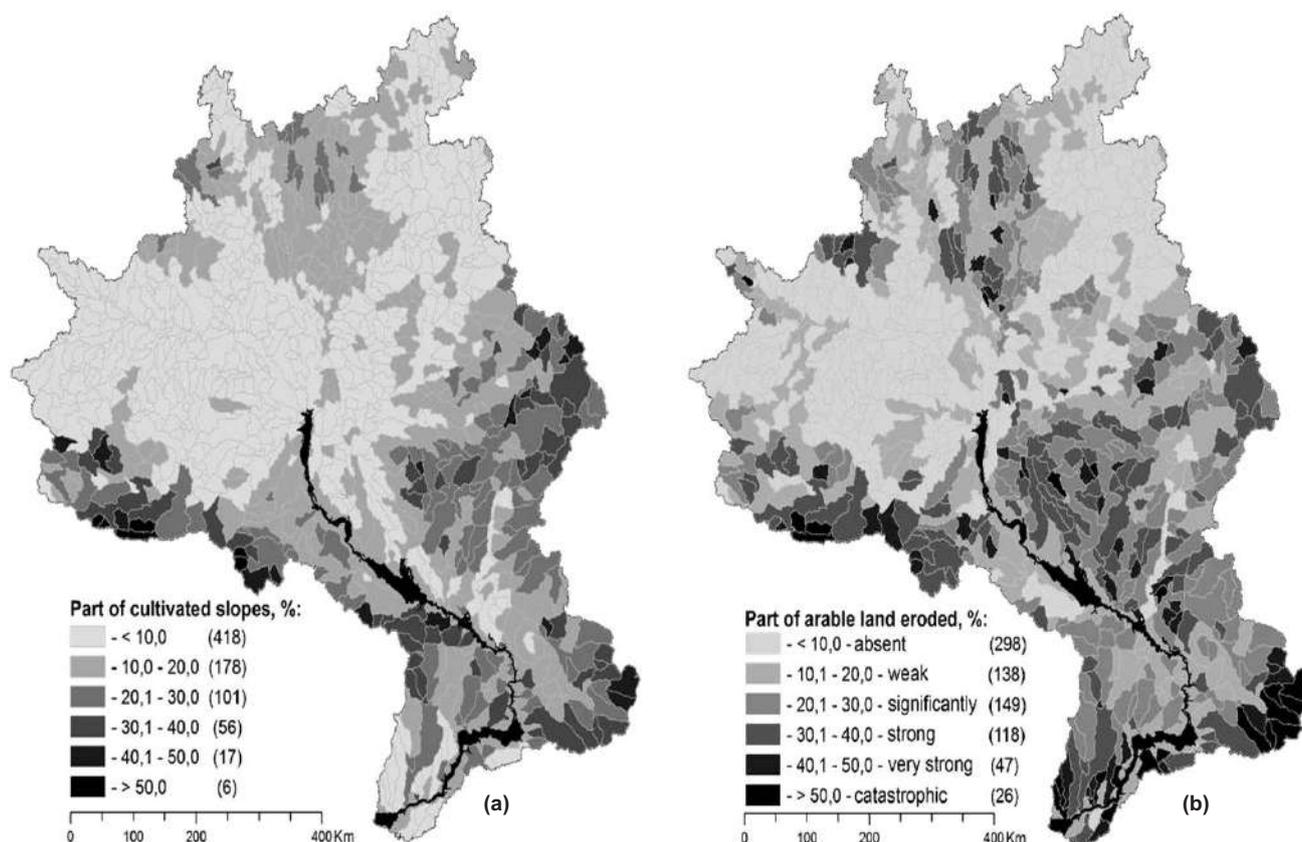


**Fig. 5.** Erosion danger and intensity of degradation of farmland in the watershed boundaries different-order Dnieper river sub-basin: a. part of cultivated slopes, (%), b. part of arable land eroded (%)

**Table 2.** Average different-order sub-basin watershed by the gradation of indicators of the agrogenic transformation of the structural and functional status of landscape ecosystems of the Dnieper river basin

| Indicators | | Share in the sub-basin watershed boundaries, % | | | | | | Total, km$^2$ |
|---|---|---|---|---|---|---|---|---|
| | | <10 | 10–20 | 20–30 | 30–40 | 40–50 | >50 | |
| Forest cover | S | 198792.70 | 125631.10 | 59463.70 | 50640.40 | 31467.00 | 45005.10 | 511000 |
| | % | 38.9 | 24.6 | 11.6 | 9.9 | 6.2 | 8.8 | 100 |
| Arable land | S | 40865.20 | 57972.20 | 66104.70 | 38574.20 | 93048.60 | 214435.10 | 511000 |
| | % | 8.0 | 11.3 | 12.9 | 7.5 | 18.2 | 42.0 | 100 |
| Slopes more 1° | S | 16612.51 | 92872.06 | 176890.40 | 134982.50 | 48567.10 | 41075.44 | 511000 |
| | % | 3.3 | 18.2 | 34.6 | 26.4 | 9.5 | 8.0 | 100 |
| Cultivated slopes | S | 223917.60 | 159686.10 | 77119.09 | 38679.22 | 9297.20 | 2300.80 | 511000 |
| | % | 43.8 | 31.2 | 15.1 | 7.6 | 1.8 | 0.5 | 100 |
| Arable land eroded | S | 151620.30 | 108608.20 | 117436.60 | 88332.11 | 29523.40 | 15479.40 | 511000 |
| | % | 29.7 | 21.3 | 23.0 | 17.3 | 5.8 | 3.0 | 100 |

**Table 3.** Indicators of agricultural development and ecological status of catchment sub-basin trans-boundary Dnieper basin
(Mean ± Standard Deviation)

| Group | Part of arable | Part of forest | Part of arable land | Part of slopes | Part of cultivated | Part of southern |
|---|---|---|---|---|---|---|
| | | | % | | | |
| I | 19.3±8.7 | 29.8±11.5 | 5.8±4.3 | 26.4±8.9 | 5.0±3.8 | 21.3±2.2 |
| II | 75.7±15.1 | 6.9±4.4 | 33.2±11.4 | 16.6±8.0 | 12.1±6.3 | 20.9±2.8 |
| III | 66.7±11.2 | 7.4±4.8 | 28.5±11.0 | 44.0±13.2 | 28.2±10.1 | 24.9±2.8 |



Grouping:
- group I (373)
- group II (198)
- group III (205)

0    100    200    400Km
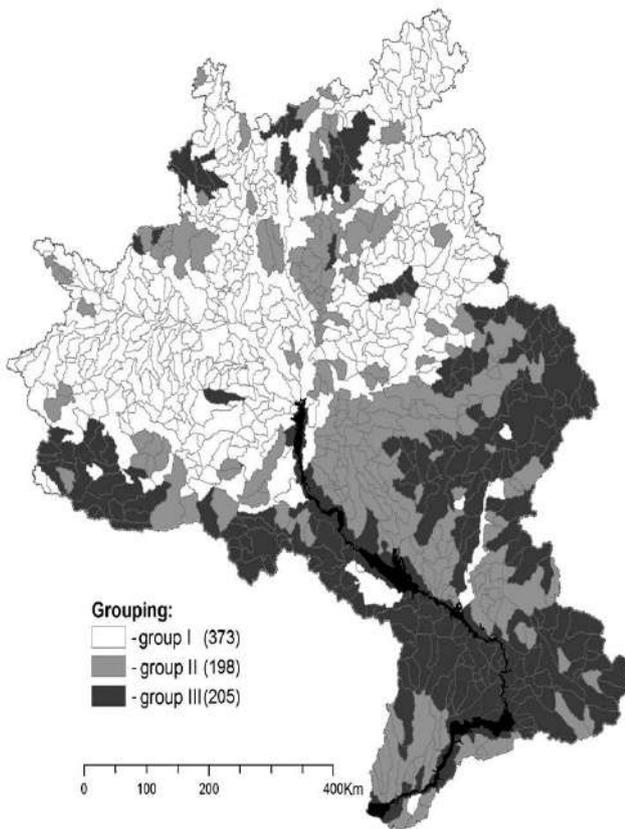
**Fig. 6.** Spatial group of the Dnieper basin by the level of the agrogenic transformation of structural and functional state of landscape ecosystems

catchment area of the rivers located in the upper reaches of the river, the total number was 373 (48.7%) sub-basin with a total area of 224.2 thousand km$^2$ (~ 43.9%).

Group II (high agro-genic transformation of landscapes) – sub-basin with high agricultural development (about 76%), a significant and strong low erodibility and forestation ~ 7%. It includes 198 (~ 25.8%) sub-basin with a total area of 116.6 thousand km$^2$ (~ 22.8%).

Group III (agrogenic transformed with high soil erosion risk) – sub-basin with high agricultural development (about 67%), strong and very strong erodibility, low forestation ~ 7%, a high proportion of cultivated slopes an average of 44% and increased erosion potential during snowmelt.

Construction of the cascade on the Dnieper of six

reservoirs with a total area of 6950 km$^2$ and total accumulated volume of 43.8 km$^3$ of water led to the flooding of 50 thousand km$^2$ and flooding of 10 thousand km$^2$ of productive land violated the ecological balance and radically altered water exchange conditions compared to natural conditions it slowed to 30 times. Taking into account these conditions the waterfront of the Dnieper basin of IX procedure was confined to the third degree of agrogenic transformation of the structural and functional status of landscape ecosystems. It includes 205 (~ 26.8%) sub-basin with a total area of 170.2 thousand km$^2$ (~ 33.3%).

In most sub-basin of the river Dnieper main criterion of destabilization of agricultural landscapes is a high level of tillage, in this case the most effective tool for improving the ecological balance of the territory trans-boundary basin study is the reduction of arable land for other land or environmental fund. As a result of the calculations, it is necessary to reduce the arable land, especially in the territories agrogenic transformed sub-basins with high soil erosion hazard, the need for reducing the extent of extensive farming agro–landscape load on the Dnieper river basin was identified (Fig. 7) the boundaries of 470 (61.4%) of the sub-basin with the total area of ~ 346.3 thousand km$^2$ ~ (~ 67.8%).

As the arable lands are the main source of agricultural production and guarantee food security in the Dnieper river basin, it is necessary to find a compromise between achieving the environmental sustainability of agricultural landscapes and cost-effective intensity of agricultural production, especially organic farming to obtain stable yields and reduce the level of pollution of surface waters.

**CONCLUSIONS**

As a result geo-modeling determined that more than 50% of the Dnieper basin has high agrogenic transformation of the structural and functional status of the landscape ecosystems. Therefore, rational land use in accordance with the strategic environmental assessment should take into account the characteristics and features of the landscape, promote the protection and reproduction of soil, vegetation and other natural components in a single agreement between the countries of trans-boundary basin. This can be achieved through the basin of nature and landscape-ecological
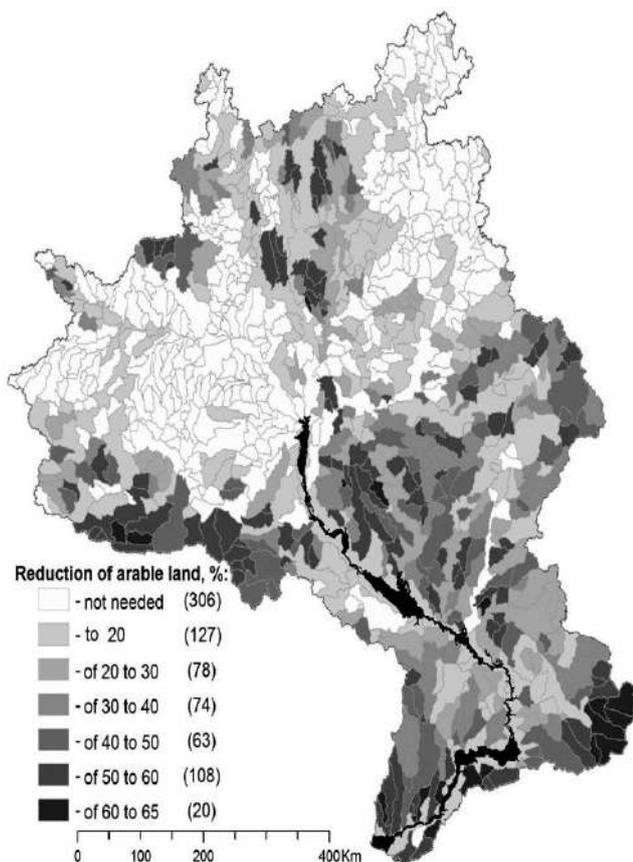
**Reduction of arable land, %:**

| | | |
|---|---|---|
| | - not needed | (306) |
| | - to 20 | (127) |
| | - of 20 to 30 | (78) |
| | - of 30 to 40 | (74) |
| | - of 40 to 50 | (63) |
| | - of 50 to 60 | (108) |
| | - of 60 to 65 | (20) |

0    100    200    400Km

**Fig. 7.** Need to reduce the burden on the agricultural basin of the river Dnieper

approach, which involves identifying relationships in ecosystems agricultural landscapes and water bodies, their record in selecting destinations and types of land use in the watershed sub‑basin boundaries. To generate environmentally sustainable agricultural landscapes and reducing erosion and cumulative processes in catchment areas of river basins, priority must provide the following set of measures: organizational and economic reclamation‑a system of soil rotation, mosaic structure of land, degraded land, creation of ecological frame area, etc. hydro‑meliorate – sustainable irrigation, erosion rates, protection zones, bottom buildings, dams, bridges, spray flow etc. land improvement‑limiting the use of heavy tillage machinery, creating buffer strips of grasses, soil protecting technologies, reduction of pesticide use, handling across slopes, etc; agroforestry‑shelter, and stock‑regulating and forest belts; continuous afforestation slopes, etc.

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# Runoff Estimation and Water Management Options for Coastal Odisha

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**Abstract**: Coastal areas are always under high stress either due to excess water availability or non-availability of water during summer season. This paper deals with estimation of runoff, which is the major component in hydrologic cycle. Runoff characteristics and the estimation of runoff were analyzed by SCS-Curve Number method with 21 years meteorological rainfall data for Kendrapara district of Odisha. On an average 34 percent of actual rainfall goes as runoff in each year, which is one of the major causes of waterlogged situation in this deltaic region. In this paper a case study was carried out by considering the water availability in creeks and water harvesting structures in different season of the year. Based on runoff from the area, spatial distribution of water availability and water quality in different irrigation sources, water controlling devices like sluice structures was designed to control drainage & check saline water entry into the creeks to improve the land and water productivity of the area.

**Keywords**: Runoff, Rainfall, Curve Number, SCS-CN method

Water is an important component for living beings. Declining per capita availability of water leads research to focus on different conservation practices. Rainfall is the major form of precipitation through which the water reaches at ground surface. Many research works are going on for the conservation of rainwater. The rainfall conservation practices should be adopted based on the quantification of runoff from the hydrological areas. Runoff is the process of draining or flowing off of precipitation from a catchment area through surface channel. For a given precipitation, the evapotranspiration, initial loss, infiltration and soil moisture storage requirement will have to be first satisfied before the commencement of runoff. When these are satisfied, the excess precipitation moves over land surface (Subramanya, 2008). A fraction of total rainfall that reaches the ground water table called as the effective rainfall. The SCS-CN method is used to calculate effective rainfall which is assessed on small mediterranean catchment. The land use aggregation and land use classification type have significant effect on hydrological modeling and in particular on effective rainfall modeling (Payraudeou, 2003). Runoff measurement is required for estimating the part of rainfall which goes as overflow. The overflowing water can be utilized as ground water recharge and also the runoff water can be stored for the future use. Such estimation is needed for design of engineering structures in the post event appraisal work for evaluation of various land use practices and for assessment of environmental impact of watershed development (Hawkins,1985, 1993).By retarding the flow of runoff water soil erosion can be minimized and conserved runoff water can be reused by irrigation as well as it can be helpful for fish farming. Runoff in volume basis is estimated by using different empirical formula for a closed catchment.United States Department of Agriculture (USDA) Soil Conservation Services (SCS) of USA in 1969 developed SCS-CN method is a simple predictable and stable conceptual method for estimation of direct runoff depth based on storm rainfall depth. It is dependent upon only one parameter, CN (Curve Number) which is determined by Hydrological Soil-cover Complex which depends upon land use cover, land treatment or practice, hydrologic condition and hydrological soil group. In this paper, standard SCS-Curve Number method was used for computation of runoff depth for a coastal irrigated area of Odisha.

## MATERIAL AND METHODS

The study was conducted in Kendrapara district of Odisha. The study area is having of 640 ha cropping land. The area receives an average annual rainfall of 1507mm. The area is dominated by clayey soil, which restricts the downward entry of water and creates waterlogging situation during monsoon season. According to the soil property it is in hydrological soil group C, having low infiltration rate when thoroughly wetted and a low rate of water transmission

(Ritzema, 1996). Major cropping system adopted in the study area is Rice‑Green gram/pulses‑fallow throughout the year i.e. June to September (*kharif*), November to February (*rabi*) and March to May (summer) respectively. Monthly rainfall data for the period of 1994 to 2014 was considered for estimation of effective rainfall by using the FAO CROPWAT model. Runoff was computed by using SCS curve number method. Curve number method being used for measuring the depth of runoff from rainfall depth developed by Soil Conservation Service (SCS, 1964; 1972). The initial accumulation of rainfall represents interception, depression storage and infiltration before the start of runoff which is called as initial abstraction. After the runoff started additional runoff started in form of infiltration is called as actual retention. With increase in rainfall actual retention also increases up to the maximum value: potential maximum retention. Curve Number method mathematically is the ratio of actual retention to potential maximum retention equal to the ration of actual runoff to potential maximum runoff.

$$\frac{F}{S} = \frac{Q}{P - I_a}$$

Where, F = actual retention, mm; S = potential maximum retention, mm; Q = accumulated runoff depth, mm; P = accumulated rainfall depth, mm; $I_a$ = initial abstraction, mm

By simplifying

$$Q = \frac{(P - 0.2S)^2}{P + 0.8S} \text{for} P > 0.2S$$

Potential maximum retention S has been converted to Curve Number. This relationship is

$$CN = \frac{25400}{254 + S}$$

Curve number is decided relating to land use cover, land treatment or practice, hydrologic condition and hydrological soil group. These parameters together called as Hydrological Soil‑cover Complex. The CN value varies from 0 to 100.This runoff estimation procedure was followed for study area. The soil type of the area was found to be clay soil with low infiltration rate which comes under Hydrological soil group C.

Other parameters for deciding CN are land use and land cover for which the land use pattern was classified into three growing seasons as mentioned above. As per the field survey, it was observed that farmers grow paddy and legumes or pulses in *kharif* and *rabi* season respectively. Mostly fallow lands were observed during summer season due to non‑availability of required amount of water. Generally, conventional straight row method is being adopted by the farmers. The curve number (CN) values were decided based on this field condition (Table1).

**RESULTS AND DISCUSSION**

Rainfall data of 21 years (1994 to 2014) showed that average annual rainfall and runoff of Kendrapara is 1507mm and 510mm respectively (Table 1) with 59 numbers of rainy days. The highest annual rainfall of 2832 mm occurred during the year 2003 with highest runoff of 1239mm (Fig. 1). The lowest rainfall of 582 mm occurred during the year 2000 with lowest runoff of 78 mm. It showed the positive correlation between the rainfall and runoff. Similarly highest monthly average rainfall was recorded during the month July, August, September and October with 300mm, 411mm, 237mm and 238mm respectively. The highest runoff was also recorded for same months with 101, 152, 66 and 93mm respectively. On an average 72.7 per cent of the annual rainfall is received during monsoon season. Pre and post monsoon rainfall contributed only 7.2 and 19.0 per cent of the total annual rainfall in the study area. Rest 0.9 per cent rainfall occurred during winter season (Table 2). It also showed that pre monsoon rainfall contributed maximum rainfall (89.1%) as effective rainfall followed by monsoon and winter rainfall. Monthly rainfall/runoff analysis was shown in Table 3. July month receives highest average rainfall of 410.9 mm (27.3% of annual rainfall) and runoff of 152 mm (29.7 % of annual runoff). December receives lowest average rainfall (3.3 mm) and no runoff. Maximum rainfall and runoff occurred in August with 894.9 and 416.0 mm respectively (Table 4).

In this analysis, CN method with basic parameters like hydrologic soil group, land use, land cover and condition of soil were considered. From the field survey, it was observed that rice‑pulses (green gram)‑fallow is the common cropping

**Table 1.** CN values for study area soil condition relating to different crop growing seasons

| Crop growing season | Land use | Treatment/ Practices | Hydrological condition | Hydrological soil group | CN value | *S* |
|---|---|---|---|---|---|---|
| Monsoon *kharif* (June–September) | Paddy | Straight row | Good | C | 83 | 52.02 |
| Post‑monsoon *rabi* (October–February) | Legumes | Straight row | Good | C | 81 | 59.58 |
| Pre‑monsoon summer (March–May) | Fallow | Straight row | Good | C | 91 | 25.12 |

pattern in the study area. The cultivation practices adopted by the farmers are conventional straight row cropping system for rice and green gram. During the summer season mostly the land remains fallow. The water availability status showed that the area received excess amount of water only during the monsoon periods i.e. July to October, whereas rest all the season, there is deficit rainfall which could not fulfill the crop water requirement (Fig. 2). Monthly rainfall, runoff and PET status (Table 5) showed that 72 per cent of annual rainfall occurred in monsoon season. Nearly 68 per cent of annual runoff occurred in this season only where the potential ET is only 33 per cent of annual PET. Hence additional storage structures could tap the excess rainfall during monsoon season, which could be utilized in post monsoon and summer season. But, the area is located very close to sea and waterlogging is the common problem in most of the deltaic region of the State.

Hence detail study in one of the representative area of the coastal ecosystem was identified for the detail study. The area was surrounded by the network of creeks, which very common in most of the coastal areas. From the preliminary study, it was observed that in study locations (Sunity) of 3800 ha of area, there is presence of 65 numbers of water bodies and a network of 19 numbers of creeks within the length of
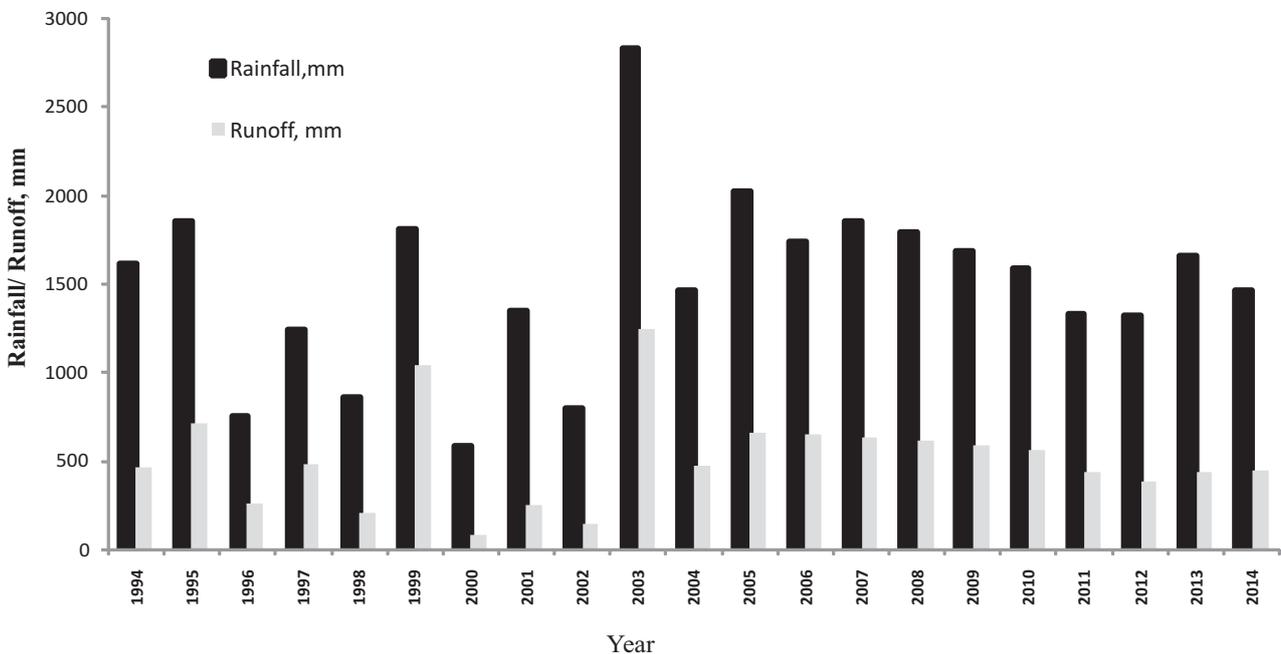


**Fig. 1.** Annual rainfall, runoff of Kendrapara

**Table 2.** Rainfall/runoff analysis of Kendrapara

|  | Minimum rainfall, mm | Maximum rainfall, mm | Average rainfall, mm | Standard Deviation, mm | Coefficient of variation |
|---|---|---|---|---|---|
| Rainfall | 582.3 | 2831.8 | 1507.1 | 491.5 | 0.3 |
| Runoff | 77.5 | 1238.7 | 510.5 | 267.2 | 0.5 |

**Table 3.** Rainfall, effective rainfall distribution in Kendrapara district

| Seasons | Average rainfall (mm) | Percentage of total rainfall | Average effective rainfall, mm | Effective rainfall (% of rainfall) |
|---|---|---|---|---|
| Pre-monsoon (March–May) | 109.4 | 7.2 | 97.0 | 89.1 |
| Monsoon (June–September) | 1096.1 | 72.7 | 909.7 | 82.8 |
| Post-monsoon (October–December) | 287.3 | 19.0 | 146.4 | 50.6 |
| Winter (January–February) | 13.8 | 0.9 | 11.5 | 82.3 |
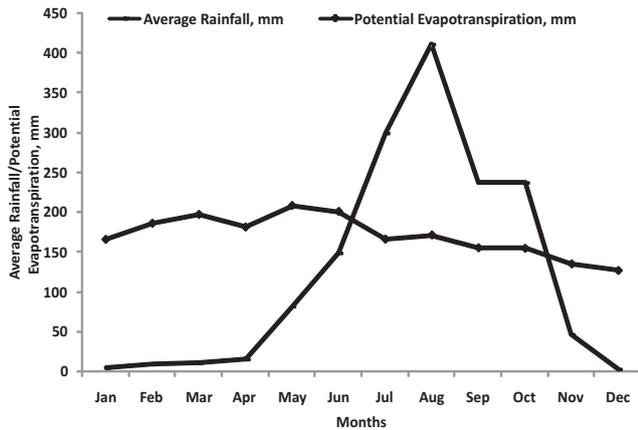
**Fig. 2.** Water availability status of Kendrapara

0.43 to 23 km. Statistics showed that the area of the water bodies varied within 823–3568 m² with average area of 1800m² and 900–1300 m² water harvesting structures were quite common in this area.

In order to study the hydraulics of water harvesting structures, depth-capacity curve of two water harvesting structures were plotted. It showed that out of 52 standard weeks, the water harvesting structures remains dried within 14 to 28th week i.e. during summer season. These structures could be utilized to store the runoff water during monsoon period, which could be utilized during non-monsoon season to meet the crop water demand. Hence appropriate locations should be identified based on the land use land pattern of the

**Table 4.** Monthly rainfall and runoff analysis

| Month | Minimum, mm | | Maximum, mm | | Average, mm | | % contribution to annual, % | | Standard Deviation, mm | | Coefficient of variation | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R | RO | R | RO | R | RO | R | RO | R | RO | R | RO |
| January | 0.0 | 0.0 | 37.3 | 1.0 | 4.2 | 0.0 | 0.3 | 0.0 | 8.6 | 0.2 | 2.1 | Nil |
| February | 0.0 | 0.0 | 108.6 | 42.0 | 9.6 | 2.0 | 0.6 | 0.4 | 24.1 | 8.9 | 2.4 | 4.4 |
| March | 0.0 | 0.0 | 93.5 | 52.0 | 10.8 | 4.0 | 0.7 | 0.8 | 21.3 | 11.3 | 1.9 | 2.8 |
| April | 0.0 | 0.0 | 128.4 | 98.0 | 16.3 | 7.0 | 1.1 | 1.5 | 31.9 | 22.5 | 2.0 | 3.2 |
| May | 0.0 | 0.0 | 513.2 | 350 | 82.3 | 38.0 | 5.5 | 7.5 | 110.2 | 77.2 | 1.3 | 2.0 |
| June | 42.7 | 0.0 | 355.8 | 99.0 | 148.6 | 32.0 | 9.9 | 6.3 | 93.3 | 28.8 | 0.6 | 0.9 |
| July | 40.1 | 0.0 | 717.5 | 380 | 299.5 | 101 | 19.9 | 19.7 | 163.9 | 98.5 | 0.5 | 1.0 |
| August | 75.6 | 3.0 | 894.9 | 416 | 410.9 | 152 | 27.3 | 29.7 | 204.9 | 120 | 0.5 | 0.8 |
| September | 30.0 | 0.0 | 500.0 | 287 | 237.0 | 66.0 | 15.7 | 12.9 | 141.1 | 67.4 | 0.6 | 1.0 |
| October | 15.0 | 0.0 | 760.3 | 262 | 238.0 | 93.0 | 15.8 | 18.3 | 231.2 | 136 | 1.0 | 1.5 |
| November | 0.0 | 0.0 | 218.4 | 86.0 | 46.0 | 15.0 | 3.1 | 2.9 | 72.1 | 26.9 | 1.6 | 1.8 |
| December | 0.0 | 0.0 | 38.0 | 4.0 | 3.3 | 0.0 | 0.2 | 0.0 | 8.8 | 0.8 | 2.9 | Nil |

**Table 5.** Season wise rainfall/runoff analysis

| Season | Minimum (mm) | | Maximum (mm) | | Average (mm) | | Contribution to annual (%) | | Standard deviation | | Coefficient of variation | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R | RO | R | RO | R | RO | R | RO | R | RO | R | RO |
| Pre-monsoon | 36.0 | 0.9 | 513.0 | 349.8 | 109.4 | 49.9 | 7.3 | 9.8 | 103.4 | 76.4 | 0.9 | 1.5 |
| Monsoon | 462 | 71.4 | 2130 | 963.1 | 1096 | 350.3 | 72.7 | 68.6 | 411.7 | 211.9 | 0.4 | 0.6 |
| Post-monsoon | 15.0 | 0.0 | 760.0 | 567.1 | 284 | 108.0 | 18.8 | 21.2 | 230.9 | 134.0 | 0.8 | 1.2 |
| Winter | 0.0 | 0.0 | 109.0 | 41.8 | 17.1 | 2.3 | 1.1 | 0.5 | 25.5 | 8.9 | 1.5 | 3.9 |

R= Rainfall, RO=Runoff

**Table 6.** Monthly rainfall, runoff and PET status of the Kendrapara

| Month | January | February | March | April | May | June | July | August | September | October | November | December |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Average rainfall, mm | 4.2 | 9.6 | 10.8 | 16.3 | 82.3 | 148.6 | 299.5 | 410.9 | 237.0 | 238.0 | 46.0 | 3.3 |
| Average runoff, mm | 0.1 | 2.1 | 4.1 | 7.4 | 38.4 | 32.0 | 100.8 | 151.6 | 66.0 | 93.3 | 14.7 | 0.2 |
| Potential ET, mm | 166.0 | 186.1 | 197.1 | 181.4 | 208.2 | 200.8 | 166.3 | 171.5 | 155.5 | 154.8 | 135.4 | 127.2 |

area to store the runoff water before flowing to the sea. This will facilitate to increase the crop coverage during post and summer season by increasing overall productivity of the area.

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# Evaluation of Saturated Hydraulic Conductivity Methods for Different Land Uses

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**Abstract**: Saturated hydraulic conductivity (Ks) is one of the important soil properties and essentially required for designing irrigation, drainage and waste water systems, and modelling infiltration, runoff, erosion, seepage, solute transport and migration of pollutant to groundwater. However, the accuracy of Ks is highly dependent on the method used, soil and surface characteristics. In this study, saturated hydraulic conductivity (Ks) methods such as *in-situ* [single ring infiltrometer (SRI), Double ring infiltrometer (DRI), air-entry permeameter (AEP)], *laboratory* [constant head permeameter (LPM)] and a *pedotransfer function* (PTF) based (ROSETTA[(R)]) methods were evaluated for five different land uses such as residential area (RESA), paddy field (PADF), mango field (MANF), cashew field (CASF) and playground (PLAG). The observed Ks by using SRI, DRI, AEP, LPM and PTF methods were observed for performance and evaluation of measuring methods on land uses and suitability of a method for a land use. The measured Ks data using different methods follow a lognormal distribution. The mean Ks was significantly different for both Ks method and the land use. The AEP and LPM resulted highest and lowest Ks, respectively for all land uses, whereas the Ks was highest and lowest for CASF and PLAG, respectively. For all land uses, the mean Ks were the highest for AEP followed by SRI, PTF and LPM methods. The order of Ks obtained for the land uses were CASF, RESA, MANF, PADF and PLAG. Spatial variability of Ks was observed for DRI method and PLAG. The AEP method was quicker and the PTF method was cheaper.

**Keywords**: Saturated hydraulic conductivity, Ring infiltrometer, Air-entry permeameter, Pedo-transfer function, Land use

Saturated hydraulic conductivity (Ks) is the movement of water in soil surface when it is at saturated condition (Shukla and Lal, 2004). Ks is helpful in designing irrigation, drainage and waste water systems, studying runoff characteristics and groundwater recharge, modelling of movement of water and pesticides from agricultural land (Jarvis *et al*., 2013). Ks at the top soil surface is an essential parameter to be measured as soil properties like depth, land use, land cover and land management affect most of the soil hydrological processes and enables development of hydrogeological models (Rossiter and Jetten, 2011). For accurate measurement of Ks, one should have proper knowledge on soil properties, selection of site, appropriate method and protocol are essential. The Ks methods have been classified into laboratory, in-situ and correlation based methods (Bagarello *et al*., 2013a). The in-situ methods generally yield substantially different Ks values for the same location as they have different operating ranges, boundary conditions, underlying assassins (Bagarello *et al*., 2004). Air entry permeameter method cannot be applicable to wet soils as the wetting front determination will be difficult (Bouwer, 1966).

Spatial and temporal variability of Ks is generally affected by porosity of soil media (macrospores, stones, fissures, cracks and root holes) and fluid media (viscosity and temperature), methods of measurement (Deb and Shukla, 2012). Therefore, accurate measurement of Ks is difficult because it has spatial and seasonal variability and scaled dependency (Bormann and Klaassen, 2008). It is important to know the particular soil and site conditions to select the proper application procedure and the method for getting reliable conductivity values (Hu *et al*., 2013). From several studies it can be concluded that many of the methods may not be accurate or appropriate for all soil conditions. Thus the selection for selecting suitable Ks-methods for a particular land uses must consider some important aspect covering suitability of method, initial cost, labour and time requirement, as well as accuracy (Lee *et al*., 1985). Furthermore, there is no standard benchmark for measuring Ks and thus comparison of methods is essential source of information for choosing a suitable method for specific circumstances (Jacka *et al*., 2014). In order to considering some limited studies focused on measuring Ks for different land uses and no study has been done (to the authors' knowledge) on comparison of air-entry permeameter and ring infiltrometer, which share the same operating principle. Therefore, the objective was to study the performance of air entry permeameter and ring infiltrometer methods for various land use practices and compare them with laboratory and

pedotransfer function based methods.

## MATERIAL AND METHODS

**Study area:** The study of field and laboratory experiments on Ks measurements were performed at Indian Institute Technology Kharagpur (IIT KGP) campus, which has an area of 2100 acres (latitude 22˚31'N longitude 87˚31'E). The climatic condition is humid subtropical carrying an average rainfall of around 1700 mm annually out of which 80% of the total rainfall occurs in June to September (monsoon period). Winters are brief but chilly, lasting from December to mid−February ($10^0$ to $25^0$C), summer (March to June) is hot ($25^0$ to $40^0$C) and sometimes humid (50 to 95%). Five land uses VSRC student hall as residential area (RESA) as an agricultural area Paddy field (PADF) as orchard area Mango field (MANF) and Cashew field (CASF) and Tata Sports Complex as playground (PLAG) were selected for measuring Ks from top 0−15 cm depth of soil. The five years' past record of these land uses is presented in Table 1.

**Soil physical properties:** Soil core samples were collected and analyzed from the top15 cm depth from three locations of each land use. The soil samples were analysed for determining soil texture (percent sand, silt and clay) and bulk density using standard soil analysis protocols (Carter and Gregorich, 2008). It was observed that the soils for all land uses were mostly of sandy loam type. The mean sand and silt contents were not much different among the land uses at 5% level of significance, but the clay content was significantly different (P=0.037), and highest for PADF with 18.33 % and lowest for RESA with 12.67%. The highest clay content in PADF was mainly due to soil puddling and addition of organic carbon during cultural operations. The bulk density was not significantly different among the land uses and varied from 1.72 to 1.85 g $cm^{-3}$. Similarly, the porosity was not significantly different for the land uses and varied from 33 to 35% (Table 2).

**Methods of Ks measurement:** The Ks was measured at three random locations for each land use using single ring infiltrometer, double ring infiltrometer, air−entry permeameter and laboratory permeameter. The pedotransfer function based method was used for estimation of Ks using the soil physical properties.

### In-situ measuring techniques

**Single ring infiltrometer:** Single ring infiltrometer (SRI) device and Green and Ampt (1911) model were used together for estimation of Ks. The SRI consisted of a cylindrical ring of 30 cm diameter, 29 cm height and 2 cm thickness. The cylinder was driven about 15 cm deep in to soil at the study site with taking care the ring sides vertical and minimum soil disturbance. Water was subjected to fill the ring up to a depth of 7 cm which was marked by a point gauge to measure the reduced water level due to infiltration. After reaching at a steady state of water level, observations were made for time taken for a decrease in water level of 1 cm. The observations were used to generate data sets for infiltration rate and infiltration depth values with respect to different time intervals.

The infiltration data were fitted with a physically existing Green and Ampt (1911) infiltration model, to estimate Ks (Rao *et al*., 2006). The Green and Ampt infiltration equation can be written as:

$$f = K_s(1 + \frac{nS_c}{F}) \qquad (1)$$

Where, F is the cumulative infiltration [L]; *f* is infiltration capacity [$LT^{-1}$]; n is porosity of soil; $S_c$ is capillary suction at the wetting front and $K_s$ is field saturated hydraulic conductivity [$LT^{-1}$].

Equation 1 could be considered as a straight line equation;

$$f = m + \frac{n}{F} \qquad (2)$$

Where, F is the cumulative infiltration [L $T^{-1}$]; f is the infiltration capacity [$LT^{-1}$] and *m* and *n* are the Green−Ampt parameters of infiltration model. The values of *f* are plotted against (1/*F*) and the best fit straight line was drawn through the plotted points. The intercept of the best fit line gives the Ks (Rao *et al*., 2006; Machiwal *et al*., 2006; Bagarello and Sgroi, 2007).

**Double ring infiltrometer:** The double ring infiltrometer (DRI) also works on the same principle as SRI. The Ks was estimated similar to the procedure described above using Green and Ampt equation. The DRI consists of an inner ring

**Table 1.** Soil condition for different land uses of the study area

| Land use | Land use history for past 5 years | Ground condition |
| --- | --- | --- |
| PADF | *Kharif*: paddy and *Rabi*: wheat/paddy | Presence of cracks, paddy roots, stems and weeds |
| MANF | Not tilled | Completely shaded, and very light weeds between plants |
| CASF | Not tilled | Loose soil, with long weeds (4−12 cm), many worm holes |
| RESA | Not disturbed | Covered with grass (3−5cm), compact and mixed with gravels/plastics |
| PLAG | Soil compacted annually | Covered with grass (3−5cm), compact and mixed with gravels/plastics |

of 30 cm diameter and an outer ring of 60 cm diameter inserted into the ground at 15 cm depth. Each ring of the infiltrometer was filled up with a constant head of water level and the outer ring helps to check the lateral flow from the inside ring which can estimate better Ks reducing losses. The Ks can be estimated when water flow rate inside the inner ring comes to a steady state. The infiltration data obtained from the DRI method were further utilized to fit into the Green and Ampt infiltration equation and Ks values were estimated by using equations 1 and 2.

**Air entry permeameter:** The operating mechanism of air entry permeameter (AEP) is almost equivalent to the SRI in design and operation. The vertical saturated hydraulic conductivity was measured in this method relating to volumetric flux of water entering into the soil. The major differences between SRI and AEP methods are the instrument used in AEP method completely penetrates in to the deeper layer of the soil which also measures the pressure

of air inside the soil. The AEP consisted of a single permeameter ring of 30 cm in diameter and 25 cm long, closed with an air-tight cover at the top, which was forced into soil depth up to 15 − 25 cm and infiltrates water through the cylinder under a positive pressure head about 1 m (Lee *et al.*, 1985). The pressure head was used for determination of hydraulic gradient and Ks measurement. Water was introduced into permeameter through stand pipe and allowed freely to infiltrate into the soil. The rate of infiltration was observed and continued up to a flow rate of relatively stable in nature. The air-entry value is calculated when there is a minimum pressure was being measured over the standing water inside the permeameter ring.

The air entry value was calculated as:

$$P_a = G + L + P_{min} \qquad (3)$$

where, $P_a$ is the air entry value of soil, expressed as pressure head in cm at the point of entry are negative value [L]; $P_{min}$ is the minimum pressure in cm water as determined

**Table 2.** Descriptive statistics of the selected soil properties for different land uses

| Soil properties | Statistic | Land use type | | | | |
|---|---|---|---|---|---|---|
| | | PADF | MANF | CASF | RESA | PLAG |
| Sand percentage (%) | Mean (%) | 64.33 | 68.67 | 69.67 | 69.33 | 63.33 |
| | *SD (%) | 3.51 | 1.15 | 8.96 | 5.03 | 1.15 |
| | **CV (%) | 5 | 2 | 1.3 | 7 | 2 |
| | #SE (%) | 2.03 | 0.67 | 5.17 | 2.91 | 0.67 |
| | @SV (%) | 12.33 | 1.33 | 80.33 | 25.33 | 1.33 |
| | Range | 7 | 2 | 16 | 10 | 2 |
| Clay percentage (%) | Mean (%) | 18.33 | 14.00 | 15.67 | 12.67 | 16.00 |
| | *SD (%) | 1.53 | 1 | 2.08 | 1.15 | 3.46 |
| | **CV (%) | 8 | 7 | 1.3 | 9 | 2.2 |
| | #SE (%) | 0.88 | 0.58 | 1.20 | 0.67 | 2.00 |
| | @SV (%) | 2.33 | 1 | 4.33 | 1.33 | 12 |
| | Range | 3 | 2 | 4 | 2 | 6 |
| Bulk density (g cm⁻³) | Mean (g cm⁻³) | 1.85 | 1.78 | 1.73 | 1.72 | 1.75 |
| | *SD (g cm⁻³) | 0.05 | 0.08 | 0.08 | 0.04 | 0.1 |
| | **CV (%) | 2 | 5 | 5 | 2 | 5 |
| | #SE (%) | 3 | 5 | 5 | 2 | 6 |
| | @SV (g cm⁻³) | 0 | 0.01 | 0.01 | 0 | 0.01 |
| | Range | 0.1 | 0.16 | 0.159 | 0.074 | 0.19 |
| Porosity (%) | Mean (%) | 33 | 33 | 35 | 35 | 34 |
| | *SD (%) | 2 | 3 | 3 | 2 | 4 |
| | **CV (%) | 6 | 9 | 9 | 4 | 1.1 |
| | #SE (%) | 1 | 2 | 2 | 1 | 2 |
| | @SV (%) | 0 | 0 | 0 | 0 | 0 |
| | Range | 4 | 4 | 6 | 3 | 7 |

*Standard deviation; **=Coefficient of variation; #=Standard error; @=Sample variance

by maximum reading on vacuum gauge are negative value [L]; G is the height of vacuum gauge [L]; *L* is the depth of wetting front [L].

$$K_s = \frac{2 \times (dH \ / \ dt) \times l \times (R_r \ / \ R_c)^2}{(H_t + L + P_a \ / \ 2)} \qquad (4)$$

where, dH/dt is the rate of fall of water level in reservoir just before closing supply valve [L $T^{-1}$]; $H_t$ is the height above soil surface of water level in reservoir at time supply valve is closed [L]; $R_r$ is the radius of reservoir [L]; $R_c$ is the radius of cylinder [L].

**Laboratory permeameter method:** The laboratory permeameter method (LPM) was carried out using the new "Hardson Constant head standard soil permeameter" (Topp and Binns, 1976). Soil cores were collected from three locations vertically by pressing an open cylinder diameter of 7 cm and depth of 6 cm into the soil in each land use with minimum soil disturbance. The setup for the experiment was arranged in such that water passes through the sample while the rate of flow and the corresponding head loss can be recorded. The Ks was calculated using Darcy (1856) equation:

$$Q = -K_s \times A \times \frac{\Delta H}{L} \qquad (5)$$

$$K_s = -\frac{Q \times L}{\Delta H \times A} \qquad (6)$$

Where, $K_s$ is the saturated hydraulic conductivity [$LT^{-1}$]; Q is the rate of flow [$L^3T^{-1}$]; L is the length of the specimen [L]; A is the cross section area of specimen [$L^2$] and " H is the constant hydraulic head causing flow [L].

**Pedotransfer Function based Method:** The pedotransfer functions (PTF) are the predictive function of soil physical properties using data from soil survey. ROSETTA$^{(R)}$ implements five hierarchical PTFs conceptualized on neural network analysis combined with the bootstrap method, and it allows predicting uncertainty in Ks (Schaap *et al*., 1999; Schaap *et al*., 2001). The input parameters for the Ks estimation such as textural classes, silt and clay percentage in fraction, soil dry bulk density, and soil volumetric water content and water suction were used at five different levels (Alvarez-Acosta *et al*., 2012). ROSETTA$^{(R)}$ relates between volumetric water content (θ) and water suction at a head of (h) which is known as water retention [θ(ha)] as well as saturated hydraulic conductivity which can be better described by Mualem-Van Genuchten equation (Schaap and Van Genuchten, 2006) given as:

$$\theta(h) = \theta_r + \frac{\theta_s - \theta_r}{[1 + (a \times h)^n]^m} \qquad (7)$$

where, θ(h) is soil volumetric water content at suction head *h* [$L^3 L^{-3}$]; $\theta_s$ and $\theta_r$ = saturated and residual water content [$L^3 L^{-3}$]; is the inverse air entry suction; *n* is a measure of the pore-size distribution and m=1−1/n. The hydraulic conductivity can be described by Mualem-Van Genuchten model in conjunction with pore-size distribution model (Schaap and Van Genuchten, 2006) as:

$$K(S_e) = K_0 S_e^L [1 - (1 - S_e^{\frac{n}{n-1}})^m]^2 \qquad (8)$$

Where, $K_o$ is a fitted value of K at saturation [$L T^{-1}$], which is similar but not considered as $K_s$ and L is a pore connectivity factor and $S_e$ is the effective saturation given by:

$$S_e = \frac{(\theta(h) - \theta_r)}{(\theta_s - \theta_r)} = \left[ \frac{1}{1 + (a \times h)^n} \right]^m \qquad (9)$$

**Analysis of Ks data:** The Ks data measured or estimated by five methods (SRI, DRI, AEP, LPM and PTF) from five land uses (PADF, MANF, CASF, RESA and PLAG) were pooled together and divided into three groups; (1) *Ks data-method* (5 land uses x 3 trials = 15 data points for each method), (2) *Ks data-land use* ( 5 methods x 3 trials = 15 data points for each land use) and (3) *Ks data-total* (5 methods x 3 trials x 5 land use= 75 data points). These data groups were analyzed for normality test and best fit frequency distribution by using the Shapiro and Wilk (1965) test at 5% significant level and investigated the numerical (Skewness and Kurtosis) and visual ( Q-Q plots) outputs. Based on the best fit frequency distribution, the Ks obtained from the SRI, DRI, AEP, LPM and PTF methods were compared relatively based on descriptive statistics, analysis of variance (ANOVA) and Tukey's pair-wise mean comparison test.

**RESULTS AND DISCUSSION**

**Characteristics of Ks data:** The nature of Ks-data were analysed using Shapiro-Wilk test (Table 3). The AEP and LPM methods rejected the null hypothesis (data is normally distributed) at 5% significant level (P < 0.05) and hence, the Ks data (*Ks data-methods*) for these methods were approximately not normally distributed. This is evident from the skewness and kurtosis values, which are not close to zero. This is also evident from the normal Q-Q plot of AEP given in Figure 2a (the plots for other methods are not shown here), in which the data points do not lie on a straight line. The null hypothesis was not rejected for the Ks data of SRI, DRI and PTF and thus the Ks data for these methods were approximately normally distributed. With the log-transformed Ks data, the AEP and LPM methods did not reject the null hypothesis and they were approximately normally distributed. This was also observed from the skewness and kurtosis values, which are close to zero and the Q-Q plot (

Figure 2b), in which maximum data points lie on the straight line.

The CASF and PLAG land uses rejected the null hypothesis (data is normally distributed) at 5% significant level (P < 0.05) and hence, the Ks data (*Ks data-land use*) for these land uses were approximately not normally distributed. This is also evident from the skewness and kurtosis values, which are not close to zero and the normal Q-Q plot of PLAG (Figure 2c), for which most of the data points did not follow a straight line. The Ks data for all other land uses including PADF, MANF and RESA did not reject the null hypothesis and distributed normally. With the log-transformed Ks data, all land uses were not rejected the null hypothesis and they were approximately normally distributed. This is also evident from the skewness and kurtosis values, which are close to zero and the Q-Q plot of PLAG shown in Figure 2d, in which maximum data points lie on the straight line.

The pooled Ks data (*Ks data-total*) rejected the null hypothesis (data is normally distributed) at 5% significant level (P < 0.05) and hence, the *Ks data-total* were approximately not normally distributed. This was also evident from the skewness and kurtosis values, which are not close

to zero and normal the Q-Q plot given in Figure 2e, in which the data points did not follow the trend of a straight line. With the log-transformed Ks data, Ks data-total did not reject the null hypothesis and they were approximately normally distributed (Table 3). This was also evident from the skewness and kurtosis values, which are close to zero and the Q-Q plot (Figure 2f), in which maximum data points lie on the straight line. It was understood that the Ks data were performed better according to the nature of log-normal frequency distribution as it was also observed by several other researchers (Lee *et al*., 1985, Jacka *et al*., 2014). Therefore, the log transformed Ks data were used in studying the performance of the methods for different land uses on the basis of the descriptive statistics and ANOVA as explained in the previous section.

**Effect of measuring technique on Ks:** The effect of the method on Ks was significantly different for all land uses at 5% significant level (Table 4). Mostly, the mean Ks obtained by each method for a land use was significantly different from other methods (Table 5). That means the methods measure different Ks values for a particular land use as different methods have different operating process and different

**Table 3.** Normality test results for Ks data collected from five landuses using five Ks measuring techniques

| Data group | Particulars | | Shapiro-Wilk | | | Skewness | Kurtosis |
|---|---|---|---|---|---|---|---|
| | | | Statistic | Df | Sig. | | |
| Ks-methods | Ks (mm h$^{-1}$) | SRI | 0.99 | 15 | $ | 0.21 | 0.04 |
| | | DRI | 0.97 | | $ | 0.06 | -0.39 |
| | | AEP | 0.88 | | * | 1.21 | 0.96 |
| | | LPM | 0.77 | | ** | 1.51 | 1.36 |
| | | PTF | 0.93 | | $ | 0.05 | -1.35 |
| | lnKs (mm h$^{-1}$) | SRI | 0.97 | | $ | -0.62 | 0.66 |
| | | DRI | 0.93 | | $ | -0.75 | -0.74 |
| | | AEP | 0.96 | | $ | 0.50 | -0.08 |
| | | LPM | 0.85 | | $ | 0.72 | -0.01 |
| | | PTF | 0.91 | | $ | -0.48 | -1.10 |
| Ks-landuses | Ks (mm h$^{-1}$) | PADF | 0.94 | 18 | $ | 0.13 | -1.22 |
| | | MANF | 0.95 | | $ | 0.48 | -0.25 |
| | | CASF | 0.79 | | *** | 1.60 | 1.80 |
| | | RESA | 0.94 | | $ | 0.82 | 0.27 |
| | | PLAG | 0.87 | | * | 1.27 | 1.41 |
| | lnKs (mm h$^{-1}$) | PADF | 0.91 | | $ | -0.50 | -1.07 |
| | | MANF | 0.94 | | $ | -0.30 | -0.89 |
| | | CASF | 0.90 | | $ | 0.98 | 0.37 |
| | | RESA | 0.98 | | $ | 0.12 | -0.66 |
| | | PLAG | 0.95 | | $ | 0.43 | -0.77 |
| Ks-total | Ks (mm h$^{-1}$) | | 0.89 | 75 | *** | 1.51 | 5.95 |
| | lnKs (mm h$^{-1}$) | | 0.97 | | $ | -0.25 | -0.98 |

$ > 0.05; * < 0.05; ** < 0.01; *** < 0.001
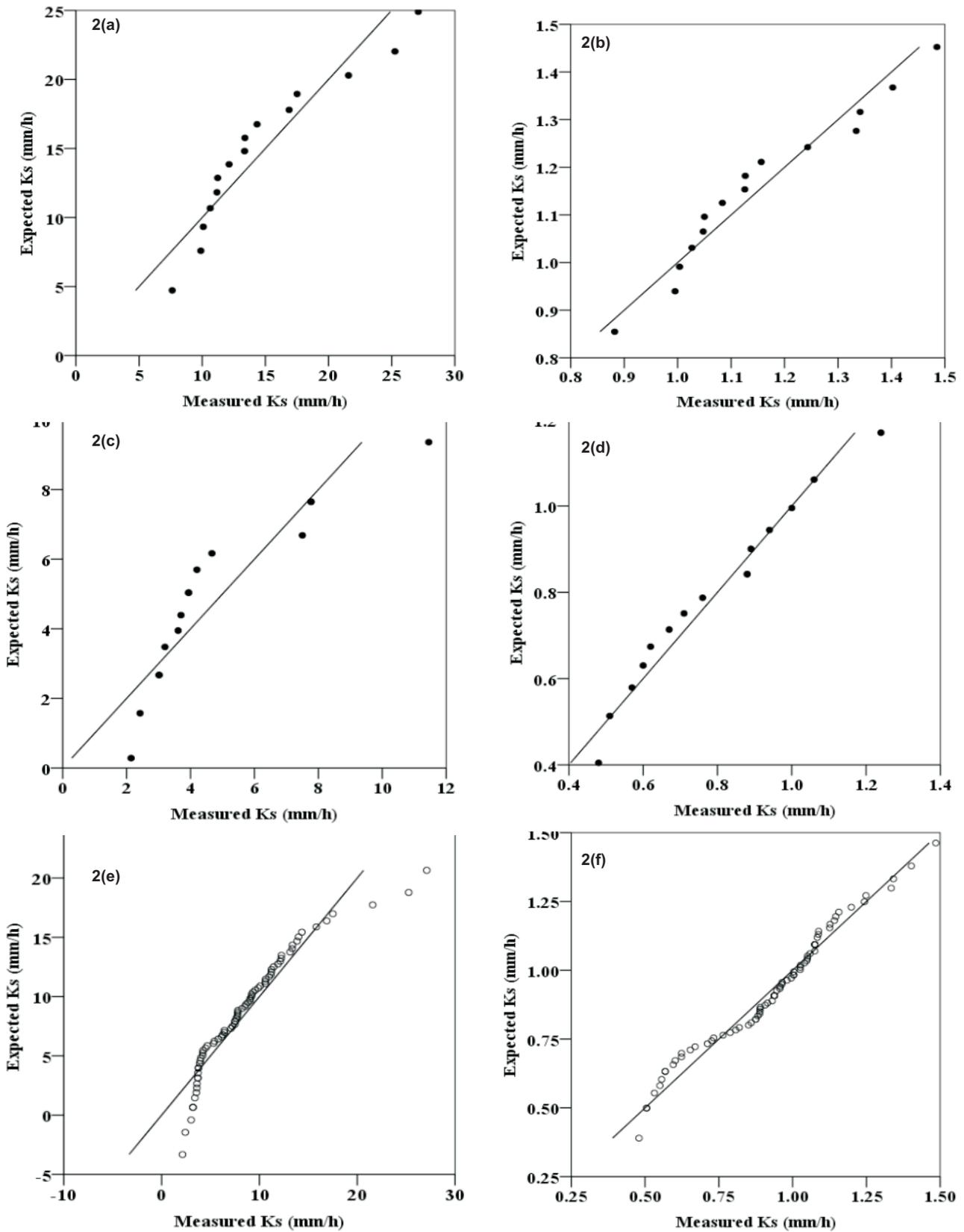
**Fig. 2.** Normality test results using Q-Q plots for a) and b) Ks data–AEP method, c) and d) Ks data–land use, and e) and f) Ks data–total

**Table 4.** Two-way ANOVA test for the Ks data to analyze the effect of measuring technique and land uses on Ks

| Source | Sum of squares (SS) | df | Mean square (MS) | F | Sig. |
|---|---|---|---|---|---|
| Method | 12.49 | 4 | 3.12 | 45.92 | *** |
| Land use | 6.53 | 4 | 1.63 | 24 | *** |
| Method land use | 2.80 | 20 | 0.14 | 2.05 | ** |
| Error | 3.20 | 47 | 0.06 | | |
| Total | 389.94 | 75 | | | |

* < 0.05; ** < 0.01; *** < 0.001

**Table 5.** Pair-wise comparisons the mean Ks of different methods

| Method (i) | Methods (j) | Mean difference (i-j) | Sig. | 95% confidence interval | |
|---|---|---|---|---|---|
| | | | | Lower bound | Upper bound |
| SRI | DRI | 0.25 | ** | 0.09 | 0.42 |
| | AEP | -0.37 | *** | -0.53 | -0.20 |
| | LPM | 0.71 | *** | 0.54 | 0.88 |
| | PTF | 0.65 | *** | 0.48 | 0.82 |
| DRI | SRI | -0.25 | ** | -0.42 | -0.09 |
| | AEP | -0.62 | *** | -0.79 | -0.46 |
| | LPM | 0.45 | *** | 0.28 | 0.62 |
| | PTF | 0.39 | *** | 0.23 | 0.56 |
| AEP | SRI | 0.37 | *** | 0.20 | 0.53 |
| | DRI | 0.62 | *** | 0.46 | 0.79 |
| | LPM | 1.08 | *** | 0.91 | 1.25 |
| | PTF | 1.02 | *** | 0.85 | 1.19 |
| LPM | SRI | -0.71 | *** | -0.88 | -0.54 |
| | DRI | -0.45 | *** | -0.62 | -0.28 |
| | AEP | -1.08 | *** | -1.25 | -0.91 |
| | PTF | -0.05 | 0.51 | -0.22 | 0.11 |
| PTF | SRI | -0.65 | *** | -0.82 | -0.48 |
| | DRI | -0.39 | *** | -0.56 | -0.23 |
| | AEP | -1.02 | *** | -1.19 | -0.85 |
| | LPM | 0.05 | 0.51 | -0.11 | 0.22 |

*<0.05; ** < 0.01; *** < 0.001

sample dimensions. It showed that AEP method was highly variable with other methods as it rejected the null hypothesis at high level of significance. The LPM and PTF methods were not significantly different from each other which show the least difference between them.

For all land uses, the mean Ks was maximum and minimum for AEP and LPM methods, respectively. The AEP method estimated highest values of Ks for all land uses (maximum in CASF and minimum in PLAG) with an average of 2.64 mm h$^{-1}$. The SRI method resulted second highest values of Ks (maximum in CASF and minimum in PLAG) with

an average of 2.25 mm h$^{-1}$. Similarly, the DRI method resulted the third highest Ks values (maximum in CASF and minimum in PLAG) with an average of 1.99 mm h$^{-1}$. The Tukey's pair-wise comparison showed that there was a close correlation between LPM and PTF methods; therefore, it was seen that the average estimated Ks for all the land uses were 1.54 and 1.60 mm h$^{-1}$ for LPM and PTF methods, respectively.

The box plot (Figure 3) suggested that the mean Ks obtained from different methods were different from each other for different land uses (Table 4). The AEP method estimated Ks had high level of agreement with each other next to that of SRI method. The PTF estimated Ks showed more variation and highly different from other methods. Furthermore, it can be interpreted that mean difference between the AEP and LPM methods were much significant. In case of LPM method, it was observed that the Ks measured values had similar views at lower quartile region whereas the scale of Ks values were more variable. In lower whisker of LPM, the Ks varied amongst the positive quartile group and very similar for the least positive quartile group. However the mean Ks was different for all methods mean Ks in DRI method (1.99 mm h$^{-1}$) is closely related to the overall mean of Ks for IIT Kharagpur campus (2.00 mm h$^{-1}$). The ranges of estimated/measured Ks were higher for PTF method and comparatively lower in SRI method than the other methods (Table 6). The estimated error was highest in PTF (28.20 %) and lowest in case of DRI method (5.4 %). The variance obtained for different methods implied that the highest was in AEP method and lowest in LPM method. The deviation from the mean was highest in DRI method and lowest in LPM method. The higher Ks for AEP method was possibly due to presence of higher water head and completely air tight system (Nemati *et al.*, 2002). The smaller sample volume tested under controlled conditions might
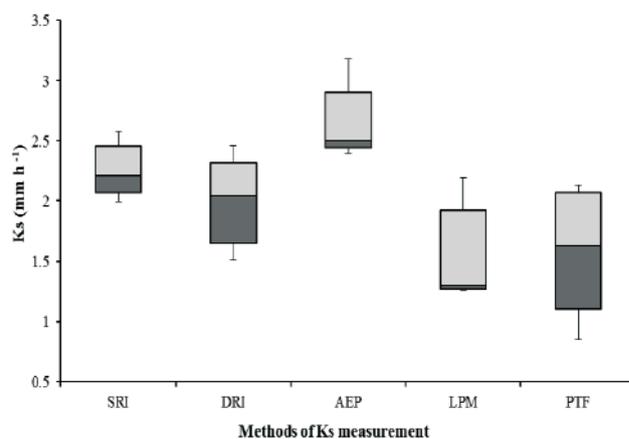


**Fig. 3.** Box plot showing the performance of Ks to different measurement methods

**Table 6.** Descriptive statistics of log transformed Ks value for different methods

| Ks-method | Statistics | Land use | | | | |
|---|---|---|---|---|---|---|
| | | PADF | MANF | CASF | RESA | PLAG |
| SRI | Mean (mm h^{-1}) | 2.33 | 2.15 | 2.58 | 2.21 | 1.99 |
| | *SD (mm h^{-1}) | 0.08 | 0.11 | 0.16 | 0.39 | 0.45 |
| | **CV (%) | 4 | 5 | 6 | 1.8 | 2.2 |
| | #SE (%) | 5 | 6 | 9 | 22 | 26 |
| | @SV(mm h^{-1}) | 0.01 | 0.01 | 0.02 | 0.15 | 0.2 |
| | Range | 0.16 | 1.43 | 0.29 | 0.77 | 0.89 |
| DRI | Mean (mm h^{-1}) | 2.17 | 1.79 | 2.46 | 2.04 | 1.51 |
| | *SD (mm h^{-1}) | 0.04 | 0.3 | 0.13 | 0.35 | 0.49 |
| | **CV (%) | 0.02 | 0.17 | 0.05 | 0.17 | 0.32 |
| | #SE (%) | 7 | 0.05 | 0.04 | 0.04 | 0.07 |
| | @SV (mm h^{-1}) | 0 | 0.09 | 0.02 | 0.12 | 0.24 |
| | Range | 0.08 | 0.54 | 0.26 | 0.71 | 0.94 |
| AEP | Mean(mm h^{-1}) | 2.50 | 2.49 | 3.18 | 2.62 | 2.39 |
| | *SD (mm h^{-1}) | 0.14 | 0.18 | 0.15 | 0.19 | 0.42 |
| | **CV (%) | 0.05 | 0.07 | 0.05 | 0.07 | 0.32 |
| | #SE (%) | 8 | 1 | 8 | 11 | 24 |
| | @SV(mm h^{-1}) | 0.02 | 0.31 | 0.02 | 0.04 | 0.18 |
| | Range | 0.27 | 0.35 | 0.28 | 0.39 | 0.83 |
| LPM | Mean(mm h^{-1}) | 1.26 | 1.28 | 2.19 | 1.66 | 1.30 |
| | *SD (mm h^{-1}) | 0.04 | 0.11 | 0.17 | 0.15 | 0.14 |
| | **CV (%) | 0.03 | 0.09 | 0.08 | 0.09 | 0.1 |
| | #SE (%) | 2 | 6 | 9 | 89 | 8 |
| | @SV(mm h^{-1}) | 0 | 0.01 | 0.03 | 0.02 | 0.02 |
| | Range | 0.08 | 0.22 | 0.35 | 0.31 | 0.27 |
| PTF | Mean(mm h^{-1}) | 0.85 | 1.63 | 2.13 | 2.01 | 1.36 |
| | *SD (mm h^{-1}) | 0.22 | 0.27 | 0.15 | 0.15 | 0.32 |
| | **CV (%) | 0..25 | 0.16 | 0.07 | 0.07 | 0.24 |
| | #SE (%) | 12 | 16 | 9 | 85 | 19 |
| | @SV(mm h^{-1}) | 0.05 | 0.08 | 0.02 | 0.02 | 0.11 |
| | Range | 0.39 | 0.51 | 0.28 | 0.29 | 20.62 |

*=Standard deviation; **=Coefficient of variation; #=Standard error; @=Sample variance

have resulted the lower Ks in case of LPM. Furthermore, the LPM method does not consider the field actual conditions and it was observed that the Ks value decreased when the soil moisture content and the time of experiment increased (Bagarello and Sgroi, 2004; Fallico *et al.*, 2006). The Ks values resulted from DRI method was lower than SRI method as the lateral flow was controlled by the outer ring in DRI. The SRI method resulted more error due to the issue of lateral flow. The PTF method of Ks measurement requires more accuracy in primary input data regarding the soil physical properties thus it may get erroneous result due to availability of inadequate soil data.

**Effect of land use on Ks:** The effect of the land use on Ks was significantly different for all methods (Table 4). The pair−wise mean comparison of the measured /estimated Ks for the individual land uses. Mostly, the mean Ks of each land use considered was significantly different with that of other land use. That means the land uses had different values of Ks. However, the Ks was not significantly different for PADF, MANF and PLAG (Table 7). Whereas the Ks measured/estimated was significantly different for other land uses such as CASF and RESA. The Ks varied between the land uses as they have different land management practices and presence of macrospores (created by earthworms, roots holes etc.). The Ks was lowest in PLAG with PTF and highest in CASF with AEP (Figure 4). The Ks for CASF was highest with AEP and lowest with LPM with an average of 2.51 mm h^{-1}. CASF had loose soil profile and large macropores

**Table 7.** Pair-wise comparisons of the mean Ks of different land uses

| Land use (I) | Land uses (j) | Mean difference (i-j) | Sig. | 95% confidence interval | |
|---|---|---|---|---|---|
| | | | | Lower bound | Upper bound |
| PADF | MANF | -0.10 | 0.19 | -0.25 | 0.05 |
| | CASF | -0.66 | *** | -0.81 | -0.50 |
| | RESA | -0.31 | *** | -0.47 | -0.16 |
| | PLAG | 0.09 | 0.24 | -0.06 | 0.24 |
| MANF | PADF | 0.10 | 0.19 | -0.05 | 0.25 |
| | CASF | -0.56 | *** | -0.71 | -0.40 |
| | RESA | -0.21 | ** | -0.37 | -0.06 |
| | PLAG | 0.19 | * | 0.03 | 0.34 |
| CASF | PADF | 0.66 | *** | 0.50 | 0.81 |
| | MANF | 0.56 | *** | 0.40 | 0.71 |
| | RESA | 0.34 | *** | 0.19 | 0.49 |
| | PLAG | 0.75 | *** | 0.60 | 0.90 |
| RESA | PADF | 0.31 | *** | 0.16 | 0.47 |
| | MANF | 0.21 | ** | 0.06 | 0.37 |
| | CASF | -0.34 | *** | -0.49 | -0.19 |
| | PLAG | 0.40 | *** | 0.25 | 0.56 |
| PLAG | PADF | -0.09 | 0.24 | -0.24 | 0.06 |
| | MANF | -0.19 | * | -0.34 | -0.03 |
| | CASF | -0.75 | *** | -0.90 | -0.60 |
| | RESA | -0.40 | *** | -0.56 | -0.25 |

$>0.05; *< 0.05; ** <0.01; *** < 0.001

(presence of earth worms) to result in higher values of Ks (Chapuis, 2004; Park and Smucker, 2005). The PLAG showed the lowest Ks among all the land uses as it compacted regularly to make the field suitable for playing football and cricket (Zhou *et al*., 2008; Hu *et al*., 2009; Matthews *et al*., 2010). The compaction resulted the variation in Ks for top soil were found minimum with LPM and maximum with AEP with the mean Ks of 1.71 mm h$^{-1}$. The Ks for RESA land use was ranging from LPM to AEP with an average Ks of 2.11 mm h$^{-1}$. The RESA land uses consisted of the soil with small gravels and plastics which might have caused the variability in Ks. From the textural analysis it was observed that clay percentage and bulk density were found higher in PADF, hence the measured Ks was found to be lower in PADF next to PLAG among all the land uses ranging from PTF to AEP with an average of 1.82 mm h$^{-1}$. The bulk density of MANF was found to be higher next to PADF and the measured Ks was also resulted closer to PADF. The Ks for MANF was ranging from 1.28 to 2.49 mm/h with an average value of 1.87 mm h$^{-1}$ (SD=0.19 mm h$^{-1}$). Zhang and Norton (2002) and Dusa (2013) were also found decrease in Ks with increase of bulk density and clay content which was similar

as of our study for CASF and MANF land uses. The order of Ks obtained for the land uses were CASF, RESA, MANF, PADF and PLAG from higher to lower due to nature of porosity, organic and clay matter content, land management practices.

The box plot (Figure 4) suggested that the mean Ks values obtained for different land uses were significantly different from each other. The range (spread) of Ks was observed to be highest for PADF and lowest for RESA. The Ks values of RESA land uses have high level of agreement with each other. For PADF land use, the Ks values were different from each other. There was an obvious difference existed among the groups of Ks values for all five land uses by comparing pair-wise. In case of PADF most of the Ks values were lying in only one quartile of the box which shows the more variation in other part. The mean Ks of PADF and MANF were closely related. The mean Ks value of RESA is close match to the overall mean Ks.
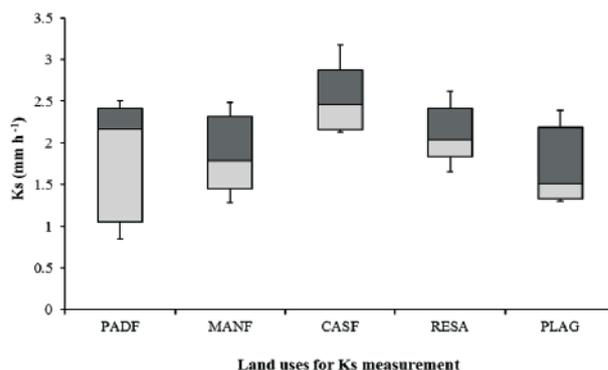


**Fig. 4.** Box plot showing the performance of Ks to different land uses

**Feasibility of Ks methods:** Based on cost of operation, the maximum and minimum instrument cost was observed in AEP and PTF method, respectively (Table 8). Operating time was highest in LPM and lower in AEP methods among all the methods. Both the methods take equal time to reach steady state. The SRI and DRI methods were simple and easier and utilize same manpower and operating time. Proper installation with minimum soil disturbances for these instruments is important. The DRI method reduced the lateral flow of water from the inner ring which requires more water and little costlier than SRI method and resulted better measurement of Ks. The AEP method installation requires skilled labour and knowledge required to operate the instrument, and requires 30 minute time for total operation of the instrument. The LPM method was easy and portable instrument, but high initial setup cost for the instrument. It was a time consuming Ks measurement technique in which

**Table 8.** Feasibility of the Ks methods for different land uses

| Performance criteria | SRI | DRI | AEP | LPM | PTF |
|---|---|---|---|---|---|
| Instrument/ Equipment required | Cylindrical ring, scale, leveller, wooden piece, mug, water bucket , hook gage, stopwatch | Two cylindrical rings, wooden piece leveller, hook gage, stopwatch, scale, water | Cylindrical ring, reservoir, suction plate, stopwatch, scale, mug, water bucket, wooden piece leveler, manometer | Hammer, scale, core, shovel, Constant head permeameter set, wooden piece | None |
| Instrument cost ($) | 170.00 | 250.00 | 280.00 | 850.00 | None |
| Manpower | Skilled–1 Labour–1 | Skilled–1 Labour–1 | Skilled–1 Labour–1 | Skilled–1 Labour–1 | Skilled–1 |
| Transportation | Laborious | Laborious | Laborious | Soil core transport | No |
| Installation time | 30 minute | 30 minute | 45 minute | 10 minute | No |
| Time to reach steady state | 2–3 minutes | 2–3 minutes | 1–2 minutes | 1–2 minutes | Not applicable |
| Operating time(hours) | 3 to 6 hours | 3 to 6 hours | 75 minutes | 8–10 hours | 6–7 hours |
| Water requirement (litre) | 20 – 30 | 40–50 | 25–35 | 20 | 2 |
| Operation difficulties | Noisy installation and soil disturbance | Noisy installation and soil disturbance | Wetting front detection and Noisy installation and soil disturbance | Extracting a core sample | Textural analysis: chemical spill |

steady state can be achieved very rapidly than the other methods but operating time is more, not laborious, but extraction of core sample is a major constraint for the method. The PTF method is an easier method among all which require less manpower, cost of operation but dependent upon accurate textural analyses. This is not surprise since each method has a characteristics different sampling volume, its own flow geometry, different method of applying water to water to the soil and different boundary conditions. It can be realised that every method has its characteristics of sampling volume, own flow pattern or geometry, method of application of water to soil with certain initial and boundary conditions.

## CONCLUSIONS

The Ks data were approximately fitted well into log–normal distribution. The mean Ks was significantly different among the measuring or estimating techniques and the selected land uses. For all land uses, the mean Ks was highest for AEP followed by SRI, DRI, PTF and LPM methods. The estimated error in Ks was more for PTF and low for DRI methods. The PTF estimated Ks showed more variation and highly different from other methods. For the land uses, the mean Ks was highest for CASF followed by RESA, MANF, PADF and PLAG. In respect to land uses it was concluded that, the Ks measurement were highly affected by soil textural properties, porosity, clay content in the soil, bulk density and land management practices for PADF, MANF and PLAG land uses. Furthermore, the effect of

interaction of method and land use on Ks was also significantly different at 5% level of significant. The –selection of best suitable method for a particular situation can be obtained by optimizing the interdependent parameters, including method to be used, accuracy in instrument and measurement methods, soil condition and the numbers of practical constraints of the investigation (e.g. cost, availability of manpower, time requirement, portability of estimate, simplicity in measuring technique, operating condition). The overall performance of the methods suggested that the AEP method was quicker and PTF method was cheapest among all the methods.

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# Daily Pan Evaporation Modeling in Hilly Region of Uttarakhand Using Artificial Neural Network

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**Abstract**: Evaporation is one of the major processes in the hydrologic cycle, and its accurate estimation is essential for hydrologic water-balance, irrigation, and water resources planning and management. In the present study an attempt has been develop artificial neural network (ANN) for estimation of daily pan evaporation (Ep) for Hawalbagh, Uttarakhand, India. The daily data of temperature (T), relative humidity (Rh), wind velocity (W), sunshine hours (S) and evaporation (Ep) data of years 2010-2012 were used to train the models and remaining data of year 2013 were used for test the models of ANN. The daily meteorological data of 4 years such as temperature, relative humidity, wind velocity and sunshine hours as input parameters and evaporation as output. The artificial neural networks were used for designing of models based on different learning algorithm (DBD, L-M, Step, Momentum, C-G and Quickprop) activation function TanhAxon, sigmoid and linear sigmoid were used evaporation estimation. The performances indices of the models were evaluated by; viz. normalized mean square error (NMSE), correlation coefficient (r) and coefficient of efficiency (CE). The comparisons of the different combinations of ANN models and select a best suited model for estimating daily pan evaporation of the Hawalbagh Uttarakhand.

**Keywords:** Activation functions, ANNs, Learning algorithms, Meteorological parameters

Water resources of a country constitute one of its vital assets. The evaporation process represents a major component of the energy and water balance of bare soil and green cover (i.e. forest and farm) ecosystems. Estimation of the water loss by evaporation is of primary importance for monitoring, survey and management of water resources, design of irrigation and drainage systems and irrigation scheduling (Molina Martinez *et al.,* 2005; Gundekar *et al.,* 2008). It is generally made by some commonly used approaches such as mass transfer or water balance methods. One of the direct methods for Ep measurements is the pan evaporation (Eslamian *et al.,* 2008). Pan performance is affected by instrumental limits and operational problems such as human errors, instrumentation errors, turbidity of water, watering of birds or other animals, as well as other maintenance problems which can affect the accuracy of Ep measurements. Accurate estimation of evaporation is essential for the balancing of irrigation water use in arid and semiarid regions, highly conditioned by water shortages, where responsible irrigation engineering and watershed management is mandatory. It is a key factor for irrigation system design and management, crop production, environmental assessment water resources management and planning.

Estimation of evaporation for remote rural areas where no evaporation data are available is of great attention to the hydrologists and meteorologists (Kisi 2006). The most important factors affecting evapotranspiration are solar radiation, air temperature, air humidity and wind speed, vapour pressure deficit (Allen *et al.,* 1998). Sudheer *et al*. (2002) showed that the neural computing technique could be employed successfully in modelling the evaporation process from the available climatic data set. Evaporation estimation by multilayer perceptron based artificial neural network and multiple linear regression techniques (Bhagwat *et al.,* 2017). However, an analysis of the residuals from the ANN models developed revealed that the models showed significant error in predictions during the validation, implying loss of generalization of ANN models unless trained carefully. Recent experiment have reported that ANN may offer a promising alternative in the hydrological context developed feed forward ANN models for modelling daily evaporation and found that the ANN model performed better the conventional method. An attempt has been made to develop models for computation of evaporation by the available wheatear data of Hawalbagh. The objectives to develop the ANN based evaporation estimation models with different learning algorithms and to evaluate performance and adequacy of the developed models.

## MATERIAL AND METHODS

**Study Area:** Hawalbagh is located in Almora district of Uttarakhand, India at $29^0$ 36' N latitude and $79^0$ 40' E longitudes at an elevation of 1250 m from the mean sea level. The climate of the study area is cool temperate with annual maximum, minimum and average temperatures in the area stands at 25.77, 13.50 and 19.635°C respectively. Maximum rain is received from south-west monsoon during four months rainy season from June to September. The May is the hottest month when the mean maximum temperature rises up to 31.50°C and January is the coldest month when the mean minimum temperature drops down to 5.04°C. The maximum and minimum temperatures gradually decrease between July and October.

**Data Acquisition:** The weather data used to develop the ANN models were acquired from the Meteorological observatory of Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS) Almora, Uttarakhand. The daily weather data of maximum and minimum temperature, wind velocity, relative humidity ($Rh_1$) was recorded in the morning at 7 am and relative humidity ($Rh_2$) was recorded in afternoon at 2 pm at Indian Standard Time, sunshine hour and evaporation. The data set consisted of four years of daily records from 2010 to 2013.

**Development of models for study area:** The data set formulation was carried out with standard meteorological weather data of, mean of maximum and minimum temperature, mean of relative humidity, sunshine hours and wind velocity as input and remaining evaporation data was used for output. Total number of data for each year's period comes out to be 365. Then the whole numbers of data of 4 year were 1460. The daily meteorological data of years 2010 to 2012 (75%) were used for training of the models and remaining data of 2013 (25%) were used for testing of the models.

**Artificial Neural Networks (ANNs):** In the present study, the various algorithms (i.e. Levenberg–Marquardt, Delta–Bar–Delta, Step, Momentum, Conjugate Gradient and Quickprop) were applied in order to identify the one which best network. Two different functions (Sigmoid and TanhAxon) were used in order to identify the one which gives the best results in depicting the non-linearity of the model system. Also, the best learning algorithm, activation function and architecture of the network (the number of hidden layers and neurons in hidden layers) were determined by trial and error. The study examined various combinations of the ANN (Algorithm and Activation function) as inputs to the models so as to evaluate the degree of effect of each of these models on evaporation. The signals flowing on the connections are scaled by adjustable parameters known as weights (Principe *et al.,*

2000). It describes a nonlinear relationship between the input and output of a complex system using historic process data. Fig. 1 is a general architecture of a Feed Forward ANN, with one and more than one hidden layer. Most ANNs have three layers or more: an input layer, which is used to present data to the network; an output layer, which is used to produce an appropriate response to the given input; and one or more intermediate layers, which are used to act as a collection of feature detectors. There are a wide variety of algorithms available for training a network and adjusting its weights.
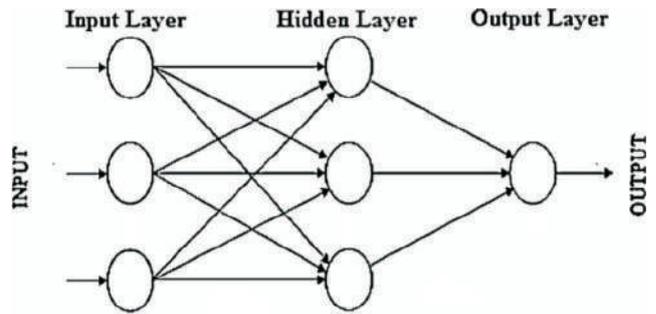


**Fig. 1.** Architecture of multilayer feed forward neural network

Let $x_i$ (i = 1,2,... n) are inputs and $w_i$ (i = 1,2,… n) are respective weights. The net input to the node can be expressed as−

$$net = \sum_{i=1}^{n} x_i w_i \qquad .................................................(1)$$

The net input is then passed through an activation function *f (net)* and the output *y* of the node is computed as−

$y = f (net)$.................................................................... (2)

In the feed-forward calculation, the nodes in the input layer receive the input signals which are passed to the hidden layer and then to the output layer. The signals are multiplied by the current values of weights, and then the weighted inputs are added to yield the net input to each neuron of the next layer. The net input of a neuron is passed through an activation or transfer function to produce the output of the neuron.

The net input to $j^{th}$ node of the hidden layer is given by−

$$neth_j = \sum_{i=1}^{n} wh_{ji} x_i \qquad .....................................................(3)$$

Where, *ni* is the number of neurons in the input layer and $wh_{ji}$ is the connection weight between $i^{th}$ node of the input layer and $j^{th}$ node of the hidden layer. The output of $j^{th}$ node of the hidden layer $h_j$ is−

$hj = f (neth_j)$ .................................................……(4)

In this study activation functions TanhAxon, Sigmoid and linear sigmoid along with different learning algorithms of artificial neural network are used and they are briefly described below.
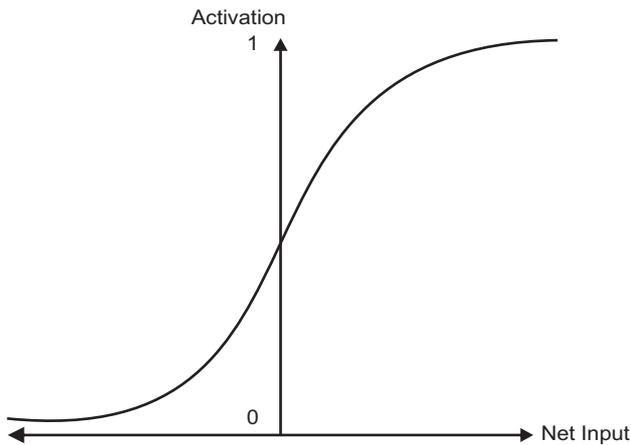
**Fig. 2.** Sigmoid Axon function
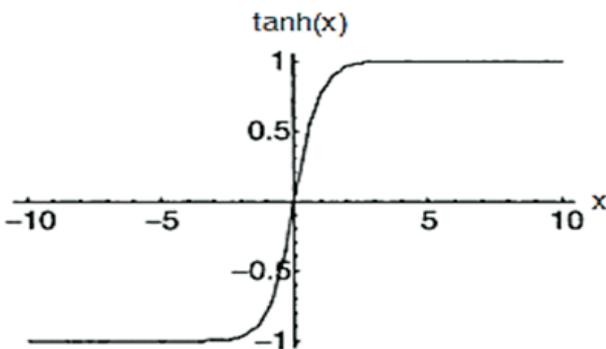


**Fig. 3.** Tanh Axon function

**Activation Functions:** The activation function is the formula used to determine the output of a processing neuron. The Linear function implements a linear axon with slope and offset control. The Sigmoid function applies a scaled and biased sigmoid function to each neuron in the layer. The range of values for each neuron in the layer is between zero and 1. This research, selecting the best activation function was carried out by a process of trial and error. The main selection criterion here was to increase the neural network accuracy.

**Sigmoid Axon:** It is a mathematical function having an S shape (sigmoid curve). Sigmoid Axon function refers to the special case of the logistic function shown in figure and defined by the formula−

$$S(t) = \frac{1}{1 + e^{-t}} \quad \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(5)$$

**TanhAxon :** The TanhAxon applies a bias and tanh function to each neuron in the layer. This will squash the range of each neuron in the layer to between −1 to 1. Such nonlinear elements provide a network with the ability to make soft decisions.

$$Tanh\ (x) = \frac{\sinh(x)}{\cosh(x)} = \frac{e^{x} - e^{-x}}{e^{x} + e^{-x}} \quad \dots\dots\dots\dots\dots\dots(6)$$

**LEARNING ALGORITHMS**

**Delta-Bar-Delta:** The Delta−Bar−Delta is an adaptive step−size procedure for searching a performance surface. Step size and momentum are adapted according to the previous values of the error at the neurons. If the current and past weight updates are both of the same sign, it increases the learning rate linearly. The reasoning is that if the weight is being moved in the same direction to decrease the error, then it will get there faster with a larger step size. If the updates have different signs, this is an indication that the weight has been moved too far. When this happens, the learning rate decreases geometrically to avoid divergence (Haciismailoglu *et al.,* 2009).

**Levenberg–Marquardt :** The Levenberg−Marquardt (LM) algorithm is one of the most appropriate higher−order adaptive algorithms known for minimizing the MSE of a neural network. It is a member of a class of learning algorithms called "pseudo second− order methods". Standard gradient descent algorithms use only the local approximation of the slope of the performance surface (error vs. weights) to determine the best direction to move the weights for lowering the error. A key advantage of the LM approach is that it defaults to the gradient search when the local curvature of the performance surface deviates from a parabola, which may happen often in neural computing (Neuro Solutions 2003).

**Step:** Step learning rules (e.g. back propagation and real−time recurrent learning) provide first−order gradient information about the network's performance surface. The most straightforward way of reaching the bottom (the minima), given which way is up, is to move in the opposite direction. With this scenario, the only variable is the step size (i.e. how far should it move before obtaining another directional estimate). If the steps are too small, then it will take too long to get there. If the steps are too large, then it may overshoot the bottom, causing it to rattle or even diverge. The Step uses this procedure to adapt the weights of the Activation component that it is stacked on (Neuro Solutions 2003).

**Momentum:** Step components try to ﬁnd the bottom of a performance surface by taking steps in the direction estimated by the attached back propagation component. The Momentum provides the gradient descent with some inertia, so that it tends to move along a direction that is the average estimate for down. The higher the momentum, the more it smoothest the gradient estimate and the less effect a single change in the gradient has on the weight change. The major

bene"t is the added ability to break out of local minima. The momentum parameter is the same for all weights of the attached component. An access point has been provided for the step size and momentum allowing access for adaptive and scheduled learning rate procedures (NeuroSolutions 2003).

**Conjugate Gradient:** This technique not only focuses on the local gradient of the error function but also makes use of its second derivative. The first derivative measures the slope of the error surface at a point, while the second one measures the curvature of the error surface at the same point. This information is very important for determining the optimal update direction. Since this method makes use of the second derivatives of the function to be optimized, it is typically referred to as the second-order methods (Jalali−Heravi *et al.,* 2008).

**Quickprop:** Quickprop uses information about the curvature of the error surface. This requires the computation of the second order derivatives of the error function during training. Quickprop assumes the error surface, a function of connection weights, to be locally quadratic (i.e., a parabola) and attempts to jump in one step from the current position directly into the minimum of the parabola. Quickprop computes the derivatives in the direction of each weight. After computing the first gradient as in regular back-propagation, a direct step to the error minimum is attempted by changing the weight (Chakraborty and Chakraborty 2002). Quickprop is an iterative method for determining the minimum of the loss function of an artificial neural network. The procedure requires only local information of the artificial neuron to which it is applied.

## CRITERIA OF EVALUATION

**Normalize mean square error (NMSE):** It is computed by following formula−

$$NMSE = \frac{1}{n} \Sigma_i \frac{(Yj - Yej)^2}{e.j.e} \quad .....................................(7)$$

$$ej = \frac{1}{n} \Sigma_i Yeji \ , e = \frac{1}{n} \Sigma_i Yei$$

**2.5.2 Correlation coefficient (r):** The correlation coefficient (r) is computed by the following equation−

$$r = \frac{\Sigma_{i=1}^{n} (Yj - j) - (Yej - ej)}{\sqrt{\Sigma_{i=1}^{n} (Yj - j)^2 - (Yej - ej)^2}} \quad .......................(8)$$

**2.5.3 Coefficient of efficiency (CE):** The coefficient of efficiency (CE) in percentage is computed by following equation−

$$CE = \left(1 - \frac{\Sigma_{i=1}^{n} (Yj - Yej)^2}{\Sigma_{i=1}^{n} (Yj - j)^2}\right) \times 100 \quad .......................(9)$$

Where, $Y_j$ and $Y_{ej}$ observed and estimated values respectively, "*j* and *ej* are the average of $Y_j$ and $Y_{ej}$, and n is the total number of data.

## RESULTS AND DISCUSSION

Multilayer perceptron based Artificial neural network were applied for the models; the results of all the models are shown in Table 1. All the learning rule (DBD, L-M, Step, Momentum, C-G and Quickprop), activation function TanhAxon, sigmoid and linear sigmoid were used for the artificial neural networks. Various networks of single and two hidden layers were trained for a maximum iteration of 1000, step size 1, additive 1, multiplicative 0.10 and smoothing 0.50 with different hidden neurons to choose best suited network was selected based on the minimum value of Normalize mean square error (NMSE), maximum value of correlation coefficient (r) and coefficient of efficiency (CE) by trial and error method. Different ANN architectures were tried using these inputs and the appropriate model structures were determined for each input combination. Different ANN models were tested and the results were compared by means of correlation coefficient, coefficient of efficiency and normalize mean square error statistics. 1095 data sets were used for training and 365 data sets were used for testing for all the ANN models.

In all the models, pan evaporation estimation was evaluated by the comparing ordinates of observed and estimated graphs. The normalize mean square error

**Table 1.** Performance indices of ANN based daily pan evaporation estimation models

| Model Name | Networks | Training | | | Testing | | |
|---|---|---|---|---|---|---|---|
| | | NMSE | CE (%) | r | NMSE | CE (%) | r |
| Delta Bar Delta and Tanh Axon | 4-3-1 | 0.16 | 95.85 | 0.91 | 0.30 | 93.44 | 0.85 |
| Levenberg–Marquardt (L-M) and Tanh Axon | 4-12-1 | 0.12 | 96.72 | 0.94 | 0.34 | 92.95 | 0.85 |
| Momentum and Liner Sigmoid Axon | 4-15-20-1 | 0.54 | 85.96 | 0.80 | 0.53 | 88.31 | 0.77 |
| Conjugate gradient (C-G) and Linear sigmoid Axon | 4-8-10-1 | 0.22 | 94.20 | 0.89 | 0.45 | 90.13 | 0.83 |
| Quick-prop and Tanh Axon | 4-5-1 | 0.17 | 95.53 | 0.90 | 0.32 | 92.94 | 0.84 |
| Step and Tanh Axon | 4-25-1 | 0.17 | 95.85 | 0.91 | 0.30 | 93.30 | 0.86 |

**Fig. 4.** Observed and estimated evaporation for Delta Bar Delta and TanhAxon combination of ANN model (4–3–1) during training period
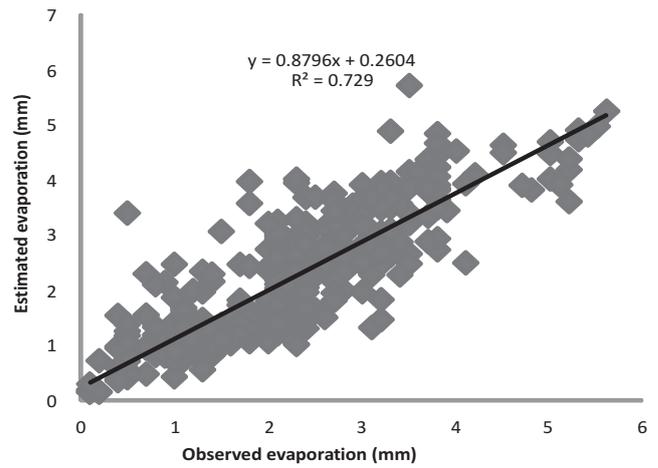


**Fig. 5.** Observed and estimated evaporation for Delta Bar Delta and TanhAxon combination of ANN model (4–3–1) during testing period
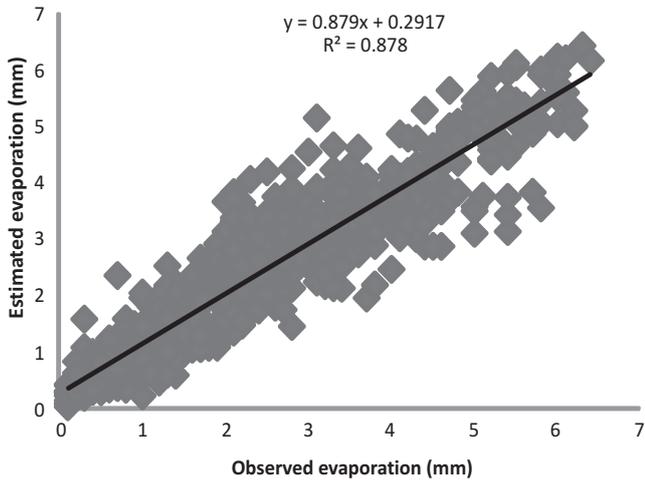


**Fig. 6.** Observed and estimated evaporation for Levenberg–Marquardt and TanhAxon combination of ANN model (4–12–1) during training period
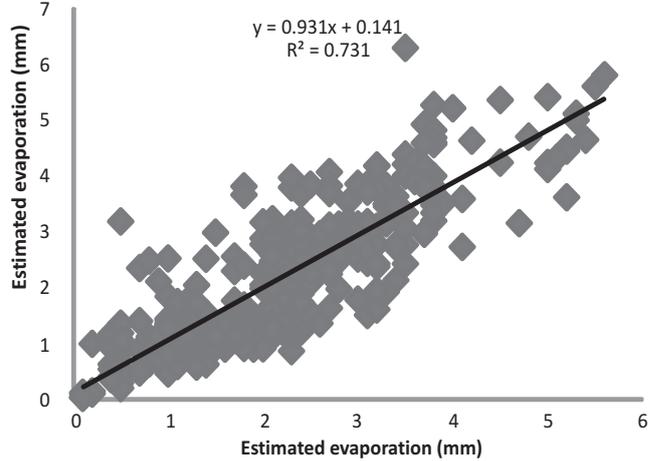


**Fig. 7.** Observed and estimated evaporation for Levenberg–Marquardt and TanhAxon combination of ANN model (4–12–1) during testing period
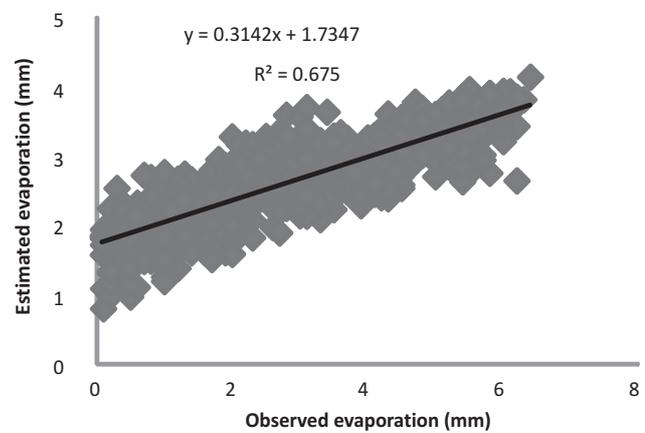


**Fig. 8.** Observed and estimated evaporation Momentum and Liner Sigmoid Axon combination of ANN model (4–15–20–1) during training period
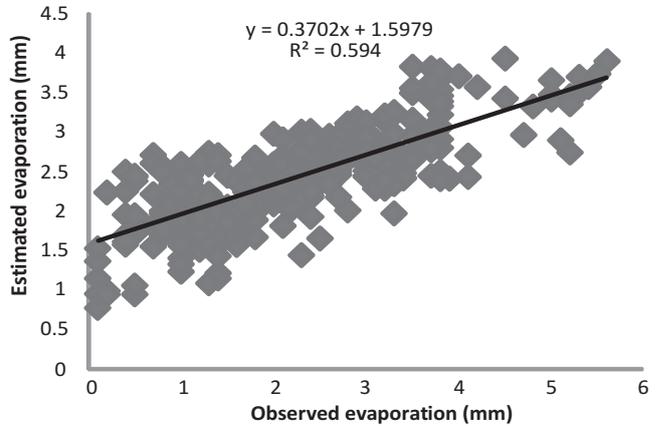


**Fig. 9.** Observed and estimated evaporation Momentum and Liner Sigmoid Axon combination of ANN model (4–15–20–1) during testing period
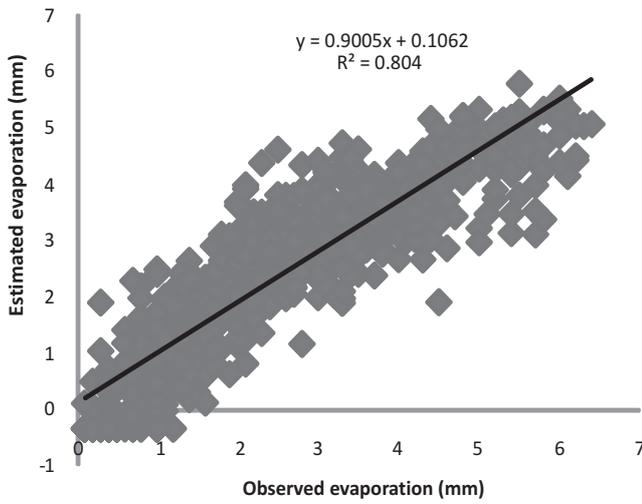
**Fig. 10.** Observed and estimated evaporation Conjugate gradient and linear sigmoid Axon combination of ANN model (4‑8‑10‑1) during training period
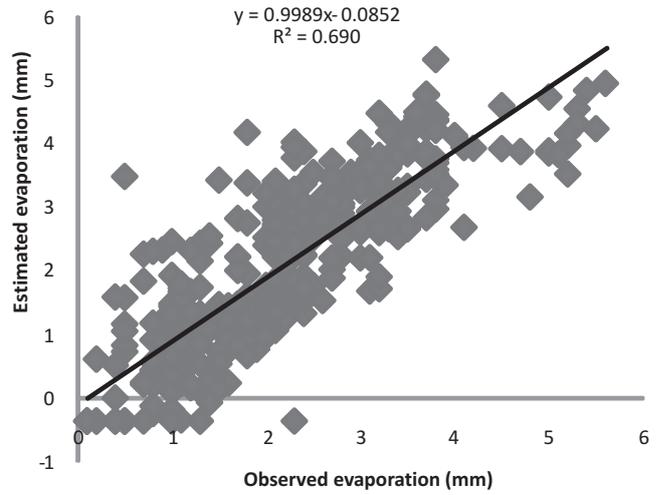


**Fig. 11.** Observed and estimated evaporation Conjugate gradient and linear sigmoid Axon combination of ANN model (4‑8‑10‑1) during testing period



**Fig. 12.** Observed and estimated evaporation Quick‑prop and TanhAxon combination of ANN model (4‑5‑1) during training period
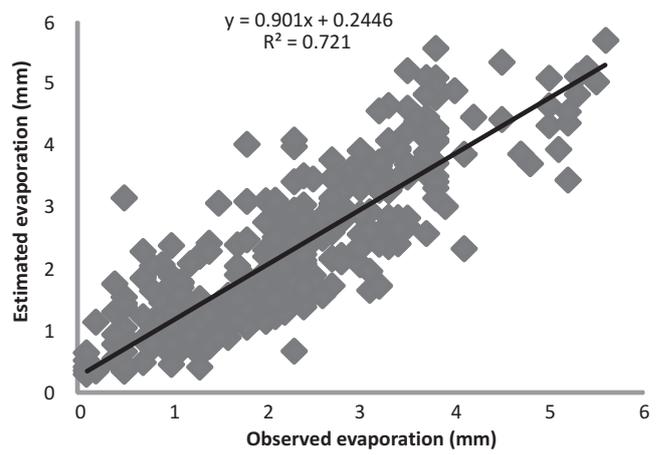


**Fig. 13.** Observed and estimated evaporation Quick‑prop and TanhAxon combination of ANN model (4‑5‑1) during testing period
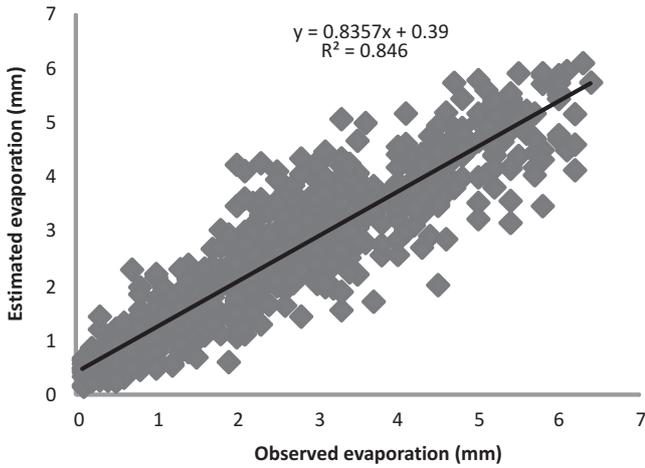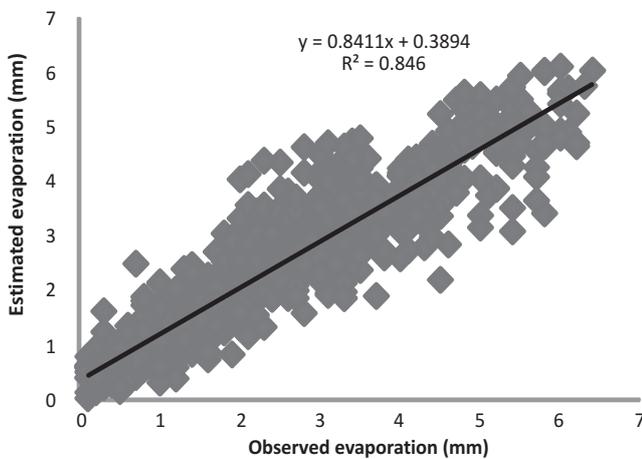


**Fig. 14.** Observed and estimated evaporation Step and TanhAxon combination of ANN model (4‑25‑1) during training period
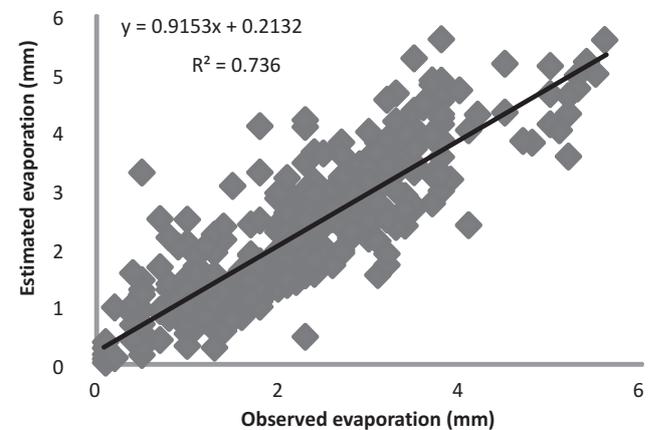


**Fig. 15.** Observed and estimated evaporation Step and TanhAxon combination of ANN model (4‑25‑1) during testing period
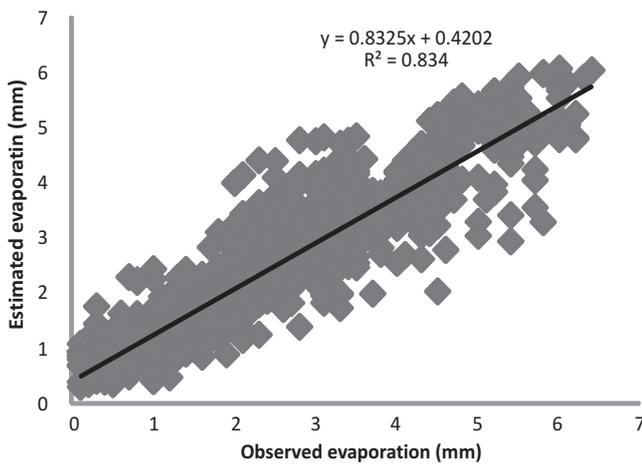
(NMSE), correlation coefficient (r) and coefficient of efficiency (CE) were evaluated by equation 7, 8 and 9 respectively Table 1. There is a close agreement between observed and predicted evaporation and over all shape of the plot of estimated evaporation is similar to that of the observed evaporation. In the model Delta Bar Delta and TanhAxon, the values of NMSE, r and CE are 0.16, 0.91 and 95.85 respectively during training period and 0.30, 0.85 and 93.44 in testing period. Similarly in the model L-M and TanhAxon, the values of NMSE, r and CE are 0.12, 0.94 and 96.72 respectively during training period and 0.34, 0.85 and 92.95 in testing period. Same as the next model Momentum and Liner Sigmoid Axon the values of NMSE, r and CE are 0.54, 0.80 and 85.96 respectively during training period and 0.53, 0.77 and 88.31 in testing period. Same as the model C-G and Linear sigmoid Axon the values of NMSE, r and CE are 0.22, 0.89 and 94.20 respectively during training period and 0.45, 0.83 and 90.13 in testing period. Same as the next model Quick-prop and TanhAxon the values of NMSE, r and CE are 0.17, 0.90 and 95.53 respectively during training period and 0.32, 0.84 and 92.94 in testing period. Finally in last model Step and TanhAxon the values of NMSE, r and CE are 0.17, 0.91 and 95.85 respectively during training period and 0.30, 0.86 and 93.30 in testing period. By visual inspection of all the figures shows that, there is a fairly good agreement between observed and predicted evaporation values for all ANN models. But by performance indices it is concluded that DBD and TanhAxon models is better than other models for evaporation estimation of Hawalbagh.

## CONCLUSIONS

This study has been undertaken to develop and evaluate different artificial neural networks (ANNs) for daily estimation of evaporation for Hawalbagh. In this study different combinations of ANN with different learning algorithms and activation functions were used to select best suited network by trial and error. But in all six models the best model is selected DBD and TanhAxon by different parameters such as NMSE, CE and r.

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# Floral Diversity of A Sacred Grove Nai-Ka-Nath Bassi Jaipur, Rajasthan

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**Abstract**: The present study was carried out in the Nai-Ka-Nath sacred grove, Bassi Jaipur, Rajasthan during the period of 2013 to 2015. There are 11 villages include in this grove and divided into 4 zones. Total 180 plants species were identified during this period. Of these 167 in North and North-East Zone, 174 in Middle Zone, 148 in South and South-East Zone and 130 species in North Zone were documented . These include 41,37,92 44 and 60 percent tree, shrubs, herbs, climbers and tuber species are common in these zones. The topography and religious beliefs of local people are the main causes of species variation. Plants *Azadirachta indica, Abutilon indicum, Citrullus colocynthis, Ocimum sanctum, Momordica balsamina, Tinospora cordifolia, Asparagus racemosus, Gloriosa superba,* and *Urginea indica* were commonly used in the treatment of cough, cold, skin diseases, stomach problems, fever, piles, diabetes, arthritis, snakebite, poisonous insect, and weakness. Majority (80%) local tribal people use traditional medicinal sources for primary health care.

**Keywords**: Sacred Grove, Biodiversity, Religious, Sacred

Sacred groves play a significant role in maintaining the ecological balance of rural areas and even urban settings. Ambient data of the floral species of Rajasthan were published by Botanical survey of India in three volumes (Shetty and Singh, 1987, 1991, 1993), "The Flora of Rajasthan (Sharma, 2002) and "Flora of the Indian Desert" (Bhandari, 1990) It is estimated by World Health Organization that 80 percent of the world people are based on traditional medicinal system to meet their primary health care. (Arya *et al,* 2008). Ancient historic sites of Eastern Rajasthan's were surveyed by Archaeological Survey of India, including Nai-Ka-Nath. In terms of architecture, the temple is supposed to be about a one and half thousand years old. (Mamodiya, 1987) and is one of the finest sacred grove in Rajasthan. This sacred groove play a role of lifeline for the local tribal population. They used these plants in sustainable manner to meet their primary medicinal requirements. The forest of the grove undulating with plateaus, lands and rolling sand dunes. The present study highlighted the plant species and their traditional medicinal uses. Many medicinal plants that have become extinct in neighboring areas are still well conserved in this grove and also highlight the importance of sacred groves for local people.

## MATERIAL AND METHODS

**Study area:** The sacred grove Nai-Ka-Nath is situated in the Bassi Tehsil of Rajasthan is bound in the north by village Jhar, to the north-east by the villages Khori, Banskho and Nimora,

to the east and south-east by the villages Chitodi, Charangarh and Tekchandpura, to the south by the village Kishanpura, to the south-west by the villages Gudhameena and Charanwas, and to the west and north-west by the village Nai-Ka-Mahadev and Jhar. Total area of the grove is 1515.51 Hectare. Latitude of 26.819 and longitude 76.1382. (My NASA Data, 2016) There are 11 villages include in Nai-Ka-Nath grove, named-Jhar, Khori, Nimora, Banskho, Chitodi, Tekchandpura, Charangarh, Kishanpura, Gudhameena, Charanwas, and Nai-Ka-Mahadev. (Apna khata, Rajasthan Sarkar 2013)

The topography of the grove includes mountains, hillocks, deep gorges, forest Regions, sand dunes, rugged and ravines etc. The area is harbour with sedimentary rocks like limestone, clay stone, sandstone and chalk. Entire forest divided in two main categories Dry tropical deciduous and dry tropical thorn forest. The soil and vegetation of the area characterized by grasses, thorny herbs & shrubs, and forest. (Aggarwal *et al*., 2011) There are more than 10 natural water bodies in the area, 4 are big and 6 small. There are 7 small artificial water pond are present near by the temple and 5 are present around the grove. 16 handpumps and 5 tube-wells are present in the area. The ground water level of the area is very low due to less rainfall. The ground water level of the area at 26.70 mbgl in the year of 2006, in present scenario (2015) the level of ground water is reached at 37.80 mbgl. (Regional Director of Ground water board Jaipur, 2016). The climate of the grove is Sub-tropical/Semi-Arid. There are four climatic seasons. The highest temperature is 47°C on Jun 08

2014 and lowest is $2.3^0$C on January 07 2013 during the study. The average annual rainfall is 576.05 mm in 36.3 rainy days of last ten years (2006 to 2015). (Director of India Metrological Department, 2016)

Filed survey were conducted in all parts of the study area to collect the valuable information about the floral species. Of these 63 trips in winter (October to March), 32 trips in monsoon (July to September) and 23 tours were conducted in summers (April to June). Spot identification of possible species were done with the help of the local people and forest officers. The identification is mainly based on morphological observations and close up high-resolution photos (Sony cyber shot dsc-hx400v). The identification of remaining species and confirmation of all species were done with the help of herbarium, botanical garden and available appropriate data (Sharma, 2002; Bhandari, 1990). To know the ethno-medicinal use of floral species of the area, group and single interviews of local experienced people of various communities' people and forest staff members were conducted. Total floral diversity of the grove is calculated by the formula (Somvanshi and Dhupper, 2013)

$\gamma = s_1 + s_2 - c.$

$\gamma = s_1 + s_2 + s_3 + s_4 - c.$

$s_1 + s_2 + s_3 + s_4 =$ the total number of species recorded in the North and North-East Zone, Middle Zone, South and South-East Zone and North Zone, c = the number of species common.

## RESULTS AND DISCUSSION

**North and North-East Zone:** Jhar, Khori, Nimora, and Chitodi villages are included in North and North-East zone. The north boundary of the grove is made up by Jhar village and Chitodi is situated on the North-East border of the zone. Nimora and Khori are present in the middle part of the zone. Total forest area under this zone is 485.23 ha. Maximum area (310.94 ha ) is covered by Jhar village. *Butea Monosperma, Cassia fistula, Diospyros melanoxylon, Hesperethusa crenulata, Wrightia tinctoria, Barleria prionitis, Euphorbia caducifolia, Adhatoda vasica, Grewia hirsuta, Rhus mysorensis, Pupalia lappacea*, and *Xanthium strumarium* are the dominant species of this zone. Total 167 plant species were documented in this zone (Fig. 1).

**Middle Zone:** Banskho and Nai-Ka-Mahadev present in the middle zone of the grove. Jhar village situated on the north boundary, Chitodi on East boundary, Charanwas on west and the lower part of the zone is covered by Kishanpura and Gudhameena villages. Nai-Ka-Mahadev covers 462.18 ha out of total area 692.27 ha. *Acacia leucophloea, A. senegal, A. catechu, A. ferruginea, Butea Monosperma, Cordia dichotoma, Melia azedarach, Prosopis juliflora, Sterculia*

*urens, Adhatoda vasica, Actiniopteris radiata, Cassia tora, Euphorbia caducifolia, Sesamum indicum, and Urginea indica* are the dominant species of the area. One hundred and seventy four plant species were collected from this zone (Fig. 2).

**South and South-East Zone:** Charangarh, Tekchandpura and Kishanpura villages are included in South and South-East zone. The south side of the area covered by Gudhameena and Charanwas and the upper boundary is made up by Chitodi and Nai-Ka-Mahadev. Total area of this zone is (Charangarh, 59.10 Ha. The dominant species of this area includes *Acacia senegal, A. nilotica, A. tortilis, A. catechu, Anogeissus pendula, Prosopis juliflora, Calotropis procera, Achyranthes aspera, Citrullus colocynthis, Solanum surattense, Tribulus terrestris, Saccharum bengalense,* and *Xanthium strumarium*. Total 148 plant species were identified in this zone (Fig. 3). North Zone:
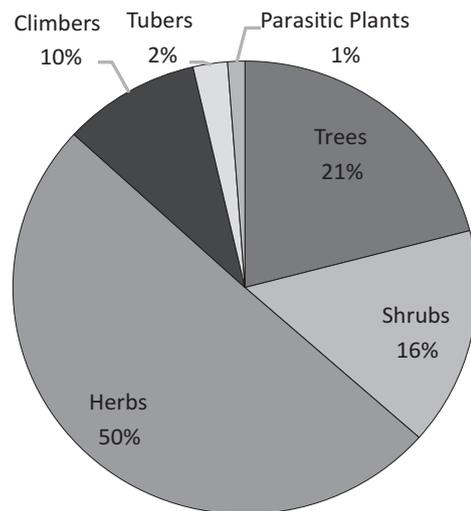


**Fig. 1.** North and North-East zone
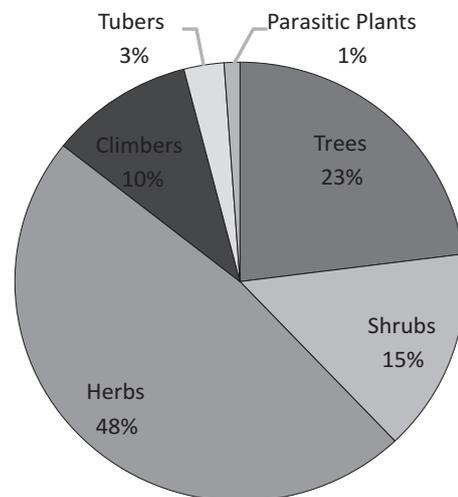


**Fig. 2.** Middle zone

**Table 1.** Zone wise list of floral species and their traditional uses

| Botanical Name | Family | A | B | C | D | Traditional Uses by Local People |
|---|---|---|---|---|---|---|
| **Trees** | | | | | | |
| *Acacia leucophloea* | Mimosaceae | + | + | + | + | Bark decoction for of fever and stomach pain. |
| *A. senegal* | ,, | + | + | + | + | Gum for diarrhea |
| *A. nilotica (indica)* | ,, | + | + | + | + | Bark is boiled with milk or water and used in cough and cold |
| *A. nilotica (cupressiformis)* | ,, | − | + | + | − | As in 3 |
| *A. tortilis* | ,, | − | + | + | + | Fodder (leafs, pods, young stems), timber, gum as food and making glue, firewood |
| *A. catechu* | ,, | **+** | + | + | + | Root pest for treatment of skin diseases |
| *A.ferruginea* | ,, | **-** | + | + | - | Fodder (leafs, pods, young stems), timber, gum as food and making glue, firewood |
| *Azadirachta indica* | Meliaceae | + | + | + | + | Leaves and bark used for the treatment of skin diseases, fresh leaves for stomach problems. Twig are used as tooth brush |
| *Anogeissus pendula* | Combretaceae | + | + | + | + | Fodder (leafs, pods, young stems), timber, gum as food and making glue, firewood |
| *Aegle marmelos* | Rutaceae | + | + | − | − | Fruit is used in the treatment of stomach problems, and bark & root is used in fever. |
| *Ailanthus excelsa* | Simaroubaceae | + | − | + | − | Leafs as fodder |
| *Alstoni scholaris* | Apocynaceae | − | − | + | − | Firewood |
| *Balanites aegyptiaca* | Simarubaceae | + | **+** | − | + | Bark pest for treatment of dog bite. |
| *Butea Monosperma* | <u>Fabaceae</u> | + | + | + | − | Females used the fruit for correcting menstruation |
| *Boswellia serrata* | Burseraceae | − | + | − | − | Gum used in skin & urinary problems and leaf juice in eye problems |
| *Capparis decidua* | Capparidaceae | + | + | + | + | Bark and other parts of ker is useful in treatment of coughs, asthma, boils etc. |
| *Cordia dichotoma* | Boraginaceae | + | + | − | − | Food (fruit), odder (fruit, flower), |
| *Cassia fistula* | Fabaceae | + | + | + | − | Leaves forn skin problems |
| *Commiphora wightii* | Burseraceae | + | + | + | + | Gum for cough |
| *Diospyros melanoxylon* | Ebenaceae | + | + | + | + | Flowers in urinary and blood diseases |
| *Dalbergia sissoo* | Fabaceae | + | + | + | + | Juice of leaves used in ulcer |
| *Dichrostachys cinerea* | ,, | − | + | + | − | Fodder (pods, leafs), food (gum), firewood |
| *Eucalyptus camaldulensis* | Myrtaceae | + | + | + | − | Timber |
| *Ficus benghalensis* | Moraceae | **+** | + | − | − | Leaves eaten by women after menses, bark and leaves for skin problems. |
| *Ficus religiosa* | ,, | **+** | + | + | + | Bark-inflammations and glandular swelling of the neck, roots− stomac &, ulcers, fruit-digestion, latex-skin diseases. |
| *F. racemosa* | ,, | **-** | + | + | − | Fruits & leaves are used in abortions, urinary problems, diarrhea, dyspepsia. Bark and latex decoction used in ulcers, cough, boils & skin problems. |
| *Helicteres isora* | Sterculiaceae | **+** | + | − | − | Dried pods pest for stomach problems and also in fever & dysentery |
| *Hesperethusa crenulata* | Rutaceae | + | − | − | − | Bark and leaves for skin disorders |
| *Holoptelea integrifolia* | Ulmaceae | **+** | + | + | + | Bark and leaves for skin disorders |
| *Leucaena leucocephala* | Fabaceae | + | **-** | − | − | Fodder (leafs), firewood |
| *Lannea coromandelica* | Anacardiaceae | + | + | − | − | Timber, firewood |
| *Maytenus emarginata* | Flacourtiaceae | + | + | + | + | Stem & leaves used to treat mouth ulcers, bark and leaves −skin problems, fruit −purify blood |

**Cont...**

| Species | Family | | | | | Uses |
|---|---|---|---|---|---|---|
| *Morus alba* | Moraceae | + | + | + | + | Fruit and leaves to treat sore throat problems |
| *Melia azedarach* | Meliaceae | + | + | + | + | Bark, leaves and fruits in skin problems |
| *Millettia pinnata* | Fabaceae | + | + | − | − | Timber, firewood |
| *Moringa oleifera* | Moringaceae | + | + | + | + | Fodder (leafs, pods),firewood |
| *Prosopis juliflora* | Mimosaceae | + | + | + | + | Leaves juice used to treat eye infection |
| *Prosopis cineraria* | ,, | + | + | + | + | Religious,fodder (leafs, pods), food (pods, bark), timber, firewood, worship (stem) |
| *Prosopis chilensis* | ,, | - | + | − | + | Fodder (leafs, pods),timber, firewood |
| *Phoenix sylvestris* | Arecaceae | - | + | − | − | Fruit for stomach problems |
| *Sterculia urens* | Sterculiaceae | + | + | + | − | Timber |
| *Salvadora persica* | Salvadoraceae | + | + | − | − | Latex used in the treatment of sores, body pain, & stomach aches |
| *Wrightia tinctoria* | Apocynaceae | + | + | − | − | Fodder (leaves) |
| *Ziziphus mauritiana* | Rhamnaceae | + | + | + | + | Root powder used by female after pregnancy |
| Shrubs | | | | | | |
| *Adhatoda vasica* | Acanthaceae | + | + | + | + | Leaves used for cold, cough & piles |
| *Annona squamosa* | ,, | + | + | − | − | Religious, food (fruit), fodder (leafs, fruit), worship (fruit) |
| *Barleria prionitis* | ,, | + | − | + | − | Fodder |
| *Bougainvillea spectabilis* | Nyctaginaceae | + | + | + | + | Fodder (leaf, flowers), firewood |
| *Calotropis procera* | Asclepiadaceae | + | + | + | + | Latex for skin problems |
| *Cassia occidentalis* | Fabaceae | + | + | + | + | Used in cough and cleansers throat |
| *Capparis sepiaria* | Capparaceae | + | + | − | − | Leaves for skin diseases |
| *Datura inoxia* | Solanaceae | + | + | + | + | Hot leaves used to treat body pain and swelling, it also used in dog–bites and poisonous insect bites. |
| *Datura metel* | ,, | + | + | + | + | Leaves for skin problems |
| *Dyerophytum indicum* | Plumbaginaceae | + | + | − | − | − |
| *Euphorbia caducifolia* | Euphorbiaceae | + | + | + | + | − |
| *Grewia flavescens* | Tiliaceae | − | + | + | − | Leaves stomach problems |
| *Grewia hirsuta* | ,, | + | + | − | − | Firewood |
| *Grewia occidentalis* | Malvaceae | + | + | + | − | − |
| *Grewia tenax* | Tiliaceae | + | + | − | − | − |
| *Jasminum sambac* | Oleaceae | + | + | − | − | Religious, fodder (leafs), worship (flowers) |
| *Leptadenia pyrotechnica* | Asclepiadaceae | + | + | + | + | Latex and young leaves are used to treat skin diseases |
| *Lantana camara* | Verbenaceae | + | + | + | + | Firewood |
| *Lantana indica* | ,, | + | + | + | − | Firewood |
| *Ricinus communis* | Euphorbiaceae | + | + | + | + | Hot leaves used to treat body pain and swelling |
| *Rhus mysorensis* | Anacardiaceae | + | + | + | + | Food (fruit), F fodder (leafs, fruit) |
| *Solanum indicum* | Solanaceae | + | + | + | + | Used in the treatment of respiratory disorders |
| *Sarcostemma acidum* | Asclepiadaceae | + | + | + | + | − |
| *Thevetia peruviana* | Apocynaceae | + | + | + | + | Firewood |
| *Tephrosia purpurea* | Fabaceae | + | + | + | + | Firewood |
| *Withania somnifera* | Solanaceae | + | + | + | + | Used to treat sexual and general weakness, root for ulcers, leaves to treat body pain and swelling |
| *Ziziphus nummularia* | Rhamnaceae | + | + | + | + | Root powder used by female after pregnancy |
| **Herbs** | | | | | | |
| *Achyranthes aspera* | Amaranthaceae | + | + | + | + | Useful in stomach ache, bowel complaints, piles, skin eruptions, vomiting etc. |

**Cont...**

| | | | | | | |
|---|---|---|---|---|---|---|
| *Aloe vera* | Xanthorrhoeaceae | + | + | + | + | Used in skin diseases and juice in body pain |
| *Alternanthera sessilis* | Amaranthaceae | + | + | + | + | – |
| *Argemone mexicana* | Papaveraceae | + | + | + | + | Leaf and oil for skin problems and ulcers |
| *Abutilon indicum* | Malvaceae | + | + | + | + | Leaves to treat piles and stomach problem |
| *Adiantum caudatum* | Pteridaceae | + | + | + | – | – |
| *Aerva lanata* | Amaranthaceae | + | + | + | + | – |
| *Amaranthus spinosus* | ,, | + | + | + | + | Vegetable |
| *Amaranthus viridis* | ,, | + | + | + | + | Fodder |
| *Actiniopteris radiata* | Pteridophyta | + | + | + | + | As in |
| *Aerva javanica* | Amaranthaceae | + | + | + | + | – |
| *Acalypha indica* | Euphorbiaceae | + | + | + | + | Fodder |
| *Amaranthus polygonoides* | Amaranthaceae | + | + | + | + | Fodder |
| *Aristida adscensionis* | Poaceae | + | + | + | + | Fodder |
| *Bacopa monnieri* | Plantaginaceae | + | + | + | + | Used for improving memory |
| *Boerhavia diffusa* | Nyctaginaceae | + | + | + | + | Leaves used to reducing swelling & urinary burning, and roots used in night blindness. |
| *Bidens biternata* | Asteraceae | + | + | + | + | Fodder |
| *Blainvillea acmella* | ,, | + | + | + | + | Fodder |
| *Borreria articularis* | Rubiaceae | + | + | + | – | Fodder |
| *Brachiaria reptans* | Poaceae | + | + | + | + | Fodder |
| *Citrullus colocynthis* | Cucurbitaceae | + | + | + | + | Indrayan used in treatment of diabetes, metabolic disorder, fever, jaundice, arthritis, snakebite, tumors and urogenital disorders |
| *Cynodon dactylon* | Poaceae | + | + | + | + | Fodder |
| *Cassia tora* | Fabaceae | + | + | + | + | Fodder |
| *Chenopodium album* | Chenopodiaceae | + | + | + | + | Fodder, V |
| *Chenopodium murale* | ,, | + | + | + | + | Used in skin fungal infection |
| *Cenchrus ciliaris* | Poaceae | + | + | + | + | Fodder |
| *Cenchrus spinifex* | ,, | + | + | + | + | Fodder |
| *Cenchrus biflorus* | ,, | + | **+** | + | + | Fodder |
| *Crotalaria medicaginea* | Fabaceae | + | **+** | + | + | Fodder |
| *Croton bonplandianus* | Euphorbiaceae | + | + | + | + | – |
| *Convolvulus microphyllus* | Convolvulaceae | + | + | + | + | Fodder |
| *Cassia obtusifolia* | Fabaceae | **+** | + | + | + | – |
| *Commelina benghalensis* | Commelianaceae | **+** | + | + | + | Fodder |
| *Corchorus aestuans* | Malvaceae | + | + | + | + | – |
| *Desmostachya bipinnata* | Poaceae | + | + | + | + | Religious |
| *Digitaria sanguinalis* | ,, | + | + | + | + | Fodder |
| *Digera muricata* | Amaranthaceae | + | + | + | + | Fodder |
| *Dipteracanthus patulus (Synonym Ruellia patula)* | Acanthaceae | + | – | – | – | – |
| *Dactyloctenium aegyptium* | Poaceae | + | + | + | + | Fodder |
| *Digitaria adscendens* | ,, | + | + | + | + | Fodder |
| *Eleusine indica* | ,, | + | + | + | + | Fodder |
| *Eclipta prostrata* | Asteraceae | + | + | + | + | Fodder |
| *Echinochloa colonum* | Poaceae | + | + | + | + | Fodder |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Eragrostis pilosa* | ,, | + | + | + | + | Fodder |
| *Euphorbia hirta* | Euphorbiaceae | + | + | + | + | Used for asthma, chronic nasal, diarrhoea |
| *Echinops echinatus* | Asteraceae | + | + | + | + | Fodder |
| *Glinus lotoides* | Molluginaceae | + | + | + | + | Fodder |
| *Hemidesmus indicus* | Apocynaceae | + | + | + | + | Fodder |
| *Heliotropium strigosum* | Boraginaceae | + | + | + | + | Fodder |
| *Indigofera cordifolia* | Papilionaceae | + | + | + | + | Fodder |
| *Leucas aspera* | Lamiaceae | **+** | + | + | + | Fodder |
| *Launaea sarmentosa* | Asteraceae | **+** | + | + | + | Fodder |
| *Lepidagathis trinervis* | Acanthaceae | + | + | + | + | Fodder |
| *Lindenbergia muraria* | Scrophulariaceae | + | + | + | + | Fodder |
| *Mollugo cerviana* | Molluginaceae | + | + | + | + | Fodder |
| *Phyllanthus urinaria* | Phyllanthaceae | + | + | + | + | Fodder |
| *Parthenium hysterophorus* | Asteraceae | + | + | + | + | – |
| *Peristrophe bicalyculata* | Acanthaceae | + | + | + | + | Fodder |
| *Portulaca oleracea* | Portulacaceae | **+** | + | + | + | Fodder |
| *Portulaca pilosa* | ,, | + | + | + | + | Fodder |
| *Polygala erioptera* | Polygalaceae | + | + | + | + | Fodder |
| *Pedalium murex* | Pedaliaceae | + | + | + | + | Leaf and roots decoction and fruit powder used to treat S sexual diseases and general weakness. It also used in ulcers, fever, wounds |
| *Physalis minima* | Solanaceae | + | + | + | + | – |
| *Pupalia lappacea* | Amaranthaceae | + | + | + | + | – |
| *Ocimum canum* | Lamiaceae | + | + | − | − | Tulsi to treat colds, headaches, stomach problems, inflammation, heart disease, asthma, malaria, diarrhea, skin problems, arthritis, eye diseases, fever |
| *Ocimum sanctum* | Lamiaceae | + | + | − | − | As above |
| *Oxalis corniculata* | Oxalidaceae | + | + | − | − | Fodder |
| *Rumex dentatus* | Polygonaceae | + | + | + | + | Fodder |
| *Sorghum halepense* | Poaceae | + | + | + | + | Fodder |
| *Solanum nigrum* | Solanaceae | + | + | + | + | Juice of the leaf and fruits used to treat cough, asthma, fever and liver diseases |
| *Solanum surattense* | ,, | + | + | + | + | Root Kantakari is used in the treatment of phlegmatic cough and fever |
| *Sesamum indicum* | Pedaliaceae | + | + | + | − | Fodder, Food |
| *Sonchus oleraceus* | Asteraceae | + | + | + | + | Fodder |
| *Schismus barbatus* | Poaceae | + | + | + | + | Fodder |
| *Sida cordifolia* | Malvaceae | + | + | + | + | Roots used to treat paralysis and wounds |
| *Sesbania bispinosa* | Fabaceae | + | + | + | + | – |
| *Saccharum bengalense* | Poaceae | + | + | + | + | Making houses |
| *Tridax procumbens* | Asteraceae | + | + | + | + | Leaves are used to treat various skin injury |
| *Tribulus terrestris* | Zygophyllaceae | + | + | + | + | Used to treat sexual diseases, general weakness and urinary problems |
| *Trianthema Portulacastrum* | Aizoaceae | + | + | + | + | Making houses |
| *Triumfetta pentandra* | Tiliaceae | + | + | + | + | – |
| *Triumfetta rhomboidea* | ,, | + | + | + | + | – |
| *Verbesina encelioides* | Asteraceae | + | + | + | + | – |
| *Xanthium strumarium* | ,, | + | + | + | + | Treatment of high fever |

**Cont...**

**Climbers**

| | | A | B | C | D | |
|---|---|---|---|---|---|---|
| *Argyreia nervosa* | Convolvulaceae | + | + | − | − | − |
| *Abrus precatorius* | Fabaceae | + | + | − | − | Seeds to treat swelling of throat, roots used to treat head-ache |
| *Ampelocissus latifolia* | Vilaceae | + | + | + | − | Decoction of bark and stem for stomach pain and past to treat |
| *Cucumis pubescens* | Cucurbitaceae | + | + | + | + | Dried fruit powder fort stomach problems |
| *Cardiospermum halicacabum* | Sapindaceae | + | + | − | − | − |
| *Cissampelos pareira* | Menispermaceae | + | + | + | − | Roots powder used in body pain |
| *Cocculus hirsutus* | ,, | + | + | + | + | − |
| *Cocculus pendulus* | ,, | + | + | + | + | − |
| *Ipomoea pes tigridis* | Convolvulaceae | + | + | − | − | − |
| *Ipomoea obscura* | ,, | + | + | − | − | − |
| *Ipomoea nil* | ,, | − | + | − | − | − |
| *Mukia maderaspatana* | Cucurbitaceae | + | + | + | + | − |
| *Momordica balsamina* | ,, | + | + | + | + | For poisonous insect and snake bite |
| *Pergularia daemia* | Asclepiadaceae | + | + | + | + | Leaf juice used to treat body pain, cough, asthma, liver problems and constipation and latex treat boils and wounds |
| *Rhynchosia minima* | Fabaceae | + | + | + | + | Fodder |
| *Rivea hypocrateriformis* | Convolvulaceae | − | + | − | − | − |
| *Rivea ornata* | ,, | + | + | + | + | − |
| *Tinospora cordifolia* | Menispermaceae | + | + | + | + | Used to treat body pain, arthritis, fever, jaundice, diabetes, urinary disorders, asthma, poisonous insect, snake bite, and eye disorders |

**Tuberous**

| | | A | B | C | D | |
|---|---|---|---|---|---|---|
| *Asparagus racemosus* | Liliaceae | + | + | + | + | Roots used for improving physical stamina, increases secretion of breast milk, increasing body weight, improving reproductive system, maintains healthy hormonal balance, normal function of the immune and digestive system |
| *Cyperus rotundus* | Cyperaceae | + | + | + | + | Tubers used to treat stomach disorder, jaundice, fiver, body pain, cold, health weakness, skin problems, and sores wounds. Root pest applied on breast to increase size |
| *Cyperus compressus* | ,, | + | + | + | + | As in 2 |
| *Gloriosa superba* | Liliaceae | − | + | + | − | Used for snake bites, scorpion stings, parasitic skin diseases, urological pains, piles, gonorrhoea, infertility, wounds, arthritis |
| *Urginea indica* | ,, | + | + | − | − | Used for cardiac diseases, cough, arthritis, tumors, swellings |

**Parasitic Plants**

| | | A | B | C | D | |
|---|---|---|---|---|---|---|
| *Cuscuta reflexa* | Convolvulaceae | + | + | + | + | Used for skin diseases, fevers, body pain, jaundice, coughs, and eye infections |
| *Cuscuta chinensis* | ,, | + | + | + | + | As in 1 |

A − North and North-East Zone, B − Middle Zone, C − South and South-East Zone, D − North Zone

Charanwas and Gudhameena are included in North zone. North-East boundary of the area covered by Nai-Ka-Mahadev, East and South-East border covered by Kishanpura and Charangarh villages. Total area of North Zone is 99.65 Ha. The dominant floral species of the area are *Acacia senegal, A. nilotica, A. tortilis, A. catechu, Prosopis juliflora, Ziziphus nummularia, Argemone mexicana, Citrullus colocynthis, Lepidagathis trinervis, Pupalia lappacea, Saccharum bengalense* and *Cyperus compressus*. Total 130 plant species identified in this zone (Fig. 4).

**Total floral diversity:**

$\gamma = 167 + 174 + 148 + 130 - 439$

$\gamma = 180$ species

During the study 180 plant species of 80 families were identified. Of these 24.44 percent trees of 20 families, 15 percent shrubs of 17 families, 46.66 percent herbs of 33 families, 10 percent climbers of 7 families, 2.78 percent tubers of 2 families, and 1.1 pe rcent parasitic plants of 1 family. (Fig. 5, 6, 7)
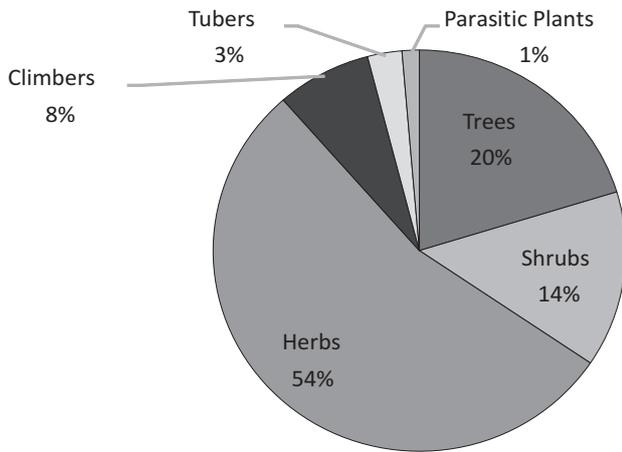
**Fig. 3.** South and South-East zone
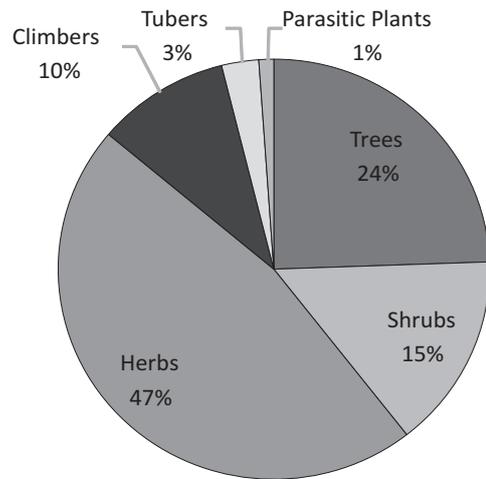


**Fig. 6.** Total plants – 180
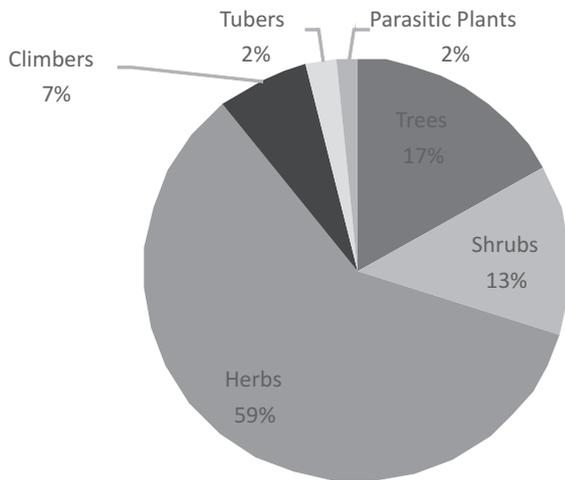


**Fig. 4.** North zone
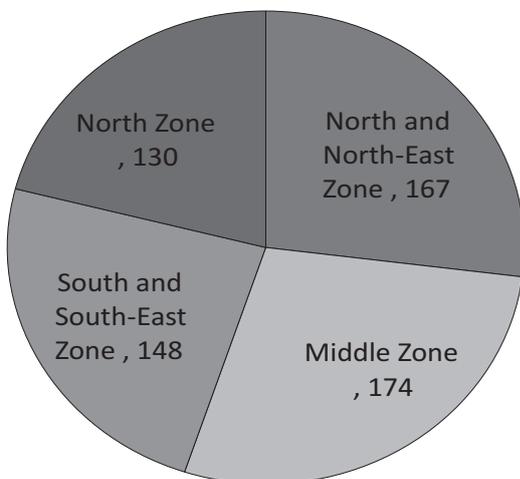


**Fig. 5.** Zone wise total species

There are two main reasons behind the variation in the number of species in these zones. First socio-religious tradition, sacredness and religious beliefs of local people and second is topography. Daath Mata temple is situated in North and North-East Zone, Nai-ka-Nath temple is in Middle Zone and Bharthari Temple is in the South and South-East Zone, because of the sacredness of these temples the rate of anthropogenic interventions is low. There are no temple in North Zone, and the rate illegal human activities is high in this zone. Most part of the North Zone and the upper part of South and South-East Zone is infertile sand dunes. The infertility of these sand dunes reduce the number of species in these zones.

In tree species *A. senegal, A. tortilis, A. ferruginea, Azadirachta indica, Dichrostachys cinerea, Prosopis juliflora*, and *Wrightia tinctoria* are the dominant species. F*icus benghalensis, Ficus religiosa, Ficus racemosa,* and *Prosopis cineraria* are the sacred plants and are worshiped. Bark of Acacia nilotica is boiled with milk or water and used in the treatment of cough and cold. Dried pods pest of *Helicteres isora* give to childs in stomach problems and also in fever & dysentery, leaf or bark pest in skin problems. *Adhatoda vasica, Euphorbia caducifolia, Grewia hirsuta, Withania somnifera and Tephrosia purpurea are the dominant shrubs in the area.* Hot leaves of *Datura inoxia* and *Ricinus communis* are used to treat body pain and swelling, it also used in dog-bites and poisonous insect bites.

*Actiniopteris radiata, Dipteracanthus patulus, Lepidagathis trinervis, Pedalium murex, Ocimum canum, Sesamum indicum, Tridax procumbens,* and *Tribulus terrestris* are the dominant herbs. Leaf and oil of *Argemone mexicana* used to treat skin problems and ulcers. Leaves of *Abutilon indicum* used to treat piles and stomach problem.
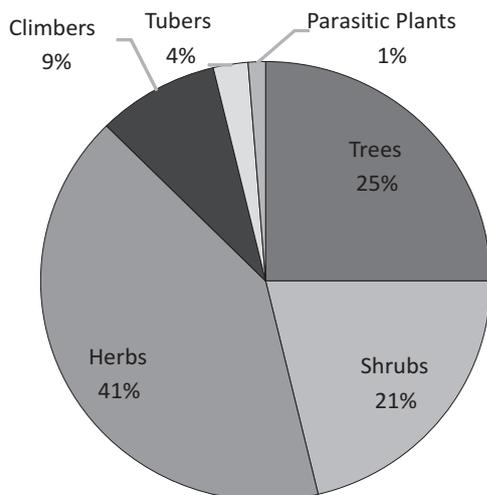
**Fig. 7.** Total families

*Citrullus colocynthis* used in treatment of diabetes, arthritis, snakebite and urogenital disorders. Leaf and roots decoction and fruit powder of *Pedalium murex* used to treat sexual diseases and general weakness. *Ocimum canum* used to treat colds, headaches, stomach problems, asthma, fever etc. Leaves of *Tridax procumbens* are used to treat various skin injury. *Tinospora cordifolia* used to treat body pain, arthritis, fever, and jaundice etc. Roots of *Asparagus racemosus* used for improving physical stamina and sexual power. *Gloriosa superba* used to treat snake and insect bites.

## CONCLUSION

Nai‑ka‑Nath grove protect the unique floral species that have been extinct in neighboring areas. Illegal human activities like hunting, logging, and developmental activities are strictly prohibited in the grove. Here, medicinal plants are used by some selected authorized persons to treat local people. It is a perfect place for the in‑situ conservation, because socio‑religious tradition, sacredness and religious beliefs of local peoples are still alive. Few people around the grove try to utilize the natural resources of the area for their personal and commercial use. There is need to conserve the valuable biodiversity of the grove.

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# Farmland Biodiversity: Seasonal Variation in Phytosociological Attributes of Vegetation in District Budgam, Kashmir

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**Abstract**: The present study was conducted in Boundary plantation of District Budgam of Kashmir valley, with an aim to assess the composition, structure, and vegetation indices of life forms growing in the system. Results of the study revealed that floristic composition of the system consists of 4 genera, 3 species of 3 families of cultivated plants (herbage), 30 genera having 31 species belonging to 17 families of wild plants (herbage) and trees represented2 genera, 4 species belonging to a single family. Seasonal variation in phytosociological parameters of cultivated species explicated dominance of *Brassica campestris* in spring achieving dominance over other species followed by *Avena sativa* as its co-dominant. The dominant species during summer was *Oryza sativa*. Among wild species, *Cynodon dactylon* dominated the system achieving highest IVI value of 45.09 during autumn season, whileas, *Prunella vulgaris* demonstrated lowest IVI attaining the maximum value during summer. Shannon-Weiner and Simpson diversity index for cultivated and wild herbaceous species showed decreasing and increasing trend respectively with advancing season. Among tree species, *Populus deltoides* attained maximum Importance value index (IVI) of 118.01.

**Keywords:** Farmland, Biodiversity, Cultivated, Wild, Phytosociological attributes, Seasons

Biodiversity is crucial for survival, health and well-being of humans. It is giving greater resilience to ecosystems and organisms. Complex, diversified and highly traditional rooted part of plant biodiversity conservation and utilization are components in agroforestry systems (Amberber *et al*., 2014). Forest modifications have negative impacts on biodiversity (Bobo *et al*., 2006), and 1 per cent reduction of natural area will cost about 1 per cent of steady-state diversity (Rosenzweig, 2003). To improve biodiversity conservation, it becomes crucial to redesign anthropogenic habitats so that their use is compatible with the use by a broad array of other species. The new strategy of conservation biology called "reconciliation ecology" has grown out of a thorough understanding of species-area relationships (Bobo *et al*., 2006). In general, agro-ecosystems that are more diverse, more permanent, isolated, and managed with low input technology (e.g., farmlands, traditional polycultures) take complete advantage of ecological processes associated with higher biodiversity than highly simplified, input-driven and disturbed systems (Atta-Krah *et al*., 2004). Thus, agroforestry system research and development has evolved dramatically with much emphasis on how they function, diversity from local, landscape to regional level, local knowledge systems, economic valuation and environmental services they provide (Beer *et al*., 2005). The great public support for the conservation of biodiversity and the work must provide opportunities for meaningful interactions with the natural world. In the past, the conservation of biodiversity has been mostly understood in terms of the management of protected areas and natural forests, ignoring the possible role of farm areas and the ways through which rural communities have promoted biodiversity in their subsistence agricultural production systems (Fifanou *et al*., 2011). Retention of forest species in agricultural landscapes enhances biodiversity conservation at both species and landscape level (Herve and Vidal, 2008). The practice of maintaining trees on the farm makes farmers the custodians of biodiversity (Acharya, 2006). The traditional agroforestry systems identified in Kashmir Valley include; boundary plantations, agri-silviculture on sloping lands, agri-silviculture in plains, horti- silviculture, horti-silvi-pasture, horti-silvi-agriculture and homegardens (Mughal and Bhattacharya, 2002). Since different research programmes have been undertaken with respect to district Budgam no work has been reported regarding farmland biodiversity and evaluation. So, in order to evaluate and summarize the phytosociological parameters in boundary plantation, the present investigation was taken up.

## MATERIAL AND METHODS

The present research study was conducted in Budgam District of Kashmir Valley during the year 2013 and2014. The experimental site is located between 34˚1'12"N latitude and 74˚46'48"E longitude at an altitude of 1610 m above mean

sea level, roughly 15 km south east of Srinagar city . The topography of the district is mixed with both mountainous and plain areas. The climate is of the temperate type with the upper-reaches receiving heavy snowfall during winter. The average annual precipitation of the district is 585 mm. Preliminary information regarding number of tehsils, blocks within tehsils,  villages within blocks, land use systems, land holdings and number of households were obtained from District Agriculture Department. After thorough reconnaissance of the said district, three tehsils namely: Budgam, Beerwah and Chadoora were selected on the basis of large of villages and households. Multistage stratified random sampling was used to select the blocks; villages within tehsils and then farmers within villages. A total of 252 farmers were selected and interviewed through pre-tested questionnaire regarding different land use patterns (agriculture, agroforestry, horticulture) and their socio-economic status. The methodology of the experimental study is given in Table 1.

**Table 1.** Methodology for the selection of sample areas

| Selected Tehsils | Beerwah | Budgam | Chadoora |
|---|---|---|---|
| Selected blocks (06) | 2 | 2 | 2 |
| Selected villages (06 per block) | 6×2 = 12 | 6×2 = 12 | 6×2 = 12 |
| Selected farmers (7 per village) | 6×2×7 = 84 | 6×2×7 = 84 | 6×2×7 = 84 |
| Grand total | | 252 | |

**Vegetation analysis:** Random quadrats of 10m × 10 m size for trees and within each of these quadrats and two 1m × 1m quadrats for herbs (cultivated & wild) were laid down and replicated three times for each life form respectively. Herbarium specimens (herbaceous plants) were collected for three consecutive seasons *viz*; spring, summer and autumn and identified from the Division of Environmental Sciences, SKUAST-Kashmir and Centre for Biodiversity & Taxonomy Department of Botany, University of Kashmir. The data on vegetation were quantitatively analyzed for density and basal area as per the methods given by Phillips (1959). The relative values of these indices were summed up to get importance value index (IVI) of individual species following Curtis and Mc Intosh (1950). For species diversity (H) and species evenness were calculated by Shannon-Weiner's method (1963).Simpson's diversity was measured following Simpsons diversity index (Simpson, 1949) separately for each life form (herbs and trees).

**Statistical Analysis:** All the data sets were analyzed using descriptive statistics(MS excel work sheet) and by standard procedures followed by Gomez and Gomez (1984).

## RESULTS AND DISCUSSION

From the present investigation, it was assessed that 184 farmers out of 252 in the study area practice different agroforestry systems of which 52 farmers adopt boundary plantation as one of the agroforestry land use system, covering about 6.90 hectares of land area.

**Floristic diversity, community structure and composition in boundary plantation:** Floristic composition in the present study revealed the presence of 4 genera, 3 species of 3 families of cultivated plants (herbage), 30 genera having 31 species belonging to 17 families of wild plants (herbage)and trees represented by 2 genera, 4 species of a single family (Table 2). Less vegetation diversity may be due to selective approach of landholders to grow plants that are required for their primary needs, uses and benefits that they render to the household and for income generation. It is also reported that the regional patterns of species richness are consequences of many interacting factors, such as regional species dynamics, regional species pool, environmental variables and human activity (Neelamegam *et al*., 2015; Amberber *et al*., 2014; Deb *et al*., 2014; Man *et al*., 2012; Mahmoud, 2009; Zobel, 1997).

**Seasonal variation in phyto-sociological attribute (IVI) of cultivated plants:** The importance value index (IVI)revealed that highest IVI values (122.90) was exhibited by *Brassica campestris* in spring achieving dominance over other species followed by *Avena sativa* (106.58) as its co-dominant (Table 3). On the other hand, the dominant species during summer was *Oryza sativa* explicating IVI value of 300.Absenceof cultivated herbage during autumn season owing to the reason that the land is left fallow for few months in order to regain its lost nutrients. Our present findings are in consonance with Krishnal and Weerahewa (2014) for traditional home gardens in Batticaloa District, Sri-Lanka; Hasanuzzaman *et al*. (2013) for cropland agroforest in south-western Bangladesh.

**Seasonal variation in phyto-sociological attributes of wild plants:** Phytosociological attributes i.e. density (individuals/$m^2$), basal area (cm$^2$/$m^2$) and IVI (importance value index)of wild herbage (plants) showed consistent rise from spring season i.e. with advancement of growing season, attained peak values and species number during summer season and decreased abruptly in autumn (Table 4 to 6). Among all herbaceous plants encountered, *Cynodon dactylon* explicated maximum values for evaluated growth attributes as 36 (density), 1.19 (basal area), 27.42 (IVI) during spring; 95 (density),1.98 (basal area), 27.91 (IVI) during summer and 31.83 (density), 1.06 (basal area), 45.09 (IVI) in autumn. However, *Prunella vulgaris* recorded minimum values as1.66 (density), 0.06 (basal area), 1.78 (IVI) during spring, 4.33 (density), 0.10 (basal area) and 1.96

(IVI) in summer and 0.33 (density), 0.01 (basal area),1.32 (IVI) in autumn. The marked variation among seasons in prevalent agroforestry systems may be attributed to the conducive growth and development conditions, amount of litter and rate of litter decomposition which may be influenced by tree density (Lebret *et al*., 2001), availability of soil moisture for optimum nutrient flow in soil-plant system and other environmental factors i.e. humidity and solar radiation from spring onwards which declined with the commencement of autumn. During autumn season, the rate of sprouting of root/seed stock is diminished and species number declined owing to adverse climatic conditions (Alhassan *et al*., 2006); Sharma and Upadhyay (2002).

The growth attributes of few wild plants i.e. *Cynodon dactylon*, *Polygonum hydropiper* and *Oenothera rosea* were comparatively higher than other species in different seasons

**Table 3.** Importance value index (IVI) of different cultivated plants in boundary plantation of the study area

| Plant species | Importance value index | |
|---|---|---|
| | Spring | Summer |
| | Mean±S.E | Mean±S.E |
| *Avena sativa* L. | 106.58±1.70 | – |
| *Brassica campestris* L. | 122.90±2.46 | – |
| *Oryza sativa* L. | – | 300 |
| *Pisum sativum* L. | 70.51±3.99 | – |
| Total | 300 | 300 |

***Note***: Absence of cultivated herbage was during autumn season

of sampling. Their better adaptation to prevailing environmental conditions i.e. suitable edaphic and climatic conditions that favors growth and survival of species in a particular agroforestry system. The dominance of few

**Table 2.** Diversity recorded in boundary plantation in the study area

| Plant names & family | Global IUCN status | English/Local name |
|---|---|---|
| **Cultivated herbage** | | |
| *Avena sativa* (Poaceae) | N.A. | Oats/Khaseeli |
| *Brassica campestris* (Brassicaceae) | N.A | Mustard/Tiligogul |
| *Oryza sativa* (Poaceae) | N.A | Paddy/Daani |
| *Pisum sativum* (Fabaceae) | N.A | Peas/Matar |
| **Wild herbage** | | |
| *Amaranthus caudatus* (Amaranthaceae) | Not evaluated | Tassel flower/Lissi |
| *Anthemis cotula* (Asteraceae) | Not evaluated | Mayweed/Stinking chamomile/Faki-gaasi |
| *Artemisia absinthium* (Asteraceae) | Vulnerable | Worm wood/Tethwan |
| *Cannabis sativa* (Cannabaceae) | Not evaluated | Hemp/Bhang |
| *Clinopodium vulgare* (Lamiaceae) | Not evaluated | Wild basil |
| *Capsella-bursapastoris* (Brassicaceae) | Not evaluated | Shepherd's purse/Kralmond |
| *Cynodon dactylon* (Poaceae) | Not evaluated | Durva grass/Dramun |
| *Conyza Canadensis* (Asteraceae) | Not evaluated | Canadian horseweed/Gur loute |
| *Daucus carota* (Apiaceae) | Vulnerable | Wild carrot/Bird's nest/Jangli-gazir |
| *Equisetum arvense* (Equisetaceae) | Not evaluated | Field horsetail/Gandum-gund |
| *Euphorbia helioscopia* (Euphorbiaceae) | Not evaluated | Mad woman's milk/Gur-duode |
| *Equisetum arvense* (Equisetaceae) | Not evaluated | Field horsetail/Gandum-gund |
| *Galinsoga parviflora* (Asteraceae) | Not evaluated | Gallant soldier |
| *Gallum aparine* (Rubiaceae) | Not evaluated | Goose grass/Zoa ghasi |
| *Geranium polyanthes* (Geranaceae) | Not evaluated | Cranes bills |
| *Medicago polymorpha* (Fabaceae) | Not evaluated | Toothed bur clover/Poshi-gassi |
| *Nepeta cataria* Lamiaceae) | Not evaluated | Cat mint/Byari-gassi or Gand soi |
| *Oenothera rosea* (Onagraceae) | Not evaluated | Evening primrose |
| *Plantago major* (Plantaginaceae) | Not evaluated | Broadleaf plantain/Greater plantain/Veuth-gulli |
| *Polygonum hydropiper* (Polygoniaceae) | Least conceren (Stable) | Water-pepper/Marchagban gassi |
| *Potentilla reptans* (Rosaceae) | Least concern | Creeping tormentil |
| *Prunella vulgaris* (Lamiaceae) | Not evaluated | Self-heal/Kalevueth |
| *Rorripa islandica* (Brassicaceae) | Not evaluated | Yellow watercress |
| *Rumex dentatus* (Polygonaceae) | Least concern | Toothed dock/Obuj |

**Cont...**

| | | |
|---|---|---|
| *Scandix pectenveneris* (Apiaceae) | Least concern | Shepherd's needle |
| *Sonchus oleraceus* (Asteraceae) | Not evaluated | Sow thistle |
| *Setaria viridis* (Poaceae) | Least concern | Foxtail or Green bristle grass/Gassi |
| *Sysiumbrium irio* (Brassicaceae) | Least concern | London rocket/Khub-kalan |
| **Trees** | | |
| *Populus deltoides* (Salicaceae) | Not evaluated | Eastern cottonwood/Fras |
| *Populus nigra* (Salicaceae) | Least concern | Black poplar/Kashur fras |
| *Salix alba* (Salicaceae) | Least concern | White willow/Boti veer |
| *Salix fragils* (Salicaceae) | Not evaluated | Crack willow/Veer |

**Table 4.** Density of different wild plants in boundary plantation of the study area

| Plant species | Density m$^2$ | | |
|---|---|---|---|
| | Spring | Summer | Autumn |
| | Mean±S.E | Mean±S.E | Mean±S.E |
| *Amaranthus caudatus* L. | 5.66±1.96 | 14.33±1.94 | 2.83±0.42 |
| *Anthemis cotula* L. | 7.00±1.08 | 18.00±1.60 | 3.33±0.76 |
| *Artemisia absinthium* L. | 4.66±1.66 | 12.5±1.29 | 2.16±0.69 |
| *Cannabis sativa* L. | 23.66±3.17 | 57.66±0.31 | 8.5±0.28 |
| *Capsella bursa pastoris* L. | 6.16±3.08 | 16.33±1.23 | 3.16±0.16 |
| *Clinopodium vulgare* L. | 10.50±1.52 | 26.66±2.03 | 4.83±1.07 |
| *Conyza canadensis* L. Cronquist | 9.50±0.14 | 24.83±2.60 | 4.00±1.01 |
| *Cynodon dactylon* L. | 36.00±1.62 | 95.00±1.11 | 31.83±1.16 |
| *Daucus carota* L. | 19.83±1.65 | 50.16±2.52 | 8.16±1.20 |
| *Equisetum arvense* L. | 6.00±2.01 | 16.00±1.04 | – |
| *Euphorbia helioscopia* L. | 2.16±0.09 | 7.83±1.36 | 0.50±0.50 |
| *Galinsoga parviflora* Cav. | 4.50±0.29 | 10.33±1.48 | 1.66±1.06 |
| *Galium aparine* L. | 7.83±0.92 | 18.33±1.30 | 3.83±0.83 |
| *Geranium polyanthes* Edgew & Hook | 16.00±3.5 | 38.83±4.23 | 7.83±1.16 |
| *Impatiens glandulifera* Royle | 2.66±1.33 | 5.66±2.84 | 0.66±0.001 |
| *Lithospermemium arvense* L. | 3.33±0.13 | 8.83±1.91 | 1.00±0.001 |
| *Medicago sativa* L. | 9.16±1.19 | 23.33±2.43 | 5.00±1.56 |
| *Nepeta cataria* L. | 5.33±1.72 | 14.50±1.60 | 2.33±0.33 |
| *Oenothera rosea* L'Her. ex. Aiton | 25.33±6.78 | 67.00±8.28 | 17.16±7.21 |
| *Plantago major* L. | 8.16±1.20 | 13.00±6.50 | 1.33±0.33 |
| *Polygonum hydropiper* L. | 32.16±9.32 | 89.16±11.81 | 21.16±4.74 |
| *Potentilla reptans* L. | 8.50±1.44 | 20.00±1.32 | 4.00±2.17 |
| *Prunella vulgaris* L. | 1.66±0.06 | 4.33±1.33 | 0.33±0.002 |
| *Rorripa islandica* L. | 8.00±1.75 | 18.83±2.18 | – |
| *Rumex dentatus* L. | 11.83±0.83 | 31.16±2.63 | 4.00±1.08 |
| *Scandix pectenveneris* L. | 4.50±0.29 | 12.00±0.28 | 1.83±0.03 |
| *Sonchus oleraceus* L. | 7.66±0.96 | 19.66±2.20 | 1.66±0.06 |
| *Setaria viridis* L. | 13.16±1.08 | 22.33±1.93 | 8.33±1.01 |
| *Sysiumbrium irio* L. | 1.83±0.83 | 4.50±1.75 | – |
| *Tragopogan kashmirianus* GS | 2.16±0.16 | 4.66±2.35 | – |
| *Trifolium pratense* L. | 25.16±0.56 | 60.66±4.44 | 9.50±0.50 |
| Total | 330.16 | 826.50 | 161.00 |

**Table 5.** Basal area of different wild plants in boundary plantation of the study area

| Plant species | Basal area (cm$^2$/m$^2$) | | |
|---|---|---|---|
| | Spring | Summer | Autumn |
| | Mean±S.E | Mean±S.E | Mean±S.E |
| *Amaranthus caudatus* L. | 0.36±0.12 | 0.54±0.05 | 0.22±0.01 |
| *Anthemis cotula* L. | 0.54±0.16 | 0.70±0.10 | 0.46±0.16 |
| *Artemisia absinthium* L. | 0.11±0.01 | 0.20±0.07 | 0.10±0.05 |
| *Cannabis sativa* L. | 0.67±0.11 | 0.94±0.02 | 0.34±0.082 |
| *Capsella bursa-pastoris* L. | 0.11±0.06 | 0.19±0.005 | 0.099±0.009 |
| *Clinopodium vulgare* L. | 0.89±0.07 | 1.03±0.09 | 0.74±0.10 |
| *Conyza canadensis* L. Cronquist | 0.31±0.08 | 0.43±0.03 | 0.21±0.001 |
| *Cynodon dactylon* L. | 1.19±0.02 | 1.98±0.05 | 1.06±0.02 |
| *Daucus carota* L. | 0.38±0.02 | 0.44±0.03 | 0.23±0.083 |
| *Equisetum arvense* L. | 0.38±0.10 | 0.43±0.04 | – |
| *Euphorbia helioscopia* L. | 0.23±0.01 | 0.32±0.01 | 0.05±0.01 |
| *Galinsoga parviflora* Cav. | 0.25±0.03 | 0.33±0.04 | 0.16±0.06 |
| *Galium aparine* L. | 0.25±0.04 | 0.46±0.16 | 0.16±0.01 |
| *Geranium polyanthes* Edgew & Hook | 0.22±0.03 | 0.27±0.02 | 0.12±0.01 |
| *Impatiens glandulifera* Royle | 0.23±0.11 | 0.27±0.05 | 0.05±0.003 |
| *Lithospermemium arvense* L. | 0.31±0.09 | 0.35±0.09 | 0.26±0.02 |
| *Medicago sativa* L. | 0.12±0.06 | 0.13±0.003 | 0.10±0.05 |
| *Nepeta cataria* L. | 0.17±0.08 | 0.19±0.004 | 0.12±0.03 |
| *Oenothera rosea* L'Her. ex. Aiton | 0.70±0.15 | 1.04±0.018 | 0.57±0.05 |
| *Plantago major* L. | 0.42±0.20 | 0.95±0.06 | 0.10±0.04 |
| *Polygonum hydropiper* L. | 1.02±0.006 | 1.69±0.15 | 0.96±0.017 |
| *Potentilla reptans* L. | 0.26±0.13 | 0.32±0.032 | 0.11±0.05 |
| *Prunella vulgaris* L. | 0.06±0.01 | 0.10±0.06 | 0.01±0.003 |
| *Rorripa islandica* L. | 0.25±0.08 | 0.57±0.17 | – |
| *Rumex dentatus* L. | 0.33±0.19 | 0.72±0.16 | 0.16±0.08 |
| *Scandix pectenveneris* L. | 0.11±0.06 | 0.52±0.19 | 0.10±0.015 |
| *Sonchus oleraceus* L. | 0.17±0.06 | 0.23±0.008 | 0.12±0.01 |
| *Setaria viridis* L. | 0.36±0.11 | 0.58±0.10 | 0.34±0.13 |
| *Sysiumbrium irio* L. | 0.10±0.001 | 0.28±0.024 | – |
| *Tragopogan kashmirianus* GS | 0.12±0.01 | 0.27±0.04 | – |
| *Trifolium pratense* L. | 0.41±0.05 | 0.51±0.08 | 0.34±0.06 |
| Total | 11.15 | 16.98 | 7.45 |

species in plant communities has also been reported by Sharma *et al*. (2014) in Sangla Valley, Northwest Himalaya; Dutt and Gupta (2005) in Chir pine stands in sub-tropical region in India. Changes in basal area of wild herbage in different seasons can be related to corresponding changes in density (Pappoe *et al*., 2010). Also, high IVI of few species indicated their dominance and ecological success, their good power of regeneration and greater ecological amplitude, availability of optimum conditions for their growth, better adaptability of a particular species under prevailing environmental conditions (Bijalwan *et al*., 2011; Shameem *et al*., 2010). A close observation of IVI of different species

showed that there was irregular increase and decrease of this parameter in subsequent seasons with highest IVI values during autumn. It may be due to the reason that most of the available resources are being utilized by that species (having high IVI) and left over are being trapped by another species as the competitors and the associates i.e. their inter-relationships with ambient environment and associate species, light availability etc. Other factors affecting the vegetation distribution include biotic such as dispersal limitation, competition, and predation (Munzbergova and Herben, 2005).

**Vegetation indices of cultivated and wild herbaceous**

**Table 6.** Importance Value Index (IVI) of different wild plants in boundary plantation of the study area

| Plant species | Importance Value Index | | |
| --- | --- | --- | --- |
| | Spring | Summer | Autumn |
| | Mea±S.E | Mean±S.E | Mean±S.E |
| *Amaranthus caudatus* L. | 7.16±1.85 | 8.88±0.89 | 7.82±3.91 |
| *Anthemis cotula* L. | 12.04±3.68 | 10.26±0.74 | 12.24±6.13 |
| *Artemisia absinthium* L. | 3.19±0.19 | 5.94±2.09 | 4.47±2.24 |
| *Cannabis sativa* L. | 17.64±1.54 | 16.16±2.18 | 15.37±0.56 |
| *Capsella bursa-pastoris* L. | 5.06±2.23 | 7.11±1.60 | 3.76±0.76 |
| *Clinopodium vulgare* L. | 14.85±1.40 | 12.03±1.00 | 13.99±1.15 |
| *Conyza canadensis* L. Cronquist | 8.14±1.56 | 8.32±1.37 | 8.52±4.29 |
| *Cynodon dactylon* L. | 27.42±2.41 | 27.91±1.42 | 45.09±5.02 |
| *Daucus carota* L. | 14.56±3.56 | 12.22±1.42 | 14.25±2.43 |
| *Equisetum arvense* L. | 7.45±3.73 | 6.83±0.38 | − |
| *Euphorbia helioscopia* L. | 4.88±2.44 | 6.03±0.73 | 3.74±0.74 |
| *Galinsoga parviflora* Cav. | 5.83±2.92 | 7.18±0.88 | 6.19±0.19 |
| *Galium aparine L.* | 8.20±0.72 | 7.72±0.95 | 5.76±0.76 |
| *Geranium polyanthes* Edgew & Hook | 10.75±1.05 | 9.44±1.09 | 13.33±0.34 |
| *Impatiens glandulifera* Royle | 5.16±2.60 | 4.61±0.56 | 2.64±0.64 |
| *Lithospermemium arvense* L. | 5.33±0.33 | 6.38±0.37 | 5.80±0.80 |
| *Medicago sativa* L. | 8.05±0.47 | 6.74±1.43 | 7.46±3.74 |
| *Nepeta cataria* L. | 5.95±2.97 | 5.25±0.08 | 4.73±0.73 |
| *Oenothera rosea* L'Her. ex. Aiton | 17.82±0.22 | 17.74±1.87 | 25.78±5.26 |
| *Plantago major* L. | 9.66±0.87 | 10.25±0.77 | 3.97±0.97 |
| *Polygonum hydropiper* L. | 25.01±4.06 | 24.87±2.26 | 35.80±1.07 |
| *Potentilla reptans* L. | 8.46±0.73 | 7.49±0.73 | 8.11±0.20 |
| *Prunella vulgaris* L. | 1.78±0.78 | 1.96±0.03 | 1.32±0.03 |
| *Rorripa islandica* L. | 8.24±1.06 | 9.18±2.59 | − |
| *Rumex dentatus* L. | 9.97±1.70 | 11.50±2.34 | 8.93±0.77 |
| *Scandix pectenveneris L.* | 4.59±0.34 | 7.73±0.78 | 7.40±0.40 |
| *Sonchus oleraceus* L. | 7.42±1.61 | 6.51±0.66 | 4.32±0.32 |
| *Setaria viridis* L. | 12.31±4.48 | 10.13±1.12 | 13.53±3.83 |
| *Sysiumbrium irio* L. | 4.09±0.09 | 5.37±1.17 | − |
| *Tragopogan kashmirianus* GS | 3.11±0.11 | 3.78±1.92 | − |
| *Trifolium pratense* L. | 15.81±2.64 | 14.35±1.80 | 15.60±1.17 |
| Total | 300 | 300 | 300 |

**Table 7.** Vegetation indices of cultivated and wild plants (herbage) in boundary plantation

| Plant species | Vegetation indices | Seasons | | |
| --- | --- | --- | --- | --- |
| | | Spring | Summer | Autumn |
| Cultivated | Shannon Weiner index | 1.06 | 0 | − |
| | Simpson's diversity | 0.33 | 1 | − |
| | Species evenness | 0.96 | 0 | − |
| Wild | Shannon Weiner index | 2.90 | 3.05 | 2.52 |
| | Simpson's diversity index | 0.066 | 0.060 | 0.09 |
| | Species evenness | 0.84 | 0.88 | 0.76 |

**Table 8.** Phytosociological attributes of trees in boundary plantation of the study area

(Mean ±S.E)

| Quantitative attributes | Density (plants ha$^{-1}$) | Basal area (m$^2$ ha$^{-1}$) | Importance value index (IVI) |
| --- | --- | --- | --- |
| *Populus deltoides* Bartr. | 100.00±0.00 | 2.41±0.24 | 118.01±18.76 |
| *Populus nigra* Bartr. | 83.33±16.66 | 1.34±0.33 | 79.40±13.04 |
| *Salix alba* L. | 66.66±13.34 | 1.02±0.51 | 52.72±16.36 |
| *Salix fragilis* L. | 50.00±8.86 | 0.84±0.44 | 49.86±14.98 |
| Total | 300 | 5.61 | 300 |

**plants: Shannon-Weiner index**: Seasonal Shannon−Weiner index of cultivated herbaceous vegetation were 1.06 in spring, zero during summer and for wild plants 2.90 in spring, 3.05 in summer and 2.52 in autumn (Table 7).During autumn season, there exist no cultivated herbage due to the fact that farmers use to left their land fallow for few months in order to recapture the lost nutrients and H index was 0 (zero) in summer.

**Simpson's diversity index:** The Shannon−Weiner index was 0.33(spring), 1.00 (summer) and 0.066 (spring), 0.060 (summer) and 0.09 (autumn) for cultivated and wild herbaceous plants respectively. From the above data, it was observed that the maximum value for this diversity index was for autumn season (Table 7).

**Species evenness:** Species evenness was maximum during summer season and minimum in autumn in this agroforestry systems for both cultivated and wild plants in the evaluated agroforestry system (Table 7).

Seasonal Shannon−Weiner index, species evenness and Simpson index of cultivated and wild plants displayed similarity with density, basal area and IVI with respect to seasons i.e. highest and lowest values exhibited during summer and autumn respectively. Seasonal Shannon −Weiner index and species evenness showed rise from spring season with advancement of growing season, attained peak values during summer and abruptly decreased in autumn season. The important and possible cause for higher values during summer season could be higher species diversification maintained by farmers in case of cultivated plants, congenial growth conditions in terms of nutrient availability, soil moisture, humidity, light and temperature for a large number of plants as reported by Kharkwal *et al*. (2004) and Peyre *et al*. (2006). Decreased diversity index of plants during autumn could be due to lower rate of evolution and diversification of communities (Man *et al*., 2012) and severity in environment (Bhandari *et al*., 1998). Simpson index and concentration of dominance showed a reverse trend in the systems with respect to Shannon−Weiner index, species evenness and seasons achieved lowest values during spring and summer season and highest in autumn. Compatible results of inverse relationship between diversity and dominance were also reported by Pokhrel *et al*. (2015) in home gardens of Gulmi and Palpa Districts, Western Nepal; Singh and Singh (2013) for riparian corridors of Gomati at Rouza Ghat in District Jaunpur, U.P, India; Singh (2012)vegetations around sewage drains in Jaunpur, India.

**Phytosociological attributes of Trees:** Boundary plantation was represented by four tree species characterizing broad leaved category *viz*., *Populus deltoides*, *Populus nigra*, *Salix alba* and *Salix fragilis*.

Boundary plantation with density, basal area and IVI of 100ha$^{-1}$, 2.41 m$^2$ha$^{-1}$and 118.01was dominated by *Populus deltoids* with minimum achieved by *Salix fragilis* as 50 ha$^{-1}$, 0.84 m$^2$ha$^{-1}$and 49.86 respectively. Several researchers have documented the similar ranges for these quantitative parameters in tropical evergreen and deciduous forests in Uttara Kannada district, South India (Murthy *et al*., 2016), *Oxytenanthera abyssinica* based homestead agroforestry systems of Serako, Northern Ethiopia (Darcha *et al*., 2015); in woody vegetation of Garhwal Himalaya (Bhandari *et al*., 1998).

Quantitative information on distribution and abundance of vegetation is of key significance to understand the form and structure of a community for planning and implementation of conservation strategy of the community (Raturi, 2012). The higher values for quantitative or phytosociological attributes indicated by certain species like *Populus deltoids* may be due to ecological/environmental adaptability, farmers preference for their subsistence requirement fulfillment and variety of multipurpose uses such as fuel wood, timber, soil fertility, easy propagation and management, commercial purposes (Gupta and Prasad, 2013; Rawat *et al*., 2010).

**CONCLUSION**

This system holds a number of valuable plant species which are either vulnerable or threatened as per the global IUCN status. This system if adapted on large scale can prove worth for conserving those species at sustainable scale. The farmers must be encouraged to be the custodians of large agroforestry land holdings, acknowledged and rewarded so that they will take keen and firm interest further to give his best in conserving the natures unsurpassed gift −" the agro−biodiversity".

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# Phytoplankton Assemblages in the River Barak, South Assam, India: A Study to Know its Composition in Changing Space and Time

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**Abstract:** A study was carried out to investigate the phytoplankton community composition in accordance with changing period at different sites of the River Barak, a major river of South Assam. Analyses of major environmental variables of water, phytoplankton identification and enumeration were done by standard methods. All total 45 species belonging to 32 families, 18 orders, and 4 classes of phytoplankton were recorded from the river. The values of Shannon_H$'$ were in a range of 1.96–2.78 and highest Shannon_H$'$ was recorded in site 2. This site was considered to be least polluted as per PCA and CCA results. Dominant taxa like *Chaetophora* sp. was found significantly abundant in different sites whereas *Microthamnion* sp. was significantly abundant in different periods. This study showed that riverine ecosystems can provide better β- diversity of the phytoplankton.

**Keywords**: Phytoplankton, River Barak, Diversity, Canonical Correspondence Analysis (CCA)

Phytoplanktons are the microscopic plant communities occur in water throughout the world. Phytoplankton community properties are linked with changes in biological, physical, and chemical hydrologic conditions (Cloern and Jassby, 2010). The qualitative and quantitative analyses of phytoplankton of an ecosystem provide distribution and diversity status of that system. The river Barak located in the biodiversity hotspot is a major river of North East India. It originates from Barail range of Naga hills in the state of Manipur, flows through Mizoram, enters the Cachar district of Assam (Das Gupta and Biswas, 2000; Das *et al.,* 2007; Nandy, 2001) and finally travels to Bangladesh. Studies on water quality and biodiversity of this river is scanty (Rajkumar and Sharma, 2013; Roy and Gupta, 2012). Phytoplankton is the primary source of food for fishes and food base for all the subsequent trophic levels. Phytoplankton is also a bio−indicator of water pollution and indicates the quality of water (Panigrahi and Patra, 2013; Wu, 1984). In spite of its importance there is no study on phytoplankton of river Barak. In southern Assam, studies on phytoplankton were mainly from lentic systems (Dalal and Gupta, 2013; Devi *et al.,* 2016; Laskar and Gupta, 2013). Against this backdrop the present study is the first attempt to explore the diversity, density and distribution of phytoplankton community in the river Barak of southern Assam and their role as bio indicator of pollution. This study also attempts to point out the changes in phytoplankton community composition in different sites in different periods.

## MATERIAL AND METHODS

**Study Area:** The study was conducted on the river Barak in southern Assam. The river enters Cachar district of Assam (24 ˚ 49'47" N, 92 ˚ 46'80" E) at Puranagram area under Lakkhipur subdivision, travels through Silchar, the main city of south Assam and after travelling 35 km it forms two rivers namely Surma and Kushiyara. At Cachar district the river meanders forming many oxbow lakes. The river is known for the presence of endangered Gangetic River Dolphin *Platanista gangetica* (Biswas, 1995; Singha, 2000) and fish like Mahseer (Barbuiya, 2012). Phytoplankton samples were collected from four different sites (site 1 2, 3 and 4) mainly from pool area taking into consideration the low flow of water and probability of occurrence of phytoplankton. The site 1 is at Puranagram, Cachar which is the entry point of the river at Assam (24º47'12.34"N and 93º1'29.91"E), site 2 is at Dayapur(24º49'8.03"N and 92º50'48.18"E), site 3 is at Kathigara area (24º52'30.72"N and 92º34'40.68"E) and site 4 is at Tukargram, which falls under Karimganj district (24º52'31.73"N and 92º29'27.43"E) near Indo-Bangladesh border (Fig. 1).

**Field sampling of phytoplankton and water samples:** Both phytoplankton and water samples were collected at four different time periods at an interval of fifteen days from January to March, 2015. For the collection of phytoplankton, three replicate samples were taken from each site where 50 litres of water was filtered through a standard plankton net (mesh size 40 µm) and preserved in 4% formaldehyde
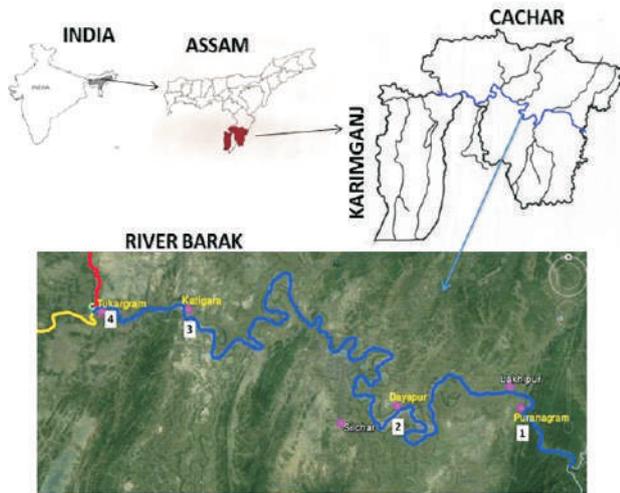
**Fig. 1.** Map of River Barak, southern Assam showing four different locations of the sampling sites

solution (Lund and Davies, 2000).The qualitative analysis of phytoplankton was done by identification of species using standard literatures   to the lowest possible taxa (Anand, 1998; Arulmurugan *et al.,* 2011; Edmondson, 1959). The quantitative analysis of phytoplankton was done by Lackey's drop method (APHA, 2005).

Organisms per litre (N)= R x At x 103/As x S x V

Where, R = Number of organisms counted per sub sample ; At = Area of coverslip, $mm^2$; As = Area of one strip, $mm^2$; S = Number of strips counted ; V = Volume of sample under the coverslip in ml.

Total organisms per litre = N X 1/C

Concentration factor C=   Volume of original sample (ml)

Volume of concentrated sample (ml)

All the water samples were collected in PET bottles. Environmental variables of water like air temperature (AT), water temperature (WT), transparency (TR), pH, electrical conductivity (EC), dissolved oxygen (DO), free carbon dioxide ($FCO_2$), total alkalinity (TA), nitrate ($NO_3^-$) and phosphate ($PO_4^{3-}$) were estimated. For estimation of DO the samples were collected in cleaned BOD bottles to the brim, without leaving any space in order to prevent the premature release of dissolved gases during the transit period. DO was estimated by Winkler method, while TA and $FCO_2$ content were analysed by titrating with strong acid and alkali, respectively (Ramesh and Anbu, 1996; APHA, 2005). pH and EC were measured by pH meter (Systronics µ pH System 361) and conductivity meter (Systronics Conductivity-TDS Meter 308), respectively. By using Secchi's disk TR was estimated by taking appearance and disappearance measurement (Michael, 1984). $NO_3^-$ was estimated by using UV spectrophotometer (APHA, 2005) and $PO_4^{3-}$ was with light

spectrophotometer (Michael, 1984).

**Statistical analysis:** To examine the environmental impacts of different sites and periods on the phytoplankton composition, two-way analysis of variance (ANOVA) was done where sites and sampling periods were the main factors. To correct the problem of multiple comparisons Bonferroni test was used. Tukey (HSD) tests were also done to detect the significant difference among the sites and as well as the periods. Two-way ANOVA for ten dominant taxa were also performed where dominance status were checked based on Engelmann's scale of dominance (1978) and where dominant taxa were selected which had more than 2% in the sample. Shapiro-Wilk test of normality was done to check whether data were normally distributed or not. Those data that are not normally distributed were improvised by transformation of the data using $\log_{10}$ (X + 1). Similarity percentages (SIMPER) were calculated to check the average percentage contribution of each phytoplankton taxa to dissimilarity between sites based on a Bray-Curtis dissimilarity matrix (Clarke, 1993). The composition of the phytoplankton assemblage combining four sites and periods were examined with non-metric multidimensional scaling (NMDS) to check if any taxa behaved differently from the rest in terms of abundance. Vector fittings of the environmental variables of water were done using principle correspondence analysis (PCA) values where *p*-values were used to identify the significant environmental variables of water. Canonical component analysis (CCA) was done to find out the different parameters of environmental variables of water responsible for phytoplankton assemblage and their distribution. All the statistical analysis were done using R studio (R core team 2014). Diversity indices were analysed using free software PAST (ver. 3.11).

**RESULTS AND DISCUSSION**

From the four sites and four collections, 589 individuals and 45 species were recorded from 31 families, 18 orders and 4 classes (Bacillariophyceae, Chlorophyceae, Cyanophyceae, Euglenophyceae) (Table 1). The values of Shannon_$H'$ was in a range of 1.96-2.78 (lowest in site 1 and highest in site 2) while values of Evenness_e^H/S was varied form 0.28 to 0.62 (lowest in site 1 and highest in site 2). The values of Margelef index was in between 5.76-8.09 (lowest in site 3 and highest in site 2) while Berger-Parker index of dominance was in a range of 0.23-0.57 (lowest in site 2 and highest in site 1). Shannon_$H'$ values recorded in this study were within proper range (1.5-3.5) according to Turkmen and Kazanci (2010). Similar Shannon_$H'$diversity was recorded from Sharavati River Basin, Central Western Ghats (Desai *et al.*, 2008). According to Algae genus pollution index (Palmer,

**Table 1.** Phytoplankton list, its taxa code and mean density (no.L$^{-1}$× 10$_2$) (mean±SD)

| Species | Taxa code | Site 1 | Site 2 | Site 3 | Site 4 |
|---|---|---|---|---|---|
| *Fragilaria* sp. | Fra1 | 0.39±0.46 | 1.00±0.66 | 0.33±0.14 | 0.56±0.57 |
| *Diatoma* sp.1 | Dia1 | – | 0.33±0.14 | – | 1.33±0.14 |
| *Diatoma* sp.2 | Dia2 | 0.33±0.14 | – | – | – |
| *Synedra* sp. | Syn | – | – | 0.33±0.14 | – |
| *Amphipleura* sp. | Amp1 | 0.56±0.72 | 0.33±0.14 | 0.50±0.43 | 0.67±0.81 |
| *Frustulia* sp. | Fru1 | 0.50±0.28 | 0.33±0.28 | – | 0.33±0.14 |
| *Gyrosigma* sp. | Gyr | 0.33±0.14 | 0.67±0.57 | 0.33±0.14 | – |
| *Stauroneis* sp. | Sta | 0.33±0.14 | – | – | – |
| *Navicula* sp. | Nav | 0.50±0.17 | 0.33±0.14 | – | – |
| *Pleurasigma* sp. | Ple | – | – | 0.33±0.14 | – |
| *Amphora* sp. | Amphi | 0.33±0.14 | 0.33±0.14 | – | 0.33±0.14 |
| *Gomphonema* sp. | Gom | 0.33±0.28 | 0.33±0.28 | – | 0.33±0.14 |
| *Cymbella astula* | Cym1 | 0.30±0.25 | 0.35±0 | 0.20±0 | 0.50±0 |
| *Cymbella* sp. | Cym2 | 0.45±0.39 | 0.35±0.43 | 0.36±0.28 | 1.00±0.67 |
| *Achnanthes* sp. | Ach | – | – | 0.33±0.14 | – |
| *Nitzschia* sp. | Nitz | – | – | – | 0.33±0.28 |
| *Meridion circulare* | Meri | 0.33±0.14 | 0.33±0.43 | – | – |
| *Cyclotella* sp. | Cyclot | 0.33±0.14 | – | 0.33±0.14 | 0.33±0.14 |
| *Melosira* sp. | Melo | 1.92±2.27 | 0.67±0.39 | 3.67±2.30 | 0.67±0.28 |
| *Crucigina tetrapedia* | Cruci | 0.33±0.14 | 0.33±0.14 | – | – |
| *Scenedesmus quadricauda* | Sce | – | – | 0.33±0.14 | – |
| *Chlorococcum* sp. | Chloro | 0.33±0.14 | – | – | – |
| *Oedogonium* sp. | Oedo | – | 0.33±0.14 | 10.67±4.61 | – |
| *Microspora*sp.1 | Micro | 0.33±0.14 | – | – | – |
| *Pediastrum simplex* | Ped | – | – | 0.33±0.14 | – |
| *Microspora* sp.2 | Micro1 | – | – | 0.67±0.28 | – |
| *Spirogyra* sp. | Spiog | 1.50±0.43 | – | 2.50±3.17 | 0.67±0.67 |
| *Sirocladium* sp. | Sirocl | 0.33±0.14 | 2.00±1.36 | 0.33±0.14 | – |
| *Mougeotia* sp. | Mou | 0.83±0.43 | 2.00±0.66 | – | – |
| *Temnogametum* sp. | Tem | – | 0.33±0.14 | – | – |
| *Spirotaenia* sp. | SpiroN | – | – | – | 0.33±0.14 |
| *Mesotaenium* sp. | Mesot | – | – | – | 0.33±0.14 |
| *Netrium* sp. | Netri | – | – | – | 0.33±0.14 |
| *Micrasterias* sp. | Micras | 0.33±0.14 | – | 0.33±0.14 | – |
| *Closterium* sp. | Closter | 0.67±0.39 | 0.33±0.28 | 0.33±0.14 | 0.33±0.14 |
| *Oocystis* sp. | Ooc | 0.33±0.14 | – | – | – |
| *Microthamnion* sp. | Micro | – | 2.11±1.64 | – | 0.67±0.28 |
| *Hormidium* sp. | Hormi | – | 0.33±0.14 | 0.67±0.28 | – |
| *Chaetophora sp.* | Chae1 | 17.22±16.74 | 5.17±4.47 | 10.67±2.30 | 6.33±2.74 |
| *Nostoc* sp. | Nos | 0.67±0.28 | – | – | – |
| *Anabaena* sp. | Ana | – | 0.33±0.14 | – | – |
| *Oscillatoria* sp. | Osc1 | – | 0.50±0.43 | – | 0.50±0.43 |
| *Dactylococcopsis* sp. | Dac | – | 0.67±0.28 | – | – |
| *Hyella caespitosa* | Hye1 | – | – | – | 1.67±0.72 |
| *Euglena* sp. | Eugl | – | 1.00±0.25 | – | |

1969), the tolerant species recorded in the River Barak were *Melosira* sp., *Closterium* sp., *Gomphonema* sp., *Cyclotella* sp., *Navicula* sp., *Oscillatoria* sp., *Euglena* sp*., Synedra* sp., and *Nitzschia* sp. According to algae genus pollution index (Palmer, 1969), all the sites were found under "not high organic pollution" status and when compared, site 4 was found to have highest score and site 1 lowest (Table 2).

The environmental variables of any aquatic ecosystem and the nature and distribution of its biota are directly related to and influenced by each other and controlled by a multiplicity of natural regulatory mechanisms (Bhat *et al.*, 2009). Both AT and WT did not show much fluctuation. DO values ranged from 6.32 to 8.23 mg $L^{-1}$ whereas pH ranged from 7.33 to 7.62. pH of river water in all the sites was alkaline and within the desirable limit of BIS (2012). The alkaline pH is of importance for proper growth and development of fishes and aquatic organisms (Islam, 2007). The values of DO and pH declined gradually from site 1 to site 4 indicating deterioration of water quality. TA ranged from 102.54±6.62 to 114.56±5.22 mg $L^{-1}$ indicating high organic pollution. Concentration of $PO_4^{3-}$ and $NO_3^-$ ranged from 0.08 to 0.21 mg $L^{-1}$ and 0.08 to 0.46 mg $L^{-1}$ respectively. The range of TR values of River Barak was similar to the TR of River Yamuna in Doon valley (Ishaq and Khan, 2013) (Table 3).

The phytoplankton density revealed significant difference in different periods whereas phytoplankton richness did not reveal significant difference. Post hoc test (Tukey HSD) for multiple comparisons showed no significant effects between the sites and the periods individually based on its density and richness. The analysis of 10 dominant phytoplankton taxa density revealed that *Chaetophora* sp. was more abundant in site 1 and site 2 as compared to site 3 and site 4 ($p< 0.05$). *Microthamnion* sp. was more abundant during the initial period as compared to rest of the periods ($p < 0.05$). Sites ×period interactions of 10 dominant taxa density, overall density of taxa and its richness revealed no significant differences (Fig. 2; Table 4). NMDS did not reveal much

difference among the different sites but site 3 was little isolated from the rest of the sites (Fig. 3). Second collection was isolated from rest of the collections in NMDS among different periods (Fig. 4). SIMPER based on Bray-Curtis analysis at different sites (S) and periods (P) revealed *Chaetophora* sp. (S1 vs. S2= 42.04 S1 vs. S3= 41.07; S1 vs. S4= 49.21; S2 vs. S3= 21.07; S2 vs. S4= 21.53; S3 vs. S4= 18.08; P1 vs. P2= 33.3; P1 vs. P3= 26.71; P1 vs. P4= 31.71; P2 vs. P3= 30.63; P2 vs. P4= 45.67; P3 vs. P4= 24.82%) was responsible for contributing highest dissimilarity percentage among the different sites and periods followed by *Oedogonium* sp. (S1 vs. S3= 10.85; S2 vs. S3= 13.62; S3 vs. S4= 15.64; P2 vs. P3= 16.6; P2 vs. P4= 21.19%).

The phytoplankton community composition changed with the different periods of short intervals. NMDS graph further supported the evidence where period 2 was found isolated from rest of the periods (Fig. 4). Rapid growth and decline of the phytoplankton might be the result of simultaneous reproduction and losses (Sommer, 2002). In the present study, *Chaetophora* sp. was highest density taxa as compared to the other taxa recorded. This supported the SIMPER analysis where this taxon showed highest dissimilarity in phytoplankton composition between any two sites and seasons of the river. In stream macroalgal communities, the family Chaetophoraceae is a prevalent taxonomic group since their gelatinous thallus are favoured by the increment of current velocity (Branco and Necchi Junior, 1996a, 1997b; Sheath and Burkholder, 1985; Sheath and Cole, 1992). Report of this genus in the lentic systems are scanty, mostly from open type wetlands connected with river (Bordoloi *et al.,* 2013; Devi and Gupta, 2014). Studies on Umiew stream of Meghalaya and River Mahi of Gujarat, India also showed occurrence of this genus (Ramanujam and Siangbood, 2009; Sharma and Bhardwaj, 2011).

Vector fitting of the environmental variables of water was done using PCA ordination values to detect the significant variables contributing to the phytoplankton composition.

**Table 2.** Algae genus pollution index (Palmer 1969)

| Site 1 | | Site 2 | | Site 3 | | Site 4 | |
|---|---|---|---|---|---|---|---|
| Genus | Pollution index | Genus | Pollution index | Genus | Pollution index | Genus | Pollution index |
| *Melosira* sp. | 1 | *Melosira* sp. | 1 | *Melosira* sp. | 1 | *Melosira* sp. | 1 |
| *Closterium* sp. | 1 | *Closterium* sp. | 1 | *Closterium* sp. | 1 | *Closterium* sp. | 1 |
| *Gomphonema* sp. | 1 | *Gomphonema* sp. | 1 | *Euglena* sp. | 5 | *Gomphonema* sp. | 1 |
| *Cyclotella* sp. | 1 | *Oscillatoria* sp. | 5 | *Cyclotella* sp. | 1 | *Nitzschia* sp. | 3 |
| *Navicula* sp. | 3 | *Navicula* sp. | 3 | *Synedra* sp. | 2 | *Cyclotella* sp. | 1 |
| Total score | 7 | Total score | 11 | Total score | 10 | *Oscillatoria* sp. | 5 |
| | | | | | | Total score | 12 |

0–14= Organic pollution not high; 15–19= Probable high organic pollution; 20 or more = Confirms high organic pollution
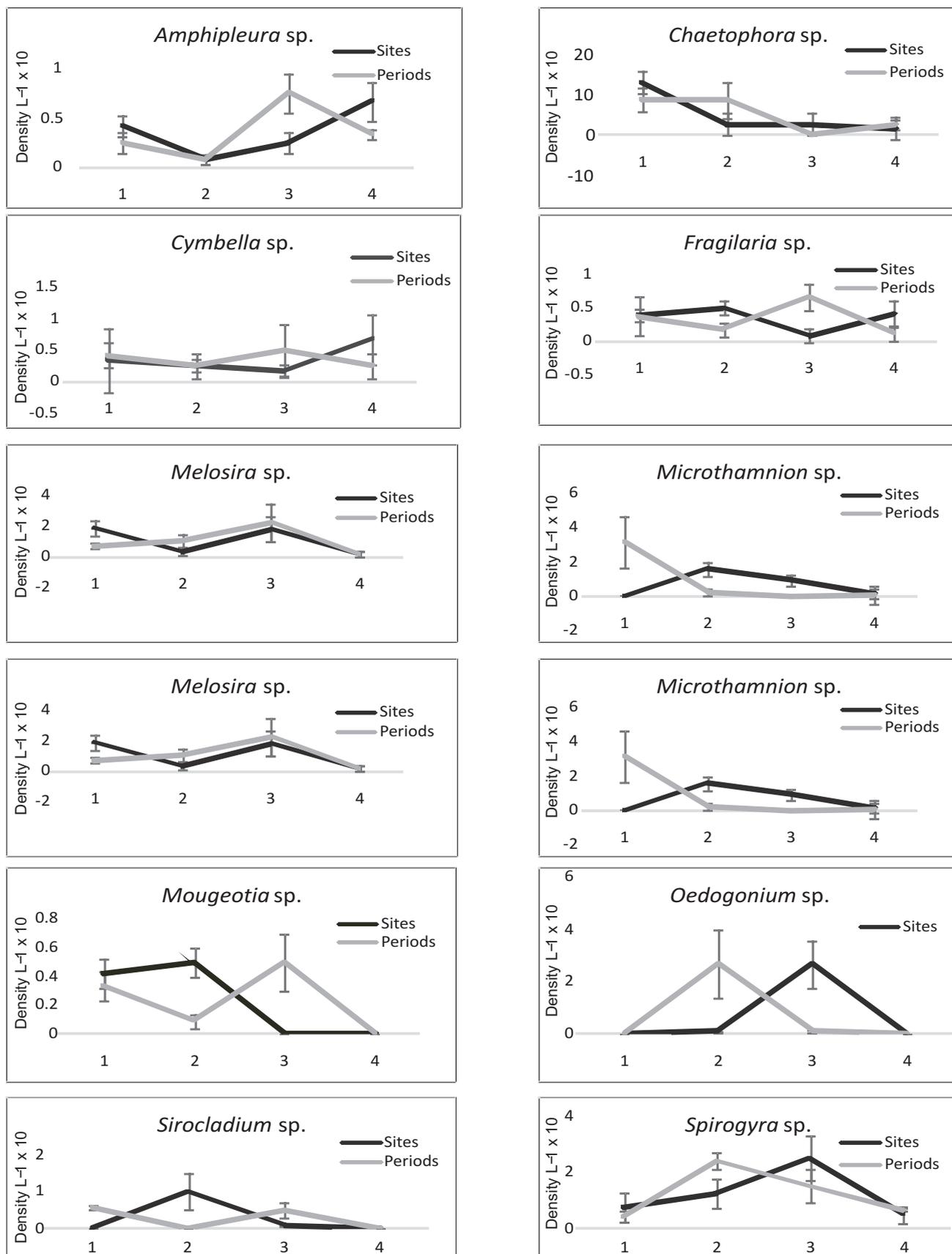
**Fig. 2.** Mean density (±SD) of the 10 dominant taxa in four different sites and periods

**Table 3.** Environmental variables of water of four different sites (Mean±SD)

| Sites | | AT(°C) | WT(°C) | TR (cm) | pH | EC (µS.cm⁻¹) | TDS (ppm) | DO (mg L⁻¹) | FCO₂ (mg L⁻¹) | TA (mg.L⁻¹) | PO₄³⁻ (mg L⁻¹) | NO₃⁻ (mg L⁻¹) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site 1 | Mean±SD | 26±0.81 | 22.5±0.577 | 34.25±14.45 | 7.62±0.15 | 119.69±7.81 | 77.59±4.66 | 8.23±1.10 | 6.45±1.95 | 102.54±6.62 | 0.10±0.04 | 0.08±0 |
| Site 2 | Mean±SD | 23.75±1.70 | 18±0.81 | 21.73±7.63 | 7.41±0.10 | 124.84±22.79 | 81.41±14.88 | 7.91±1.05 | 13.40±3.33 | 113.5±10.15 | 0.08±0.02 | 0.19±0.12 |
| Site 3 | Mean±SD | 26.25±2.06 | 23.5±2.08 | 27.10±5.92 | 7.38±0.05 | 166.52±8.74 | 107.58±6.07 | 6.96±0.46 | 14.24±4.58 | 118.42±5.40 | 0.21±0.21 | 0.46±0.07 |
| Site 4 | Mean±SD | 23.25±3.59 | 22±4.39 | 20.91±2.28 | 7.33±0.02 | 167.55±8.69 | 106.62±6.29 | 6.32±0.48 | 17.82±4.03 | 114.56±5.22 | 0.10±0.02 | 0.37±0.31 |

Here, AT, WT, EC and TDS were the most significant ($p< 0.001$), followed by pH and FCO2 ($p< 0.01$), and DO and TA ($p< 0.05$). 56.4% of the factors were explained by both axes 1 and 2 in PCA (Table 5). PCA ordination plot revealed that AT, WT, EC, TDS, pH, FCO2, DO and TA were the important variables controlling the river microclimate and overall environment to sustain the living organisms. PC1 explained the 38% of the explained variables. DO, FCO2, TA, EC and TDS were found close to PC1. Here the factor responsible might be the organic pollution loadings where respiration might be higher along with the ionic contents. Site 4 was found to be associated with PC1 which indicated the most polluted site, further supported by algae genus pollution index. PC2 explained the 18. Per cent of the variables. Associated variables close to PC2 are AT and WT along with

**Table 4.** Two−way ANOVA for 10 dominant phytoplankton taxa with overall species density and richness

| Dominant taxa | $p$: Sites | $p$: Periods | $p$: Sites x Periods |
|---|---|---|---|
| *Amphipleura* sp. | 0.33 | 0.33 | 0.77 |
| *Chaetophora* sp. | 0.04* | 0.11 | 0.48 |
| *Cymbella* sp. | 0.43 | 0.82 | 0.35 |
| *Fragilaria* sp. | 0.97 | 0.73 | 0.67 |
| *Melosira* sp. | 0.41 | 0.89 | 0.53 |
| *Microthamnion* sp. | 0.96 | 0.04* | 0.94 |
| *Mougeotia* sp. | 0.20 | 0.66 | 0.36 |
| *Oedogonium* sp. | 0.70 | 0.70 | 0.85 |
| *Sirocladium* sp. | 0.58 | 0.46 | 0.84 |
| *Spirogyra* sp. | 0.88 | 0.93 | 0.63 |
| Species density | 0.15 | 0.05* | 0.47 |
| Species richness | 0.21 | 0.89 | 0.31 |

*=$p<0.05$

**Table 5.** Vector fitting of the environmental variables of water based on PCA ordination analysis

| Environment variables | PC 1 | PC 2 | $p$–value |
|---|---|---|---|
| AT (°C) | −0.023 | 0.999 | 0.001*** |
| WT (°C) | 0.196 | 0.981 | 0.001*** |
| TR (cm) | −0.654 | 0.755 | 0.081 |
| pH | −0.681 | 0.732 | 0.009** |
| EC (µScm⁻¹) | 0.99 | 0.11 | 0.001*** |
| TDS (mg L⁻¹) | 0.994 | 0.104 | 0.001*** |
| DO (mg L⁻¹) | −0.997 | 0.063 | 0.027* |
| FCO₂ (mg L⁻¹) | 0.990 | −0.139 | 0.002** |
| TA (mg L⁻¹) | 0.991 | 0.133 | 0.003* |
| PO₄³⁻ (mg L⁻¹) | 0.579 | 0.814 | 0.592 |
| NO₃⁻ (mg L⁻¹) | 0.891 | 0.453 | 0.139 |

*= $p <0.05$, **= $p<0.01$, ***=$p<0.001$
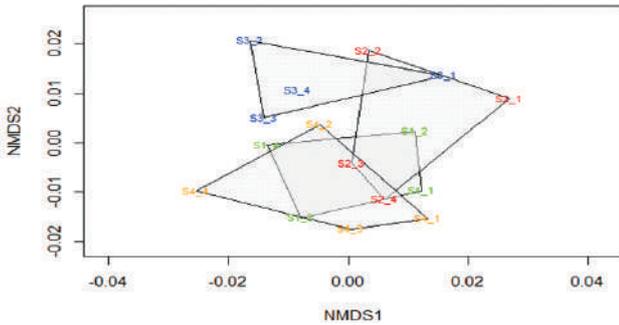
**Fig. 3.** Nonmetric multidimensional scaling (NMDS) ordination of phytoplankton assemblages for the four different sites. Final stress of the NMDS is 0.101. (S1_1–S1_4= Site 1, S2_1–S2_4= Site 2, S3_1–S3_4= Site 3, S4_1–S4_4= Site 4)
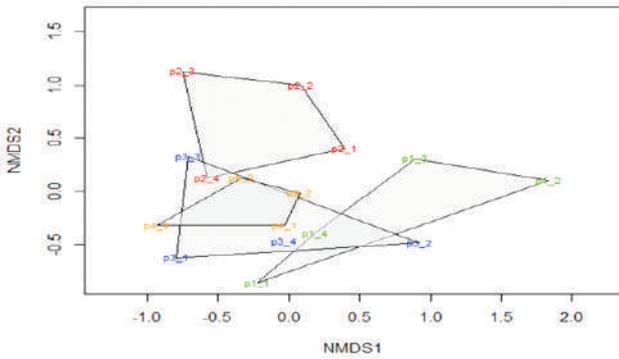


**Fig. 4.** Nonmetric multidimensional scaling (NMDS) ordination of phytoplankton assemblages for the four different periods. Final stress of the NMDS is 0.198. (P1_1–P1_4= Period 1, P2_1–P2_4–Period 2, P3_1–P3_4= Period 3, P4_1–P4_4= Period 4)
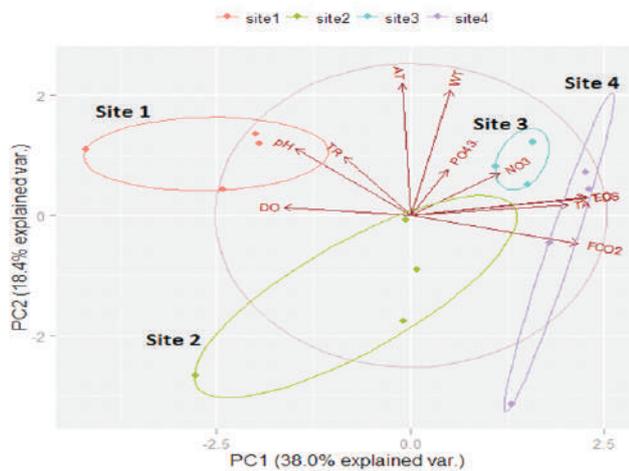
site 2. Here temperature increase was the governing factor which was mainly due to seasonal change from late winter to early summer (Fig. 5).

The eigen values of the first two axes of CCA ordination were 0.402 and 0.304, respectively. The species–environment correlations for first two axes were 1. Sum of all eigenvalues and canonical eigenvalues was 0.945. The DO, $PO_4^{3-}$, $NO_3^-$, $FCO_2$, EC and TDS were the major regulating vectors effecting species composition at different sites.The CCA ordination diagram showed that site 1 was strongly related to DO and association of non−tolerant genera indicated better quality of water as compared to other sites. Strong association of site 3 with $PO_4^{3-}$(close to CCA1) ($PO_4^{3-}$ and $NO_3^-$ were also associated with site 3 observed in PCA) and tolerant taxa *Synedra* sp., *Melosira* sp. along with *Oedogonium* sp., *Pleurasigma* sp., *Achnanthes* sp., *Scenedesmus quadricauda*, *Pediastrum simplex*, and *Microspora* sp. 2 showed sign of organic pollution. Therefore, organic pollution loading was the main factor for CCA1. This site of the river is near Silchar city, the highly populous area of south Assam which receives effluent of the whole city and a few tributaries. AT, WT, TR and $NO_3^-$ in association with site 2 were close to CCA2. This revealed that in site 2 the above mentioned parameters governed the water quality of the river. Same result was found in PCA as well. This was the reason for both highest Shannon_H$^/$ and Evenness_e^H/S of phytoplankton taxa in site 2 along with Margalef Diversity Index which complimented with lowest Berger–Parker index of dominance (Fig. 6).

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**Fig. 5.** Principle component analysis (PCA) ordination diagram for all the environmental variables of water and four different sites



**Fig. 6.** Canonical correspondence analysis (CCA) triplot ordination diagram for phytoplankton taxa, sites and environmental variables of water

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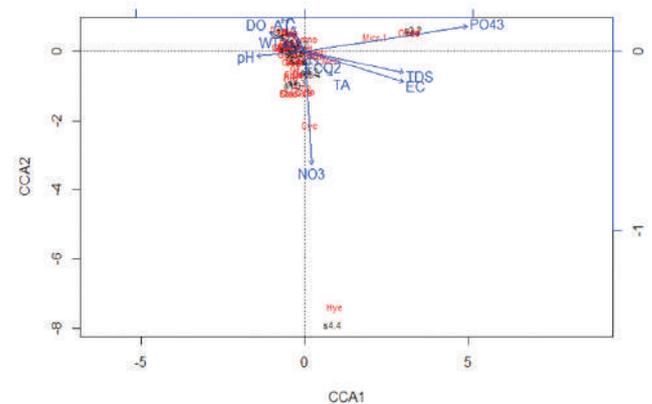
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# Water Properties as Influenced by Abattoir Wastes in Abakaliki and Ezzamgbo Southeastern Nigeria

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**Abstract**: Selected water bodies near abattoir dumpsites in Abakaliki and Ezzamgbo South-Eastern Nigeria were studied to determine their properties as influence by abattoir wastes in 2015 and 2016. Water collected were analysed for colour, conductivity, total solids, total dissolved solids, total suspended solids, pH, $NO_3^-$, $Cl^-$, Ca hardness, Mg hardness, total hardness, Pb, Cd, Cu and Zn. Most of physical and chemical parameters and heavy metals studied were higher than control and standards for domestic water use. Therefore, for water near abattoir waste dumpsite to be fit for usage, it must be treated in order to avoid the health problems associated with such water.

Abattor Acts (1985) gave a definition of abattoir as any area or vicinity where cattle, sheep, cow and other animals are slaughtered for human consumption. Public abattoir was dated back in ancient Roman Empire and French nation by 1500 and 1600 centuries (Oyedemi, 2000). However, in 1890 in Italy, laws was promulgated that public abattoir be provided in all the nooks and crannies of more than six thousand occupants. Also, the same records were noted in the Scandinavian countries and Romania (Jode *et al.,* 1996; Oyedemi, 2000). Robert (2005) indicated that in England, abattoirs performed pivotal measures in buying of animals and the butchering for human consumption. In Nigeria, almost all the cities and neighbourhood has the provision of slaughter slab. Oyedemi (2000) studied on the state and wellbeing of abattoir tools, equipments and instruments which he noted that slaughter houses were cited in the cities, villages and selected industrial location which possess merit and demerit. Based on his facts, the merits of the local locations supersede other locations and suggested that local site be given due consideration where possible. However, he suggested that slaughter houses be constructed on a strong calm sloping land far from other structures, neighbourhood and industries. Furthermore, recommendation on the sighting of slaughter houses out rightly far from urban boundaries was made. Oyedemi (2000) also reported that the animal butchering in slaughterhouse of third world countries was done in unpalatable areas by skilled butchers without the prior knowledge of sanitary regulations; with wastes generation by slaughter houses poses threat to environmental quality. Improper management and supervision of the activities of abattoir operators in Nigeria is a source of great risk to water properties. Most abattoirs are located near water bodies because of the high demand for water needed for the processing (Adelegan, 2002). Therefore, the objective of the study was to determine water properties as influence by abattoir wastes in Abakaliki and Ezzamgbo Southeastern Nigeria.

## MATERIAL AND METHODS

**Study Area:** The study was carried out in Abakaliki and Ezzamgbo of Ebonyi State, South Eastern Nigeria. The area lies approximately between latitude $06^0 14^'$ and $06^0 30^'$N with longitude $08^0 0^'$ and $08^0 15^'$E. The rainfall pattern is bimodal (April to July and September to November), with a quick dry spell in August normally referred to as "August Break". It has annual rainfall of 1700 to 2000 mm and annual mean of 1800mm. Abakaliki and Ezzamgbo have high temperature of $27^{\circ}C$ and the topmost mean daily temperature of $31^{\circ}C$ that is within the year. Humidity is high (80%) during rainy season and low (60%) during dry season. Geologically, the research site is sedimentary rock which is obtained from straight seawater retainer of the cretaceous periods and quaternary periods. As stated by Federal Department of Agriculture and Land Resources (1987), agricultural zone of areas remains within 'Asu River group' and made up of olive brown sandy shale, small particles of mudstones and sandstone. The soil is not very deep with unconsolidated parent substances within 1 m of the sand uppermost layer.

**Field Method:** A reconnaissance survey of the study area was carried out and the water body's sites selected were: 1=non-dumpsite at Mile-4 Ishieke, 2=10 m away from donkey abattoir wastes dumpsite at Ezzamgbo, 3=100 m away from donkey abattoir wastes dumpsite at Ezzamgbo, 4=10 m away from donkey abattoir wastes dumpsite at Abakaliki. 5=100 m away from donkey abattoir wastes dumpsite at Abakaliki

**Water Sample Collection:** Sterilized and cleaned Ivy bottle water was used to collect four replicate water samples in each location for three months intervals in 2016.

**Laboratory Analysis**

**Determination of physical parameters:** Total solids, Total dissolved solid and total suspended solid were determined using methods described by Bhagure (2010). The colour of each sample was determined in terms of percentage transmittance of light. The instrument used was photo electronic colorimeter model AE-11 M. The instrument was calibrated using distilled water and transmittance of each sample were read and digitally recorded in percentage (Hossain *et al.,* 2001). The conductivity of each sample was determined using SANXIN SX723 conductivity meter. The probe was dipped into each sample in a beaker, mixed and allowed to stabilize and the result digitally read and recorded (Hossain *et al.,* 2001).

**Determination of chemical parameters:** The water pH was determined using pH meter (Jung, 2001). The nitrate ($NO_3^-$) was determined by the turbidimetric method as proposed by (Stavrianou, 2007) and chloride, calcium, magnesium and total hardness were determined using estimation method described by Jung (2001).

**Determination of heavy metals:** Heavy metals (Pb, Cd, Cu and Zn) were therefore analysed in the digested sample using Flame Atomic Absorption Spectrophotometer, model 10 VGP and Buck Scientific (American Public Health Association, 1998).

**Data and Analysis:** The data obtained from this research was analysed using analysis of variance (ANOVA) for RCBD and the differences between means were dictated using F-LSD at P=0.05 (SAS Institute Inc., 1999) and compared with World Health Organisation standards (Alloways, 1996).

**RESULTS AND DISCUSSION**

**Effect of abattoir wastes on water physical properties:** There was a significant difference in water physical properties among the different locations studied in colour, conductivity, total solids, total dissolved solids and total suspended solids (Table 1). The orders colour increase in 1st and 2nd year were 1 < 5 < 4 < 3 < 2 and 1 < 2 < 5 < 3 < 4, respectively. The colour of sites near abattoir waste dumpsites had higher colour transmittance when compared to control. This may be attributed to the dissolution effect caused by wastes water from the abattoir. Control recorded the lowest conductivity value of 0.06 µS cm⁻¹ in the 1st year of study while conductivity in the sites near abattoir wastes dumpsites ranged between 0.10-0.32 µS cm⁻¹. In the 2nd year also, control had the lowest conductivity value of 0.04 µS cm⁻¹ whereas the conductivity in the sites near abattoir wastes dumpsites ranged between 0.11-0.16 µS cm⁻¹. Higher conductivity values in sites near abattoir waste dumpsites may be due to concentration and mineralization of organic matters and nutrients from the abattoir wastes. Though, these values were within the set standard. The order of increase in TS in the 1st and 2nd year of the study was 1 < 2 < 3 < 5 < 4. Lowest TDS was observed in control (32 mg l⁻¹ and 55 mg l⁻¹ in 1st and 2nd year, respectively) while TDS in sites near abattoir wastes dumpsites ranged between 50-161.20 mg l⁻¹ and 74-113.10 mg l⁻¹ for the corresponding years. Control recorded the lowest TSS value of 28.45 mg l⁻¹ in the 1st year of study. This observed TSS in control was lower than TSS in 2, 3, 4 and 5 by 270, 586, 757 and 772%, respectively. In the 2nd

**Table 1.** Effect of abattoir wastes on water colour (% transmittance), conductivity (µScm⁻¹), TS (mgl⁻¹), TDS (mgl⁻¹) and TSS (mgl⁻¹)

| Site | Colour | | Conductivity | | TS | | TDS | | TSS | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| 1 | 0.03 | 0.01 | 0.06 | 0.04 | 60.45 | 112.00 | 32.00 | 55.00 | 28.45 | 57.00 |
| 2 | 0.11 | 0.03 | 0.10 | 0.17 | 155.20 | 156.00 | 50.00 | 88.00 | 105.20 | 68.00 |
| 3 | 0.08 | 0.10 | 0.12 | 0.11 | 3331.25 | 165.00 | 136.00 | 74.00 | 195.25 | 91.00 |
| 4 | 0.07 | 0.11 | 0.31 | 0.04 | 400.35 | 251.00 | 156.00 | 113.10 | 243.75 | 137.90 |
| 5 | 0.05 | 0.06 | 0.32 | 0.16 | 378.35 | 273.15 | 161.20 | 108.45 | 217.15 | 164.70 |
| FLSD (p<0.05) | 0.04 | 0.07 | 1.60 | 0.03 | 5.78 | 1.08 | 81.80 | 1.88 | 13.81 | 2.55 |
| Standard | 50 | | 500 | | 500 | | 500 | | 150 | |

***Note:*** 1 = Control; 2 = 10m away from donkey abattoir waste dumpsite at Ezzamgbo; 3 = 100m away from donkey abattoir waste dumpsite at Ezzamgbo abattoir; 4 = 10m away from cow abattoir waste dumpsite at Abakaliki and 5 = 100m away from cow abattoir waste dumpsite at Abakaliki;TS = total solids; TDS = total dissolved solid; TSS = total suspended solids; 1st = 2015 and 2nd = 2016.
Standards according to Alloways (1996)

**Table 2.** Effect of abattoir wastes on water pH, $NO_3^-$ and $Cl^-$

| Site | pH | | $NO_3$ (mg $l^{-1}$) | | $Cr$ (mg $l^{-1}$) | |
|------|-----|-----|-----|-----|-----|-----|
| | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| 1 | 4.28 | 7.29 | 8.25 | 5.60 | 88.73 | 66.18 |
| 2 | 6.28 | 7.81 | 145.60 | 44.80 | 106.60 | 269.40 |
| 3 | 4.73 | 8.33 | 56.00 | 14.00 | 223.34 | 861.75 |
| 4 | 5.01 | 8.23 | 75.60 | 8.40 | 153.10 | 413.76 |
| 5 | 5.31 | 8.40 | 75.60 | 14.00 | 276.61 | 294.57 |
| FLSD (p<0.05) | 0.79 | 0.99 | 27.54 | 2.48 | 8.98 | 5.09 |
| Standard | 6.5-8.5 | | 10 | | 250 | |

See Table 1 for treatment details

**Table 3.** Effect of abattoir wastes on water Ca, Mg and total hardness (mg$l^{-1}$)

| Site | Ca hardness | | Mg hardness | | Total hardness | |
|------|-----|-----|-----|-----|-----|-----|
| | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| 1 | 24.05 | 52.10 | 9.73 | 17.02 | 33.78 | 69.12 |
| 2 | 88.18 | 120.12 | 36.50 | 24.31 | 124.68 | 144.43 |
| 3 | 72.14 | 180.36 | 34.05 | 48.63 | 106.19 | 228.99 |
| 4 | 105.48 | 100.20 | 19.46 | 48.63 | 129.94 | 148.83 |
| 5 | 108.80 | 128.27 | 51.06 | 53.50 | 159.86 | 181.77 |
| FLSD (p<0.05) | 1.95 | 4.12 | 1.04 | 5.75 | 7.98 | 3.09 |

See Table 1 for treatment details

year of the study control recorded the lowest TSS value of 57mg $l^{-1}$ while TSS in sites near abattoir wastes dumpsites ranged 68-64 mg $l^{-1}$. Total solids, total dissolved solids and total suspended solids were significant higher in soils near abattoir dumpsites than control. Colour, conductivity, total dissolve solids and total solids were within range (Alloyways, 1996) whereas apart from site 2, all the other sites recorded total suspended solids that were above the standard in the first year of study. Whereas total suspended solids in the 2nd year of study were within the standard apart from site 5 which recorded 164.70 mg $l^{-1}$ as against the standard of 150 mg $l^{-1}$. These higher values of total solids, total dissolved solids and total suspended solids in sites near abattoir waste dumpsites might be as a result of leaching of abattoir wastes into the water bodies in the study sites. American Public Health Association (1998) noted that total solids measurement can be useful as an indicator of the effects of run-off from construction, agricultural practices, abattoir sites and other sources. Total dissolved solids and total solids were to low when compared to the set standard of 500 mg $l^{-1}$ (Alloways, 1996). Total suspended solids are the filterable particles in water that may be of organic origin (algae, zooplankton and bacteria) or inorganic origin (silt, clay, calcium etc), this when in excess may reduce light penetration, hence reducing the ability of algae to photosynthesize.

**Effect of Abattoir Wastes on water chemical properties:**
There were significant differences in water pH, $NO_3^-$ and $Cl^-$ among the difference sites studied. The orders of increase in pH in the two years of the study were 1 < 3 < 4 < 5 < 2 and 1 < 2 < 4 < 3 < 5 for year 1 and 2, respectively. The pH observed in sites near abattoir waste dumpsites were significantly higher than the pH observed in control throughout the two years of the study. The rise in the pH level was attributed to the dissolution effect caused by the waste water produced in abattoir. These sites before water collection were used severally for washing roasted and unroasted meat coupled with the leachate from the abattoir waste dumpsites that leached to the water bodies studied. The pH levels of the water bodies near abattoir waste dumpsites and control were within the stipulated standard limit of 6.5 − 8.5 by Alloways (1996). The higher values in sites near abattoir waste dumpsites than control. They were both higher than set standard. Lowest $NO_3^-$ values of 8.25 mg $l^{-1}$ and 5.60 mg $l^{-1}$ were observed in conrol in first and second year, respectively while $NO_3^-$ values in sites near abattoir wastes dumpsites ranged between 56.00-145.00 mg $l^{-1}$ and 8.40-4.80 for the corresponding years of study. The higher nitrate observed could be attributed to the natural process of organic mineralization that converts abattoir wastes to nitrate in the sites near abattoir dumpsites. Control recorded the lowest value of $Cl^-$ in the first year of study. The observed $Cl^-$ in the control in 1st year of study was lower than $Cl^-$ in 2, 3, 4 and 5 by 20, 152, 73 and 212%, respectively. Similarly, control had the lowest $Cl^-$ (66.18 mg $l^{-1}$) in the 2nd year of study while $Cl^-$ values in sites near abattoir wastes dumpsites ranged between 269.40-861.75 mg $l^{-1}$, respectively. There was a significant change in Ca hardness, Mg hardness and total hardness among the different sites studied. The orders of increase in Ca hardness in 1st and 2nd year of study were 1 < 3 < 2 < 4 < 5 and 1 < 4 < 2 < 5 < 3, respectively. In the 1st and 2nd year of the study control recorded the lowest Mg hardness value of 9.73mg$l^{-1}$ and 17.02 mg $l^{-1}$ whereas Mg hardness in the sites near to abattoir waste dumpsites ranged between 19.46-51.06 mg $l^{-1}$ and 24.31-53.50 mg $l^{-1}$, respectively. Also, in the 1st year of the study control recorded the lowest total hardness value of 33.78 mg $l^{-1}$. This observed total hardness in control was lower than total hardness in 2, 3, 4 and 5 by 269, 214, 270 and 373%, respectively. Similar trend was observed in second year. Sites near abattoirs wastes dumpsites had higher $Cl^-$, Ca and Mg than control. Chlorine, calcium and magnesium are essential element for all organisms and are incorporated into the shells of many aquatic invertebrates as well as the bones of vertebrates. Calcium and magnesium also serves as an important

**Table 4.** Effect of abattoir wastes on water Pb, Cd, Cu and Zn (mgl$^{-1}$)

| Site | Pb | | Cd | | Cu | | Zn | |
|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| 1 | 0.001 | 0.009 | 0.001 | 0.001 | 0.13 | 0.63 | 2.31 | 2.47 |
| 2 | 0.050 | 0.016 | 0.004 | 0.005 | 0.43 | 7.62 | 4.81 | 5.01 |
| 3 | 0.081 | 0.012 | 0.003 | 0.005 | 0.39 | 7.62 | 4.02 | 4.99 |
| 4 | 0.076 | 0.005 | 0.006 | 0.003 | 1.24 | 2.08 | 4.09 | 4.18 |
| 5 | 0.069 | 0.019 | 0.006 | 0.009 | 0.91 | 4.66 | 3.96 | 3.81 |
| FLSD (p<0.05) | 0.002 | NS | 0.005 | 0.900 | 0.011 | 0.002 | 0.009 | 0.267 |

See Table 1 for treatment details

element required for the optimal growth and productivity in plants and animals. Also, when these elements are in excess in water bodies, it tends to contribute to the hardness of water (Norton *et al.*, 2002). The total hardness of water shown higher values of total hardness in water near abattoir waste dumpsites than total hardness in control. In the 1st year of study, sites 3 and 5 recorded the total hardness value that are above the standard whereas it was only site 3 that had higher total hardness in the 2nd year than the standard

**Effect of abattoir wastes on water heavy metals:** The effect of abattoir wastes on heavy metals showed significant difference among the difference sites. The orders of increase in Pb in 1st and 2nd year of study were 1 < 2 < 5 < 4 < 3 and 1 < 4 < 3 < 2 < 5, respectively. Control recorded the lowest Cd. This observed Cd value in the 1st year of study was higher than Cd in 2, 3, 4 and 5 by 300, 200, 500 and 500%, respectively. Also, in the 2nd year of the study control recorded the lowest Cd value of 0.001 mg l$^{-1}$ while Cd in sites near abattoir waste dumpsites ranged between 0.003–0.009 mg l$^{-1}$. The orders of increase in Cu in 1st and 2nd year of study were 1 < 3 < 2 < 5 < 4 and 1< 3 < 5 < 4 < 2 for the corresponding year. The lowest Zn value of 2.31mgl$^{-1}$ was observed in control in 1st year of study. This observed Zn in control was lower than Zn in 2, 3, 4 and 5 by 108, 74, 77 and 71%, respectively. Similar trend was observed in the second year. The heavy metals–Pb, Cd, Cu and Zn were higher in water near abattoir waste dumpsites than control in the both two years of the studied. The observed heavy metals were higher than the standard in the sites near abattoir wastes dumpsites. According to (Agayarko *et al.,* 2010), heavy metals in water cause severe vomiting, diarrhoea, bloody urine, liver, kidney failure, anaemia, inhibition of haemoglobin synthesis, cardiovascular system. It also causes death in aquatic life and disturbance in variety of crop production. According to Njoku (2014) and World Health Organization (1993) Zn is not known to cause any health problem in animals or human being.

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# Qualitative and Quantitative Study of Epiphytic Diatoms on Two Macrophytes In A lotic Ecosystem, Iraq

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*Abstract:* The study aimed to determine the quantitative and qualitative variations of diatoms epiphytic on two macrophytes in Al‑Shamiyah river  (Euphrates river) as lotic ecosystems. The samples were taken from four sites along the river. Epiphytic diatoms of *Certatophyllum demersum* and *Phragmites australis* and some environmental parameters were recorded from March 2013 to February 2014. The results showed a high number of diatoms taxa and quantity values recorded on *C. demersum* (142 taxa and 227.67 cell x $10^4$ /g WW),  while on *P. australis*  were recorded a total of 136 epiphytic diatoms taxa  and 223.09 cell x $10^4$ /g WW. The pennate diatoms were  predominated among the study periods and sites. Temporal and spatial variation of epiphytic diatoms and physicochemical parameters were noticed in this study. The results of environmental factors and the biodiversity indices indicated that the Al‑Shamiyah river water quality is considered to be moderately polluted to clean water.

*Keywords*:  Lotic Ecosystems, Epiphytic Algae, Diatoms, Al−Shamiyah River, Macrophytes

Epiphytic algae is the algae that grows on vascular plants submersible and the flowering plants (Wehr and Sheath, 2003), or in general are the algae that grows on the aquatic plant surfaces or on the immersed parts in water (Graham *et al*., 2009). Diatoms are considered as important components in the algal community in most aquatic ecosystems, especially in a lotic systems, in addition to their role in aquatic food chains (Bere and Tundisi, 2010) as primary products, and a refuge for many invertebrates and fish (Furey *et al*., 2012).

Epiphytic microalgae have a big role in implication for the lotic ecosystem processes such as environmental balance, water quality (Bere and Tundisi,  2010), alarm for the environmental alteration and its role in nutrient cycling (Dong *et al*., 2007; Mann, 1999). Diatoms were considered as an important part of biota in a lotic ecosystem (Azim *et al*., 2005; Taylor *et al*., 2007). Many diatoms are important in the study of water quality assessment and monitoring due to its fast response to the environmental changes in different aquatic systems (Zalack *et al*., 2010; Reavie *et al*., 2010; Wu *et al*., 2012 a, b; Wu, *et al*., 2014).  The river ecosystem is affected by different pollutants during their passing from upstream to downstream, in addition to river morphological change, therefore all physicochemical and biological characteristics were altered (Vasanthy and Velmurugan, 2009; Nahar *et al*., 2016; Wu *et al*., 2016). Moreover, different

human activities have accelerated the deterioration of water quality of the river (Singh and Choudhary, 2013).

A few studies were conducted on the Al‑Shamiyah River. This part of Euphrates river is the main source of drinking water and other agricultural and industrial activities of three towns along the river. Authors started to study the phytoplankton and physicochemical properties of the river (Hassan and Shawaat, 2015a). Also, studied the epipelic algae communities and its used to assess the river (Hassan and Shawaar, 2015b, 2016). This study aimed to provide  the information and fill the gap on the epiphytic diatom communities in the Al‑Shamiyah River and its temporal and spatial variations.

## MATERIAL AND METHODS

**Study area:** Al‑Shamiyah River represents branch II of the Euphrates River after Kufa branch where enters the territory of Qadisiyah province of the northern part of western heading towards the south. Then is passing the cities of AL‑Salahiya town at Kilometer (23.5), and the center of Al‑Shamiyah town at Kilometer (42), and Ghammas town at Kilometer (71.40). Its length is (120 km) and the discharge capacity 180 m³ /s, and up to the land irrigated by (384000 space). Four sites were selected along the river for the period from  March 2013 to February, 2014. The first site is located after the branching of the Al‑Hindia River (Euphrates River) into two branches

(Kufa and Al-Shamiyah Rivers) and represented as an upstream. The second and third sites are located at Salahiya town and at the center of the Shamiyah town. The fourth site was located at Ghammas town and represented as a downstream. Sites (1, 2, 4) which were characterized by dense macrophytes and agricultural activities (cultivation of rice and wheat and barley). While the third site affected agricultural and human activities where was a little aquatic macrophyte compared with other sites (Figure 1, Table 1)

**Physical and chemical analysis:** Some environmental parameters were the flow meter for water flow, pH –meter, turbidity meter, Secchi disc and electrical conductivity meter were used for temperature (air and water), water flow, pH, turbidity, light penetration and electrical conductivity. The dissolved oxygen was collected by Winkler bottles followed APHA (2003). The other parameters were measured in the lab followed APHA (2003) for total dissolved solids (TDS), total suspended solids (TSS), total alkalinity (TA), total hardness (TH), calcium ($Ca^{+2}$), magnesium ($Mg^{+2}$), sodium ($Na^{+1}$), potassium ($K^{+1}$), sulphate ($SO_4$), chloride ($Cl^{-1}$) and biochemical oxygen demand (BOD). Moreover, salinity (S‰) and percent of saturation of dissolved oxygen followed APHA (2003), While nutrients (nitrite ($NO_2$), nitrate ($NO_3$), phosphate ($PO_4$) and silicate ($SiO_3$) followed APHA (2005)). Total organic carbon was measured by titration with ammonium ferrous sulfate (Salman, 2014).

**Epiphytic microalgae analysis:** The sample of the epiphytic microalgae on two aquatic plants (*C. demersum* and *P. australis*) was collected from each site. The epiphytic microalgae were determined on *C. demersum* by shaking strongly with distilled water to remove the microalgae from the aquatic plant surface. The attached microalgae on *P. australis* was treated manually by toothbrush and shaking then preserved with Lugol's iodine solution (Zimba and Hopson, 1997). The clearing of diatom frustules was done by concentrated sulfuric acid and potassium dichromate method of Patric and Reimer (APHA 2003). The preparation for counting methods of diatoms were followed Furet and Benson-Evans and micro-transect method used for counting (Hotzel and Croome, 1999). Diatoms were identified according to the following references (Hassan et al., 2012; Al-Hassany and Hassan, 2014; Burliga and Patrick, 2016 ).

**Biodiversity Indices:** Two indices (Richness Index (D) and Shannon and Weaver Index) were used in this study. The Richness Index (D) was calculated using the following formula according to Magurran (2004):

D=S-1/InN

Where the D is Richness,

S = Total number of different species and N= total number of individuals

While the Shannon and Weaver Index was computed according the formula (Magurran, 2004):

$$H' = \sum_{n=1}^{s} Pi \; log \; 2 \, Pi$$

H' = Shannon and weaver Index; S= Total number of species and $P_i$= the proportion of species about the total number of species.

**Data analysis:** The SPSS (Statistical Package for Social Science, 2011) and Canonical correspondence analysis (CCA) program version 4.5 (Ter Braak and Smilauer, 2002) were used for statistical analysis.
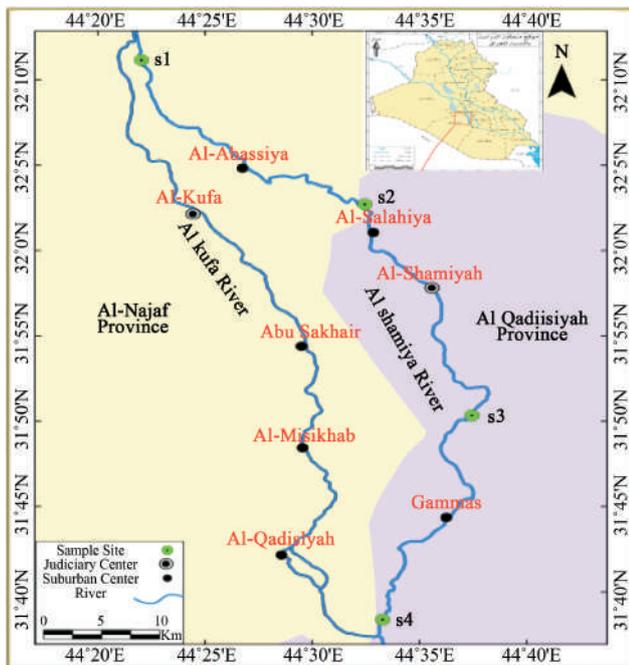


**Fig. 1**. Map of the Al-Shamiyah River and the locations of the sampling sites

**Table 1**. The geographical positions GPS) of the sampling sites

| Sites | Longitude (eastwards) | Latitudes (northward) |
|---|---|---|
| 1 | 44 "36' 68" | 32 "18' 53" |
| 2 | 44 "53' 83" | 32 "04' 45" |
| 3 | 44 "62' 40" | 31 "83' 92" |
| 4 | 44 "55' 39" | 31 "63' 92" |

**RESULTS AND DISCUSSION**

The results showed that the air temperature was ranged between 13.3 $^{\circ}$C at site 1 in spring 2014 and 34.4$^{\circ}$C at site 4 in summer 2013. The lowest value of water temperature was 11.8$^{\circ}$C at site 1 in winter 2014 and the highest value was 31.2$^{\circ}$C at site 4 in summer 2013 (Table 2).

The average value of the water flow was ranged from

24.74 m/min in the spring 2013 to 48.07m/min in winter 2014, both values were at site 4. The highest average value (20.68 NTU) of turbidity was at the site 1 in winter 2014, while the lowest value was 10.38 NTU at site 4 in the spring 2013. The results of light penetration showed the lowest value (25.75 cm) was in winter2014 and the highest value (56.25cm) was in spring 2013 at sites 1 and 3, respectively. Both electrical conductivity, salinity and total dissolved solids were shown the same seasonal variation trend, the lowest values (1130 µs/cm, 0.72 PUS and 723 mg/l, respectively) were in spring 2014 in site 2, while the highest values (1384 µs/cm, 0.99 PUS, and 886mg/l, respectively) in autumn 2013 at site 4.

The average pH value ranged from 7.73 to 8.22 at sites 4 and 1 in summer 2013 and spring 2014, respectively. The dissolved oxygen concentration in the river was not recorded less than 6 throughout the study period. The percentage of saturation of dissolved oxygen was ranged from 84.80% at site 4 in spring 2014 to the highest percentage (109.23%) at site 1 in spring 2013. All results of BOD were not exceeded

than 2.3 mg $l^{-1}$ and the lowest concentration (1.13mg/l) were recorded in both the summer 2013and winter 2014 at site. Total available carbon dioxide ranged from 125.40 mg $l^{-1}$ in spring 2013 at site 1 to 187.00 mg $l^{-1}$ in spring 2014 at site 3.

The lowest value of total alkalinity was 108.00 mg $CaCO_3$/l in the autumn 2013 at site 2, while the highest valve was 151.50 mg $CaCO_3$/l in the winter 2014 at site 4. Total hardness, calcium and magnesium were ranged as follows: 296.00−522.00 mg $CaCO_3$/l, 79.36−129.28 mg $l^{-1}$, and 12.57−55.01 mg $l^{-1}$ in the spring and summer 2013, respectively, except the highest value of Mg recorded in the autumn 2013. The lowest concentration of sodium (93.05 mg $l^{-1}$) was recorded in the summer 2013 and the highest value (148.35 mg $l^{-1}$) in autumn 2013 at site 4. The average values of potassium ranged from 4.82 mg $l^{-1}$ to 8.35 mg $l^{-1}$ at sites 1 and 4 in the winter 2014 and the autumn 2013, respectively. The highest concentrations of chloride (111.34 mg $l^{-1}$)  were recorded in the summer 2013 while the lowest concentration was  54.44 in the winter 2014, the concentration of sulfates

**Table 2**. Mean±SD of Physicochemical factors in Al-Shamiyah River during the study period

| Parameters | Spring 2013 | Summer 2013 | Autumn 2013 | Winter 2014 | Spring 2014 |
|---|---|---|---|---|---|
| Air Temperature (C°) | 25.42 ±1.39 | 32.79 ± 1.93 | 27.39 ± 1.30 | 18.04 ±1.68 | 15.13 ± 2.02 |
| Water Temperature (C°) | 22.30 ± 1.26 | 30.33 ±1.02 | 24.31 ±2.18 | 13.56 ±1.22 | 13.21 ±0.58 |
| Water flow (m $min^{-1}$) | 28.38 ± 4.29 | 33.21 ±3.78 | 33.05 ±2.34 | 36.05 ± 8.13 | 40.09 ±2.19 |
| Light penetration (Cm) | 45.18 ±11.23 | 38.66 ±5.64 | 38.87 ±2.86 | 36.18 ±8.65 | 37.53 ± 6.97 |
| Turbidity (NTU) | 12.18 ±1.79 | 14.47 ±1.40 | 14.25 ±1.18 | 16.69 ±3.03 | 16.65 ±2.19 |
| pH | 8.21 ±0.01 | 7.78 ±0.05 | 8.14 ±0.01 | 7.92 ±0.05 | 7.89 ±0.07 |
| E.C(µs $cm^{-1}$) | 1157.00 ±26.09 | 1294.63±53.17 | 1282.00±84.48 | 1186.88±37.58 | 1208.25±103.59 |
| Salinity (PSU) | 0.74 ±0.02 | 0.83 ±0.03 | 0.82 ±0.05 | 0.76± 0.0 | 0.77 ±0.07 |
| T.D.S(mg $l^{-1}$) | 740.38 ±16.96 | 28.63±34.08 | 820.58±54.19 | 759.63±24.00 | 773.00±66.21 |
| T. S.S(mg $l^{-1}$) | 24.41 ± 3.65 | 30.08 ±4.74 | 28.49 ±2.83 | 31.08 ±4.14 | 33.79 ±3.95 |
| Dissolved oxygen mg $l^{-1}$ | 8.68 ±0.72 | 7.19 ±0.57 | 8.09 ±0.52 | 9.41 ±0.63 | 9.75 ±0.71 |
| DO % | 99.71 ± 7.88 | 91.57 ± 5.58 | 97.33 ± 3.35 | 91.95 ± 4.27 | 92.47 ± 5.96 |
| BOD | 1.46 ± 0.12 | 1.98 ± 0.33 | 1.61 ± 0.23 | 1.32 ± 0.20 | 1.42 ± 0.17 |
| Total Available carbon dioxide mg $l^{-1}$ | 130.63 ± 5.11 | 180.01 ± 5.74 | 141.66 ± 1.81 | 153.10 ± 2.56 | 170.78±12.75 |
| Total Alkalinity mg $CaCO_3$ $l^{-1}$ | 126.88 ± 0.63 | 144.72 ± 4.51 | 110.92 ± 3.50 | 139.88 ± 7.95 | 143.42 ± 6.64 |
| Total Hardness mg $CaCO_3$ $l^{-1}$ | 339.00 ± 36.24 | 495.00±28.23 | 26.33±75.32 | 417.00±35.80 | 326.00±21.79 |
| Calcium mg $l^{-1}$ | 82.77 ± 2.65 | 123.16± 6.15 | 108.08 ± 2.02 | 102.80 ± 1.78 | 96.44 ± 2.09 |
| Magnesium mg $l^{-1}$l | 31.77 ± 7.95 | 45.57 ± 4.88 | 43.19 ± 9.02 | 38.94 ± 8.54 | 20.69 ± 6.20 |
| Sodium mg $l^{-1}$ | 107.92 ± 8.35 | 96.94 ± 2.70 | 142.95 ± 4.24 | 125.06 ± 6.45 | 120.08±14.48 |
| Potassium mg $l^{-1}$ | 6.43 ± 0.35 | 6.90 ± 0.37 | 7.81 ± 0.82 | 5.45 ± 0.49 | 6.68 ± 0.68 |
| Nitrite µg $l^{-1}$ | 1.39 ± 0.10 | 1.81 ±0.10 | 1.36 ±0.13 | 0.83 ±0.18 | 0.47 ±0.31 |
| Nitrate µg $l^{-1}$ | 59.56 ±3.61 | 46.44 ± 3.25 | 60.84 ±8.40 | 89.16 ±11.43 | 102.73±12.47 |
| Phosphate µg $l^{-1}$ | 0.05 ± 0.03 | 0.14 ± 0.01 | 0.05 ± 0.02 | 0.13 ± 0.01 | 0.15 ± 0.01 |
| Silicate µg $l^{-1}$ | 135.58 ± 67.89 | 134.76±11.20 | 132.70 ±5.55 | 137.05±13.27 | 135.03±12.16 |
| Sulphate mg $l^{-1}$ | 248.24 ± 27.16 | 392.74±27.91 | 313.06±29.06 | 265.64±37.02 | 88.67±14.86 |
| chloride mg $l^{-1}$ | 64.12 ±7.05 | 101.73 ±7.11 | 79.71 ±8.72 | 71.69 ±11.86 | 72.98 ±2.85 |
| Total organic Carbon mg $l^{-1}$ | 0.57 ±0.15 | 1.11 ±0.38 | 0.60 ±0.16 | 0.66 ± 0.06 | 0.40 ± 0.18 |

shown the same variation trend as in chloride and ranged from 216.25 mg l⁻¹ at the site 1to 420.82mg/l at site 4.

The results of nutrient concentrations showed the temporal variation (Table 2). The nitrite and nitrate were ranged as follows: 0.16- 1.94 µg l⁻¹ and 42.57–116.80 µg l⁻¹, respectively. Phosphate and silicate were 0.02–0.16 µg l⁻¹ and 99.15–237.33 µg l⁻¹, respectively. The lowest value of total organic carbon (0.24 mg/l) was recorded in spring 2014 at site 2 while high value (1.55 mg/l) was in the summer 2013a t the site 4. The sediment texture of the river was as follows: clay (44.00−55.00%), sand (30.00-40.00%) and silt (11.00–16.00%).

A total of 142 taxon of diatoms was identified on *C. demersum* which these taxa belonged to 32 genera. The centric diatoms were represented about 9.2% of the total identified diatoms by 13 species belonged to 4 genera. While pennate diatoms was 90.8 % of the total identified diatoms by 129 species belonged to 28 genera. The total identified diatoms that attached on *P. australis* were 136 taxa belonged to 31 genera. The centric diatoms represented about 8.8% of the total epiphytic diatoms by 12 species belonged to 4 genera, while the pennate diatoms were 91.2% by 124 species belonged to 27 genera (Table 3). The tables 4 illustrated the variety of identified diatoms among the study sites. The epiphyte diatoms in site 1 on both aquatic plants were higher than other sites, whereas, the sites 2 and 3 were recorded the lowest number of identified diatoms.

The epiphytic diatoms on *C. demersum* observed only in one site and absent from another site. The following species observed only in site 1: *Cymbella microcephala, Diploneis elliptica, Fragillaria acus, Pseudostaurosira brevistriata, Fragillaria virescens, Gomphonema acuminatum, Mastogloia elliptica, Navicula gregaria, Rhopalodia gibba* and *Stauroneis anceps* . While the other species emerged only in site 2 such as: *Cymatopleara ellipitca, Cymbella tumidula, Diploneis simithii, Fragillaria construnes, Navicula viridula var. rostellata, Nitzschia vitrea, Fragilaria capucina var. rumpens*. Whereas the following species found only in site 3: *Aulacoseira italica, Cyclotella stelligera, Geissleria simillis, Tryblionella acuta, Nitzschia commutate, Nitzschia microcephala, Nitzschia umbonata, Nitzschia tryblionella* and *Caloneis leptosome. Anomoeoneis exilis, Navicula secretary and medium iridis* were found only at site 4. In the same trend, some epiphyte diatom species on *P. australis* observed only in one site. The following species were found only in site1: *Amphiprora alata, Amphora ovalis, Cyclotella glomerata, Cymbella aspera, Diatoma hiemale var. mesodon, Diploneis puella (Fragilaria construens, Gomphonema constrictum, Tryblionella acut, Nitzschia fasciculate, Nitzschia inconspicua, Nitzschia sigma,*

*Caloneis leptosoma, Surirella. turgida, Fragilaria capucina sub sp. rumpens*. Other species found only at site 2 such as: *Cymatopleura elliptica, Cymbella leptoceros, Diploneis smithii, Gyrosigma peisonis, Mastogloia muradi, Homoeocladia amphibia* . While these species as follows found only at site 4: *Navicula rhynchocephala Kuetzing, Navicula, Stauroneis anceps, Tabellaria fenestrate* and *Tabellaria flocculosa*.

The results of total number of epiphytic diatoms on both aquatic plants varied among seasons and showed two peaks, the first is in spring (the highest) and the second is in autumn (Figure 2). The lowest number (17.01 cell x 10⁴ /g WW) of epiphytic diatoms on *C. demersum* recorded at the site 2, while the higher number was 227.67 cell x 10⁴ /g WW at site 1 (Table 5, Figure 2). Whereas, for *P. australis* the total number of epiphytic diatoms were ranged from 9.08 cell x 10⁴ /g WW at the site 1 to 223.09 cell x 10⁴ /g WW at the site1 too (Table 5, Figure 2).

The results showed that the highest percentage of epiphytic diatoms was belonged to pennate diatoms on both *C. demersum* and *P. australis* among the study period. The representage of epiphytic centric diatoms on *C. demersum* was ranged from 0.86% at site 4 in the autumn 2013 to 11.81% at site 3 in the autumn 2013. Whereas that for the pennate diatoms was ranged from 88.19% at site 3 in the autumn to 99.14% at site 4 in the autumn 2013 (Table 5; Figure 2).

The results of epiphytic diatoms on *P. australis* were shown the lowest percentage of centric diatom was 0.77% in summer 2013 at site 3 while for pennate diatoms was 82.53% in the spring 2013 at site 3. The highest percentage of centric diatom was (17.47%) in the spring 2014 at site 3, while the pennate diatom was 99.23% at the same site in summer 2013. (Table 5; Figure 2). Richness index showed different values for the epiphytic algae on the host plants. The index values ranged from 6.71 in autumn 2013 at site 3 to 11.11 in spring 2014 at site 1for the attached algae on *C. demersum* (Table 5 and Fig 3). The index values of epiphytic algae on *P. australis* recorded lowest value (5.82) in summer 2013 at site 1 and the highest value was 12.69 in spring 2014 at site 3 (Table 5 and Fig 3).

The lowest values of the Shannon and Weaver Index were recorded in winter 2014 for the epiphytic algae on both host plants. These values were 1.42 and 1.35 for *C. demersum* and *P. australis* at sites 1and 2 in winter 2014, respectively (Tables 6 and 7). While the highest values were 2.72 (on *C. demersum*) and 2.75 (on *P. australis*) at sites 2 and 3 in spring 2014, respectively (Figures 3). The statistical analysis showed a significant correlation between D and H' Indices with the studied variables (Table 5).

**Table 3**. List and total number (cell×10$^4$/g) of identifying epipelic diatoms during the study period in Al-Shamiyah river

(0=not present; C=*C. demersum*; P=*P. australis*)

| Taxa | Site | | | | Taxa | Site | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 2 | 4 | | 1 | 2 | 3 | 4 |
| *Bacillariophyceae*-centrles | | | | | *C. placentula* Ehr | C+P | C+P | C+P | C+P |
| *Aulacoseira ambigua* O. Muller | C+P | C+P | C+P | C+P | *C.placentula var lineate* (Ehr) Cleve | C+P | C+P | C+P | C+P |
| *A. dicikiei* (Thwaites) Ktz | 0 | 0 | C | C | *Cymatopleura elliptica* (Breb) W. | 0 | C+P | 0 | 0 |
| *A. distanse* (Ehr.) Ktz | C | C+P | C+P | C+P | *C. solea* (Breb) W.Smith | C+P | C+P | C+P | C+P |
| *A. granulate* (Ehr.) Ralfs | C=P | C+P | C+P | C+P | *Cymbella affinis* Kützing | C+P | C+P | C+P | C+P |
| *A. italica* Ktz | 0 | 0 | C | 0 | *Cymbella aspera* (Ehr) *H.paragallo.* | P | 0 | 0 | 0 |
| *Cosindiscus lacustris* Grunow | C | C | C | C | *C. cistula* (Ehr.) Kirchn | C | C | C | C |
| *Cycllotella comta* (Ehr)Ktz | C+P | C+P | C+P | C+P | *C. gracilis*( Rabh ) Cleve | C+P | P | 0 | C |
| *C menenghiniana* Ktz | P | 0 | 0 | 0 | *C. helvetica* Ktz. | C+P | C+P | C | C+P |
| *C. ocellata* Pantocsek | C+P | C+P | C+P | C+P | *C. leptoceros* (Ehr.) Grunow | C | C+P | 0 | 0 |
| *C. stelligera* Cleve et Grun. | 0 | P | C | P | *C. microcephala* Grun | C | 0 | 0 | 0 |
| *Stephanodiscus hantzschii* Ehr) Grun. | C | 0 | C+P | C+P | *C. tumida*(Bréb.)Van. Heurck | C+P | C+P | C+P | C+P |
| *S .hantzschii* Grun. | C | 0 | C+P | C+P | *C. tumidula* Grun | P | C+P | 0 | 0 |
| *Thalassioira bramaputa* (Ehr) Hakansson and Locker | P | P | P | P | *C. turgida* (Greg.) Cleve | P | C+P | C | C |
| Pennales | | | | | *C. ventricosa* Ktz | C+P | C+P | C+P | C+P |
| *Achnanthidium affinis* Grun. | C+P | C+P | C+P | C+P | *Craticula halophila* (Grunow) D. G. Mann. | C+P | C+P | C+P | C+P |
| *A. lanceolata* Grun. | C+P | 0 | 0 | C+P | *Ctenophora pulchella* Raffs ex Ktz.) Willimas and Round | C+P | C+P | C | C |
| *A. linearis* Grun. | C | 0 | 0 | C | *Diatoma elongtoma* (Lyngb) Agardh | C+P | C+P | C | C |
| *A.microcephala* (Kütz) Grun. | C | C | C | C | *D. hiemale* var. mesodon (Eher) Grun. | P | 0 | 0 | 0 |
| *A. mintussima* Ktz | C+P | C+P | C+P | C+P | *D. vulgare* Bory | C+P | C | C+P | C+P |
| *A. saxonica* Krasska | C | 0 | 0 | C | *Denticula elegens* Ktz | 0 | 0 | p | p |
| *Amphiprora alata* (Ehr) Ktz | C+P | 0 | 0 | C | *Diploneis elliptica* (ktz) Cleve | C | 0 | 0 | 0 |
| *Amphora normanii* Rabenhorst | C+P | C | C+P | C+P | *D. ovalis* (Hisle) Cleve | C+P | C+P | C+P | C+P |
| *A. ovalis* (Ktz) Ktz | C+P | 0 | 0 | C+P | *D. puella* (Schum) Cleve | C+P | 0 | 0 | C |
| *Anomoeoneis exilis* (Ktz) Cleve. | P | P | P | C+P | *D.smithii* (Breb) Cleve | 0 | C+P | 0 | 0 |
| *Halamphora veneta* (Ktz) Levkov. | C | P | C+P | C+P | *Encyonema caespitosum* Ktz | C+P | C+P | 0 | 0 |
| *Asterionella japononica* Cl.And Muller. | 0 | 0 | P | P | *Epithemia zebra* (Ehr.) Kützing | C | 0 | 0 | C |
| *Bacillaria paxillifer* (Muller)Hendey | C+P | C+P | C+P | C+P | *Eunotia. pectinalis* var.undulate Rabenhorst | C | C | 0 | C |
| *Caloneis bacillum* (Grun) Cleve | C | 0 | 0 | C | *Fragilaria acus* Ktz | C | 0 | 0 | 0 |
| *C. ventricosa* (Ehr.) Meister | C | 0 | 0 | C | *F.capucina* Desmazieres | C+P | C+P | C+P | C+P |
| *C. leptosome* (Grun)Krammer | P | 0 | C | 0 | *F.capucina* sub sp runpens (Ktz) Lange−Bertalot | P | C | 0 | 0 |
| *Cocconies pediculus* Ehr | C+P | C+P | C+P | C+P | *F.construnes* (Ktz) Grun | P | C | 0 | 0 |
| *F..crotonensis* Kitton | C+P | C+P | C+P | C+P | *N.festiva* Krasske | C | 0 | 0 | C |
| *F.vaucheriae* (Ktz) J.B.Petersen | C+P | C+P | C+P | C+P | *N. gracilis* (Ehr.) Ktz. | C+P | C+P | C+P | C+P |
| *Fragilariforma virescens* (Ralfs) Williams and Round | C | 0 | 0 | 0 | *N. graciloides* A.Mayer | P | C+P | P | C+P |
| *Fallacia pygmaea* (Ktz) A.J.Stickle and D.G.Mann | C | 0 | C | C | *N. gregaria* Dinkin | C | 0 | 0 | 0 |

**Cont...**

| Species | 1 | 2 | 3 | 4 | Species | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|---|---|---|
| *Geissleria simillis* (Krasske) Lange−Bertalot and Metzeltin | 0 | 0 | C | 0 | *N. grimmei* Krasske | C+P | C+P | P | C+P |
| *Gomphonema acuminatum* Ehr. | C | 0 | P | P | *N. lanceolata* (Ag.) Ktzi | C+P | C+P | C+P | C+P |
| *G. acuminatum* var. turris Cleve | C | 0 | 0 | C | *N. phyllepta* Kutz | C+P | C+P | C+P | C+P |
| *Gomphonema angustatum* (Ktz) Rabenhorst | C | C+P | C+P | C+P | *N. pupula* Ktz | 0 | P | 0 | 0 |
| *G. constrictum* Ehernberg. | C+P | 0 | 0 | C | *N. radiosa* Ktz | C+P | C+P | C+P | C+P |
| *G.constrictum* var.capitat (Ehr) Cleve | C+P | C | P | P | *N. rhynchocephala* Ktz | 0 | 0 | 0 | P |
| *G. gracile* Ehrenberg | C+P | C+P | C+P | C+P | *N. salinarum* Grun. | 0 | P | P | 0 |
| *G. intricatum* Ktz | C+P | C+P | C+P | C+P | *N. schroeteri* Meister | 0 | 0 | 0 | C+P |
| *G. intricatum* var.lunata Nov | C+P | C+P | C+P | C+P | *N. viridula* Ehr. (ktz) N. | C+P | C+P | C+P | C+P |
| *G. lanceolatum* Ehrenberg | C+P | P | C+P | C+P | *N. viridula* var.*rostellata* (Ktz) Cleve | 0 | C | 0 | 0 |
| *G. parvulum* (Ktz)Grun. | C | C | 0 | 0 | *Neidium affine* (Ehr.) Pfitz | C | 0 | 0 | C |
| *G. tergistiurris* Ehrenberg | C+P | C+P | P | C+P | *N. iridis* (Ehr.) Cleve | P | 0 | 0 | C+P |
| *G.productum* (Grun) Lange−Bertalot E.Rechardt | C+P | C+P | C+P | C+P | *Nitzshia.acicularis* (Ktz.)M.Smith | C+P | C+P | C+P | C+P |
| *Gomphonies olivaceum* (Lyng.) Ktzi | C+P | C+P | C+P | C+P | *N. apiculata* (Greg.) Grun. | C+P | C+P | 0 | C |
| *Gyrosigma acuminatum* (Ktz.) Rabenhorst | C+P | C+P | C+P | C+P | *N. clausii* Hantzsch | C+P | C+P | C+P | C |
| *G.attenuatum* (Ktz.) Rabenhorst | C | C | C+P | C+P | *N. commutata* Grun | 0 | 0 | C | 0 |
| *G. peisonis* Hustedt | 0 | P | 0 | 0 | *N. dissipata* (Ktz.) Grun | C+P | C+P | C+P | C+P |
| *Halamphora veneta* (Ktz) Levkov. | C+P | C+P | C+P | C+P | *N. dubia* W.Smith | C | 0 | 0 | C |
| *Homoeocladia amphibia* (Grunow) Ktz | C | P | C | C | *N. fasciculate* (Grun.) Grun. | P | 0 | 0 | 0 |
| *Lemnicola hungarica* (Grun) Round and Bason | C | P | P | C+P | *N.filiforms* (W.Smith) Hustedt | C+P | C+P | C+P | C+P |
| *Mastogloia elliptica* (Ag) Cleve | C | 0 | 0 | 0 | *N. gracilis* Hantzsch | C+P | C+P | C+P | C+P |
| *Mastogloia muradi* Voigt | 0 | P | 0 | 0 | *N. hantzschiana* Rabenhorst | C+P | C+P | C+P | C+P |
| *M.smithii* Thw. Ex W.Sm. | C | 0 | P | C+P | *N. nana* (Grunow) | C+P | C+P | 0 | 0 |
| *Navicula angilica* Ralfs | P | P | P | P | *N. intermedia* Hantzsch ex Cleve & Grunow | C+P | C+P | C+P | C+P |
| *N. angilica* var. subsulsa Grun | 0 | 0 | 0 | P | *N. inconspicua* Grun | P | 0 | C | C |
| *N. cincta* (Ehr. )Kutzing | C+P | C+P | C+P | C+P | *N. linears* W. Smith. | P | P | C+P | C+P |
| *N. creptocyphala* Ktz | C+P | C+P | C+P | C+P | *N. longissima* Ralfs | C+P | C+P | C+P | P |
| *N. dicephala* (Ehr.) W.Smith | C | C | P | C+P | *N. microcephala* Grun | C | P | C+P | P |
| *N. palea* (Ktz. )W.Smith | C+P | C+P | C+P | C+P | *Rhoicosphenia curvata*(Kutz. ) Grunow | C+P | C+P | C+P | C+P |
| *N. obtusa* W.smith | P | 0 | 0 | P | *Rhopaladia gibba* (Ehr.) Mueller | C | 0 | 0 | 0 |
| *N. paleacea* (Grun.) Grun. | C | C | C | C | *Rossithidium Linearis* (W.Smith) Round and Bukhtiyarova | 0 | 0 | P | P |
| *N. pusilla* (Ktz.)Grun. | C+P | C+P | C+P | C+P | *Stauroneis anceps* Ehr. | C | 0 | 0 | P |
| *N. recta* Hantzsch ex Kebenh | P | C+P | C+P | P | *S. smithii* Grun. | P | P | P | 0 |
| *N.fonticola* Grunow in Van Heurck | C+P | C+P | C+P | P | *Surirella ovalis* de Brebission | C+P | C | P | C+P |
| *N. rostellata* Hustedt | P | P | P | P | *S. robusta* Ehrenberg | C+P | C+P | C+P | C+P |
| *N. sigma* (Ktz) W.Smith | C | 0 | 0 | 0 | Surirella Smith | P | 0 | 0 | 0 |
| *N. sigmoidea* (Ehr. )W.Smith | C+P | C+P | C+P | C+P | *Synedra acus* Kuetzing | C+P | C+P | C+P | C+P |
| *N. umbonata* (Ehr) Lange−Bertalot | P | P | C | 0 | *S.tabulata* var.*fasciculate* Agardh | P | C+P | C+P | P |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *N. tryblionella* Hantsch | P | 0 | C+P | P | *S. ulna* (Nitz.)Ehrenberg. | C+P | C+P | C+P | C+P |
| *N.tryblionella* var.*levidensis (W.Sm.) Grun* | P | C+P | 0 | P | *S. vaucheriae* Kuetzing | C+P | C+P | C | C+P |
| *N. vermicularis* (ktz) Hantzsch. | C+P | P | C | C+P | Sellaphora gregoryana (cleve et Grun.) Metzeltin et Lange-Bertalot | C | C | P | P |
| *N. vitrea* Norman | 0 | C | P | P | *Tabellaria fenestrate* (Lyng)Kuetzi | 0 | 0 | 0 | P |
| *Pleurosigma angulatum* W.Smith | 0 | C | C | 0 | *T. flocculosa* (Roth) Ktz. | 0 | 0 | 0 | P |
| *Pinnularia borealis* Ehr. | C+P | P | 0 | C+P | *Tryblionella acuta* (Cleve) D.G.Ma | P | 0 | C | 0 |
| *Pseudostaurosira brevistriata* (Grun.) D.M.Williams and Round | C | P | P | P | *T. hungarica* (Grun.) Frengulli | C+P | C+P | C+P | C+P |

The CCA analysis showed that some species on *C. demersum* have positive relations with environmental factors (Figure 4). *A. granulate*, *C. ocellata*, *C. tumida*, *C. placentala* var. *lineate*, *G. accuminatum*, *N. cincta*, and *N.viridula* have a significant relationship with the environmental factors as follows: $CO_2$, $PO_4$, SiO3, TOC and TA. The following epiphytic algae on *P. australis*: *Achnanthidium affinis* and *Cocconeis placentula* var. *Euglypt*, *Cymbella tumida* and *Navicula viridula* showed a significant relationship with the WT, NO2, LP, TOC, TH, Ca, TDS, EC S‰ (Figure 4). The species−environment correlations had ranged from 0.737 in the four axes to 0.880 in the first axes for *C. demersum*. While on *P. australis* was ranged 0.738-0.872. The eigenvalues were 0.332 and 0.328 for *C. demersum* and *P. australis*, respectively. The statistical analysis showed a significant correlation etween some environmental factors and epiphytic diatoms on both host plants (Table 6).

The study area is characterized by the hot desert climate, so the variations of temperature among months and seasons are well known in Iraq (Hassan and Shawaat 2015a). The water level and its flow affected on light penetration, turbidity, total suspended solids and biological factors (Ayoade *et al*., 2009; Salman *et al*., 2013). The site 4 showed high values of EC, S‰ and TDS due to its location (downstream) and the impact of illegal discharge of wastewater. The highest values of TDS might be due to mixing of the wastewater discharge with the river water (Gupta et al., 2013). pH values are important to drive many chemical and biological processes in an aquatic ecosystem. The pH results were agreed with other Iraqi aquatic ecosystem studies which are characterized by high buffer capacity (Hassan *et al*., 2014). The river was alkaline and very hard water (APAH, 2005). The $Ca^{+2}$ concentrations were higher than the $Mg^{+2}$, Na, K, and Cl concentrations during the study period and this might be due to the nature of calcareous sediment of the river (Khursid, et al., 1998). The DO values indicated that the river has a good aerated system during the study period. This resulted was a coincidence with BOD

values which were 2 mg/l. The concentrations of Total available carbon dioxide were effected with the same factors that effected on pH and TA (Wetzel, 2002). Overall the study period, the $NO_3$ concentration was higher than $NO_2$ concentration because high DO concentrations in the river. The $PO_4$ concentrations indicated that the river is oligotrophic (Wetzel, 2002). The $SiO_3$ concentrations were more than I µmole/l (76.08µg/l) which indicated that the diatoms not limited by $SiO_3$ in the river.

Diatoms are important communities hat are useful for assessment of ecological conditions in lotic systems. These algae influenced by various environmental factors as observed in this study. In particular, the diatom communities, which constitute the major part of an epiphytic community in the Iraqi river and other water bodies, are composed of a large number of species that varies in their tolerance of pollution. Thus it considered a good bio-indicators (Bellinger *et al*., 2006; Salman *et al*., 2014; Bere, 2014).

Different number of epiphytic algae taxa were recorded on the two macrophytes, where the higher number is noticed on *C. demersum* in comparing with *P. australis*, this difference is due to the architecture of these macrophytes and its effect on the availability of light and nutrients (Pettit *et al*., 2015; Messyasz *et al*., 2009). The macrophtytes have a role in nutrient availability in the water column and the sediment (Clarke and Wharton, 2001). Many authors noticed the high number of epiphytic algae found on *C. demersum* incomparable with that found on *P. australis*; in a lotic ecosystems (Kassim *et al*., 2000; Salman et al., 2014; Al−Hassany *et al*., 2014), in marshes (Al-Handal *et al*., 2014) and in pond (Messyasz *et al*., 2009). While in another study, *C. demersum* recorded similar epiphytic algae with *Typha latifolia* and lower incomparable with Nymphaea alba at Acarlar floodplain fores, Turkey (Tunca *et al*., 2014).

The richness and Shannon diversity index were recorded high value on *P. australis*. The water temperature, the light penetration and the pH effected significantly on the

**Table 4**. Total umber of species and genera of identifying diatoms (Centric (c) and pennate (P)) on the host macrophytes during the study period in Al‑Shaiyah river

| Site | | S1 | | S2 | | S3 | | S4 | |
|---|---|---|---|---|---|---|---|---|---|
| | | C | P | C | P | C | P | C | P |
| Species | *C. demersum* | 10 | 104 | 8 | 87 | 12 | 79 | 10 | 93 |
| | *P. australis* | 8 | 94 | 9 | 87 | 9 | 81 | 10 | 89 |
| Genra | *C. demersum* | 4 | 28 | 3 | 19 | 4 | 19 | 4 | 24 |
| | *P. australis* | 3 | 18 | 3 | 21 | 4 | 22 | 4 | 24 |
| Percent | *C. demersum* | 8.8 | 91.23 | 8.58 | 91.24 | 13.19 | 86.31 | 9.71 | 90.29 |
| | *P. australis* | 7.8 | 92.16 | 9.38 | 90.62 | 10 | 90 | 10.1 | 89.9 |
| Total species | *C. demersum* | 114 | | 95 | | 91 | | 103 | |
| | *P. australis* | 102 | | 96 | | 90 | | 99 | |
| Total genra | *C. demersum* | 32 | | 22 | | 23 | | 28 | |
| | *P. australis* | 21 | | 24 | | 26 | | 28 | |

**Table 5**. Range (Mean ± SD) of biological parameters for epiphytic diatoms on the host macrophytes in Al‑Shamiyah River during the study period. (the unites used for total number of epiphytic diatoms =cell × 104/ g)

| | Site | *C. demersum* | | | *P. australis* | | |
|---|---|---|---|---|---|---|---|
| | | Range | Mean | ±SD | Range | Mean | ±SD |
| Total number of  diatoms algae | 1 | 62.23–227.68 | 119.59 | 67.18 | 9.08-223.09 | 93.1 | 84.81 |
| | 2 | 17.01–169.19 | 78.61 | 56.9 | 34.58–174.19 | 94.61 | 48.41 |
| | 3 | 53.655–196.91 | 108.54 | 54.99 | 11.62–198.45 | 105.68 | 73.32 |
| | 4 | 45.68–200.38 | 105.03 | 64.64 | 10.36–218.65 | 91.39 | 81.16 |
| Total number of Centrales diatoms | 1 | 1.54–3.08 | 2.07 | 0.6 | 1.12–4.94 | 2.26 | 1.56 |
| | 2 | 0.99–3.15 | 1.83 | 0.83 | 0.95–2.24 | 11.95 | 22.51 |
| | 3 | 1.54–13.93 | 5.81 | 5.15 | 0.93–3.29 | 2.37 | 0.97 |
| | 4 | 0.98–3.47 | 2.11 | 1.02 | 0.32–25.9 | 8.19 | 10.86 |
| Total number of Pennales diatoms | 1 | 60.59–224.59 | 117.52 | (66.73 | 7.96–218.16 | 90.85 | 83.35 |
| | 2 | 16.03–167.37 | 76.79 | 56.79 | 32.34–171.96 | 82.67 | 55.27 |
| | 3 | 52.12–189 | 102.73 | 52.31 | 9.59– 195.16 | 99.61 | 76.44 |
| | 4 | 44.70–196.91 | 102.91 | 64.1 | 8.68–192.75 | 83.2 | 71.11 |
| Centrales diatoms % | 1 | 1.11–2.64 | 2 | 0.72 | 1.60–12.33 | 4.43 | 4.472 |
| | 2 | 1.08–5.95 | 3.38 | 2.3 | 1.29–6.48 | 13.99 | 26.7 |
| | 3 | 2.87–11.81 | 5.05 | 3.8 | 0.77–17.47 | 5.75 | 6.97 |
| | 4 | 0.86–3.86 | 2.38 | 1.21 | 0.95–16.22 | 8.34 | 6.67 |
| Pennals diatoms  % | 1 | 97.36–98.90 | 97.99 | 0.72 | 87.66–98.39 | 95.57 | 4.47 |
| | 2 | 94.06–98.92 | 96.63 | 2.3 | 93.62–98.71 | 86.52 | 26.7 |
| | 3 | 88.19–97.12 | 94.95 | 3.8 | 82.53–99.23 | 94.29 | 6.91 |
| | 4 | 96.14–99.14 | 97.62 | 1.21 | 83.78–99.05 | 91.6 | 6.66 |
| Richness Index(D) | 1 | 7.45 – 11.11 | 9.42 | 1.31 | 5.82 – 10.60 | 8.38 | 1.85 |
| | 2 | 7.4 – 9.53 | 8.78 | 0.82 | 7.09 – 12.01 | 9.39 | 1.98 |
| | 3 | 6.895 – 8.79 | 7.85 | 0.83 | 6.92 – 12.69 | 9.7 | 2.25 |
| | 4 | 8.89 –10.74 | 9.6 | 0.74 | 6.26 – 10.37 | 8.52 | 1.62 |
| Shannon and Weaver Index (H) | 1 | 1.42 – 2.47 | 2.01 | 0.38 | 1.61 – 2.59 | 2.25 | 0.42 |
| | 2 | 1.91 – 2.72 | 2.14 | 0.34 | 1.35 – 2.36 | 1.87 | 0.35 |
| | 3 | 1.58 – 2.15 | 1.78 | 0.23 | 1.63 – 2.75 | 2.14 | 0.44 |
| | 4 | 1.72 – 2.29 | 1.87 | 0.23 | 1.76 – 2.63 | 2.12 | 0.34 |

studied indices in this study, these factors affected the water quality of the river which limited the growth of epiphytic algae on the host plants (Salman et al., 2014). The index values were indicated that the water quality of the river ranged between moderate polluted to clean water Hasan and

Shaawiat, 2015C).

The species composition of epiphytic algae showed dominancy by pennate diatoms and differences among the sites, some species found only in one site and disappear in another site as this clearly shown in CCA analysis. These
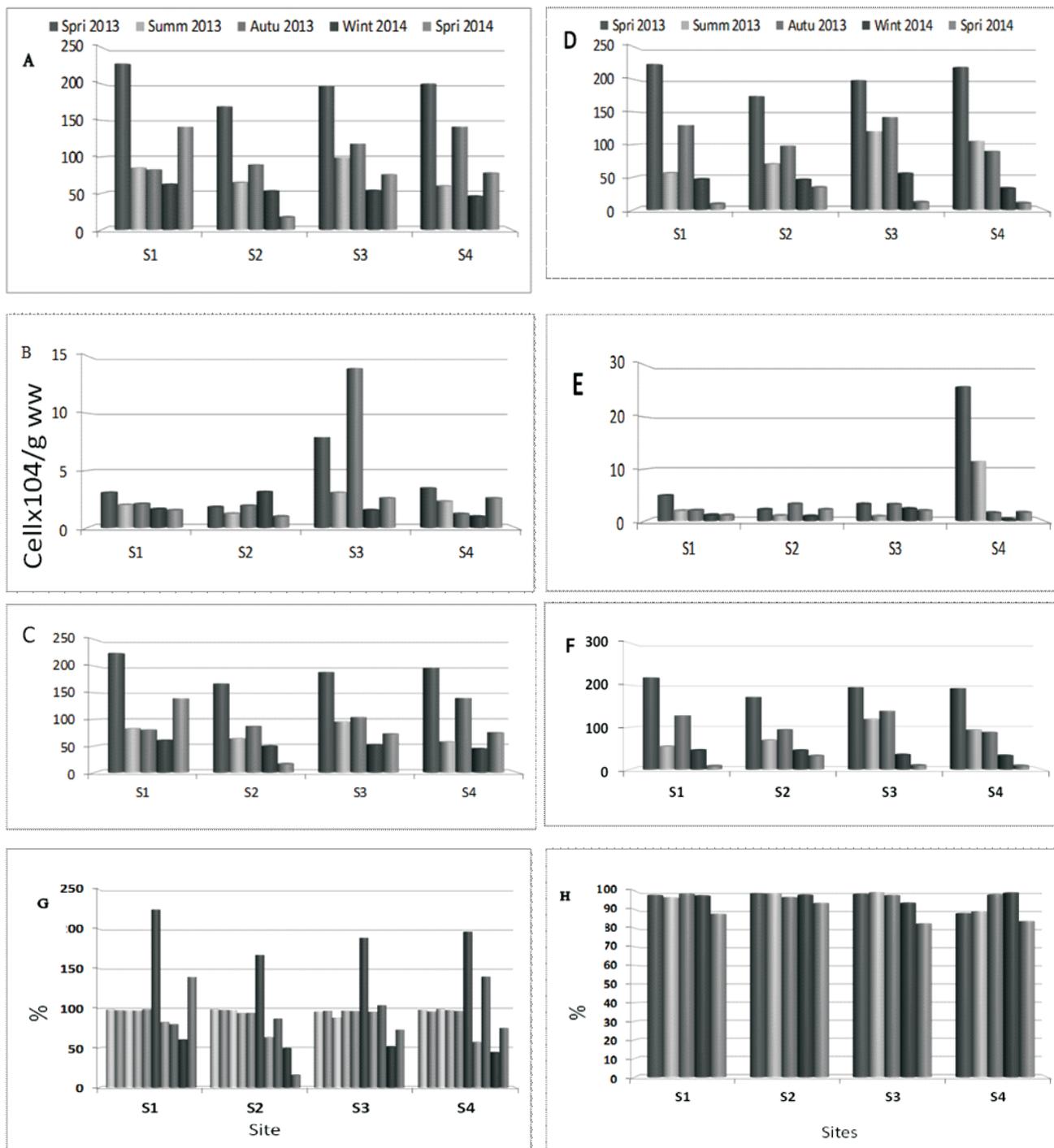


**Fig. 2**. Seasonal variations and percentage of epiphytic diatoms on *C. demersum* (A, B, C and G) and *P. australis* (D, E, F and H) During the study period in Al-Shamiyah river. A and D: total number of diatoms. B and E total number of centric diatoms. C and F: total number of pennate diatoms. G and H: the percentage of diatoms.
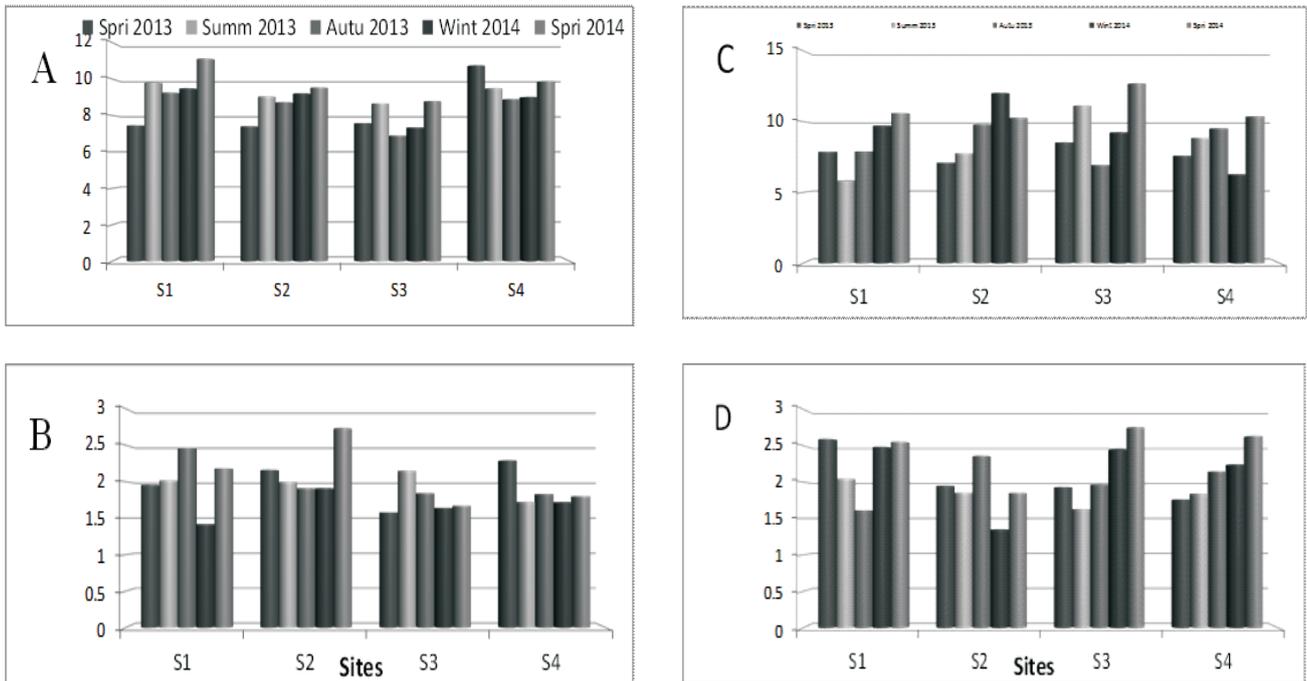
**Fig. 3**. Seasonal variations of Richness Index and Shannon and Weaver for the epiphytic diatoms on *C. demersum* (A and B) and *P. australis* (C and D) During the study period in Al‑Shamiyah river

**Table 6**. The correlation coefficient between some of the environmental factors and epiphytic diatoms (Total Number) on the host macrophytes in Al‑Shamiyah River

| Parameters | *C. demersum* | *P. australis* |
|---|---|---|
| W.T | 0.526 | 0.568 |
| C.V | -0,660 | -0.635 |
| L.P | 0.51 | 0.56 |
| Tur | -0.509 | -0.646 |
| pH | 0.73 | 0.701 |
| T.S.S | -0.501 | -0.668 |
| $CO_2$ | -0.517 | -0.622 |
| T.A | -0.539 | -0.521 |
| T.H | -0.527 | -0.524 |
| Ca | -0.569 | -0.772 |
| $NO_2$ | -0.619 | -0.519 |
| $NO_3$ | -0.755 | -0.567 |
| $PO_4$ | -0.587 | -0.728 |
| $SIO_3$ | -0.634 | -0.579 |

results may be indicated that the epiphytic algae in this study did not show preference to the host plants (Tunca *et al*., 2014). The dominance of pennate diatoms is well known in Iraqi water ecosystems (Hassan *et al*. 2007; Al‑Saadi *et al*., 2002). There was not a significant difference between the host plants based on quantities of epiphytic algae during the study period.

## CONCLUSION

The study results indicated that the river might be at risk of pollution if there no attention to improve the river monitoring program. The same results are obtained by the author when used epipelic algae as bio‑indicators in the same river (Hassan and Shawaat 2015). Different species found in the studied sites which they indicated to deterioration in the water quality in each site.

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**Fig. 4**. CCA analysis results between the diatom species on the host plants (*C. demersum* (A) and *P. australis* ( B)) and environmental factors

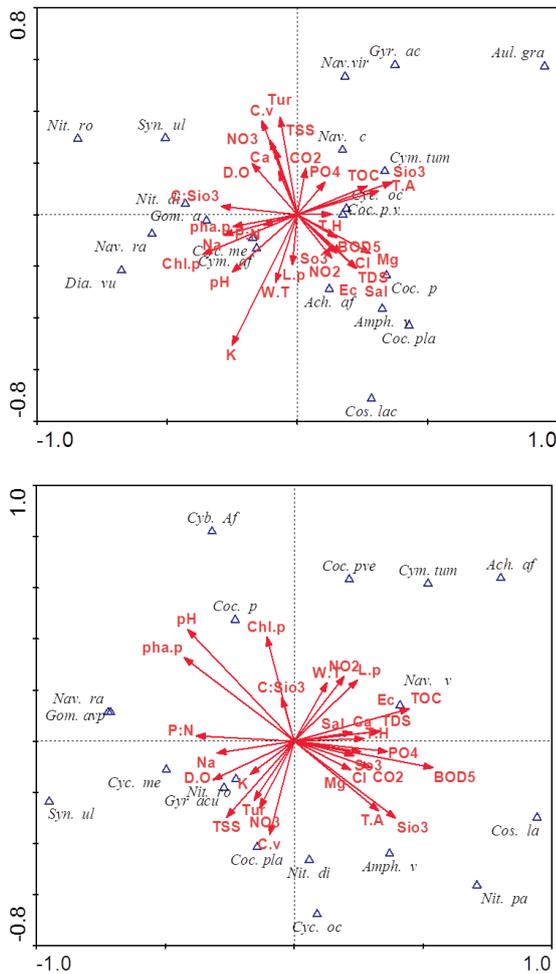Physicochemical Features and Plankton of Two Regulated High Altitude Rivers Garhwal Himalaya, India. *European Journal of Scientific Research* **27** (1): 77–92.

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# Phosphorus, Potassium and Sulphur Uptake by Rice as Influenced by Different Levels of Nitrogen and Silicon in *Typic Ustochrepts* Soil

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**Abstract**: Field experiments were carried out on rice cultivar GAR 13 to evaluate the effects of nitrogen and silicon fertilization on yield and nutrient uptake during *kharif* 2014 and 2015.The experiment encompassed four levels of nitrogen *viz.,* 0, 75, 100 and 125 kg N ha$^{-1}$ from ammonium sulphate and four levels of silicon *viz.,* 0, 200, 400 and 600 kg Si ha$^{-1}$ from calcium silicate. The application of nitrogen @125 kg ha$^{-1}$ and silicon @ 600 kg ha$^{-1}$ produced the highest rice grain and straw yields. The effect of different levels of N and Si significantly influence the phosphorus, potassium and sulphur uptake by both rice grain and straw. The maximum P and K uptake by rice grain and straw was due to application of N at 100 kg ha$^{-1}$ and Si at 600 kg ha$^{-1}$. The maximum S uptake by rice grain and straw was at 100 and 125 kg N ha$^{-1}$, respectively. Interaction effect between N and Si was significant on P, K and S uptake by grain and straw, except K uptake by rice grain.

**Keywords**: Rice, Nitrogen, Silicon, Yield, Nutrient uptake

Silicon is considered an agronomically essential element for sustainable rice production (Savant *et al.,* 1997) and is believed to play the following roles in rice plants as a Si accumulator and is absorbed as PAS by rice plants in far larger quantities than the macronutrients. Si uptake is 108 percent higher than nitrogen uptake. Rice crop producing a yield of 5000 kg ha$^{-1}$ removes 230‒470 kg Si ha$^{-1}$ and an adequate supply of Si increases the number of panicles, the number of grains per panicle, the percentage ripening and the light‒receiving posture of rice plants, thereby improving photosynthesis resulting increase fertilizer efficiency. The Si also improves the availability and utilization of P by rice plants (Savant *et al.*1997). Moreover, Si supplied rice plants can tolerate Fe, Al and Mn toxicities and the increased mechanical strength of the culm helps reduce crop lodging (Savant *et al.,* 1997; Takahashi, 1995). Nitrogen fertilization increased the number of stems and panicles per square meter and the total number of spikelet's, reflecting on grain productivity. Excessive tillering caused by inadequate nitrogen fertilization reduced the percentage of fertile tiller, filled spikelet percentage and grain mass (Mauad *et al.,* 2003). Nitrogen application significantly increased grain yield largely through an increased biomass and grain number (Mauad *et al.,* 2003).

Rice is considered to be a Si accumulator plant and tends to actively accumulate Si to tissue concentrations of 5 per cent or higher (Epstein, 1999). Application of N fertilizers is an important practice for increasing rice yields. However, when applied in excess may limit yield because of lodging, promote shading and susceptibility to insects and diseases. These effects could be minimized by the use of Si (Munir *et al.,* 2003). Information on the importance of Si in Indian rice farming system is limited (Prakash, 2002). Rice is prone to various stresses if the available soil silicon is low for absorption. Production of 5 t ha$^{-1}$ of grain yield of rice is estimated to remove about 230‒470 kg elemental Si from soil, depending upon soil and plant factors. Absorption will be about 108 % more than the N content. Adequate supply of silicon to rice from tillering to elongation stage increases the number of grains panicle‒1 and the percentage of ripening (Korndorfer *et al.,* 2001). Silicon effects the growth under P deficiency, stress by decreased Mn and Fe uptake, and increased P availability in P‒deficiency plants. Si can also decrease P uptake when P supply is high. This phenomenon has been observed in some non Si‒accumulators such as strawberry. Excess internal inorganic P has a negative effect on growth by causing inactivation of metals such as zinc, inhibiting enzyme activity, and creating abnormal osmotic pressure in the cell. Therefore, P‒excess stress can be alleviated by Si‒induced decrease of its uptake (Ma *et al.,* 2001). Hence the present study was undertaken to the phosphorus, potassium and sulphur uptake by rice as influenced by different levels of nitrogen and silicon in *Typic Ustochrepts* soil.

## MATERIAL AND METHODS

Field experiments were conducted during the Kharif season for two years 2014 and 2015 at Anand Agricultural University, Jabugam, Gujarat. Geographically, Jabugam is situated at $22^0 17'37.70"$ north latitude, $73^0 46'41.02"$ east longitude with an elevation of 92 meters above mean sea level. The climate of Jabugam region is semi-arid and sub-tropical with hot summer and cold winter. In this region, generally monsoon commences in the month of June and retreats from the end of September. Most of the rainfall is received from south-west monsoon currents. July and August are the months of heavy showers. The soil type of Gujarat varies from medium black to loamy sand with a good drainage capacity. The total rainfall of the region is about 800-1000 mm. Average minimum and maximum temperature of both the year of study was 19.6°C and 33.3°C, respectively. The soil was loamy sand, with a sand, fine sand, silt and clay composition of 49.85, 26.6, 10.0 and 12.1 per cent, respectively. The soil chemical analysis indicates: pH 6.32 and available N, Si, $P_2O_5$, $K_2O$ was 313, 190, 88 and 221kg $ha^{-1}$ respectively, S-3.39 mg $kg^{-1}$, OC -6.32 g $kg^{-1}$ and EC-0.43 dS $m^{-1}$. The experiment was in randomized block design with factorial concept and three replications and sixteen combined treatments on Gujarat Anand Rice 13 variety with plot size of 5.0 × 3.6 m. The entire dose of phosphorus as per recommendation was applied through single super phosphate. Four levels (0, 75, 100 and 125 kg) of N were applied through ammonium sulphate and four levels (0, 200, 400 and 600 kg) of Si through calcium silicate at the time of sowing. Nitrogen was applied as per the treatments as scheduled in the form of Ammonium sulphate in 3 equal splits (1/3 basal, 1/3 at active tillering stage and 1/3 at panicle initiation stage).

Available phosphorus, potassium and Sulphur was analyzed as per the standard procedures advised by Jackson (1973) and Williams and Steinberg (1959), respectively.

## RESULTS AND DISCUSSION

**Grain and straw yield:** The application of nitrogen had significant effect on grain and straw yields of rice. The treatment 125 kg N $ha^{-1}$ gave significantly higher grain (6445 kg $ha^{-1}$) and straw (8658 kg $ha^{-1}$) yields over the control. The increase in yield as a result of nitrogen application could be due to marginal nitrogen content of soil, improvement in root development and vegetative growth as well. The improvement in yield attributing traits may be ascribed to the improved vegetative growth due to N fertilization, facilitating photosynthesis, thereby increasing translocation of organic food materials towards the reproductive organs; which enhanced the formation of panicles with fertile grains. Sudhakar *et al.* (2006) also observed 16.7 per cent increase in grain yield with application of N at 160 kg $ha^{-1}$ as compared to 80 kg N $ha^{-1}$. Above all, excess N also prolongs the vegetative growth at the cost of reproductive growth, thus, diminishing the production of carbohydrates (Mauad *et al.,* 2003). The rice grain and straw yields per plot was significantly influenced by silicon application. The significantly higher grain (6163 kg $ha^{-1}$) and straw (8536 kg $ha^{-1}$) yields was recorded due to silicon application at 600 kg $ha^{-1}$. The increase in rice yield might be due to increased availability of silicon. The lower yield in the control compared to silicon fertilized plots, might be due to leaching and fixation loss of native silicon in submerged conditions which is inadequate in meeting the Si requirement by the crop for producing higher grain yield. The increase in yield with Si application could be due beneficial effects *viz.,* decreasing mutual shading by improving leaf erectness, decreasing susceptibility to lodging, decreasing the incidence of infections with root parasites and pathogens, leaf pathogens and preventing manganese and iron toxicity or both. Chen *et al.* (2011) stated that silicon application increased grain yield by increase of spikelet number, filled spikelet percentage and 1000-seed weight. Many earler workers also reported that grain yield increased by silicon application (Ma and Takahashi, 2002; Mauod *et al.* 2003; Malav *et al.,* 2015; Meshram *et al.,* 2015).

**Phosphorus uptake (kg $ha^{-1}$):** The varying levels of N significantly influenced the P uptake by rice grain and straw (Table 1). The 100 kg N $ha^{-1}$ gave significantly higher P uptake by rice grain and straw. The application of silicon had significant effect on P uptake by grain and straw of rice. The maximum P uptake by rice grain and straw was due to application of 600 kg Si $ha^{-1}$ which gave significantly higher P uptake by grain and straw. Interaction effect between N and Si was significant on P uptake by grain and straw with different combinations of N and Si application (Table 2). Combination of 75 kg N $ha^{-1}$ and 600 kg Si $ha^{-1}$ recorded significantly highest P uptake by grain (16.15 kg $ha^{-1}$) and straw (8.88 kg $ha^{-1}$) over all other combination. This increase in P uptake by silicon application might be due to increase in soil available P, as both of these nutrients are absorbed by plants. Phosphorus use efficiency also enhances by silicon application and the beneficial effect of silicon when available P is low, it may due to partial substituting of silicon for P or an improvement of P availability in soil. On mineral soils with low soil pH, phosphorus present as complex with Al and Fe phosphate may become available to plant with addition of silicon thereby increasing crop yield. The increase in soil

available P when silicate and phosphate were added together could be explained on the basis of formation of alumino silicates or phosphates which decreased P fixation.

Presence of silicon increased phosphorus concentration and P uptake due to enhanced phosphate absorption and it was attributed to the availability of silicate ions to displace the

**Table 1.** Effect of nitrogen and silicon on yield and nutrients uptake by rice under low land conditions (Pooled data of 2 years)

| Treatments | | Nutrients uptake (kg ha$^{-1}$) | | | | | | Yield (kg ha$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|
| | | P | | K | | S | | Grain | Straw |
| | | Grain | Straw | Grain | Straw | Grain | Straw | | |
| **Nitrogen levels (kg ha$^{-1}$)** | | | | | | | | | |
| $N_0$ | | 10.78 | 5.75 | 56.59 | 105 | 8.73 | 12.86 | 5241 | 6961 |
| $N_{75}$ | | 12.74 | 7.00 | 68.12 | 126 | 10.24 | 15.27 | 6040 | 7982 |
| $N_{100}$ | | 13.35 | 7.37 | 71.71 | 136 | 11.05 | 16.36 | 6163 | 8541 |
| $N_{125}$ | | 11.97 | 5.73 | 65.69 | 123 | 11.30 | 15.42 | 6445 | 8658 |
| CD (p=0.05) | | 0.87 | 0.49 | 4.23 | 8.0 | 0.66 | 1.07 | 221 | 422 |
| **Silicon levels (kg ha$^{-1}$)** | | | | | | | | | |
| $Si_0$ | | 11.15 | 5.06 | 59.60 | 106 | 9.43 | 11.81 | 5693 | 7319 |
| $Si_{200}$ | | 11.89 | 5.87 | 63.86 | 117 | 9.97 | 14.60 | 5944 | 7934 |
| $Si_{400}$ | | 12.33 | 6.77 | 67.47 | 127 | 10.53 | 16.39 | 6091 | 8354 |
| $Si_{600}$ | | 13.47 | 8.15 | 71.18 | 140 | 11.39 | 17.11 | 6163 | 8536 |
| CD (p=0.05) | Si | 0.87 | 0.49 | 4.23 | 8 | 0.66 | 1.07 | 221 | 422 |
| | N x Si | S | S | NS | S | NS | S | NS | NS |
| CV( %) | | 12.3 | 13.1 | 11.2 | 11.4 | 11.1 | 12.3 | 6.2 | 9.5 |

**Table 2.** Interaction effect of N x Si on P and S uptake by grain and straw at harvest (Pooled data of 2 years)

| Silicon (kg ha$^{-1}$) | | Nitrogen (kg ha$^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 75 | | 100 | | 125 | |
| | | Grain | Straw | Grain | Straw | Grain | Straw | Grain | Straw |
| **Phosphorus uptake by grain and straw (kg ha$^{-1}$)** | | | | | | | | | |
| $Si_0$ | | 9.33 | 4.12 | 11.00 | 6.91 | 12.10 | 5.39 | 12.15 | 3.81 |
| $Si_{200}$ | | 10.18 | 4.36 | 11.45 | 4.28 | 14.15 | 8.14 | 11.79 | 6.69 |
| $Si_{400}$ | | 11.59 | 5.79 | 12.35 | 7.91 | 13.93 | 7.30 | 11.46 | 6.07 |
| $Si_{600}$ | | 12.02 | 8.74 | 16.15 | 8.88 | 13.22 | 8.64 | 12.49 | 6.33 |
| | | **Grain** | | | | **Straw** | | | |
| CD (p=0.05) | | 1.74 | | | | 0.98 | | | |
| CV (%) | | 12.3 | | | | 13.1 | | | |
| **Sulphur uptake by grain and straw (kg ha$^{-1}$)** | | | | | | | | | |
| $Si_0$ | | 6.81 | 9.79 | 11.14 | 10.78 | 9.78 | 12.93 | 10.02 | 13.73 |
| $Si_{200}$ | | 8.52 | 12.00 | 10.11 | 14.34 | 10.38 | 15.58 | 10.88 | 16.48 |
| $Si_{400}$ | | 9.60 | 14.01 | 8.82 | 16.59 | 12.23 | 19.04 | 11.48 | 15.92 |
| $Si_{600}$ | | 10.01 | 15.62 | 10.91 | 19.37 | 11.81 | 17.88 | 12.81 | 15.55 |
| | | **Grain** | | | | **Straw** | | | |
| CD (p=0.05) | | 1.33 | | | | 2.13 | | | |
| CV( %) | | 11.1 | | | | 12.3 | | | |

fixed phosphorus ions in the soil leading to increased phosphorus uptake. Depressing effect of silicate on P retention capacity of soil may be the reason to increase the level of water soluble P in the soil. Hence, it can be inferred that the increase in the uptake of P with the application of silicon might be attributed to enhanced availability and uptake of nutrients from soil which is made possible by desorption of P (Subramaniyan and Gopalaswarmy, 1991). The overall beneficial effect of Si may be attributed to a higher P: Fe and P: Mn ratio in the shoot inactive due to the decreased Mn and Fe uptake thus indirectly improved P utilization within rice plants. Singh *et al.* (2006) observed that 180 kg ha$^{-1}$ of silicon increased nitrogen and phosphorus levels in the grain and straw of rice and suggested that silicon application at lesser amounts can be beneficial in increasing grain yield and growth of cereal crops. Phosphorus use efficiency also enhances by silicon application and the beneficial effect of silicon when available P is low, it may due to partial substituting of silicon for P or an improvement of P availability in soil. On mineral soils with low soil pH, phosphorus present as complex with Al and Fe phosphate may become plant available with addition of silicon thereby increasing crop yield. Presence of silicon increased phosphorus concentration and P uptake due to enhanced phosphate absorption and it was attributed to the availability of silicate ions to displace the fixed phosphorus ions in the soil leading to increased phosphorus uptake. Depressing effect of silicate on P retention capacity of soil may be the reason to increase the level of water soluble P in the soil. Hence, it can be inferred that the increase in the uptake of P with the application of silicon might be attributed to enhanced availability and uptake of nutrients from soil which is made possible by desorption of P .

***Potassium* uptake (kg ha$^{-1}$):** Thre altering levels of N and Si significantly influenced the K uptake by rice grain and straw. The individual application of nitrogen at 100 kg ha$^{-1}$ and silicon at 600 kg ha$^{-1}$ gave significantly higher K uptake by rice grain and straw (Table 1). Interaction effect between N and Si was found significant on K uptake by straw with different combinations of N and Si application (Table 3). Combination of 75 kg N ha$^{-1}$ and 600 kg Si ha$^{-1}$ recorded significantly the highest K uptake (156 kg ha$^{-1}$) by straw over all other combination. Interaction of applied K and Si in soil seems to have beneficial effects on rice yield as silification of cell linked with K and its deficiency reduced by the accumulation of Si in the epidermal cells of the leaf blades, thus increasing susceptibility of plant to rice blast. Combined use of various levels of Si along with K @ 40 kg ha$^{-1}$ increased the total K uptake, and the treatment which recorded the highest total K uptake showed 60.51 per cent increase over control. Field

**Table 3.** Interaction effect of N x Si on potassium uptake by straw of rice at harvest (Pooled data of 2 years)

| Silicon levels (kg ha$^{-1}$) | Nitrogen levels (kg ha$^{-1}$) | | | |
|---|---|---|---|---|
| | 0 | 75 | 100 | 125 |
| **Potassium uptake by straw (kg ha$^{-1}$)** | | | | |
| Si$_0$ | 86 | 111 | 123 | 103 |
| Si$_{200}$ | 101 | 107 | 130 | 131 |
| Si$_{400}$ | 113 | 131 | 143 | 122 |
| Si$_{600}$ | 118 | 156 | 150 | 135 |
| CD (p=.05) | 16.0 | | | |
| CV( %) | 11.4 | | | |

experiment conducted at Varanasi also observed increased NPK concentration and uptake due to Si application @180 kg Si ha$^{-1}$ full as basal application (Singh *et al.,* 2006; Malav *et al.,* 2015).

**Sulphur uptake (kg ha$^{-1}$):** The varying levels of N and Si significantly influenced the S uptake by grain and straw. The treatment 125 kg N ha$^{-1}$ gave significantly higher S content by grain. However, significantly the maximum S uptake by straw was observed at 100 kg N ha$^{-1}$. On the other hand the significantly higher sulphur uptake by grain and straw was with the application of silicon at 600 kg ha$^{-1}$. The interaction of N x Si on S uptake by rice grain revealed that N application at 125 kg ha$^{-1}$ combined with 600 kg Si ha$^{-1}$ application showed highest significant S uptake by rice grain (12.81 kg ha$^{-1}$) and in straw was observed with N application at 75 kg ha$^{-1}$ combined with 600 kg Si ha$^{-1}$. The present observations are in accordance with the study of earlier workers ( Jawahar and Vaiyapuri 2013; Singh *et al*., 2006 and Malav *et al*., 2015).

From the forgoing results, it can be concluded that individual application of nitrogen @125 kg ha$^{-1}$ and silicon @ 600 kg ha$^{-1}$ produced the highest rice grain and straw yields as well as P, K and S uptake by rice grain and straw.

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# Response of Irrigation Regimes and Nitrogen Doses on Consumptive Water Use, Water Use Efficiency and Moisture Extraction Pattern of Late Sown Wheat

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*Abstract:* To find out response of irrigation regimes and nitrogen doses on consumptive water use, water use efficiency and moisture extraction pattern of late sown wheat was carried out at CCS Haryana Agricultural University, Hisar for two seasons. The experiment consisting three irrigation regimes *viz.* one irrigation at CRI ($I_1$), two irrigations at CRI and heading ($I_2$) and four irrigations at CRI, late tillering, heading and milking ($I_3$) in main plots and five nitrogen doses *viz.* control ($F_0$ −0 kg N ha$^{-1}$), 50 kg N ha$^{-1}$ ($F_1$), 100 kg N ha$^{-1}$ ($F_2$), recommended dose of nitrogen ($F_3$ −150 kg N ha$^{-1}$) and 200 kg N ha$^{-1}$ ($F_4$) in sub-plots was laid out in strip plot design with four replications. The water use increased with increased irrigation regimes. 200 kg N ha$^{-1}$ crop consumed markedly higher water than 0 kg N ha$^{-1}$. The moisture extraction from surface layer increased slightly and deeper profile layers decreased slightly with increased irrigation regimes. 200 kg N ha$^{-1}$ consumed more moisture from deeper profile than control. WUE decreased with increased irrigations, whereas, N application improved WUE at all irrigation regimes. The increase irrigation number from one to two, two to four and one to four irrigations increased the grain yield of wheat by about 25.1, 20.4 and 50.6 % during 2010−11 and 21.0, 21.9 and 47.5 % during 2011−12, respectively. Grain yield increased significantly upto 150 kg N ha$^{-1}$.

*Keywords:* Irrigation, Nitrogen, WUE, Moisture extraction pattern, Late sown wheat

Wheat is the most important food crop in India and improvement in its productivity has played a key role in making the country self-sufficient in food grain. However, in the past decade a general slowdown in increase the productivity of wheat has been noticed, particularly under environments relatively unfavourable for growth and development of wheat (Jat *et al.*, 2011). Late sowing of wheat is a major problem in the rice-wheat and cotton-wheat cropping system (Khan *et al.*, 2010; Yasmeen *et al.*, 2012). Irrigation water and fertilizers are the two vital but costly inputs in irrigated farming. Irrigation is necessary to grow crops because of insufficient amount of rain water and high atmospheric evaporative demand by crops. Proper growth and development of wheat needs favourable soil moisture in the root zone. Extractable water capacity of soil has significant influence on wheat grain yield and water productivity response to irrigation (Arora *et al.,* 2007). Nitrogen is a key element in plant nutrition and is important constituent of chlorophyll and plays a vital role in all metabolic processes. Proper use of nitrogen fertilizer is also considered for farm profitability and environment protection (Ali *et al*., 2012). Irrigation water dissolved the fertilizers and was made available to the crop for proper growth and development. Synergistic interactions between irrigation and nitrogen levels on grain yield have been reported by Shirazi *et al.*,

2014. Therefore, an attempt has been made to evaluate the response of irrigation regimes and nitrogen doses on consumptive water use, water use efficiency and moisture extraction pattern of late sown wheat.

## MATERIAL AND METHODS

Experiment was conducted during 2010−11 and 2011−12 at CCS Haryana Agricultural University, Hisar (India) located in Indo-Gangetic plains of North-West India with latitude of $29^0$10' North and longitude of $75^0$46' East at 215.2 meters above mean sea level. The soil of the field was sandy loam, slightly alkaline in pH (7.9), low in organic carbon, poor in available nitrogen and medium in available phosphorus and available potassium. The experiment consisting of three irrigation regimes *viz.* one irrigation at CRI ($I_1$), two irrigations at CRI and heading ($I_2$) and four irrigations at CRI, late tillering, heading and milking ($I_3$) in main plots and five nitrogen levels *viz.* control ($F_0$ −0 kg N ha$^{-1}$), 50 kg N ha$^{-1}$ ($F_1$), 100 kg N ha$^{-1}$ ($F_2$), recommended dose of nitrogen ($F_3$ −150 kg N ha$^{-1}$) and 200 kg N ha$^{-1}$ ($F_4$) in sub-plots was laid out in strip plot design with four replications. Late sown wheat variety (WH 1021) was sown with seed drill @ 125 kg/ha on 23$^{rd}$ Dec. during both the years. Half N (urea) and full P (DAP) were applied as basal dose. Remaining half urea was top dressed

after 1st irrigation. SSP fertilizer was applied in Control treatment as basal dose. To control weeds, one hand weeding was done at 30 days after sowing (DAS). Other management practices were adopted as per recommendations of wheat crop. The consumptive use of water for different treatments was determined by using the following formula given by Dastane 1972.

$$WUE = \frac{\text{Grain yield (kg/ha)}}{\text{Consumptive use of water (cm)}}$$

$U = \Sigma u$; and $u = (E_0 \times 0.8) + \Sigma (M_{1i} - M_{2i}) + GWC + ER$

Where,

$U$ = Total seasonal consumptive use;

$u$ = Consumptive use during a given interval;

$E_0$ = Pan evaporation (mm) from USWB class I open pan evaporimeter during the interval from the day of irrigation to the day when sampling in wet soil is possible;

0.8 = Pan coefficient

$M_{1i}$ = Soil moisture content (mm) of $i$th soil layer on the day when sampling in irrigated soil is possible;

$M2_i$ = Soil moisture (mm) of $i$th soil layer on the day just before the next irrigation;

GWC = Ground water contribution during the interval; the groundwater contribution was considered negligible during the crop growth season;

ER = Effective rainfall (mm) during the interval

Water use efficiency (kg ha$^{-1}$ cm$^{-1}$) was calculated by dividing grain yield (kg ha$^{-1}$) to evapotranspiration (cm) (Scott 2000).

Soil moisture extraction pattern was calculated on the basis of soil moisture data recorded from different layers at monthly interval. The small amount of rainfall received during crop season has been added to the first upper soil layer of the profile. The fraction of each layer was computed from the total water use of whole profile. Data was analysed statistically with OPSTAT software.

## RESULTS AND DISCUSSION

**Weather:** Mean weekly maximum temperature ranged between 11.2 °C (1st SMW, late emergence stage) to 41.1 °C (18th SMW, at maturity) in 2010‒11 and 17 °C (2nd SMW, at CRI) to 36.8 °C (18th SMW, at maturity) in 2011‒12, respectively. However, mean minimum temperature ranged between 3.2 °C (3rd SMW, tillering) to 23.7 °C (18th SMW) in 2010‒11 and 1.2 °C (52nd SMW, at emergence) to 18.2 °C (18th SMW) in 2011‒12, respectively. Amount of rainfall as well as its distribution during the crop growth season is very important for growth and development of the crop. During *Rabi* season of 2010‒11, total rainfall received was 116.3 mm

with 12 rainy days. The vegetative phases received 80.3 mm rainfall. The highest rainfall was received during 52nd SMW which coincide with emergence of crop. Rest of the rainfall was received from flag leaf to physiological maturity. During Rabi 2011‒12, total rainfall was only 47.7 mm with 3 rainy days. Only one rainy day was occurred during tillering phase and rest two was at late maturity phase. The vegetative and reproductive phases received 14.4 and 33.3 mm rainfall, respectively in 2011‒12. The highest rainfall of 25.5 mm was recorded during dough stage. *Rabi* 2010‒11 received more rainfall than normal (63.7 mm) than that of *Rabi* 2011‒12. However, the distribution of rainfall was very poor in both the years. The mean weekly highest sunshine hours were recorded at physiological maturity (9.9 and 9.8 hrs) and lowest just after emergence (1.2 and 1.8 hrs) during 2010‒11 and 2011‒12, respectively. The seasonal cumulative pan evaporation values were 331 mm in 2010‒11 and 383 mm in 2011‒12, respectively. The mean weekly wind speed values remained in the range of 2.5 to 9.2 km hr$^{-1}$ in 2010‒11. However, the range of mean weekly wind speed was low (1.2 to 7.3 km hr$^{-1}$) in 2011‒12.

**Consumptive Water Use:** The seasonal water use increased in all the treatments with advancement of crop season during both the years. The maximum consumption of water was recorded in four irrigations crop at CRI, late tillering, heading and milking ($I_3$) followed by two irrigations at CRI and heading ($I_2$) and minimum consumption of water was recorded with one irrigation crop. This might be due to application of more irrigation in $I_3$ than $I_2$ and $I_1$. Frequent recharging of profile through irrigation in $I_3$ kept the soil surface wet, resulted in more loss of water through process of evaporation (Table 1 and 2). Similar findings were also observed by Dhaka *et al*., 2007; Damdar *et al*., 2014. Applied nitrogen had marginal influence on seasonal water use. The maximum consumption of water was with the application of 200 kg N ha$^{-1}$ and consumption decreased with decreased N doses. Maximum consumption of water recorded with 200 kg N ha$^{-1}$ might be due to more evapo‒transpiration by the crop which is primarily a function of weather thus, once the soil surface is covered completely by foliage, there will be practically no or little increased in it (Viets, 1962). In treatment combinations, maximum consumption of water was observed in crop with four irrigations (CRI, late tillering, heading and milking) and 200 kg N ha$^{-1}$ *i.e.* $I_3F_4$ treatment and minimum consumption with one irrigation and 0 kg N ha$^{-1}$ *i.e.* $I_1F_0$. Crop water use increased with the increase in water supply in every N level. This is because of the relative decrease in WUE at higher levels of irrigation compared with WUE at deficit irrigation (Wang *et al*., 2012).

**Water Use Efficiency:** The water use efficiency decreased with the increased irrigation regimes during both the years of experimentation (Table 1 and 2). Frequent recharging in $I_3$ provided the plenty of water to crop and the loss of water was at potential rate, this is also visible from the crop water use and cumulative pan evaporation data. These findings are in harmony with the scientific approaches that supposed the plant roots could be extract more soil water from a greater depth under stress conditions as compared to those irrigated at a relatively wet. Several studies have reported WUE values that were higher under water deficit than high irrigation condition, especially when irrigation is applied in the critical stages of plant development (Mandal *et al.*, 2005; Afzal Ahmad, 2016). However, in contrary to the irrigation influence, the N rate positively influenced the WUE of wheat (Wang *et al.*, 2012). Water use efficiency increased with increased nitrogen dose. It was highest with treatment 200 kg N ha$^{-1}$, followed by RDF (150), 100, 50 kg N ha$^{-1}$ and lowest with control (0 kg N ha$^{-1}$). WUE is mainly dependent on two factors viz., grain yield and consumptive use of water. The higher water use efficiency at higher nitrogen doses was mainly due to higher grain yield of crop with similar water use at higher nitrogen doses. These results are in agreement with Pradhan *et al.*, 2013. The marked increase in water use efficiency with increasing dose of nitrogen over control was because of poor crop growth right from the beginning of crop season and drastic reduction in crop yield due to non availability of nutrient in control treatment. Maximum WUE was found in $I_1F_4$ and minimum in $I_1F_0$ during both the years. The results of the current experiment showed that the highest WUE was obtained at 200 kg N ha$^{-1}$ application rate combined with low irrigation during both the season. These obtained trends are in general agreement with those reported by Dhaka *et al.*, 2007; Wang *et al.*, 2012; Abdelraouf *et al.*, 2013.

**Table 2.** Effect of irrigation regimes and nitrogen doses combinations on the crop water use and water use efficiency of wheat

| Treatments | Crop water use (cm) | | WUE (kg ha$^{-1}$ cm$^{-1}$) | |
|---|---|---|---|---|
| | 2010–11 | 2011–12 | 2010–11 | 2011–12 |
| $I_1F_0$ | 18.61 | 16.88 | 7.61 | 8.87 |
| $I_1F_1$ | 18.74 | 17.42 | 12.51 | 14.30 |
| $I_1F_2$ | 18.91 | 17.90 | 14.81 | 16.67 |
| $I_1F_3$ | 19.05 | 18.17 | 16.01 | 17.83 |
| $I_1F_4$ | 19.23 | 18.48 | 16.18 | 17.89 |
| $I_2F_0$ | 23.49 | 22.50 | 8.29 | 9.11 |
| $I_2F_1$ | 23.85 | 23.06 | 12.39 | 13.16 |
| $I_2F_2$ | 24.30 | 24.43 | 14.29 | 14.59 |
| $I_2F_3$ | 24.24 | 24.67 | 15.35 | 15.41 |
| $I_2F_4$ | 24.66 | 24.82 | 15.50 | 15.75 |
| $I_3F_0$ | 28.97 | 28.26 | 8.41 | 8.96 |
| $I_3F_1$ | 29.56 | 30.51 | 11.98 | 12.01 |
| $I_3F_2$ | 29.42 | 31.38 | 14.11 | 13.77 |
| $I_3F_3$ | 30.15 | 31.83 | 14.73 | 14.56 |
| $I_3F_4$ | 30.85 | 31.98 | 14.90 | 14.98 |

*Whereas, $I_1$ –one irrigation at CRI, $I_2$ –two irrigations at CRI and heading, $I_3$ – four irrigations at CRI, late tillering, heading and milking, $F_0$ –0 kg N ha$^{-1}$, $F_1$ –50 kg N ha$^{-1}$, $F_2$ –100 kg N ha$^{-1}$, $F_3$ –150 kg N ha$^{-1}$ and $F_4$ –200 kg N ha$^{-1}$

**Moisture extraction pattern:** The quantum of moisture extraction from different soil profile layers decreased with the increase in profile depth during both the years (Fig. 1). This low moisture extraction from the deeper profile layer was may be due to low root density in deeper layers. The highest moisture extraction from the top layer was because of more root density and exposure of surface layer to the outer atmosphere which is more influenced by the weather parameters like air temperature, sunshine hours and wind speed. In terms of percentage, the moisture extracted from 15-45 cm layer was around 33 and 30% during 2010–11 and

**Table 1.** Effect of irrigation regimes and nitrogen doses on the crop water use and water use efficiency (WUE) of late sown wheat

| Treatments | Crop water use (cm) | | WUE (kg ha$^{-1}$ cm$^{-1}$) | |
|---|---|---|---|---|
| | 2010–11 | 2011–12 | 2010–11 | 2011–12 |
| **Irrigation levels** | | | | |
| $I_1$– One irrigation (CRI) | 18.91 | 17.77 | 13.46 | 15.22 |
| $I_2$ – Two irrigations (CRI and heading) | 24.11 | 23.90 | 13.20 | 13.69 |
| $I_3$– Four irrigations (CRI, late tillering, heading and milking) | 29.79 | 30.79 | 12.86 | 12.95 |
| **Nitrogen levels** | | | | |
| $F_0$–0 kg N ha$^{-1}$ | 23.69 | 22.55 | 8.16 | 8.99 |
| $F_1$–50kg N ha$^{-1}$ | 24.05 | 23.66 | 12.25 | 12.95 |
| $F_2$–100 kg N ha$^{-1}$ | 24.21 | 24.57 | 14.35 | 14.75 |
| $F_3$–150 kg N ha$^{-1}$ | 24.48 | 24.89 | 15.27 | 15.64 |
| $F_4$–200 kg N ha$^{-1}$ | 24.91 | 25.09 | 15.43 | 15.95 |

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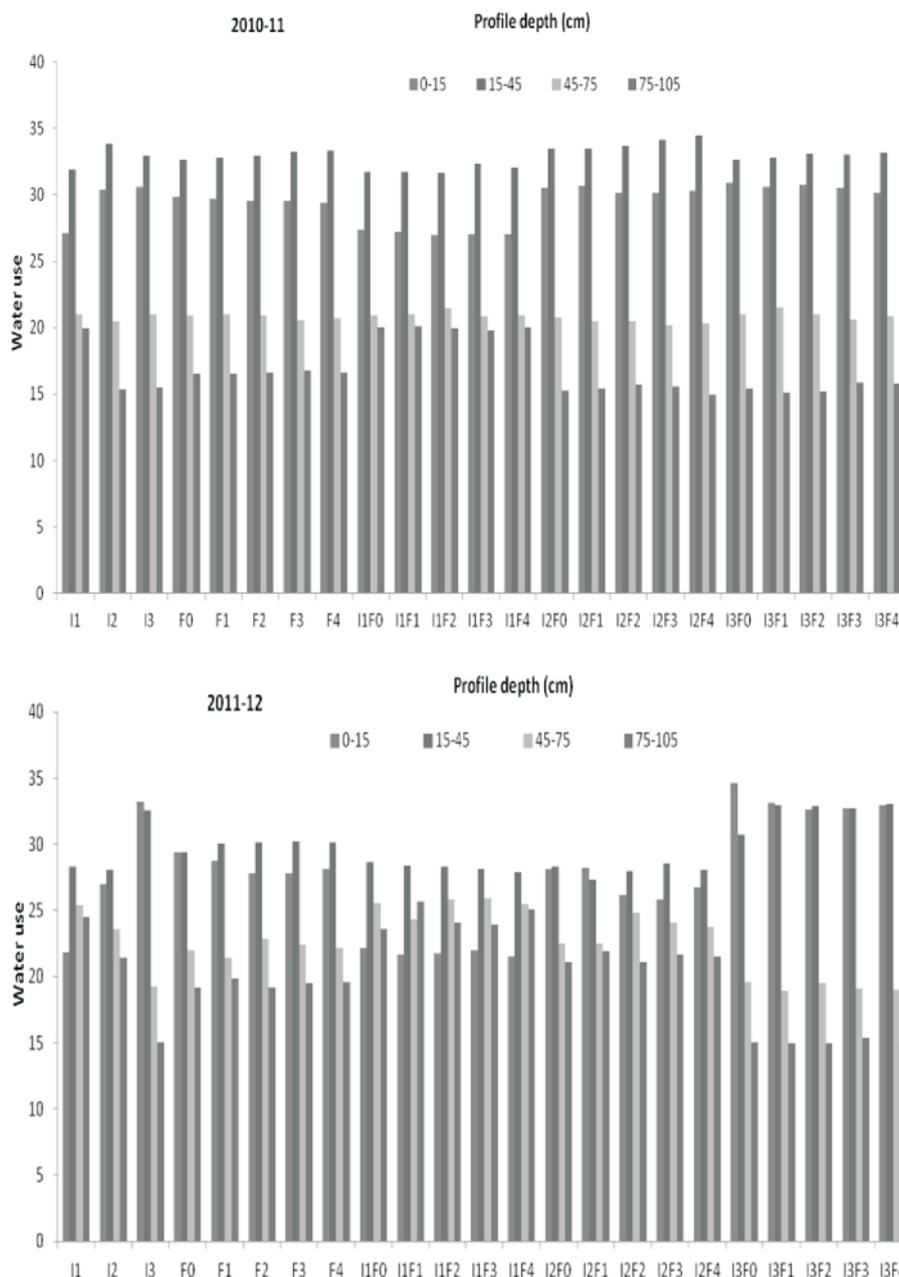


**Fig. 1.** Effect of irrigation regimes and nitrogen doses on profile moisture extraction pattern (%) of late sown wheat

2011–12, respectively, whereas, the water use from the 0–15 cm surface layer was around 29 and 27%, water use from 45–75 cm was around 21 and 23% and from 75–105 cm was around 17 and 20% during 2010–11 and 2011–12, respectively. This low percentage of moisture extraction from top layer *i.e.* 0–15 cm surface layer in comparison to 15-45 cm layer was because of lesser layer depth. This fact can be further fortified by calculating the water use per cm of soil depth as in 0–15 cm layer. It was 0.42 and 0.36 cm cm$^{-1}$ in $I_1$ and 0.49 and 0.50 cm cm$^{-1}$ in $I_3$ during 2010–11 and 2011–12 in

comparison to 15-45 cm layer the respective values for $I_1$ and $I_3$ are 0.25 and 0.26 cm cm$^{-1}$ in 2010–11 and 0.23 and 0.25 cm cm$^{-1}$ in 2011–12, respectively. However from 75–105 cm layer, the respective values for $I_1$ and $I_3$ are 0.16 and 0.12 in 2010–11 and 0.20 and 0.11 in 2011–12. The moisture extraction pattern in different treatments of irrigation indicate that with the increase in irrigation regimes the moisture extraction from surface layer has been increased and declined the moisture extraction from the deepest profile layer by 1–2 %, this may be because of more wetting of surface layer due to increased

**Table 3.** Interaction between irrigation regimes and nitrogen doses on grain yield (kg ha$^{-1}$) of late sown wheat (2010–11 and 2011–12)

| Treatments | 1 Irrigation (I$_1$) | | 2 Irrigations (I$_2$) | | 4 Irrigations (I$_3$) | | Mean | |
|---|---|---|---|---|---|---|---|---|
| | 2010–11 | 2011–12 | 2010–11 | 2011–12 | 2010–11 | 2011–12 | 2010–11 | 2011–12 |
| 0 kg N ha$^{-1}$ (F$_0$) | 1415 | 1498 | 1947 | 2050 | 2435 | 2531 | 1932 | 2026 |
| 50 kg N ha$^{-1}$ (F$_1$) | 2344 | 2491 | 2955 | 3035 | 3540 | 3665 | 2946 | 3064 |
| 100 kg N ha$^{-1}$ (F$_2$) | 2801 | 2984 | 3472 | 3564 | 4150 | 4321 | 3474 | 3623 |
| 150 kg N ha$^{-1}$ (F$_3$) | 3051 | 3240 | 3720 | 3803 | 4441 | 4635 | 3737 | 3893 |
| 200 kg N ha$^{-1}$ (F$_4$) | 3110 | 3305 | 3823 | 3910 | 4596 | 4791 | 3843 | 4002 |
| Mean | 2544 | 2704 | 3183 | 3272 | 3832 | 3989 | 3187 | 3322 |
| CD (p=0.05) | 252 (2010–11) | | | | 191 (2011–12) | | | |

frequency resulted in more evaporation losses from the surface layer. Similar declining contribution of deeper profile layer to total crop water use trend was also seen by Dhaka *et al.*, 2007; Afzal Ahmad, 2016. The increase in dose of nitrogen also consumed more moisture by about 1 % from the deepest soil layer than control (0 kg N ha$^{-1}$) may be because of better root development and better plant growth which was able to extract more moisture from the deeper profile layers. Lenka *et al.*, 2009 also reported similar findings where they found that soil water extraction from deeper layers was maximum in fertilized plots than unfertilized ones.

**Grain Yield (kg ha$^{-1}$):** The grain was slightly higher in 2011–12 than 2010–11 (Table 3). This may be because of comparatively better weather conditions like lower minimum temperature during the crop season, which causes less respiration losses. Grain yield increased with increased irrigation regimes. The higher irrigation regimes fulfilled timely crop water requirement, which resulted into better growth. The increase in irrigation number from one to two, two to four and one to four irrigations increased the grain yield of wheat by about 25.1, 20.4 and 50.6 % during 2010–11 and 21.0, 21.9 and 47.5 % during 2011–12, respectively. Wang *et al.*, (2012) also reported that grain yield under high irrigation were significantly greater than that under deficit irrigation. As a result of the present high ET and limited irrigation, crops will not grow reliably under water-stressed conditions. This indicates that it is difficult to obtain satisfactory grain yield without irrigation, suggesting that supplemental irrigation is necessary. Grain yield increased significantly with increased nitrogen doses. The increased dose of nitrogen increased the grain yield in all the irrigation levels. At one and two irrigations levels wheat yield increased significantly upto 100 kg N ha$^{-1}$ in 2010–11. However, with four irrigations levels wheat grain yield increased significantly upto 150 kg N ha$^{-1}$ during 2010–11. The increase in irrigation regimes from one to four increased the grain yield significantly with all doses of nitrogen during both years. The

increased dose of nitrogen also increased the grain yield in all the irrigation regimes. At all irrigations levels wheat yield increased significantly upto 150 kg N ha$^{-1}$ during 2011–12. The grain yield at 150 and 200 kg N ha$^{-1}$ were non significant at all the levels of irrigations. The more grain yield in higher dose of nitrogen was because of more availability of nutrients for their growth and development. The higher yield with increasing levels of irrigation and N dose may be attributed to better water and nutrient availability, which gave rise to better plant growth and yield. Similar results have been reported in wheat by Bandyopadhyay *et al.*, 2010; Pradhan *et al.*, 2013.

## CONCLUSION

Consumptive water use of late sown wheat increased with increased irrigation regimes during both the years. 200 kg N ha$^{-1}$ crop consumed markedly higher water than control (0 kg N ha$^{-1}$). The moisture extraction from the surface layer (0–15 cm) was increased slightly and deeper profile layers were decreased slightly with increase in irrigation regimes. Increasing doses of nitrogen consumed more moisture from the deeper profile layer than control and vice-versa during both the years. The water use efficiency in wheat decreased with increasing number of irrigations, whereas increasing N supplies improved WUE at all irrigation regimes. Grain yield significantly increased with increased irrigation regimes and N doses. Highest grain yield was recorded in treatment combination of four irrigations with 200 kg N ha$^{-1}$ (I$_3$F$_4$), which was significantly higher than other combinations of irrigation and nitrogen but statistically at par with combinations of four irrigations with 150 kg N ha$^{-1}$ (I$_3$F$_3$).

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# Spatial Distribution for Major Nutrients through GIS Technique and Site Specific Nutrient Recommendations for Arecanut Gardens in Karnataka, India

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***Abstract***: Delineation of spatial distribution for nitrogen, phosphorus and potassium were estimated from 65 arecanut garden soils representing 232 acres in traditional areca area, extended non-traditional areas of tank fed, bore well fed and canal fed irrigation in three districts of Karnataka, India. The selected gardens were aged 15-18 years with medium management. The standard grid technique with a spacing of 50x50 m was employed to draw soil samples from the selected sites in 0-30 cm depth with GPS identity. Traditional belts are acidic while others are basic in soil reaction. In all these gardens nitrogen was in low status. Larger variations are accounted for phosphorus and potassium status. More than 60 per cent of samples accounted for medium status of phosphorus in traditional, tank fed and bore well fed situations, while canal irrigated belts accounted for > 60 per cent medium to high status. Humcha, Konandur and Chikkingala soils had > 60 per cent lower potassium while the soils of other locations showed more than 70 per cent medium to high status. Using these data in GIS environment, fertility mapping of each location was developed. Utilizing variable value of nutrients of each grid, site specific recommendations for fertilizers were calculated based on soil test values. It is inferred that arecanut garden soils across all locations are to be supplied with slightly higher (1478 kg/ha) nitrogen and potassium (750 kg/ha) with no perceptible change in application of phosphorus, thereby given re-allocation of these resources based on site specificity.

***Keywords***: Arecanut, Canal, GIS, Grid, Fertilizers, Site specific, STV

Arecanut belonging to family Palmae, is one of the economically important plantation crops grown in India. India ranks first in area (0.45 mha) and production (0.74 mt) of arecanut in the World (Anonymous, 2016). Karnataka is one of the important areca producing states contributing nearly 60 per cent (0.46 mt) to the countries total production. The crop is traditionally well acclimatized in coastal and hilly zone situations of laterite soils of undulating topography with moderate to heavy rainfall. Being a top commercial plantation crop, its cultivation is extending across all situations including clay soil belts of non-traditional areas. The influence of soil parameter is one of the main factors governing the phyto availability of nutrient elements (Kabata-Pendias, 2004). Nutrient uptake of plant is affected by many soil parameters such as nutrient concentrations, pH, cation exchange capacity, organic carbon, etc, apart from types and varieties of crops with their age. Hence, adequate nutrition to crops depends on many factors such as ability of soils to supply the nutrients, rate of absorption of nutrients by plants, distribution of nutrients to functional sites and nutrient mobility within the plant. Hartemink (2005) stated that large quantities of nutrients are immobilized in the above and below ground of

perennial crops paving way for lesser efficiency. The serious concerns in arecanut cultivation are huge yield gap from location to location, sustained nutrient requirement and low nutrient use efficiency (Bhat and Sujatha, 2009). An excess or imbalanced application of nutrients not only wastes this limited costly resource, but also pollutes the environment and hinders the uptake of other essential nutrients. Thus maintaining appropriate levels of soil fertility is of paramount importance not only for sustained growth and yield but also for its better use efficiency. In the features of economic and environmental concerns, farmers face an increasing challenge of effective soil fertility management. An approach towards justifying such concerns is site specific nutrient management wherein spatial variations are considered for exacting fertilizer applications.

At present, there is a uniform recommendation of major nutrients to areca gardens across soils in various agro-climatic zones. The present recommendation is 100:40:140 g NPK ha$^{-1}$ per palm per year for local varieties and 150:60:60 g NPK ha$^{-1}$ per palm for high yielding recently released varieties. However, farmers are practicing blanket application of fertilizers based on many other considerations

than plants requirement. The application differs with location also. Coastal and hilly tract farmers rely more on the available source of bio-wastes while farmers of transitional and dry tracts do apply tank silt, red earth, etc to get higher yields from plants. There is an uneven balanced application of fertilizers across all situations. This mis-match of fertilizers often resulted in loss of resources and higher incidence of pests and diseases. Considering these points, a study was undertaken to assess the nutrient variability in the areca garden soils of both traditional and non-traditional tracts to derive site specific recommendations. The spatial information is meaningfully synchronized using GIS for developing soil fertility maps.

### MATERIAL AND METHODS

Arecanut is grown in different eco-systems and depending on variability in topography, soils and water supply, four different situations of arecanut growing area viz., traditional areca area of hilly zone belt, extended non-traditional areas of canal fed belt, tank fed belt and bore well fed belt were chosen for the study in three different districts during 2013-14. In all these study areas local varieties are dominant and the crop was aged 15-18 years with medium management. The study area comprised 282 acres of 65 farmers from Shivamogga, Chikkamagaluru and Davanagere districts of Karnataka, India (Table 1). The standard grid technique of 50x50 m was employed to draw soil samples from the selected study area at 0-30 cm depth. Each grid is recognised by its own GPS co-ordinate to help for geo-referencing of study area. Further, using GPS co-ordinates, the base maps of the study area was developed in GIS environment. Soil samples collected from each grid were dried, powdered, sieved with 2 mm mesh and analyzed by following the standard methods. For available nitrogen, procedure of alkaline permanganate method of Subbaiah and Asija (1956) was employed. Available phosphorus was estimated by following Brays and Olsen methods (Jackson, 1973). Potassium was extracted by shaking the requisite amount of soil sample with 1 N $NH_4OAc$ solution and estimated as outlined by Jackson (1973).

Nutrient Index Values were calculated using the following formula

NIV= [(PH*3) +(PM*2)+(PL*1)]/ 100

where, PH, PM and PL are the percentage of soil sample falling in the category of high, medium and low status given weightage factor of 3, 2 and 1 respectively (Ramamurthy and Bajaj, 1967). Utilizing soil analytical results for nitrogen, phosphorus and potassium on grid basis, maps of these nutrients were prepared based on low, medium and high classification criteria (Tandon, 1993). The points having the same category were grouped into class as a polygon by using inverse distant weighed method interpolation technique. Maps for the individual nutrients were generated using GIS. Based on these variable values of nutrients, by adopting soil test values (STV), fertilizers quantity for each grid was calculated. The package recommendation and the site specific recommendation based on STV approach are compared. For each location depending on its site specific variations, nutrient application approaches are advised for farmers. For each location depending on its site specific variations, nutrient application approaches for future with respect to different micronutrients is advised for farmers.

### RESULTS AND DISCUSSION

**Traditional areca area**: This area is represented by Humcha and Konandur locations of Shivamogga district. This is a representative of hilly zone cool area receiving 2800 mm rainfall spread over from June to November. The soils are typically lateritic, acidic in reaction with leaching of cations and fixation of P and Zn is a common feature. In the soils of selected arecanut gardens, the available nitrogen status had a mean value of 224.94 kg ha$^{-1}$ in different grids. Considering <280 kg ha$^{-1}$ as critical limit for nitrogen (Mour *et al*., 1965), 88 per cent of the samples were rated low while rest were medium in status (Table 2). The low N status may be attributed to temperature fluctuations, lower turnover of organic matter, low organic carbon and continuous demand from the crop. Similar results were also obtained in the studies of Shetty *et al*. (2008) in areca gardens. The mean available phosphorus status of soils was 40.76 kg ha$^{-1}$ in

**Table 1.** Details of study area sites

| Item | Traditional area | Non-traditional area | | | | |
|---|---|---|---|---|---|---|
| | Humcha and Konandur | Tank fed area | | Borewell fed area | Canal fed area | |
| | | Banoor | Chikkingala | Kerehosalli | Agarada-Halli | Kabbala |
| No. of farmers | 14 | 6 | 8 | 11 | 19 | 7 |
| Area (acres) | 42 | 23 | 25 | 38 | 54 | 50 |
| No. of samples | 66 | 47 | 50 | 61 | 86 | 80 |
| pH range | 5.24-6.61 | 7.29-8.64 | 7.91-8.66 | 7.06-8.27 | 6.10-8.06 | 6.82-8.62 |
| Soil reaction | Acidic | Basic | Basic | Basic | Basic | Basic |

different grids. Considering <51 kg $P_2O_5$ ha$^{-1}$ as critical limit for phosphorus (Trehan *et al.*, 2008), 35 per cent of the samples were rated low, 56 per cent of samples were rated medium and remaining samples were rated high in status. The retention of phosphorus status can be attributed to regular application of phosphatic fertilizers, demand from the older plants is quite low with varying fixation rate. Vijayakumar *et al.* (2015) also reported similar trend. On the similar lines, the available potassium status of soil samples had a mean value of 163.60 kg ha$^{-1}$ in different grids. Considering <141 kg $K_2O$ ha$^{-1}$ as critical limit for potassium, 60 per cent of the samples were rated low, 26 per cent were rated medium and remaining samples were rated high in status. These variations are probably due to lesser retention of cations in

acidic nature of soil, higher rainfall during *kharif,* etc. Mahesh Kumar *et al.* (2015) also reported lesser retentivity of cations under high rainfall situations. Nutrient index value of 1.12, 1.74 and 1.53 clearly indicates that fertility status remains very low, marginal and low respectively for nitrogen, phosphorus and potassium, thereby attention needs to be exercised for application of all major nutrients to maintain soil health in a good condition.

**Tankfed area**: Tank fed area is represented by Banoor and Chikkingala location of Chikkamagaluru district, a shadow area just beside hilly zone. The tanks nearby were filled due to rains in hilly zone and the collected water sustains the perennial crops during off rainy seasons. These areas receive around 550 mm rainfall and majority of soils possess

**Table 2.** Soil status for the different arecanut garden

| Parameter | pH | N | $P_2O_5$ | $K_2O$ | pH | N | $P_2O_5$ | $K_2O$ |
|---|---|---|---|---|---|---|---|---|
| | | (kg ha$^{-1}$) | | | | (kg ha$^{-1}$) | | |
| **Humcha and Konandur** | | | | | **Kerehosahalli** | | | |
| Average | 5.91 | 224.94 | 40.76 | 163.60 | 7.92 | 210.4 | 37.77 | 267.48 |
| Maximum | 6.61 | 313.60 | 358.77 | 480.84 | 8.27 | 376.32 | 151.84 | 539.4 |
| Minimum | 5.24 | 131.71 | 11.02 | 43.32 | 7.06 | 125.44 | 10.31 | 66.72 |
| Standard deviation | 0.28 | 42.52 | 52.69 | 126.43 | 0.27 | 39.62 | 32.01 | 124.85 |
| Per cent ratings of samples | Acidic–65 | Low–88 | Low–35 | Low–60 | Acidic–00 | Low–95 | Low–38 | Low–18 |
| | Neutral–01 | Med–12 | Med–56 | Med–26 | Neutral–05 | Med–05 | Med–46 | Med–54 |
| | Basic–00 | High–00 | High–09 | High–14 | Basic–56 | High–00 | High–16 | High–28 |
| NIV | — | 1.12 | 1.74 | 1.83 | — | 1.05 | 1.79 | 2.10 |
| Fertility rating | — | Very low | marginal | Low | — | Very low | Marginal | Adequate |
| **Banoor** | | | | | **Agaradahalli** | | | |
| Average | 8.00 | 175.75 | 33.31 | 593.39 | 6.77 | 193.56 | 60.77 | 326.83 |
| Maximum | 8.64 | 269.7 | 80.92 | 2514 | 8.06 | 357.5 | 198.37 | 2016 |
| Minimum | 7.29 | 131.71 | 6.64 | 121.2 | 6.1 | 150.53 | 3.32 | 8.4 |
| Standard deviation | 0.28 | 24.02 | 17.62 | 533.01 | 0.39 | 36.57 | 41.79 | 414.49 |
| Per cent ratings of samples | Acidic–00 | Low–100 | Low–38 | Low–08 | Acidic–24 | Low–96 | Low–15 | Low–30 |
| | Neutral–3 | Med–00 | Med–52 | Med–14 | Neutral–60 | Med–04 | Med–40 | Med–42 |
| | Basic–44 | High–00 | High–10 | High–78 | Basic–02 | High–00 | High–45 | High–28 |
| NIV | — | 1.00 | 1.78 | 2.70 | — | 1.03 | 2.3 | 1.97 |
| Fertility rating | — | Very low | Marginal | Very High | — | Very low | Adequate | Marginal |
| **Chikkingala** | | | | | **Kabbala** | | | |
| Average | 8.4 | 153.54 | 45.79 | 93.6 | 8.02 | 185.73 | 103.4 | 394.55 |
| Maximum | 8.66 | 181.89 | 153.55 | 98.4 | 8.62 | 257.15 | 394.79 | 1383.6 |
| Minimum | 7.91 | 106.62 | 2.08 | 92.4 | 6.82 | 131.71 | 10.95 | 75.12 |
| Standard deviation | 0.15 | 17.89 | 33.83 | 1.67 | 0.35 | 28.43 | 5.18 | 236.09 |
| Per cent Ratings of samples | Acidic–00 | Low–100 | Low–36 | Low–100 | Acidic–00 | Low–100 | Low–12 | Low–05 |
| | Neutral–00 | Med–00 | Med–34 | Med–00 | Neutral–06 | Med–0 | Med–24 | Med–50 |
| | Basic–50 | High–00 | High–30 | High–00 | Basic–74 | High–00 | High–64 | High–45 |
| NIV | — | 1.00 | 1.94 | 1.00 | — | 1.00 | 2.50 | 2.40 |
| Fertility rating | — | Very low | Marginal | Very low | — | Very low | High | High |

alkaline reaction hence basic in nature. The virginity of soil is lost due to continuous growing situations and experiences more of abiotic stress situations like variation in temperature, relative humidity, sunshine hours etc. Both locations recorded lower status of available nitrogen (maximum of 261 and 181 kg ha$^{-1}$) due to inherent low content, faster degradation as a result of relatively higher temperature than traditional belts. Banoor and Chikkingala soils recorded maximum of 80.92 and 153.55 kg ha$^{-1}$ respectively for phosphorus status (Table 2). Out of the samples tested, 32 and 53 per cent Banoor soils are low and medium for phosphorus status while Chikkingala soils are evenly distributed (36, 34 and 30 % of soils respectively for low, medium and high status). These variations are mainly attributed to supply from various sources, as plants grow up there demand also remains low, reserved source etc. Majority of Banoor soils are high status (79 % ) for potassium content while that of Chikkingala soils possessing low status ( maximum of 98.4 kg ha$^{-1}$). Tank fed area soils are basic in nature and amount of variation exists for different nutrient largely depends on management factor, supply pattern from different sources and well supported in the studies of Sabita Soman and Byju (2013). Nitrogen and phosphorus in these two locations remained very low (NIV of 1.00) and marginal (1.78 and 1.94 for phosphorus respectively for Banoor and Chikkingala) . Banoor soils recorded NIV of 2.70 for potassium with very high fertility rating wherein on the contrary, Chikkingala soils with 1.00 NIV recorded very low rating. Barring potassium at Banoor, other nutrients needs application regularly.

**Bore well fed area**: Bore well supported areca garden is represented by Kerehosahalli of Tarikere taluk, Chickamagaluru district, almost a continued stretch of land to that of tank fed area. With similar rainfall of 510 mm and absence of any irrigation sources, farmers opt bore wells extensively for irrigating perennial crops. These soils are again basic in nature with low status (95 per cent) of available nitrogen (a maximum of 376.32 kg ha$^{-1}$) due to low inherent capacity and fertility management tuned with irrigation supply. Larger variations are noticed for phosphorus and potassium status as dictated majorly by capacity of supply to the tune of requirement, turnover of the residuals, remaining, etc. The mean available value of 37.77 kg ha$^{-1}$ in different grids indicating 41, 50 and 9 per cent of samples under low, medium and high status respectively. The available mean potassium value was of 27.48 kg ha$^{-1}$ in different grids (Table 2). Hence, 20 per cent of the samples were rated low, 59 per cent were rated medium and the remaining samples were rated high in status. The amount of variation exists for different nutrient contents as a result of management could also be evidenced from the works of Barman *et al*. (2013) and Mahesh Kumar *et al*. (2015). The fertility rating of very low (NIV of 1.05), marginal (NIV of 1.79) and adequate (NIV of 2.10) was achieved in these soils, demanding careful attention of both nitrogen and phosphorus fertilizers.

**Canal fed area**: Canal fed area is represented by Agaradahalli of Bhadravathi taluk in Shivamogga district and Kabbala of Channagiri taluk in Davangere district. Agaradahalli is blessed with Bhadra river canal while Kabbala is blessed with both Bhadra canal and as well Shantisagara tank feeder canal and hence assured source of irrigation. Agaradahalli soils are neutral to basic in reaction, wherein Kabbala soils are tending towards basicity. The available nitrogen status of Agaradahalli soils had a mean value of 193.0 kg ha$^{-1}$ in different grids. Hence, 96 per cent of the samples were rated low. On the other hand, Kabbala soil samples with a maximum available N of 257.15 kg ha$^{-1}$ remained low in status. Similar to other places of the study

**Table 3.** Fertilizer requirement (kg) for the study area based on status of nutrient on grid basis

| Locations | Nitrogen | | Phosphorus | | | Potassium | | |
|---|---|---|---|---|---|---|---|---|
| | Low status | Medium status | Low status | Medium status | High status | Low status | Medium status | High status |
| Humcha and Konandur | 2168 | 274 | 388 | 509 | 64 | 2170 | 816 | 376 |
| Kerehosahalli | 2168 | 103 | 388 | 385 | 106 | 597 | 1584 | 710 |
| Banoor | 1757 | 0 | 253 | 371 | 53 | 217 | 288 | 1545 |
| Agradahalli | 3102 | 103 | 219 | 467 | 414 | 1410 | 1728 | 1002 |
| Chikkingala | 1869 | 0 | 304 | 234 | 159 | 2713 | 0 | 0 |
| Kabbala | 3140 | 0 | 177 | 274 | 569 | 228 | 2016 | 1578 |
| Total | 14202 | 480 | 1730 | 2241 | 1366 | 7335 | 6432 | 5211 |
| Grand Total | 14682 | | 5337 | | | 18978 | | |
| Package recommended | 13224 | | 5336 | | | 18328 | | |
| Surplus application | 1458 | | 1 | | | 750 | | |

area, inherent capacity of soil nitrogen is low and as well better utilization when the element is available due to no water stress situations are the reasons apart from abiotic stresses acting. Soils recorded maximum of 198.37 and 394.79 kg ha$^{-1}$ phosphorus status respectively for Agaradahalli and Kabbala. Of the samples analysed, 40 and 45 per cent of Agaradahalli and 24 and 64 per cent of Kabbala soils are medium and high in phosphorus status. These variations are mainly attributed to low demand of the grown

crop, reserved sources, etc. Soils of Agaradahalli are 30, 41 and 29 per cent low, medium and high status for potassium while Kabbala soils are possessing medium (50 per cent) and high (45 per cent) potassium status. These variations are attributed to nutrient management supply from different sources as indicated in the studies of Dhanashekaran Pandian and Mohamed Haroon (2014). Like other locations, these sites also recorded very low nitrogen rating demanding its must application situation. Interestingly, the rating for
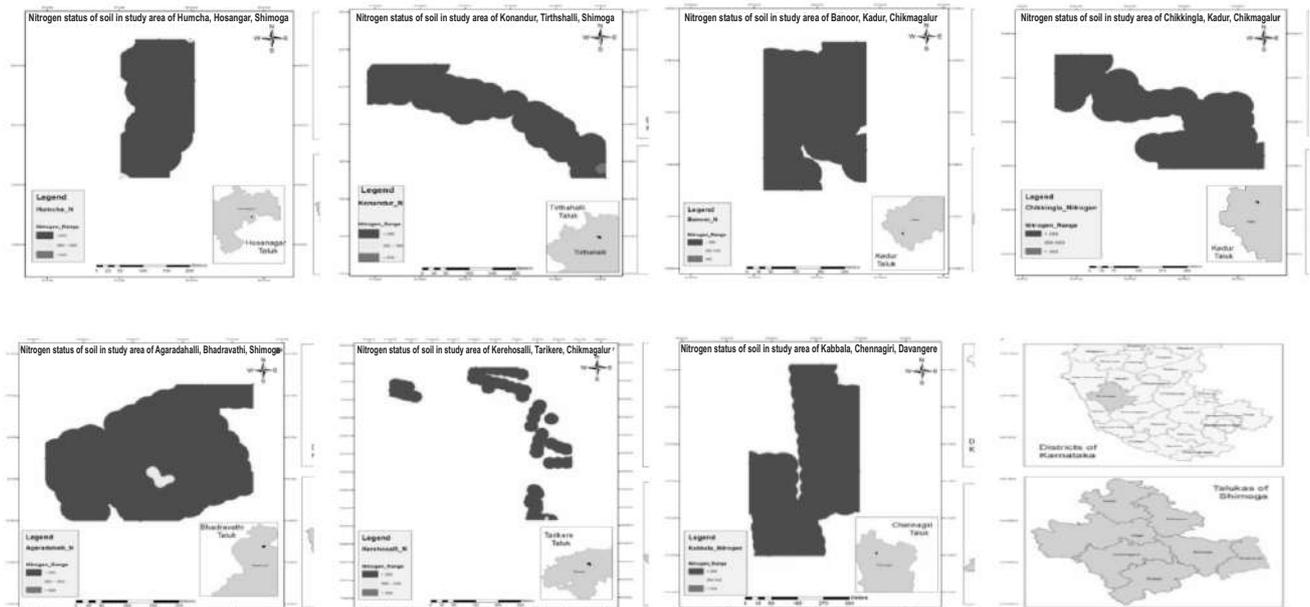


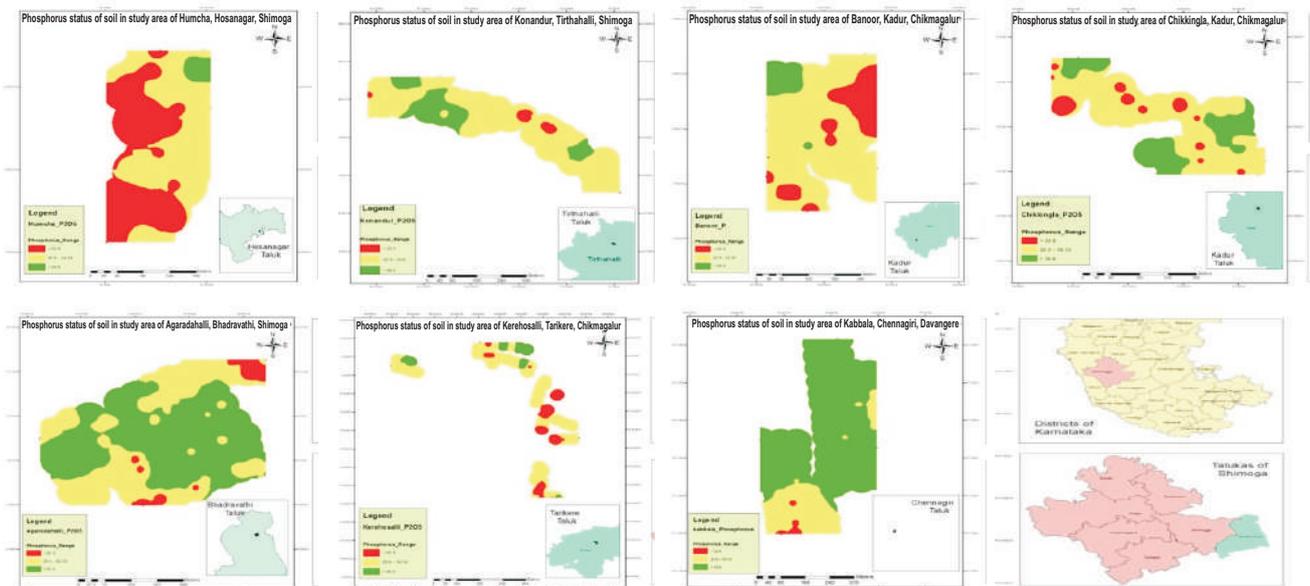**Fig. 1.** Nitrogen content of soil in different study areas of arecanut garden



**Fig. 2.** Phosphorus content of soil in different study areas of arecanut garden
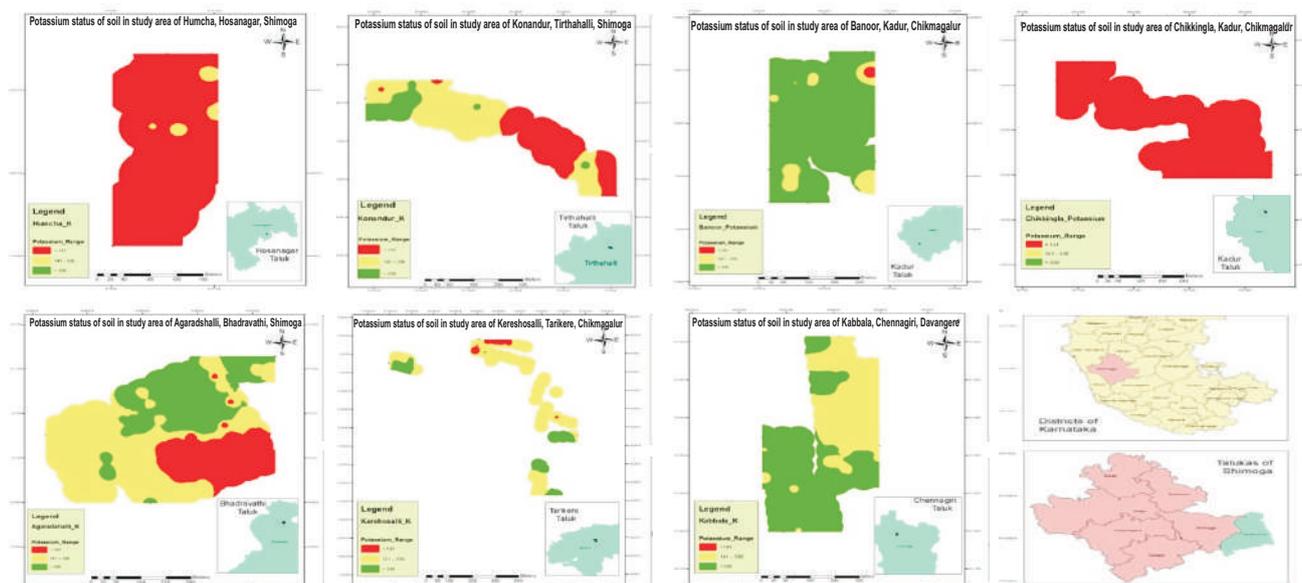
**Fig. 3.** Potassium content of soil in different study areas of arecanut garden

phosphorus remained adequate (NIV of 2.30) and high (NIV of 2.50), wherein potassium at Agaradahalli become marginal to that of high rating achieved for Kabbala soils (Table 2).

A soil variability map for major nutrients in selected study area is presented in figure 1 to 3. Across locations, for each grid a chart is prepared with respect to different macro nutrients for low, medium and high status. In the present study, based on soil test value method fertilizer recommendations (STV method) employed to make grid wise fertilizer calculations. The total requirement of fertilizers based on package recommendation for these areas is indicated in Table 3 along with calculated STV approach. It is observed from the above result that arecanut garden soils across all locations are to be supplied with slightly higher nitrogen (1478 kg/ha) and potassium (750 kg/ha) with no perceptible change in application of phosphorus thereby given re-allocation of these resources based on site specificity.

## CONCLUSION

The data permit to infer that across all locations nitrogen status remained low and hence achieved lowest fertility rating. Fertility ratings for phosphorus varied from marginal to high as samples analyzed shared 26, 42 and 32 per cent for low, medium and high status. Potassium shared 35, 34 and 31 per cent for low, medium and high status with varied fertility ratings. On the basis of soil test value, addition of 1478 and 750 kg nitrogen and potassium is required for study area of 232 acres additionally along with recommended major nutrients.

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# Effect of Foliar Sprays of 2,4-D and Frequency of Application on Preharvest Fruit Drop, Yield and Quality in Kinnow Mandarin

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**Abstract:** The effects of 2,4-D and the frequency of application on pre harvest fruit drop and quality of Kinnow mandarin (*Citrus reticulata* Blanco) at 10 and 20 ppm at monthly intervals, 1st September, 1st, October and 1st November was evaluated. Exogenous application of growth regulators significantly decreased pre harvest fruit drop percentage leading to increase in total number of fruits per plant, fruit weight, juice percentage, total soluble solids, acidity, vitamin C, reducing sugars and non reducing sugars percentage. Based on physical and bio chemical parameters 2,4-D at 20 thrice on 1st of September, October and November was most effective in checking the fruit drop and improving fruit quality and yield of Kinnow mandarin.

**Keywords:** Kinnow mandarin, Citrus, fruit drop, plant growth regulator, 2,4-D

Kinnow (*Citrus nobilis* × *Citrus deliciosa* L.) is one of the important citrus fruits known not only for its beautiful appearance and pleasing flavor but also for its high nutritive value, good yield, fresh consumption, high processing quality and better adaptation to agro-environmental conditions (Ahmed *et al.,* 2006). As per National Horticulture Board database (2015) the mandarin cover largest area (3.3 x 10$^5$ ha) among all citrus species which is 4.6% of total area under fruit crops. The common mandarins that are under commercial cultivation include Kinnow, Nagpur, Khasi and Darjeeling. Among all mandarins, Kinnow accounts for highest production and productivity. In India, Punjab, Maharashtra, Karnataka, Tamil Nadu, M.P., Rajasthan, West Bengal, Himachal Pradesh and Haryana are commercial producers of Kinnow.

Despite so many qualities of Kinnow, it is, however, beset with the problem of excessive fruit drop. Although fruit drop occurs in different waves but pre-harvest drop is of main concern as it results in direct economic loss to the growers as fruits are approaching maturity at that time. There is tremendous drop during this period and most medium sized fruits cover the area under the tree. This drop has been estimated to the extent of 50 percent of the crop load (Nawaz *et al.,* 2008). Such dropped fruits cannot be consumed despite the fact that they have already drawn metabolities from the tree and exhausted it. Preharvest fruit drop is major reason of low yield in India, this drop of fruit at various stages of fruit development is due to malnutrition, water stress, excessive insect pest attack and most importantly hormonal imbalance. Fruit drop occurs when the concentration of auxins decreases and the concentration of abscissic acid (ABA) increases as the endogenous hormones and their balance play a modulating role in the mobilization of nutrients to the developing organs (Marinho *et al.,* 2005).

Inspite of sufficient literature available on the causes and control of fruit drop in Kinnow, the problem still remains to be unsolved. Year to year variations in the extent of drop has been reported in different blocks in an orchard or trees in the same block. This shows that problem though universal in nature and location specific, needs separate control measures to be worked out under different agro-climatic conditions. Although various plant growth regulators have been successfully used for reducing fruit drop but 2,4-D (2,4-Dichlorophenoxyacetic acid) has been found to be much more potent in controlling the drop (Khalid *et al.*, 2016; Mollapur *et al.*, 2016 ) and in improving the fruit quality in Kinnow mandarin (Kaur *et al.*, 2016; Khalid *et al.,* 2016). However, till date meager information is available regarding the frequency of application of 2,4-D under varying agro-climatic and cultural conditions. Moreover, the existing findings are not applicable as such in Kinnow, due to its hybrid nature, extended fruiting period and heavy cropping. This study was, therefore, conducted to determine the effect of foliar application of 2,4-D and frequency of application on preharvest fruit drop, yield and fruit quality of Kinnow mandarin.

## MATERIAL AND METHODS

The research work was conducted at Sam Higginbottom Institute of Agricultural Technology and Sciences, Allahabad.

Seventeen years old, 45 plants of Kinnow mandarin (*Citrus reticulata* Blanco*)* grafted on rough lemon rootstock of uniform size and age were selected for this experiment. On the selected trees 2,4-D (10 and 20ppm) were sprayed at monthly intervals i.e. 1st September, 1st October and 1st November to check the effect on pre harvest fruit drop and physiochemical properties of the fruit. The experiment consisted of 15 treatments including control, replicated thrice and single tree was taken as an experimental unit. All the experimental trees were maintained under similar cultural conditions.

Numbers of fruits present on each selected tree were counted before the start of the experiment. The fruits dropped from the selected trees were counted at 5 days interval starting from 1st September and up to the 20th December and fruit drop percentage was calculated:

$$\text{Percent fruit drop} = \frac{\text{Total number of fruits dropped per tree}}{\text{Initial number of fruits per tree}} \times 100$$

Yield per tree was recorded at the time of harvesting. Fruit size was measured by measuring the length and breadth of 10 fruits per tree randomly and fruit weight was also calculated

Juice of each of 10 harvested fruit was extracted and weighed, average juice weight was calculated separately for each treatment. The average juice percentage per fruit was estimated:

$$\text{Juice \%age} = \frac{\text{Juice weight per fruit}}{\text{Average fruit weight}} \times 100$$

Total soluble solids were measured by Baush and Lamb Refractometer and the readings were calibrated at 20°C using reference table (A.O.A.C, 1985). Acidity of juice was determined by taking 10 ml of juice from each sample and diluted with distilled water in a 100 ml beaker; 2-3 drops of phenolphthalein were added for end point. The samples were titrated against N/10 NaOH (Hortwitz, 1960). The results were expressed as percent citric acid.

$$\text{Acidity \%age} = \frac{\frac{N}{10} \text{ NaOH used} \times 0.0064 \text{ 4lit}}{\text{Volume or weight of sample used}} \times 100$$

TSS/Acid ratio was computed by dividing the value of total soluble solids with that of the corresponding acidity percentage.

Vitamin-C in juice was estimated according to the method described by Ruck (1961) whereas sugars in juice were estimated by using Lane and Eyon method (1923) described by Hortwitz (1960).

## RESULTS AND DISCUSSION

**Physical analysis of fruit:** The foliar sprays of 2,4-D significantly reduced the pre harvest drop compared to control. The lowest fruit drop of (14.65%) was in the treatment 20ppm 2,4-D sprayed on 1st September + 1st October + 1st November followed by 20ppm 2,4-D sprayed on 1st September + 1st October with a fruit drop of (15.71%) whereas, maximum fruit drop (28.85%) was in Control. The 2,4-D treatments proved better when concentration was increased up to 20ppm and sprayed thrice at monthly intervals. This agrees with the findings of Modise *et al.* (2009) who observed foliar application of 2,4-D at 20 mgl–1 reduced the rate of fall in Navel oranges as compared to control. Yasin Ashraf *et al*. (2013) observed that foliar applications of 2,4-D, salicylic acid, K2 SO4 , and ZnSO4 alone or in combination significantly reduced fruit drop in 'Kinnow' mandarin. Our results were also in agreement with earlier workers (Davies and Zalman, 2006; Kaur *et al*., 2016; Mollapur *et al*., 2016) who reported that application of 2,4-D and other plant growth regulators significantly reduced the pre harvest fruit drop in citrus species. Keeping in view the above results it can safely be recommended that 2,4-D can be applied at 20ppm to control the pre harvest fruit drop in Kinnow mandarin.

Foliar sprays of 2,4-D proved very effective in improving the length, diameter, weight and juice content of retained fruits. The maximum fruit length (6.02cm), fruit breadth (6.81cm), fruit weight (149.65g) and juice content (40.94%) was in treatment 20ppm 2,4-D sprayed on 1st September + 1st October + 1st November. Improvement in physical parameters of fruit with exogenous application of 2,4-D, attributed to accelerated fruit growth and subsequently fruit size by increasing cell enlargement. Erner *et al.* (1993) reported increase in fruit size by 8-25% in Valencia orange with spraying of 2,4-D at 20 ppm, 6-8 weeks after flowering. The results were also in agreement with that of Saraswathi *et al. (*2003) who observed that growth regulators 2,4-D and GA$_3$ and their combinations significantly influenced the fruit weight. Auxins are known to affect the permeability of cell wall leading to uptake of water and expansion of cells resulting cell elongation finally. The results of all these workers proved that growth regulators increases fruit size which also encourage the results of the present investigation for these quality parameters. Results regarding juice percentage were in consonance with that of Atawia and El-Desouky (1997) and Matthew *et al. (*2002) who earlier reported that the application of growth regulators at flowering and pre harvest significantly increased the juice percentage in various citrus species. The other possible explanation for improved fruit quality under 2,4-D treatment is due to delaying in fruit maturity and provided a longer period to fruits to remain on tree during which they accumulated more food reserves and water within them.

The foliar sprays of 2,4-D proved increased fruit yield per

plant. The maximum fruit yield of (53.05kg) was in treatment 20ppm 2,4-D sprayed on 1$^{st}$ September + 1$^{st}$ October + 1$^{st}$ November and minimum (37.24 kg) per plant was in Control where growth regulator was not applied. The yield were in agreement with that of Thomas and Lovatt (2004) and Davies and Zalman (2006) who reported that pre harvest application of growth regulators like 2,4-D and GA$_3$ significantly increased the total number of fruits at the time of harvest and fruit weight per plant by reducing the pre harvest fruit drop.

**Biochemical analysis of fruit:** All the treatments significantly increased TSS, acidity, TSS-acid ratio, reducing sugar, total sugars and ascorbic acid content of fruit (Table 2). Spraying of 20 ppm 2,4-D on 1$^{st}$ September+1$^{st}$ October+1$^{st}$ November recorded the maximum TSS (11.3%), acidity (0.90%), TSS-acid ratio (12.55), reducing sugar (3.85%), total sugar (6.99%) and ascorbic acid (22.65 mg/100 ml of juice). TSS percentage were in consonance with that of Atawia and El-Desouky (1997) and Huang and Huang (2005) who reported application of growth regulators like Auxin and Gibberellins significantly increase the total soluble contents of the fruits in citrus species. Acidity percentage of fruit were found to be contrary with that of Otmani *et al. (*2004) and Xiao *et al.* (2005) who reported that by the application of 2,4-D, GA$_3$ and NAA acidity percentage was significantly reduced. The ascorbic acid observations are in agreement with that of Xiao *et al.* (2005) who also observed that pre harvest application of growth regulators increased vitamin-C contents of the citrus fruits. Increment in reducing sugars was

more under 2,4-D treated fruits as compared to control. This might be due to auxin (2,4-D) induced synthesis of invertase enzyme (Rao *et al.*, 2015). Since auxin is known to regulate the gene expression for the synthesis of invertase enzyme (Wang and Ruan, 2013). Invertase enzyme is responsible for breakdown of sucrose into fructose and glucose (Tymowska-Lalanne and Kreis, 1998). Hence results in more reducing sugars . The results regarding sugar contents were also in consonance with that of Ingle *et al. (*2001) and Wang *et al.* (2004) who find that application of 2,4-D, GA$_3$ and some other growth regulators increased the sugar contents in various mandarin and sweet orange cultivars. In Navel oranges 20 mg L-1 2,4-D application was found effective in improving fruit quality (Kassem *et al.*, 2011). Increase in biochemical characteristics of fruit juice explained that 2,4-D stimulates the functioning of a number of enzymes in physiological process which probably caused an increase in chemical composition of fruit. The other explanation for this is fruits remained on tree for longer period with 2,4-D treatments during which more accumulation of photosynthates especially carbohydrate occurred within them. The increase in ascorbic acid content of fruit by 2,4-D treatment is due to perpetual synthesis of glucose-6-phosphate throughout the growth and development of fruits which is thought to be precursor of vitamin C. Furthermore auxin caused diversion of nutrients and organic substances to the developing fruits (metabolic sink) from leaves and other parts of plant which ultimately accumulated within fruits.

**Table 1.** Effect of foliar sprays of 2,4-D and frequency of application on physical characteristics of Kinnow mandarin.

| 2,4 D concentration (ppm) | Frequency of spray | Fruit drop (%) | Fruit length (cm) | Fruit breadth (cm) | Fruit weight (g) | Fruit yield (Kg) | Fruit juice (%) |
|---|---|---|---|---|---|---|---|
| 10 | 1$^{st}$ September | 23.15 | 5.17 | 6.17 | 136.15 | 41.89 | 36.27 |
| 10 | 1$^{st}$ October | 21.86 | 5.29 | 6.27 | 136.45 | 42.13 | 36.55 |
| 10 | 1$^{st}$ November | 25.52 | 5.11 | 6.10 | 135.90 | 39.86 | 35.90 |
| 10 | 1$^{st}$ September + 1$^{st}$ October | 19.13 | 5.61 | 6.38 | 139.05 | 45.55 | 38.56 |
| 10 | 1$^{st}$ September + 1$^{st}$ November | 20.38 | 5.49 | 6.44 | 137.45 | 45.09 | 37.19 |
| 10 | 1$^{st}$ October + 1$^{st}$ November | 20.49 | 5.70 | 6.54 | 141.60 | 43.53 | 38.02 |
| 10 | 1$^{st}$ September + 1$^{st}$ October + 1$^{st}$ November | 18.06 | 5.81 | 6.60 | 143.30 | 48.29 | 38.91 |
| 20 | 1$^{st}$ September | 19.28 | 5.41 | 6.25 | 136.80 | 46.60 | 37.52 |
| 20 | 1$^{st}$ October | 18.54 | 5.67 | 6.30 | 137.80 | 45.59 | 38.04 |
| 20 | 1$^{st}$ November | 21.77 | 5.23 | 6.22 | 136.60 | 44.00 | 37.04 |
| 20 | 1$^{st}$ September + 1$^{st}$ October | 15.71 | 5.7 | 6.67 | 146.45 | 50.85 | 39.45 |
| 20 | 1$^{st}$ September + 1$^{st}$ November | 17.32 | 5.87 | 6.62 | 145.75 | 51.45 | 40.14 |
| 20 | 1$^{st}$ October + 1$^{st}$ November | 17.91 | 5.3 | 6.72 | 146.80 | 50.46 | 39.32 |
| 20 | 1$^{st}$ September + 1$^{st}$ October+ 1$^{st}$ November | 14.65 | 6.02 | 6.81 | 149.65 | 53.05 | 40.94 |
| Control ( no 2,4-D) | | 28.85 | 5.05 | 5.97 | 135.25 | 37.24 | 35.35 |
| C.D (p=0.05) | | 0.29 | 0.12 | 0.13 | 1.51 | 0.44 | 0.86 |

**Table 2.** Effect of foliar sprays of 2,4-D and frequency of application on biochemical characteristics of Kinnow mandarin

| 2,4 D concentration (ppm) | Frequency of spray | TSS (%) | Acidity (%) | TSS/ Acid Ratio | Reducing sugar (%) | Total sugar (%) | Vitamin C mg/100 ml of juice |
|---|---|---|---|---|---|---|---|
| 10 | 1st September | 9.7 | 0.82 | 11.83 | 3.61 | 6.64 | 17.32 |
| 10 | 1st October | 10.0 | 0.83 | 12.05 | 3.59 | 6.72 | 17.57 |
| 10 | 1st November | 9.6 | 0.82 | 11.71 | 3.57 | 6.67 | 17.44 |
| 10 | 1st September + 1st October | 10.3 | 0.84 | 12.26 | 3.56 | 6.79 | 20.07 |
| 10 | 1st September + 1st November | 10.2 | 0.83 | 12.29 | 3.64 | 6.76 | 18.09 |
| 10 | 1st October + 1st November | 10.3 | 0.86 | 11.98 | 3.62 | 6.78 | 19.30 |
| 10 | 1st September + 1st October + 1st November | 10.9 | 0.87 | 12.53 | 3.72 | 6.87 | 21.59 |
| 20 | 1st September | 10.5 | 0.84 | 12.50 | 3.65 | 6.80 | 19.58 |
| 20 | 1st October | 10.6 | 0.86 | 12.32 | 3.67 | 6.75 | 20.89 |
| 20 | 1st November | 10.1 | 0.84 | 12.02 | 3.70 | 6.71 | 19.49 |
| 20 | 1st September + 1st October | 11.0 | 0.87 | 12.64 | 3.82 | 6.95 | 22.21 |
| 20 | 1st September + 1st November | 10.8 | 0.90 | 12.00 | 3.79 | 6.91 | 21.79 |
| 20 | 1st October + 1st November | 11.0 | 0.89 | 12.36 | 3.75 | 6.84 | 21.85 |
| 20 | 1st September + 1st October+ 1st November | 11.3 | 0.90 | 12.55 | 3.85 | 6.99 | 22.65 |
| Control ( no 2,4-D) | | 9.1 | 0.81 | 11.23 | 3.54 | 6.61 | 16.89 |
| C D (p=0.05) | | 0.24 | 0.01 | 0.12 | 0.06 | 0.06 | 0.81 |

## CONCLUSION

2,4-Dichlorophenoxyacetic acid at 20ppm when sprayed three times at monthly intervals has ability to reduce fruit drop of Kinnow mandarin significantly when compared to the lower concentration level (10 ppm) hence increasing yield and leads to improvement of some fruit quality traits and giving good returns to the growers. Therefore 2,4-D could thus be used as foliar sprays to increase fruit yield under conditions that favor fruit drop provided it is applied in required quantity and appropriate growth stage.

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# Response of Nutrient Management Practices in Pigeonpea [*Cajanus cajan* (L.) Millsp.] and Clusterbean [*Cymopsis tetragonoloba* (L.) Taub.] Intercropping System

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**Abstract:** The nutrient uptake, yield advantages and economics of pigeonpea and clusterbean intercropping system was influenced by nutrient management practices. The intercropped treatments were significantly higher pigeonpea equivalent yield, land equivalent ratio and area time equivalent ratio when compared to sole crop. Among the intercropping system, 100 per cent RDF (25: 50: 00 kg N, $P_2O_5$ and $K_2O$ ha$^{-1}$, respectively) to pigeonpea and 100 per cent RDF (25: 75: 60 kg N, $P_2O_5$ and $K_2O$ ha$^{-1}$, respectively). to clusterbean resulted in significantly higher pigeonpea equivalent yield (29.24 q ha$^{-1}$), land equivalent ratio (1.71), area time equivalent ratio (1.54). At harvest, significantly higher uptake of nitrogen, phosphorus and potassium was observed in this treatment. The higher gross returns (Rs. 1,03,808 ha$^{-1}$) and, significantly higher net returns (Rs. 67,888 ha$^{-1}$) and benefit cost ratio (2.89) were realised with 100% RDF to pigeonpea and 100% RDF to clusterbean and next best intercropping treatment was 125% RDF to pigeonpea and no fertilizer to clusterbean.

**Keywords:** ATER, Economics, Intercropping system, LER, Nutrient uptake, PEY

Inspite of very substantial gains in agricultural production over the past few decades, the task of meeting the food grains, feed, fodder and fuel needs of increasing human and livestock population remains a formidable challenge before scientific community. It is estimated that India's population will touch nearly 1350 million by 2020 A.D. The food requirement of the country by 2020 A.D. is expected to be 300 million tonnes. This additional production has to come from existing land and water resources. In the present situation, increasing agricultural production through extensive agriculture has limited scope due to limited availability of cultivable area. The availability of land for agriculture is shrinking every day as it is increasingly utilized for non−agricultural purposes. Under this situation, one of the important strategies to increase agricultural output is the development of new high intensity cropping systems including intercropping systems. Intercropping system which involves raising of more than one crop on the same piece of land more or less simultaneously increases the cropping intensity both in space and time dimensions. Pigeonpea and clusterbean differ morphologically and physiologically in growth habit. Both of these are leguminous crops and can enriching the soil fertility due to biological nitrogen fixation in the soil. Besides, the use of temporal and spatial resource will be better by growing them under intercropping system. The studies on crop compatibility, temporal and spatial resource use will help to evolve appropriate agro techniques to achieve higher yields. There is a need for the investigations to step up the production of both the component crops. Hence, the present investigation was carried out to study the effect of nutrient management on yield advantages, nutrient uptake and economics of pigeonpea and clusterbean in an intercropping system in 1:1 row proportion.

## MATERIAL AND METHODS

A field experiment was conducted at Agricultural College Farm, Raichur during the *kharif* 2012−13. The soil of the experimental field was medium black having bulk density of 1.36 mg m$^{-3}$. The soil had 8.28, 0.18 dS m$^{-1}$ and 0.51 per cent of pH, electrical conductivity and organic carbon, respectively. The soil had fertility status of 224.42 kg nitrogen ha$^{-1}$, 36.20 kg phosphorus ha$^{-1}$ and 289.64 kg potassium ha$^{-1}$. The distribution of rainfall was normal during the crop season (468.90 mm). The experiment comprised of nine treatments (seven intercropped treatments and two sole crop treatments) in randomised block design with three replications. The gross plot was 8.1 x 4.8 m, net plot size of intercropped treatments and sole pigeonpea was 4.5 x 3.6 m and sole clusterbean was 6.3 x 4.0 m. Pigeonpea (TS−3R) and clusterbean (Pusa Naubahar) were sown in 1:1 row ratio by giving a common row spacing of 45 cm and a spacing of 90 x 30 cm for sole pigeonpea and 45 x 20 cm for sole clusterbean. In the intercropping system, 30 cm was given as intra row space for pigeonpea and 20 cm for clusterbean. The

fertilizer application was followed on the basis of plant population occupied by each crop. The nutrients *viz.,* nitrogen, phosphorus and potassium were applied in the form of urea, single super phosphate and muriate of potash, respectively. Recommended dose of fertilizers were applied for the sole crops of pigeonpea (25: 50: 00 kg N, $P_2O_5$ and $K_2O$ ha$^{-1}$, respectively) and clusterbean (25: 75: 60 kg N, $P_2O_5$ and $K_2O$ ha$^{-1}$, respectively). In the intercropping system, the fertilizers were applied to the component crops according to the treatment details. The entire quantities of fertilizers were applied to both the crops at the time of sowing as basal. The fertilizers were placed in furrows opened at 5 cm away from the seed line (crop row) and covered with soil. Data on yield parameters were collected and economics of cropping system was estimated. Pigeonpea equivalent yield, land equivalent ratio, area time equivalent ratio and benefit cost ratio were computed by using yield data to know advantage in the intercropping system. The pigeonpea equivalent yield (PEY) of intercropping system was calculated by taking into account the seed yield of both crops and the prevailing market price of both the crops. Price of pigeonpea grains and clusterbean green pods was Rs. 3,550 q$^{-1}$ and Rs. 810 q$^{-1}$) respectively.

$$PEY\ (q\ ha^{-1}) = \frac{Yield\ of\ clusterbean\ (q\ ha^{-1}) \times Price\ of\ clusterbean\ (q\ ha^{-1})}{Price\ of\ pigeonpea\ (Rs.\ q^{-1})} + Yield\ of\ pigeonpea\ (q\ ha^{-1})$$

Land equivalent ratio (LER) is defined as the relative land area under sole crops that is required to produce the yields obtained in intercropping at the same level of management (Willey, 1979).

$$LER = \frac{Yield\ of\ pigeonpea\ in\ intercropping\ system}{Yield\ of\ sole\ pigeonpea} + \frac{Yield\ of\ clusterbean\ in\ intercropping\ system}{Yield\ of\ sole\ clusterbean}$$

The limitation in the use of LER is the emphasis on the land area without consideration of time the field is dedicated to production. To correct this deficiency, the LER was modified by Hiebsch (1980) to include the duration of time of crop present on the land from planting to harvest and area time equivalent ratio (ATER).

ATER was calculated according to formula given by Hiebsch (1980).

$$ATER = \frac{(RYc \times tc) + (RYp \times tp)}{T}$$

Where,

RY = Relative yield of species c and p

$$= \frac{Yield\ of\ intercrop\ per\ hectare}{Yield\ of\ monocrop\ per\ hectare}$$

t = duration (days) for species c and p; T = duration (days)

of the intercropping system

$$Uptake\ of\ nutrient\ (kg\ ha^{-1}) = \frac{Nutrient\ concentration\ (\%) \times Biomass\ (kg\ ha^{-1})}{100}$$

The prices of the inputs that were prevailing at the time of their use were taken into account to work out the cost of cultivation. Gross returns were calculated using the pooled pigeonpea equivalent (kg ha$^{-1}$) and the prices of crop commodities at the time of marketing were taken into account. For working out gross returns for clusterbean, green pods were considered. The net returns per hectare was calculated by deducting the cost of cultivation from gross returns per hectare.

## RESULTS AND DISCUSSION

**Pigeonpea equivalent yield:** The intercropping of pigeonpea and clusterbean gave significantly higher pigeonpea equivalent yield (PEY) over the sole crop of pigeonpea. The intercropping treatments *viz.,* $T_3$, $T_4$, $T_5$, $T_8$ and $T_9$ recorded significantly higher pigeonpea equivalent yield over other treatments (Table 1). This was due to higher seed/ green pod yields of component crops owing to optimum nutrient availability coupled with higher price of both the crops which contributed to higher pigeonpea equivalent yields. Similar results have been opined by Jain *et al*. (2001), **Anup Das** *et al*. (2002) and Ranbirsingh *et al*. (2002).

**Land equivalent ratio and area time equivalent ratio:** All the intercropping fertilized treatments showed significantly higher LER over sole crop. The significantly higher LER of 1.71 was in $T_5$ followed by $T_4$, $T_3$, $T_9$ and $T_8$. The $T_6$ and $T_7$ recorded significantly lower land equivalent ratio compared to other intercropped treatments (Table 1). Thus, the yield advantage of 10 to 71 per cent was obtained due to intercropping pigeonpea and clusterbean over their respective sole crops. This indicated that, intercropping system was highly efficient in utilizing growth resources than sole cropping of component crops. Similar results of higher land equivalent ratio were reported by Koli *et al.* (2004) in inter and intra cropping of vegetables in castor and Arjun Sharma and Guled (*2012) were also found that* pigeonpea + greengram (1:2) intercropping system under set-furrow with application of vermicompost @ 2.5 t ha$^{-1}$ recorded significantly higher pigeonpea equivalent yield, LER and ATER over other intercropping systems. Similar to LER, ATER was significantly higher under the treatment, $T_5$ (1.54) followed by $T_4$, $T_3$, $T_9$ and $T_8$. The ATER under $T_6$ and $T_7$ was significantly lower when compared to other intercropped treatments (Table 1). The higher ATER values in $T_5$, $T_4$, $T_3$, $T_9$ and $T_8$ indicated that the intercropping system was highly efficient in utilizing growth resources than sole cropping of

component crops. Area time equivalent ratio obtained in $T_6$ (1.01) suggested that, only organic source of phosphorus nutrient in the intercropping system was not sufficient for getting the higher yield advantage. This revealed that intercropping without proper nutrient management was not advantageous.

**Uptake of nutrients:** The total uptake of nitrogen, phosphorus and potassium was significantly higher in all the intercropping treatments compared to sole crop of pigeonpea and clusterbean (Table 2). Among the intercropping treatments, $T_5$ (100 per cent RDF to pigeonpea and 100 per cent RDF to clusterbean) recorded significantly higher total uptake of nitrogen (224.79 kg ha$^{-1}$), phosphorus (32.14 kg ha$^{-1}$) and potassium 137.66 kg ha$^{-1}$. Further, reduction in the fertilizer level to any of the component crops from 100 per cent RDF decreased the total uptake of nitrogen, phosphorus and potassium. These results were in confirmation with the findings of Jat and Ahlawat (2003), Tolanur and Badanur (2003) and Singh *et al.* (2006)

**Economics:** The sole crop of pigeonpea with 100 per cent RDF recorded significantly lower net returns when compared to other treatments except $T_6$ which was on par with $T_1$ (Table 3). The sole crop of clusterbean with 100 per cent RDF ($T_2$) recorded significantly higher net returns of Rs. 46,801 ha$^{-1}$ compared to sole pigeonpea with 100 per cent RDF ($T_1$) (Rs. 25,927 ha$^{-1}$). Among intercropped treatments, 100 per cent RDF to pigeonpea and 100 per cent RDF to clusterbean ($T_5$) obtained significantly higher net returns (Rs. 67,888 ha$^{-1}$)

over other treatments. Among other treatments, the on par net returns were recorded between $T_3$, $T_4$ and $T_9$. Application of phosphorus only through organics (FYM) ($T_6$) recorded lowest net returns (Rs. 26,854 ha$^{-1}$) among the intercropped treatments indicating an integrated method of supplying phosphorus is important to get higher returns. These results are conformation with Rathod *et al.* (2004) who concluded that intercropping of groundnut and frenchbean with pigeonpea in 1:2 or 1:3 row ratio resulted in maximum pigeonpea equivalent yield, net returns and B:C ratio over other intercropping systems and sole pigeonpea. Similarly, higher net returns with optimum fertilization were also observed by Vishwanatha (2009) in pigeonpea + sunflower intercropping system. Among all the treatments, $T_5$ recorded significantly higher B:C ratio of 2.89, compared to other treatments. This was due to the relatively wider gap between cost of cultivation incurred and net returns accrued, compared to other treatments. Application of phosphorus only through FYM ($T_6$) recorded lowest B:C ratio of 1.76 among all the treatments indicating the futility of the system where integrated method of supplying phosphorus is lacking.

The intercropping not only helps to solve the problems of pulses and vegetable production but also helps to bring additional income to farmers and to get higher benefits with lower cost of cultivation and helps to utilize the growth resources, time (duration) very efficiently and numerically the land usage can be intensified. Therefore, pigeonpea equivalent yield, land equivalent ratio, area time equivalent

**Table 1.** PEY (q ha$^{-1}$), LER and ATER as influenced by nutrient management practices in pigeonpea and clusterbean (1:1) intercropping system

| Treatment | Treatment details | PEY (q ha$^{-1}$) | | | LER | | | ATER |
|---|---|---|---|---|---|---|---|---|
| | | PP* | CB* | Total | PP** | CB** | Total | |
| $T_1$ | Sole pigeonpea with 100% RDF | 13.14 | – | 13.14 | 1.00 | – | 1.00 | 1.00 |
| $T_2$ | Sole clusterbean with 100% RDF | – | 20.71 | 20.71 | – | 1.00 | 1.00 | 1.00 |
| $T_3$ | 100% RDF to pigeonpea and no fertilizer to clusterbean | 10.41 | 12.08 | 22.49 | 0.79 | 0.59 | 1.38 | 1.26 |
| $T_4$ | 125% RDF to pigeonpea and no fertilizer to clusterbean | 10.95 | 12.92 | 23.87 | 0.83 | 0.63 | 1.46 | 1.33 |
| $T_5$ | 100% RDF to pigeonpea and 100% RDF to clusterbean | 10.84 | 18.40 | 29.24 | 0.82 | 0.89 | 1.71 | 1.54 |
| $T_6$ | 100% RDP of pigeonpea to all rows of both crops through FYM and nitrogen through inorganic fertilizer | 8.21 | 9.81 | 18.02 | 0.62 | 0.48 | 1.10 | 1.01 |
| $T_7$ | 75% RDP of pigeonpea to all rows of both crops through FYM and rest of 25% RDP and nitrogen through inorganic fertilizer | 8.86 | 10.35 | 19.21 | 0.67 | 0.50 | 1.17 | 1.07 |
| $T_8$ | 50% RDP of pigeonpea to all rows of both crops through FYM and rest of 50% RDP and nitrogen through inorganic fertilizer | 9.87 | 11.27 | 21.14 | 0.75 | 0.55 | 1.30 | 1.19 |
| $T_9$ | 25% RDP of pigeonpea to all rows of both crops through FYM and rest of 75% RDP and nitrogen through inorganic fertilizer | 10.11 | 11.52 | 21.63 | 0.77 | 0.56 | 1.33 | 1.21 |
| | CD (P=0.05) | – | – | 1.61 | – | – | 0.09 | 0.08 |

| RDF | : | Recommended Dose of Fertilizer | RDF of pigeonpea | : | 25:50 kg ha$^{-1}$ of N and $P_2O_5$ |
|---|---|---|---|---|---|
| RDP | : | Recommended Dose of Phosphorus | RDF of clusterbean | : | 25:75:60 kg ha$^{-1}$ of N, $P_2O_5$ and $K_2O$ |
| PP* | : | Pigeonpea contribution to PEY | PP** | : | Pigeonpea contribution to LER |
| CB* | : | Clusterbean contribution to PEY | CB** | : | Clusterbean contribution to LER |

**Table 2.** Total uptake (kg ha$^{-1}$) of major nutrients and economics as influenced by nutrient management practices in pigeonpea and clusterbean (1:1) intercropping system

| Treatment | Total uptake (kg ha$^{-1}$) | | | Economics | | | |
|---|---|---|---|---|---|---|---|
| | Nitrogen | Phosphorus | Potassium | Cost of cultivation (Rs. ha$^{-1}$) | Gross returns (Rs. ha$^{-1}$) | Net returns (Rs. ha$^{-1}$) | Benefit cost ratio |
| $T_1$ | 187.64 | 27.84 | 47.21 | 20,720 | 46,647 | 25,927 | 2.25 |
| $T_2$ | 169.75 | 21.31 | 99.44 | 26,674 | 73,475 | 46,801 | 2.75 |
| $T_3$ | 201.52 | 28.72 | 122.31 | 29,800 | 78,203 | 48,403 | 2.62 |
| $T_4$ | 207.67 | 29.39 | 128.92 | 30,950 | 84,978 | 54,028 | 2.75 |
| $T_5$ | 224.79 | 32.14 | 137.66 | 35,920 | 1,03,808 | 67,888 | 2.89 |
| $T_6$ | 184.68 | 24.92 | 98.31 | 35,510 | 62,364 | 26,854 | 1.76 |
| $T_7$ | 187.19 | 25.87 | 104.55 | 33,332 | 67,393 | 34,061 | 2.02 |
| $T_8$ | 202.55 | 28.94 | 107.78 | 31,154 | 72,408 | 41,254 | 2.32 |
| $T_9$ | 208.27 | 29.52 | 123.99 | 28,976 | 76,318 | 47,342 | 2.63 |
| | 8.13 | 1.03 | 8.71 | — | — | 7,229 | 0.12 |

See table 1 for treatment details

ratio, net returns and benefit cost ratio were significantly higher in intercropping treatment fertilized with 100 per cent RDF to both the crops as compared to sole crops.

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# Long Term Effect of Integrated Nutrient Management on Distribution of Total Zn, Cu, Fe and Mn under Rice-Wheat System

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**Abstract**: Laboratory analysis was made on the soil samples collected at two intervals (April 2013 and October 2014) from an on-going long-term field experiment (in progress since *Kharif* 2009-10) at PAU, Ludhiana to investigate the effect of manure and chemical fertilizers in rice-wheat system. The organic manure through bio gas slurry (BGS) @ 6 t ha$^{-1}$ was incorporated along with nitrogen fertilizer (N @ 80 and 120 kg ha$^{-1}$), phosphorus fertilizer (P @ 30 kg ha$^{-1}$) and potassium fertilizer (K @ 30 kg ha$^{-1}$) to the rice crop. The total Zn (61.85 to 63.31mg kg$^{-1}$), Cu (11.97 to 14.07 mg kg$^{-1}$), Fe (772.8 to 778.0 mg kg$^{-1}$) and Mn (294.9 to 300.1 mg kg$^{-1}$) was observed where organic manure @ 6 t ha$^{-1}$was added along with N @ 80 kg ha$^{-1}$ and P$_2$O$_5$ @ 30 kg ha$^{-1}$before sowing of wheat. Similar trend of total Zn (68.25 to 70.25 mg kg$^{-1}$), Cu (10.30 to 11.33 mg kg$^{-1}$), Fe (1.45 to 1.47 %) and Mn (193.2 to 196.9 mg kg$^{-1}$) was observed reported organic manure @ 6 t ha$^{-1}$was added along with N @ 80 kg ha$^{-1}$ and P$_2$O$_5$ @ 30 kg ha$^{-1}$after harvesting of rice. The results of this study were of practical utility since application of manure and chemical fertilizers together increased total Zn, Cu, Fe and Mn in the soil over control.

**Keywords**: Total Zn, Cu, Fe and Mn, Biogas slurry manure, Chemical fertilizers, Rice-wheat system

Rice-wheat is the predominant cropping system, being practiced by majority of farmers in different agro-climatic zones of Punjab. During 2011-12, the net sown area under rice and wheat in Punjab was 28.3 and 35.2 lakh ha, respectively (Anonymous 2011-12, 2012). Relatively, over use of high analysis macronutrient fertilizers and less use of manures has induced secondary and micronutrient deficiencies in the Indo Gangetic Plains. In several areas with intensive cropping, Zn deficiency appeared initially and subsequently the deficiencies of Fe and Mn were observed. Herencia *et al.* (2008) reported that with the addition of organic and mineral fertilization, OM-bound fractions of micronutrients increased their availability and uptake in the soil. Sekhon *et al.* (2006) reported that Fe and Mn associated with organic matter (OM-bound fraction) increased with application of manure, which increased the yield under rice-wheat system. In a field experiment, Behera *et al.,* (2008) reported the distribution of total micronutrient, Zn fractions and their contribution toward availability and plant uptake of Zn under long-term maize-wheat cropping in an inceptisol. Dhaliwal *et al.* (2011) reported distribution of total micronutrient fractions and their contribution towards availability and plant uptake of micronutrient under long term maize-wheat cropping sequence indicated residual micronutrients as the dominant proportion of total Zn and Cu. Zhang *et al.* (2008) observed the long term effect of manure application on micronutrients availability under rice-wheat and maize-wheat cropping systems. The distribution of total Zn, Cu, Fe and Mn and their contribution towards availability and plant uptake under long-term maize-wheat system was reported(Agbenin and Henningsen2004) who indicated that the residual micronutrient fraction as the dominant portion of total Zn, Cu, Fe, and Mn fraction. Similarly, Behera *et al.* (2008) and Behera and Singh (2009) reported the distribution of total Zn fraction and their contribution toward availability and plant uptake of Zn under long-term maize-wheat cropping in an inceptisol.

Various research studies revealed that application of manure in combination with chemical fertilizers increased the OC level and micronutrients in soil. The information on surface distribution of total micronutrients with application of manure and fertilizers is meager in rice-wheat system. Taking these points into consideration the research has been conducted to study the effect of manure and fertilizers on total Zn, Cu, Fe and Mn in surface soils under rice-wheat system.

## MATERIAL AND METHODS

Laboratory analysis was conducted on the soil samples collected from an on-going long-term experiment on role of manure and fertilizers in rice-wheat cropping system (in progress since *Kharif*2009-10) at Punjab Agricultural University, Ludhiana. The experiment was conducted in a

fixed layout since its beginning with treatments combinations (Table 1). Each treatment was replicated thrice. The experiment was laid out in a split plot design with four main and three sub treatments. The organic manure through bio gas slurry (BGS) @ 6 t ha$^{-1}$ was incorporated along with nitrogen fertilizer (N @ 80 and 120 kg ha$^{-1}$), phosphorus fertilizer (P @ 30 kg ha$^{-1}$) and potassium fertilizer (K @ 30 kg ha$^{-1}$) were applied to the rice crop. Whereas in wheat crop, nitrogen fertilizer (N @ 120 kg ha$^{-1}$) with levels of phosphatic fertilizer (P @ 30 and 60 kg ha$^{-1}$) and potassium fertilizer (K @ 30 kg ha$^{-1}$) were applied.

**Total Zn, Cu, Fe and Mn:** For total elemental analysis of Zn, Cu, Fe and Mn, a 0.5 gm sample of soil was digested with 5 ml of hydrofluoric acid (HF), 1.0 ml of perchloric acid (HClO$_4$) and 5-6 drops of nitric acid (HNO$_3$) in a 30 ml capacity platinum crucibles (Page *et al.* 1982). When the soil became completely dry in the crucible the residue in the crucible was completely dissolved in 5ml of 6N HCl. The contents of the crucible were transferred to 100 ml volumetric **flask** with double distilled water. After appropriate dilutions, the concentrations total Zn, Cu, Fe and Mn fractions were measured using varian model of atomic absorption spectrophotometer (AAS FS 240 Model).

**Table 1.** Treatment detail of long term experiment in rice–wheat cropping system

| Treatments | Rice | | | Wheat |
|---|---|---|---|---|
| | Manure | N | P$_2$O$_5$ | P$_2$O$_5$ |
| | (t ha$^{-1}$) | (kg ha$^{-1}$) | | (kg ha$^{-1}$) |
| T$_1$ | 0 | 120 | 0 | 0 |
| T$_2$ | 0 | 120 | 0 | 30 |
| T$_3$ | 0 | 120 | 0 | 60 |
| T$_4$ | 6 | 80 | 30 | 0 |
| T$_5$ | 6 | 80 | 30 | 30 |
| T$_6$ | 6 | 80 | 30 | 60 |
| T$_7$ | 0 | 120 | 30 | 0 |
| T$_8$ | 0 | 120 | 30 | 30 |
| T$_9$ | 0 | 120 | 30 | 60 |
| T$_{10}$ | 6 | 80 | 0 | 0 |
| T$_{11}$ | 6 | 80 | 0 | 30 |
| T$_{12}$ | 6 | 80 | 0 | 60 |

## RESULTS AND DISCUSSION

**Distributions of total Zn, Cu, Fe and Mn in surface soil before sowing of wheat:** The total Zn ranged from 54.10 to 68.26 mg kg$^{-1}$ in the treatment combinations (Table 2). Significantly higher concentration of total Zn was observed where organic manure @ 6 t ha$^{-1}$ has been added along with N @ 80 kg ha$^{-1}$ and with and without P$_2$O$_5$ @ 30 kg ha$^{-1}$ applied

to the rice crop as compared to the treatments where no organic manure has been added and only N @ 120 kg ha$^{-1}$ and P$_2$O$_5$ @ 30 kg ha$^{-1}$ were applied to the rice crop. Similarly, significant increased concentration of total Zn was reported in the treatments where organic manure @ 6 t ha$^{-1}$ was applied along with N @ 80 kg ha$^{-1}$ without the application of phosphorus fertilizer as compared to the treatments where no organic manure was added but N @ 120 kg ha$^{-1}$ and P$_2$O$_5$ @ 30 kg ha$^{-1}$ were applied to the rice crop. Whereas in the wheat crop, the different levels of P$_2$O$_5$ (0, 30 and 60 kg ha$^{-1}$) were applied, significant decrease in concentration of total Zn has been observed with increase in levels of phosphorus fertilizer. The interaction between rice and wheat treatments is also observed as significant. Similarly, the concentration of total Cu in surface (0–15 cm) soil samples ranged from 9.73 to 19.07 mg kg$^{-1}$ in different treatment combinations (Table 2). The significant increase in total Cu was estimated in the treatments where organic manure @ 6 t ha$^{-1}$ has been incorporated along with N @ 80 kg ha$^{-1}$ and P$_2$O$_5$ @ 30 kg ha$^{-1}$ applied to the rice crop as compared to the treatments where no organic manure has been added and only N @ 120 kg ha$^{-1}$ and P$_2$O$_5$ @ 30 kg ha$^{-1}$ were applied to the rice crop. There was significantly increased concentration of total Cu was in the treatments where organic manure @ 6 t ha$^{-1}$ was incorporated along with N @ 80 kg ha$^{-1}$ without the application of phosphorus fertilizer as compared to the treatments where no organic manure was incorporated but N @ 120 kg ha$^{-1}$ and P$_2$O$_5$ @ 30 kg ha$^{-1}$ were applied to the rice crop. Further significant increase was also observed in the treatments where organic manure @ 6 t ha$^{-1}$ has been applied along with N @ 80 kg ha$^{-1}$ and P$_2$O$_5$ @ 30 kg ha$^{-1}$ applied to the rice crop as compared to the treatments where only N @ 120 kg ha$^{-1}$ was applied without the application of phosphorus fertilizer with no organic manure.

The total Fe in surface (0–15 cm) soil samples ranged from 753.77 to 788.57 mg kg$^{-1}$ in all different treatment combinations (Table 2). A significant increase in the concentration of total Fe has been observed in the treatments where organic manure @ 6 t ha$^{-1}$ has been added along with N @ 80 kg ha–1 and P$_2$O$_5$ @ 30 kg ha$^{-1}$ applied to the rice crop as compared to the treatments where no organic manure has been added and no P fertilizer was applied, only N @ 120 kg ha$^{-1}$ was applied to the rice crop. Similarly, significant increased concentration of total Zn was also noticed in the treatments where organic manure @ 6 t ha$^{-1}$ was incorporated along with N @ 80 kg ha$^{-1}$ without the application of phosphorus fertilizer as compared to the treatments where no organic manure was incorporated but N @ 120 kg ha$^{-1}$ and P$_2$O$_5$ @ 30 kg ha$^{-1}$ were applied to the rice

**Table 2.** Effect of manure and fertilizers on total Zn, Cu, Fe and Mn in surface (0–15 cm) soil before sowing of wheat

| Treatments to rice | Rates of P applied to wheat (kg $P_2O_5$ ha$^{-1}$) | | | Mean |
|---|---|---|---|---|
| | $P_0$ | $P_{30}$ | $P_{60}$ | |
| **Total Zn (mg kg$^{-1}$)** | | | | |
| $M_0 N_{120} P_0$ | 60.67 | 59.34 | 58.11 | 59.37 |
| $M_6 N_{80} P_{30}$ | 63.31 | 62.08 | 61.85 | 62.42 |
| $M_0 N_{120} P_{30}$ | 56.22 | 55.01 | 54.10 | 55.11 |
| $M_6 N_{80} P_0$ | 68.26 | 65.80 | 65.01 | 66.36 |
| Mean | 62.12 | 60.56 | 59.77 | – |
| LSD (p<0.05) | R=0.60, | W=0.38, | RxW=0.76 | |
| **Total Cu (mg kg$^{-1}$)** | | | | |
| $M_0 N_{120} P_0$ | 11.37 | 10.97 | 10.87 | 11.07 |
| $M_6 N_{80} P_{30}$ | 14.07 | 12.60 | 11.97 | 12.88 |
| $M_0 N_{120} P_{30}$ | 10.60 | 9.80 | 9.73 | 10.04 |
| $M_6 N_{80} P_0$ | 19.07 | 17.77 | 15.67 | 17.50 |
| Mean | 13.78 | 12.78 | 12.06 | – |
| LSD (p<0.05) | R=0.62, | W=0.44, | RxW=0.87 | |
| **Total Fe (mg kg$^{-1}$)** | | | | |
| $M_0 N_{120} P_0$ | 768.07 | 764.27 | 761.00 | 764.41 |
| $M_6 N_{80} P_{30}$ | 778.00 | 774.73 | 772.83 | 775.19 |
| $M_0 N_{120} P_{30}$ | 758.37 | 754.90 | 753.77 | 755.68 |
| $M_6 N_{80} P_0$ | 788.57 | 785.23 | 780.70 | 784.83 |
| Mean | 773.23 | 769.78 | 767.08 | – |
| LSD (p<0.05) | R=1.31, | W=0.57, | RxW=1.13 | |
| **Total Mn(mg kg$^{-1}$)** | | | | |
| $M_0 N_{120} P_0$ | 293.83 | 291.70 | 288.83 | 291.46 |
| $M_6 N_{80} P_{30}$ | 300.13 | 297.93 | 294.87 | 297.64 |
| $M_0 N_{120} P_{30}$ | 284.53 | 281.87 | 278.87 | 281.76 |
| $M_6 N_{80} P_0$ | 310.13 | 306.20 | 304.93 | 307.09 |
| Mean | 297.16 | 294.43 | 291.88 | – |
| LSD (p<0.05) | R=0.52, | W=0.42, | RxW=NS | |

$M_0$= No Manure, $M_6$= Manure @ 6 ton ha$^{-1}$, $N_{80}$= 80 kg N ha$^{-1}$, $N_{120}$= 120 kg N ha$^{-1}$ $P_0$= No $P_2O_5$, $P_{30}$= 30 kg $P_2O_5$ ha$^{-1}$, $P_{60}$= 60 kg $P_2O_5$ ha$^{-1}$, R= Rice, W=Wheat, RxW= Interaction

crop. Whereas in the wheat crop, the different levels of $P_2O_5$ (0, 30 and 60 kg ha$^{-1}$) were applied to the wheat crop, a significant decrease in concentration of total Fe has been observed at these levels. The interaction between rice and wheat crop is also observed as significant. Total Mn in surface (0–15 cm) soil samples ranged from 278.87 to 310.13 mg kg$^{-1}$ in all the treatments (Table 2). There was significant increase in the concentration of total Mn has in the treatments where organic manure @ 6 t ha$^{-1}$ has been added along with N @ 80 kg ha$^{-1}$ and $P_2O_5$ @ 30 kg ha$^{-1}$ applied to the rice crop as compared to the treatments where only N @ 120 kg ha$^{-1}$ was applied to the rice crop and no organic manure has been

added and no P fertilizer was applied. Similarly, significantly higher concentration of total Mn was also noticed in the treatments where organic manure @ 6 t ha$^{-1}$ was applied along with N @ 80 kg ha$^{-1}$ without the application of phosphorus fertilizer as compared to the treatments where no organic manure was added but N @ 120 kg ha$^{-1}$ and $P_2O_5$ @ 30 kg ha$^{-1}$ were applied to the rice crop. The interaction between treatments of rice and wheat crops is also observed as non significant. Dhaliwal *et al.* (2011) reported higher concentration of total micronutrient fractions and their contribution towards availability under long term maize– wheat cropping sequence indicating residual micronutrients as the dominant proportion of total Zn and Cu.

**Distributions of total Zn, Cu, Fe and Mn in surface soil after harvesting of rice:** The concentration of total Zn ranged from 62.34 to 75.03 mg kg$^{-1}$ in all the treatment combinations. Significantly higher concentration of total Zn has been observed in the treatments where organic manure @ 6 t ha$^{-1}$ has been incorporated along with N @ 80 kg ha$^{-1}$ and $P_2O_5$ @ 30 kg ha$^{-1}$ applied to the rice crop as compared to the treatments where no organic manure has been added and only N (120 kg ha$^{-1}$) and $P_2O_5$ (30 kg ha$^{-1}$) fertilizers were applied to the rice crop. Similarly, significantly higher concentration of total Zn was also noticed in the treatments where organic manure @ 6 t ha$^{-1}$ was incorporated along with N @ 80 kg ha$^{-1}$ without the application of phosphorus fertilizer as compared to the treatments where no organic manure was added but N @ 120 kg ha$^{-1}$ and $P_2O_5$ @ 30 kg ha$^{-1}$ were applied to the rice crop. There is a significant increase in the concentration of total Zn in the treatments where organic manure @ 6 t ha$^{-1}$ has been incorporated along with N @ 80 kg ha$^{-1}$ and $P_2O_5$ @ 30 kg ha$^{-1}$ applied to the rice crop as compared to the treatments where only N @ 120 kg ha$^{-1}$ was applied without the application of phosphorus fertilizer and also no organic manure was incorporated to the rice crop. Whereas in the wheat crop, the different levels of $P_2O_5$ (0, 30 and 60 kg ha$^{-1}$) were applied, significant decrease in concentration of total Zn has been observed with increase in levels of phosphorus fertilizer. The interaction between rice and wheat crop treatments is also observed as significant. Similarly, the concentration of total Cu in surface (0–15 cm) soil samples ranged from 9.33 to 11.33 mg kg$^{-1}$ in different treatment combinations (Table 3). Among the different treatments, a significant increase in total Cu was in the treatments where organic manure @ 6 t ha$^{-1}$ has been incorporated along with N @ 80 kg ha$^{-1}$ and $P_2O_5$ @ 30 kg ha$^{-1}$ applied to the rice crop as compared to the treatments where no organic manure has been added and only N @ 120 kg ha$^{-1}$ and $P_2O_5$ @ 30 kg ha$^{-1}$ were applied to the rice crop. Further

**Table 3.** Effect of manure and fertilizers on total Zn, Cu, Fe and Mn in surface soil after harvesting of rice

| Treatments to rice | Rates of P applied to wheat (kg $P_2O_5$ ha$^{-1}$) | | | Mean |
|---|---|---|---|---|
| | $P_0$ | $P_{30}$ | $P_{60}$ | |
| **Total Zn (mg kg$^{-1}$)** | | | | |
| $M_0 N_{120} P_0$ | 68.21 | 66.69 | 66.05 | 66.98 |
| $M_6 N_{80} P_{30}$ | 68.25 | 69.05 | 70.25 | 69.18 |
| $M_0 N_{120} P_{30}$ | 65.07 | 63.35 | 62.34 | 63.59 |
| $M_6 N_{80} P_0$ | 73.20 | 75.03 | 71.78 | 73.34 |
| Mean | 68.68 | 68.53 | 67.61 | – |
| LSD (p<0.05) | R=0.60, | W=0.36, | RxW=0.72 | |
| **Total Cu (mg kg$^{-1}$)** | | | | |
| $M_0 N_{120} P_0$ | 10.27 | 9.86 | 9.60 | 9.91 |
| $M_6 N_{80} P_{30}$ | 10.63 | 10.30 | 11.33 | 10.76 |
| $M_0 N_{120} P_{30}$ | 9.73 | 9.33 | 8.97 | 9.34 |
| $M_6 N_{80} P_0$ | 10.77 | 10.60 | 10.53 | 10.63 |
| Mean | 10.35 | 10.02 | 10.11 | – |
| LSD (p<0.05) | R=0.64, | W=NS, | RxW=NS | |
| **Total Fe (mg kg$^{-1}$)** | | | | |
| $M_0 N_{120} P_0$ | 1.43 | 1.40 | 1.38 | 1.40 |
| $M_6 N_{80} P_{30}$ | 1.47 | 1.47 | 1.45 | 1.46 |
| $M_0 N_{120} P_{30}$ | 1.39 | 1.36 | 1.35 | 1.37 |
| $M_6 N_{80} P_0$ | 1.51 | 1.49 | 1.48 | 1.49 |
| Mean | 1.45 | 1.43 | 1.42 | – |
| LSD (p<0.05) | R=0.01, | W=0.01, | RxW=NS | |
| **Total Mn(mg kg$^{-1}$)** | | | | |
| $M_0 N_{120} P_0$ | 192.13 | 190.13 | 187.10 | 189.79 |
| $M_6 N_{80} P_{30}$ | 196.93 | 195.53 | 193.20 | 195.22 |
| $M_0 N_{120} P_{30}$ | 188.10 | 186.83 | 185.03 | 186.66 |
| $M_6 N_{80} P_0$ | 201.50 | 198.97 | 197.00 | 199.16 |
| Mean | 194.67 | 192.87 | 190.58 | – |
| LSD (p<0.05) | R=0.30, | W=0.48, | RxW=NS | |

$M_0$= No Manure, $M_6$= Manure @ 6 ton ha$^{-1}$, $N_{80}$= 80 kg N ha$^{-1}$, $N_{120}$= 120 kg N ha$^{-1}$ $P_0$= No $P_2O_5$, $P_{30}$= 30 kg $P_2O_5$ ha$^{-1}$, $P_{60}$= 60 kg $P_2O_5$ ha$^{-1}$, R= Rice, W=Wheat, RxW= Interaction

significant increased total Cu was also observed in the treatments where organic manure @ 6 t ha$^{-1}$ has been incorporated along with N @ 80 kg ha$^{-1}$ and $P_2O_5$ @ 30 kg ha$^{-1}$ applied to the rice crop as compared to the treatments where only N @ 120 kg ha$^{-1}$ was applied without the application of phosphatic fertilizer and also no organic manure was incorporated to the rice crop. The concentrations of total Fe in surface (0–15 cm) soil samples ranged from 1.35 to 1.51 per cent in all different treatment combinations. The increase in the concentration of total Fe was significant in the treatments where organic manure @ 6 t ha$^{-1}$ has been incorporated along with N @ 80 kg ha$^{-1}$ and $P_2O_5$ @ 30 kg ha$^{-1}$ applied to the rice crop as compared to the treatments where no organic

manure has been added and no P fertilizer was applied, only N @ 120 kg ha$^{-1}$ was applied to the rice crop. Significantly increased concentration of total Zn was also observed in the treatments where organic manure @ 6 t ha$^{-1}$ was incorporated along with N @ 80 kg ha$^{-1}$ without the application of phosphorus fertilizer as compared to the treatments where no organic manure was incorporated but N @ 120 kg ha$^{-1}$ and $P_2O_5$ @ 30 kg ha$^{-1}$ were applied to the rice crop. Whereas in the wheat crop, the different levels of $P_2O_5$ (0, 30 and 60 kg ha$^{-1}$) were applied to the wheat crop, a significant decrease in concentration of total Fe has been observed at these levels. The interaction between rice and wheat crop is also observed as significant.

The concentration of total Mn in surface (0–15 cm) soil samples collected ranged from 185.03 to 201.50 mg kg$^{-1}$ in all the different treatments (Table 3).A significant increase in the concentration of total Mn (193.20 to 196.93 mg kg$^{-1}$) was observed in the treatments where organic manure @ 6 t ha$^{-1}$ was added along with N @ 80 kg ha$^{-1}$ and $P_2O_5$ @ 30 kg ha$^{-1}$ applied to the rice crop as compared to the treatments where only N @ 120 kg ha$^{-1}$ was applied to the rice crop and no organic manure has been added and no P fertilizer was applied. Significantly higher concentration of total Mn was also noticed in the treatments where organic manure @ 6 t ha$^{-1}$ was incorporated along with N @ 80 kg ha$^{-1}$ without the application of phosphorus fertilizer as compared to the treatments where no organic manure was incorporated but N @ 120 kg ha$^{-1}$ and $P_2O_5$ @ 30 kg ha$^{-1}$ were applied to the rice crop. Similarly significant increase was also observed in the treatments where organic manure @ 6 t ha$^{-1}$ has been incorporated along with N @ 80 kg ha$^{-1}$ and $P_2O_5$ @ 30 kg ha$^{-1}$ applied to the rice crop as compared to the treatments where no organic manure was incorporated but N @ 120 kg ha$^{-1}$ and $P_2O_5$ @ 30 kg ha$^{-1}$ were applied to the rice crop. In the wheat crop, the different levels of $P_2O_5$ (0, 30 and 60 kg ha$^{-1}$) were applied to the wheat crop, a significant response was observed in concentration of total Mn at these levels. The interaction between rice and wheat crop is observed as non significant. These results were in accordance with those reported by Dhaliwal *et al.* (2011) for total micronutrient fractions under long term maize-wheat cropping sequence which further indicated residual micronutrients as the dominant proportion of total Zn and Cu.

## CONCLUSION

The total Zn, Cu, Fe and Mn concentration in the soil increased with incorporation of organic manure along with chemical fertilizers. Organic matter during decomposition helps in release of Zn, Cu, Fe and Mn from the unavailable

fraction in the soil. The total Zn, Cu, Fe and Mn were significantly higher in soil where organic manure (BGS) was incorporated in combination with the chemical fertilizers.

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# Growth Pattern and Productivity of Component Crops in Intercropping of Indian Rape (*Brassica rapa* var. *Toria*) with Canola Oilseed Rape (*Brassica napus*) and Ethiopian Mustard (*Brassica carinata*)

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**Abstract:** A study was carried out at Punjab Agricultural University, Ludhiana during *rabi* 2014–15 to study the growth compatibility and production potential of Indian rape with oilseed rape and Ethiopian mustard in different row proportions and row spacing. Differences in plant height among treatments were apparent at 60 days after sowing (DAS) and later growth stages. Dry matter accumulation (DMA) by component crops at different growth stages was significantly influenced by intercropping systems (ICS). Number of days required for flowering and physiological maturity of component crops was not influenced by ICS. Differences among treatments for SPAD (soil plant analysis development) cholorophyll values at all growth stages, and for photosynthetically active radiation (PAR) interception at 60 and 90 DAS were inconspicuous. Seed and stover yields of sole crops were higher than their respective yields in ICS. The highest oilseed rape equivalent yield produced by non canola oilseed rape + Indian rape (1:1) at 22.5 cm row spacing was 22.9% higher than sole non canola oilseed rape (1849 kg ha$^{-1}$). Similarly canola oilseed rape + Indian rape (1:1 and 2:1) at 22.5 cm row spacing produced 16.9% and 20.1% higher seed yield than canola oilseed rape alone (1630 kg ha$^{-1}$). Non canola oilseed rape was more compatible with Indian rape than canola oilseed rape and Ethiopian mustard.

**Keywords:** Growth, Intercropping, Phenology, Row spacing, Row proportions, Seed yield, Stover yield

The current annual production of edible oils (9.86 million tonnes) in the country fulfils only about 48 per cent of the domestic requirements. With almost stagnation in area under oilseeds and rather diversion of area earlier under major oilseed crops to cereal crops to meet increasing demand of food grains, the increasing requirements of edible oils can be met with introduction of high yielding, superior quality cultivars and vertical growth (intercropping) of oilseed crops. Intercropping aims at higher income and judicious complementary and supplementary use of available resources (Reddy and Suresh, 2008). Higher production of rapeseed and mustard required to meet the demand of edible oils in the country can be achieved by their intercropping with other crops. Planting patterns and varying row ratios of component crops may modify the crop canopy structure and microclimate and influence light interception, rooting pattern, nutrient and moisture extraction pattern to ultimately influence the yield. Optimum row spacing ensures better light interception and its penetration in to the crop canopy and enhances light utilization efficiency in crop plants (Eberbach and Pala, 2005). Indian rape (*Brassica rapa* variety *Toria*) is an important oilseed catch crop which fits well in the major cropping systems. Recently nutritionally superior canola

cultivars of oilseed rape (*Brassica napus*) of about 145–150 days duration and dwarf–determinate cultivars of Ethiopian mustard (*Brassica carinata*) of about 160–165 days duration have been developed. The objective of this investigation was to study the agronomic feasibility of component crops in Indian rape + oilseed rape and Indian rape + Ethiopian mustard ICS vis–à–vis production potential of these ICS.

## MATERIAL AND METHODS

The field experiment was conducted with fourteen treatments at the Punjab Agricultural University, Ludhiana during *rabi* 2014–15 on loamy sand soil free from salts (EC 0.12 dS m$^{-1}$), neutral in pH (7.2), low in organic carbon content (0.28%), low in available nitrogen (188 kg ha$^{-1}$), rich in available phosphorus (28 kg ha$^{-1}$) and medium in available potassium (148 kg ha$^{-1}$) at 0–15 cm soil depth. Different treatments were: T1: Canola oilseed rape + Indian rape in 1:1 row ratio at 22.5 cm spacing, T2: Canola oilseed rape + Indian rape in 2:1 row ratio at 22.5 cm spacing, T3: Canola oilseed rape + Indian rape in 2:1 row ratio at 30 cm spacing, T4: Ethiopian mustard + Indian rape in 1:1 row ratio at 22.5 cm row spacing, T5: Ethiopian mustard + Indian rape in 1:1 row ratio at 30 cm row spacing, T6: Ethiopian mustard +

Indian rape in 2:1 row ratio at 22.5 cm row spacing, T7: Ethiopian mustard + Indian rape in 2:1 row ratio at 30 cm row spacing, T8: Ethiopian mustard + Indian rape in 1:2 row ratio at 22.5 cm row spacing, T9: Non canola oilseed rape + Indian rape in 1:1 row ratio at 22.5 cm row spacing, T10: Canola oilseed rape at 45 cm row spacing, T11: Ethiopian mustard at 30 cm row spacing, T12: Ethiopian mustard at 45 cm row spacing, T13: Non canola oilseed rape at 45 cm row spacing, T14: Indian rape at 30 cm row spacing. The study was carried out in randomized complete block design in three replications with gross plot size of 18.0 m$^2$ (5.0m x 3.6m).

The test cultivars TL 17 of Indian rape, GSC 7 of canola oilseed rape, GSL 1 of non canola oilseed rape and BJC13-4 of Ethiopian mustard were sown as per treatments on 18 September, 2014. Recommended agronomic practices were followed except for treatments. Basal dose of 62.5 kg N and 20 kg P$_2$O$_5$ ha$^{-1}$ was applied to sole crop of Indian rape whereas 50 kg N, 30 kg P$_2$O$_5$ and 15 kg K$_2$O ha$^{-1}$ were applied to each of oilseed rape and Ethiopian mustard grown as sole crops at time of sowing and another dose of 50 kg N ha$^{-1}$ was applied after first irrigation. In case of ICS, nutrients to each component crop were applied on area basis with time of application same as for sole crops of oilseed rape and Ethiopian mustard. Sources of N, P and K were urea, single super phosphate and muriate of potash, respectively. Indian rape was harvested in second fortnight of December, while oilseed rape and Ethiopian mustard were harvested in first fortnight of March.

Plant height, dry matter accumulation, interception of photosynthetically active radiation (PAR) by plants and SPAD (soil plant analysis development) readings were recorded at periodic intervals. Plant height was measured from 10 randomly selected plants from each treatment at each growth stage. For dry matter accumulation, plants were cut from base in the 0.5 metre row length from second outer most row in each plot, dried first under shade and later in oven at $65\pm2$°C till constant weight. Interception of photosynthetically active radiation (PAR) by plants was recorded with the Digital Plant Canopy Imager (Sun Scan Canopy Analyzer model CI-110/CI-120) from three random places in each plot between 11:00 am to 2:00 pm and averaged. The SPAD 502 Chlorophyll Meter was used for non-destructive estimation of amount of chlorophyll present in the leaves.

## RESULTS AND DISCUSSION

**Growth:** Indian rape attained grand growth between 30 and 60 days, canola oilseed rape between 60 and 90 days, non canola oilseed rape and Ethiopian mustard between 90 and 120 days of crop growth. Plant height of Indian rape, oilseed rape (canola and non canola) was significantly affected by different treatments of intercropping at different growth stages except at 30 DAS (Table 1). At 30 DAS, Ethiopian mustard sown as sole crop (30 cm/45 cm row spacing) or intercropped with Indian rape in 2:1 row ratio at 30 cm row spacing or in 1:2 row ratio at 22.5 cm row spacing attained

**Table 1.** Plant height (cm) of component crops at different growth stages as influenced by row proportions and row spacing in intercropping of oilseed rape and Ethiopian mustard with Indian rape

| Treatment | 30 *DAS | | | 60 DAS | | | 90 DAS | | | 120 DAS | | At maturity | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *IR | *OR | *EM | IR | OR | EM | IR | OR | EM | OR | EM | OR | EM |
| T1 | 14.0 | 12.1 | – | 112.2 | 57.6 | – | 116.4 | 118.8 | – | 167.0 | – | 179.7 | – |
| T2 | 15.2 | 15.4 | – | 111.4 | 59.1 | – | 126.1 | 122.3 | – | 178.3 | – | 192.7 | – |
| T3 | 14.1 | 13.9 | – | 109.8 | 63.9 | – | 121.2 | 125.3 | – | 177.3 | – | 188.3 | – |
| T4 | 16.6 | – | 12.6 | 113.2 | – | 75.8 | 122.4 | – | 156.0 | – | 177.7 | – | 185.7 |
| T5 | 14.8 | – | 12.7 | 113.0 | – | 73.3 | 124.1 | – | 171.3 | – | 184.0 | – | 194.7 |
| T6 | 15.6 | – | 12.5 | 109.3 | – | 76.6 | 121.9 | – | 170.7 | – | 186.3 | – | 192.7 |
| T7 | 16.9 | – | 14.2 | 110.9 | – | 70.9 | 126.6 | – | 169.3 | – | 184.3 | – | 190.3 |
| T8 | 15.4 | – | 14.2 | 115.1 | – | 74.5 | 125.4 | – | 162.3 | – | 181.7 | – | 194.0 |
| T9 | 15.3 | 12.5 | – | 116.1 | 50.7 | – | 124.1 | 63.8 | – | 147.0 | – | 179.0 | – |
| T10 | – | 15.2 | – | – | 62.5 | – | – | 132.3 | – | 189.7 | – | 202.3 | – |
| T11 | – | – | 15.2 | – | – | 70.7 | – | – | 169.0 | – | 192.3 | – | 194.0 |
| T 12 | – | – | 14.1 | – | – | 67.2 | – | – | 166.3 | – | 190.7 | – | 194.3 |
| T13 | – | 13.7 | – | – | 55.3 | – | – | 81.1 | – | 175.0 | – | 198.7 | – |
| T14 | 14.9 | – | – | 117.5 | – | – | 131.0 | – | – | – | – | – | – |
| CD (p=0.05) | NS | NS | 2.1 | 4.6 | 6.8 | 4.9 | 4.0 | 6.6 | 6.9 | 9.6 | 6.8 | 7.2 | NS |

*DAS = Days after sowing, IR = Indian rape, *OR = Oilseed rape, *EM = Ethiopian mustard

significantly more plant height than other treatments of intercropping of Ethiopian mustard with Indian rape. At 60 DAS, plant height of sole crop of Indian rape was at par with its intercropping with Ethiopian mustard (1:1 at 22.5 cm or 1:1 at 30 cm, 1:2 at 22.5 cm row spacing) and with non canola oilseed rape (1:1 at 22.5 cm row spacing) and significantly higher than other treatments of intercropping. Plant height of sole crop of canola oilseed rape (62.5 cm) was significantly higher than non canola oilseed rape (sole crop or intercropped with Indian rape in 1:1 in 22.5 cm apart rows). Plant height of Ethiopian mustard when intercropped with Indian rape (2:1 or 1:1 in 22.5 cm apart rows) was significantly higher than sole crop of Ethiopian mustard. At 90 DAS, Indian rape (sole crop) attained significantly higher plant height (131.0 cm) whereas it attained significantly lower plant height when intercropped with canola oilseed rape (1:1 at 22.5 cm row spacing) than all other treatments of Indian rape. In case of oilseed rape, plant height of sole crop (canola/non canola) was significantly higher than the respective crop sown under intercropping. However, plant height of Ethiopian mustard grown as sole crop (30 or 45 cm row spacing) and in different ICS was similar except in Ethiopian mustard + Indian rape in 1:1 row ratio at 22.5 cm row spacing. At both 120 DAS and at harvest, oilseed rape (canola/non canola) grown as sole crop attained significantly higher plant height than that grown in intercropping with Indian rape). At 120 DAS, plant height of canola oilseed rape

was significantly higher than non canola oilseed rape but at maturity, these differences were non-significant. Similarly at maturity, Ethiopian mustard grown as sole crop and in intercropping with Indian rape attained similar plant height. Kumar *et al.* (2002) also reported differences in plant height of *Brassica napus* and *Brassica carinata* under similar growth conditions.

In all crops, dry matter accumulation (DMA) increased continuously with crop age up to maturity, at a faster rate in Indian rape compared to oilseed rape and Ethiopian mustard (Table 2). The grand growth period of DMA was between 30 and 60 days of growth in Indian rape and between 60 and 90 days of growth in canola oilseed rape and Ethiopian mustard. At 30 DAS, DMA by sole crops was significantly more than their respective ICS). The DMA by Indian rape decreased with increasing row spacing as well as with decreasing row proportion of Indian rape in oilseed rape and Ethiopian mustard ICS. Dry matter of Indian rape (105 kg ha⁻¹) under intercropping with non canola oilseed rape was significantly higher than that under canola oilseed rape (87 kg ha⁻¹). Dry matter accumulation by sole crop of oilseed rape was significantly higher than its intercropping with Indian rape in alternate rows at 22.5 cm row spacing. In Ethiopian mustard, DMA by sole crop was significantly higher than that under its intercropping with Indian rape irrespective of row spacing and row proportion. Dry matter accumulation at 30 DAS by Ethiopian mustard sown at 30 cm and 45 cm row spacing was

**Table 2.** Dry matter accumulation (kg ha⁻¹) by component crops at different growth stages as influenced by row proportions and row spacing in intercropping of oilseed rape and Ethiopian mustard with Indian rape

| Treatment | 30 *DAS | | | | 60 DAS | | | | 90 DAS | | | | 120 DAS | | At maturity | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *IR | *OR | *EM | Total | IR | OR | EM | Total | IR | OR | EM | Total | OR | EM | OR | EM |
| T1 | 87 | 25 | – | 112 | 1613 | 385 | – | 1998 | 5120 | 1882 | – | 7002 | 2449 | – | 4574 | – |
| T2 | 69 | 33 | – | 102 | 972 | 707 | – | 1679 | 4148 | 2553 | – | 6701 | 3396 | – | 5859 | – |
| T3 | 51 | 31 | – | 82 | 785 | 633 | – | 1418 | 3197 | 1986 | – | 5183 | 2932 | – | 7406 | – |
| T4 | 91 | – | 42 | 133 | 1860 | – | 771 | 2631 | 4649 | – | 1478 | 6127 | – | 3761 | – | 5117 |
| T5 | 77 | – | 30 | 107 | 1576 | – | 710 | 2286 | 4863 | – | 1584 | 6447 | – | 3106 | – | 7092 |
| T6 | 74 | – | 47 | 121 | 1199 | – | 887 | 2086 | 3951 | – | 2775 | 6726 | – | 4796 | – | 7282 |
| T7 | 63 | – | 39 | 102 | 1035 | – | 778 | 1813 | 2507 | – | 2304 | 4811 | – | 3702 | – | 8241 |
| T8 | 133 | – | 27 | 160 | 2784 | – | 485 | 3269 | 5436 | – | 1397 | 6833 | – | 2452 | – | 4648 |
| T9 | 105 | 38 | – | 144 | 2144 | 498 | – | 2642 | 4260 | 1735 | – | 5995 | 2603 | – | 7241 | – |
| T10 | – | 36 | – | 36 | – | 595 | – | 595 | – | 2196 | – | 2196 | 3908 | – | 11235 | – |
| T11 | – | – | 65 | 65 | – | – | 1341 | 1341 | – | – | 5947 | 5947 | – | 8529 | – | 12704 |
| T 12 | – | – | 60 | 60 | – | – | 1075 | 1075 | – | – | 3802 | 3802 | – | 6883 | – | 11370 |
| T13 | – | 51 | – | 51 | – | 633 | – | 633 | – | 2512 | – | 2512 | 3596 | – | 1538 | – |
| T14 | 158 | – | – | 158 | 3163 | – | – | 3163 | 6245 | – | – | 6245 | – | – | – | – |
| CD (p=0.05) | 17 | 7 | 15 | 17 | 468 | 164 | 180 | 436 | 824 | NS | 798 | 931 | 515 | 1029 | 1302 | 1415 |

*DAS = Days after sowing, IR = Indian rape, *OR = Oilseed rape, *EM = Ethiopian mustard

similar. Under ICS, the highest DMA by Ethiopian mustard in Ethiopian mustard + Indian rape (2:1at 22.5 cm row spacing) was significantly higher than that accumulated under 1:1 row proportion at 30 cm row spacing and 1:2 row proportion at 22.5 cm row spacing. The highest total dry matter (160 kg ha$^{-1}$) at 30 DAS accumulated by Ethiopian mustard + Indian rape (1:2) at 22.5 cm row spacing was at par with sole crop of Indian rape (158 kg ha$^{-1}$) and significantly higher than all other treatments. The lowest dry matter (82 kg ha$^{-1}$) at this crop growth stage was accumulated by canola oilseed rape + Indian rape (2:1) at 30 cm row spacing. At 60 DAS, the trend of DMA by crops was similar to that observed at 30 DAS. The highest DM was accumulated by sole crop of Indian rape (3163 kg ha$^{-1}$) followed by intercropping of Ethiopian mustard with Indian rape (2784 kg ha$^{-1}$) in 1:2 row proportion at 22.5 cm row spacing and non canola oilseed rape intercropped with Indian rape in 1:1 at 22.5 cm row spacing (2144 kg ha$^{-1}$). The DMA by Indian rape in canola oilseed rape + Indian rape (1:1) at 22.5 cm row spacing was significantly higher than that obtained under canola oilseed rape + Indian rape (2:1) at 22.5 and 30 cm row spacing. Similarly, DMA by Indian rape in Ethiopian mustard + Indian rape (1:2) at 22.5 cm row spacing was significantly higher than other Ethiopian mustard + Indian rape ICS. In case of oilseed rape, DMA (707 kg ha$^{-1}$) at 60 DAS under canola oilseed rape + Indian rape (2:1) at 22.5 cm row spacing was significantly higher than canola oilseed rape + Indian rape (385 kg ha$^{-1}$) and non canola oil seed rape + Indian rape (498 kg ha$^{-1}$) in 1:1 row ratio at 22.5 cm row spacing. In Ethiopian mustard, DMA by sole crop sown at 30 cm row spacing was significantly higher than the crop sown at 45 cm row spacing and both these registered significantly higher DM than all ICS of Ethiopian mustard + Indian rape. The highest total DM (3269 kg ha$^{-1}$) was accumulated by Ethiopian mustard + Indian rape (1:2) at 22.5 cm row spacing which was at par with sole crop of Indian rape (3163 kg ha$^{-1}$) and significantly higher than all other treatments.

At 90 DAS also, sole crop of Indian rape accumulated highest DM (6245 kg ha$^{-1}$) which decreased with increasing row spacing and decreasing row proportion of Indian rape in the ICS (Table 2). Among different ICS, DMA by Indian rape in the Ethiopian mustard + Indian rape (1:2) at 22.5 cm row spacing was 14.9% lower than sole crop of Indian rape but it was at par with Ethiopian mustard + Indian rape in 1:1 row proportion at 22.5 cm/30 cm row spacing and canola oilseed rape + Indian rape in 1:1 row ratio at 22.5 cm row spacing. The DMA by Indian rape in canola oilseed rape + Indian rape (1:1) at 22.5 cm row spacing was significantly higher than canola oilseed rape + Indian rape (2:1) at 30 cm row spacing and non canola oilseed rape + Indian rape (1:1, 22.5 cm row spacing). In case of oilseed rape, differences in DMA

between different treatments were inconspicuous at 90 DAS. At this stage, sole crop of Ethiopian mustard at 30 cm row spacing accumulated significantly higher DM (56.4%) than at 45 cm row spacing. Irrespective of row spacing and row proportion, intercropping of Ethiopian mustard with Indian rape resulted in its significantly lower DM than sole crop of Ethiopian mustard. Intercropping of canola oilseed rape with Indian rape in 22.5 cm apart rows in 1:1 row proportion resulted in highest total DMA by ICS at 90 DAS. At 120 DAS, DMA by sole crop of canola oilseed rape was statistically at par with non canola oilseed rape. DMA by canola oilseed rape in canola oilseed rape + Indian rape at 22.5 cm row spacing at 1:1 row proportion was significantly higher than that sown in 2:1 row proportion. Sole crop of Ethiopian mustard sown at 30 cm row spacing produced significantly higher (23.9%) dry matter than that produced by the crop sown at 45 cm row spacing. Differences in DMA at 120 DAS among treatments of intercropping of Ethiopian mustard with Indian rape were significant. At maturity, sole crop of non canola oilseed rape produced (15389 kg ha$^{-1}$) significantly higher DM than canola oilseed rape (11235 kg ha$^{-1}$) and both these treatments accumulated significantly higher DM than their intercropping with Indian rape. Similarly, Ethiopian mustard sown as sole crop at 30 cm produced 11.7% higher (statistically similar) DM than its 45 cm row spacing, and both these treatments resulted in significantly higher DM than that accumulated under ICS.

Thus Indian rape attained faster growth than oilseed rape and Ethiopian mustard, and thus influenced the total DMA in the ICS up to its maturity. Growth of oilseed rape was only slightly better than Ethiopian mustard at early growth stage as is evident from plant height and due to early formation of primary branches and more number and bigger size of leaves than Ethiopian mustard. However, at later growth stages, Ethiopian mustard maintained its superiority over oilseed rape due to increase in plant height and extensive branching (particularly secondary branches) and lesser leaf fall. An obvious increase in DM of Ethiopian mustard under 30 cm row spacing over 45 cm row spacing was due to higher number of plants per unit area. Similar results were reported by Singh and Rana (2006).

Differences between treatments for PAR interception were non significant at 60 and 90 DAS (Table 3). At 120 DAS, all sole crops and non canola oilseed rape intercropped with Indian rape intercepted similar proportion of PAR except sole crop of non canola oilseed rape, which intercepted significantly higher proportion of PAR over its intercropping with Indian rape. Similarly sole crop of Ethiopian mustard intercepted significantly higher proportion of PAR than its intercropping with Indian rape at both 120 and 150 DAS. Relative growth of component crops in the ICS was less than

the relative growth of sole crop. After harvesting of Indian rape, vacant inter row space permitted more amount of PAR to reach the surface in the intercropping treatments as compared to sole crops, which intercepted more light due to better growth.

The SPAD values decreased with advancement of crop growth from 30 to 90 DAS in all species (data not shown). Ethiopian mustard showed higher SPAD values than Indian rape and oilseed rape though the differences between treatments at 30 and 60 DAS were non-significant in Indian rape and Ethiopian mustard. In case of oilseed rape, SPAD values for non canola oilseed rape were higher than canola oilseed rape and that of sole crops were higher than their intercropping with Indian rape at all growth stages. Higher SPAD values in Indian rape can be ascribed to its short duration *vis-à-vis* fast growth habit resulting in higher concentration of nitrogen. Non canola oilseed rape is reported to have slow initial growth and accumulates nutrient during its vegetative phase for use at later growth stages. At 90 DAS, the highest SPAD value recorded in Ethiopian mustard + Indian rape in 1:1 row ratio at 22.5 cm row spacing was significantly higher than all treatments of Ethiopian mustard except Ethiopian mustard + Indian rape in 2:1 row ratio at 22.5 cm row spacing. Lower SPAD values at 150 DAS compared to 120 DAS may be ascribed to senescence with advancing crop age. Yildirim and Turan (2013) also did not find differences in chlorophyll content in broccoli leaves when intercropped with lettuce.

Intercropping with Indian rape resulted in significant reduction in main shoot length at maturity of both oilseed rape and Ethiopian mustard as compared to their respective sole crops (Table 3). Reduction in main shoot length under intercropping was more under narrow row spacing (22.5 cm). These differences were obviously due to competition between component crops resulting in their restricted growth in comparison to sole crops. Main shoot length is also a genotypic trait. In Ethiopian mustard, determinate nature of this test cultivar resulted in shorter main shoot length and higher number of primary and secondary branches per plant. Nayyar (2007) also reported that main shoot length of canola cultivar of oilseed rape was significantly higher than non canola cultivar.

**Phenology**: Flowering initiation in Indian rape (mean 36.5 days) was much earlier than oilseed rape (mean 67.4 days for canola and mean 120 days for non canola) and Ethiopian mustard (mean 68.1 days). Number of days taken by different component crops for flowering initiation, completion and maturity was not significantly different among different intercropping treatments in Indian rape and Ethiopian mustard whereas non canola oilseed rape required significantly more number of days of flowering initiation, completion and maturity than canola oilseed rape (Table 3). Nayyar and Sardana (2010) reported similar differences in initiation and completion of flowering in canola and non

**Table 3.** Effect of intercropping systems on interception of photosynthetically active radiation (%) at different crop growth stages, days taken for flowering and maturity, and main shoot length

| Treatments | PAR interception (%) | | | | Days taken to | | | | | | | | | Main shoot length (cm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Days after sowing | | | | Initiation of flowering | | | Completion of flowering | | | Physiological maturity | | | | | |
| | 60 | 90 | 120 | 150 | *IR | *OR | *EM | IR | OR | EM | IR | OR | EM | IR | OR | EM |
| T1 | 83.9 | 84.7 | 65.0 | 61.3 | 36.7 | 68.7 | − | 45.3 | 81.3 | − | 95.0 | 160.3 | − | 54.1 | 68.9 | − |
| T2 | 82.7 | 85.2 | 71.9 | 69.3 | 36.7 | 67.0 | − | 43.7 | 80.0 | − | 94.3 | 160.0 | − | 56.2 | 70.3 | − |
| T3 | 84.0 | 90.1 | 71.4 | 58.9 | 37.3 | 67.0 | − | 46.3 | 81.0 | − | 95.0 | 159.0 | − | 57.5 | 73.2 | − |
| T4 | 87.4 | 90.0 | 72.9 | 63.9 | 36.7 | − | 69.3 | 45.3 | − | 83.3 | 94.3 | − | 151.3 | 55.4 | − | 27.3 |
| T5 | 74.5 | 83.4 | 71.2 | 53.4 | 37.0 | − | 68.7 | 45.3 | − | 82.7 | 95.0 | − | 152.3 | 58.5 | − | 28.5 |
| T6 | 87.3 | 90.0 | 70.9 | 57.0 | 37.0 | − | 68.0 | 45.0 | − | 81.3 | 95.0 | − | 151.3 | 56.5 | − | 24.6 |
| T7 | 78.8 | 84.4 | 71.8 | 50.6 | 35.7 | − | 67.7 | 43.0 | − | 81.3 | 94.7 | − | 151.0 | 55.7 | − | 26.3 |
| T8 | 84.1 | 82.5 | 71.2 | 43.1 | 36.3 | − | 67.7 | 42.7 | − | 82.7 | 95.0 | − | 150.3 | 57.0 | − | 24.9 |
| T9 | 85.0 | 88.4 | 74.4 | 70.2 | 36.0 | 120.3 | − | 45.3 | 138.3 | − | 94.7 | 163.0 | − | 55.6 | 73.3 | − |
| T10 | 82.5 | 90.8 | 77.0 | 71.4 | − | 67.0 | − | − | 82.7 | − | − | 160.0 | − | − | 79.0 | − |
| T11 | 89.5 | 91.8 | 83.6 | 79.5 | − | − | 67.7 | − | − | 80.7 | − | − | 150.7 | − | − | 28.2 |
| T 12 | 84.9 | 90.5 | 82.9 | 83.9 | − | − | 67.7 | − | − | 81.0 | − | − | 149.0 | − | − | 27.0 |
| T13 | 81.8 | 91.0 | 85.8 | 80.9 | − | 119.7 | − | − | 133.7 | − | − | 165.0 | − | − | 77.4 | − |
| T14 | 80.5 | 85.3 | − | − | 35.7 | − | − | 44.0 | − | − | 93.7 | − | − | 54.0 | − | − |
| CD (P=0.05) | NS | NS | 9.3 | 6.6 | NS | 1.7 | NS | NS | 4.0 | NS | NS | 3.1 | NS | NS | 3.1 | 1.3 |

*IR = Indian rape, *OR = Oilseed rape, *EM = Ethiopian mustard

canola oilseed rape. On an average, it took 95 days for maturity of Indian rape, 160 days for canola oilseed rape, 164 days for non canola oilseed rape and 151 days for Ethiopian mustard. Indian rape, non canola oilseed rape, canola oilseed rape and Ethiopian mustard attained physiological maturity in 50.1, 28.0, 78.5 and 69.0 days respectively after completion of flowering.

**Seed, stover and oilseed rape equivalent seed yield:** Seed yield of sole crop of Indian rape (1444 kg ha$^{-1}$) was significantly more than its yield under different ICS (617–1162 kg ha$^{-1}$). Seed yield of Indian rape in Ethiopian mustard + Indian rape in 1:2 row ratio at 22.5 cm apart rows was 10.2 per cent higher than 1:1 row ratio at 22.5 cm row spacing and 15.5 per cent higher than their intercropping at 30 cm row spacing in 1:1 row ratio (Table 4). Sole crop of non canola oilseed rape produced significantly higher (45.7 per cent) seed yield than its yield when intercropped with Indian rape (Table 4). Seed yields of sole crop of canola oilseed rape (1630 kg ha$^{-1}$) were 35.3–91.0 per cent higher than its yield in different ICS. The seed yields of sole crop of Ethiopian mustard under 30 cm row spacing (2245 kg ha$^{-1}$) was at par with its 45 cm row spacing (2149 kg ha$^{-1}$) but significantly higher (41.5–128.8 per cent) than its yield under different ICS of Ethiopian mustard + Indian rape. These differences in yields of component crops may be ascribed to competition between species and differences in plant population under different row proportions and row spacing. Plant population of Indian rape in ICS was lower than that of sole crop. It was

50 per cent lower in 1:1 row ratio at 22.5 cm spacing and 66 per cent lower in 2:1 row ratio at 30 cm row spacing when intercropped with oilseed rape; 25 per cent lower in 1:1 row ratio at 22.5 cm spacing and 50 per cent lower in 1:1 row ratio at 30 cm spacing when intercropped with Ethiopian mustard. Higher plant population of Indian rape in Ethiopian mustard + Indian rape ICS in 1:2 row proportion contributed to its higher seed yield as compared to other treatments of intercropping. Both canola and non canola oilseed rape differed in their growth pattern and non canola oilseed rape with slow initial growth was more compatible for intercropping with Indian rape. Gupta *et al.* (1989) obtained similar results of non canola oilseed rape + Indian rape ICS.

Intercropping of non canola oilseed rape with Indian rape in alternate rows (1:1) at 22.5 cm row spacing resulted in oilseed rape seed equivalent yield (2272 kg ha$^{-1}$) at par with sole crop of Ethiopian mustard sown at 30 cm (2245 kg ha$^{-1}$) or 45 cm (2149 kg ha$^{-1}$) row spacing. In canola oilseed rape, equivalent seed yield of canola oilseed rape + Indian rape sown at 22.5 cm row spacing in 1:1 or 2:1 row proportion was 16.9 per cent and 20.1 per cent, respectively more than that of sole crop of canola oilseed rape (1630 kg ha$^{-1}$). Similarly, intercropping of non canola oilseed rape + Indian rape (1:1) at 22.5 cm row spacing produced 22.9 per cent more oilseed rape equivalent seed yield than sole non canola oilseed rape (1849 kg ha$^{-1}$). Singh *et al.* (2014) obtained significantly more (26.5%–42.5%) canola oilseed rape seed equivalent yields with intercropping of 1–3 rows of oat (variety Kent) for fodder

**Table 4.** Seed yield and stover yield of component crops and oilseed rape equivalent seed yield as influenced by row proportions and row spacing in intercropping of oilseed rape and Ethiopian mustard with Indian rape

| Treatments | Seed yield (kg ha$^{-1}$) | | | Oilseed rape seed equivalent yield (kg ha$^{-1}$) | Stover yield (kg ha$^{-1}$) | | |
|---|---|---|---|---|---|---|---|
| | *IR | *OR | *EM | | IR | OR | EM |
| T1 | 1081 | 853 | – | 1906 | 4039 | 3721 | – |
| T2 | 966 | 1018 | – | 1958 | 3182 | 4841 | – |
| T3 | 617 | 1204 | – | 1806 | 2580 | 6201 | – |
| T4 | 1054 | – | 1091 | 2118 | 3964 | – | 4193 |
| T5 | 1007 | – | 1196 | 2176 | 3857 | – | 5897 |
| T6 | 694 | – | 1338 | 2013 | 3191 | – | 5944 |
| T7 | 648 | – | 1586 | 2216 | 2526 | – | 6656 |
| T8 | 1162 | – | 981 | 2113 | 4274 | – | 3667 |
| T9 | 1030 | 1269 | – | 2272 | 3231 | 6306 | – |
| T10 | – | 1630 | – | 1630 | – | 9605 | – |
| T11 | – | – | 2245 | 2245 | – | – | 9792 |
| T 12 | – | – | 2149 | 2149 | – | – | 9554 |
| T13 | – | 1849 | – | 1849 | – | 12873 | – |
| T14 | 1444 | – | – | 1407 | 4801 | – | – |
| CD (p=0.05) | 157 | 122 | 173 | 211 | 611 | 836 | 904 |

*IR = Indian rape, *OR = Oilseed rape, *EM = Ethiopian mustard

between two rows of canola gobhi sarson (variety GSC 6) sown at 45–90 cm row spacing than canola oilseed rape alone (1993 kg ha$^{-1}$) at Ludhiana.

Sole crop of Indian rape produced significantly more stover yield than that under different ICS except in Ethiopian mustard + Indian rape in 1:2 row ratio at 22.5 cm row spacing (Table 4). Sole crop of non canola oilseed rape produced significantly more stover yield (12873 kg ha$^{-1}$) than all other treatments of oilseed rape. Stover yield of sole crop of canola oilseed rape (9605 kg ha$^{-1}$) was 52.3–158.1 per cent more than its yield under different ICS. Stover yield of sole crop of Ethiopian mustard sown at 30 cm row spacing was significantly more than its yield under 45 cm row spacing and both these treatments out yielded Ethiopian mustard sown under ICS in different row proportions and row spacing. Singh and Dhingra (2003) also recorded increase in DMA per plant by Ethiopian mustard with increasing row spacing from 30 cm to 60 cm at both 10 and 20 cm intra row spacing.

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# Phenological Behaviour of Indian-Mustard Genotypes Under Different Sowing Environments at Hisar

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**Abstract:** A field experiment was carried out at CCSHAU, Hisar during *Rabi* season of 2014–15. The main plot treatments consisted three date of sowing ($D_1$–26th October, $D_2$–5th November and $D_3$–15th November) and sub plot treatments consisted three varieties ($V_1$–Kranti, $V_2$–RH 406 and $V_3$–RH 0749) using split plot design. The results revealed that delayed sowing reduced the length of phenophases in all varieties. The crop duration was shortest in 26th October as compared to 5th November and 15th November. Among varieties, the total crop duration was longest in RH 0749 followed by RH 406 and Kranti. Among agrometeorological indices GDD, HTU and PTU requirement were higher in 26th October followed by 5th & 15th Nov. sown crop. In late sown crop, higher day and night temperature during reproductive phase leads to forced maturity and reduced the total GDD requirement. The crop sown on 26th October was most efficient in thermal and radiation utilization in comparison with delayed sown crop.

**Keywords**: Agrometeorological indices, Indian mustard, Phenological events, Sowing dates

Indian mustard (*Brassica juncea*) is the second most important oilseed crop in India after groundnut, sharing 27.8 per cent of the total oilseed production in India. The crop occupies an area of approximately 6.90 million hectares with a production of 8.18 million tonnes per year (Shekhawat *et al*., 2012). The physiological and morphological development occurring in plants are markedly influenced by temperature and day length therefore, in any crop species the optimum sowing time becomes a major factor to achieve a potential yield (Tharranum *et al*., 2016). Every crop needs a specific amount of heat unit or growing degree days (GDD) to enter from one phase to another. Phenological development of *Brassica* is known to be manifestly influenced with temperature and photoperiod. In general, shortening in the maturity phase has been found with increase in temperature and day length (Robertson *et al*., 2002).The late sowing of mustard crop may lead to a reduction in the maturity period by 25 to 30 days, mainly due to fast accumulation of GDD which is essentially required in crops for completion of life cycle (Adak *et al*., 2011).Among crop species and cultivars, there are considerable differences in accumulated heat units and days taken to reach different growth stages. Similarly a decline in thermal use efficiency (TUE) and radiation use efficiency (RUE) also observed with delayed sowing in mustard at Hisar region (Singh, 1999). Hence, the optimization of sowing time along with selection of promising varieties through comprehensive studies on phenology and agrometeorological indices becomes a major prerequisite to provide a potential harvest to the farmers. Therefore, with this objective the present investigation was carried out with high performance varieties of mustard for their phenological behaviour under different growing environments.

## MATERIAL AND METHODS

An experiment was conducted in *Rabi* season 2014–15 at Research Farm of Department of Agrometeorology Meteorology, CCSHAU Hisar, Haryana, India at $29^0$ 10' N latitude, $75^0$ 46' E longitude and altitude of 215.2 m with split plot design. There was three date of sowing ($D_1$–26th October, $D_2$–5th November and $D_3$–15th November) as main plot treatment and three varieties ($V_1$–Kranti, $V_2$–RH 406 and $V_3$–RH 0749)as a sub plot treatment with four replication. The package and practices for Indian mustard cultivation was followed as per recommendation. The crop was closely observed at an interval of 2–3 days for the commencement of different phenological stages *viz*., $P_1$–seedling emergence, $P_2$–four leaf stage, $P_3$–first flower, $P_4$–flowering (when 50 per cent of plants in plot have at least one open flower), $P_5$–pod initiation (when 50 per cent of plants in plot have at least one pod 2.0 cm long), $P_6$–seed development (when 50 per cent of plants in plot have at least one pod with fully developed seeds) and $P_7$–physiological maturity. Daily weather data for crop season were collected from agrometeorological observatory situated adjacent to the experiment field.

**Agro-meteorological indices**

**Growing degree days (GDD):** For Brassica spp., $T_{base}$ is considered as 5 $^\circ$C for computation of GDD:

$$GDD(^\circ C \text{ day}) = \frac{(T_{max} + T_{min})}{2} T_{base}$$

**Photo thermal unit (PTU):** PTU ($^\circ$C day hours) = GDD x maximum possible sunshine hour

**Helio thermal unit (HTU):** HTU ($^\circ$C day hours) = GDD x actual bright sunshine hours

**Radiation use efficiency (RUE):**

$$RUE \text{ (g MJ}^{-1}) = \frac{\text{Biomass (g m}^{-2}) \text{ during 30 days interval}}{\text{Cumulative iPAR (MJ m}^{-2}) \text{ during 30 days interval}}$$

Daily solar radiation was computed by the expression

**Rs = Ra (1-r) (a+b (n N$^{-1}$)**

Where, Rs is solar radiation received at the surface of the earth (cal cm$^{-2}$), Ra is solar radiation received outside the atmosphere (cal cm$^{-2}$) used from Smithsonian tables corresponding to the latitude values of Hisar, ris reflection coefficient (0.25) for green vegetations, a and b are constants, a= 0.25; b = 0.56 for Hisar, (Bishnoi *et al.*, 1995).

Daily PAR

PAR = Rs x 0.48 (Oleson *et al.*, 2000)

The PAR values were converted into MJ m$^{-2}$. daily

IPAR= PAR (1–e$^{-kf}$) (Rosenthal and Gerik, 1991).

Where,

K is extinction coefficient = ln (I–Io) f$^{-1}$,

F is cumulative leaf area index of foliage layer,

Io is radiation energy at the top of the canopy,

I is radiation energy at a level inside the crop canopy.

**Thermal use efficiency (TUE):**

$$TUE \text{ (g m}^{-2\circ}C \text{ day}^{-1}) = \frac{\text{Biomass (g m}^{-2}) \text{ during 30 days interval}}{\text{Cumulative GDD (}^\circ C \text{ day) during 30 days interval}}$$

## RESULTS AND DISCUSSION

**Phenological studies:** The observations on different phenological stages of Indian-mustard crop were reflected by the influence of weather element (Table. 1). The phenological observations recorded in field experiments of three varieties in three growing environments revealed that low temperature during vegetative phase led to delayed flower appearance which consequently had delayed convergence of vegetative to reproductive phase resulting in shorter duration of reproductive phase under delayed sown conditions. Crop duration of mustard crop decreased with delay in sowing. The delayed sowing reduced the length of phenophases in all varieties, except for $P_1$ and $P_2$ which extended with delay in sown. Crop duration was shortest in 26th Oct. as compared to 5th & 15th Nov. Among varieties, the duration was longest in RH

**Table 1.** Days taken for occurrences of different phenophases in mustard crop under varying environments and varieties

| Treatment | P$_1$* | P$_2$ | P$_3$ | P$_4$ | P$_5$ | P$_6$ | P$_7$ |
|---|---|---|---|---|---|---|---|
| Phenological studies | | | | | | | |
| 26th October | 3.8 | 11.1 | 40.2 | 57.3 | 83.7 | 101.4 | 140.6 |
| 5th November | 5.0 | 14.7 | 48.0 | 58.1 | 93.5 | 105.8 | 133.8 |
| 15th November | 5.8 | 16.6 | 65.1 | 70.6 | 95.4 | 112.8 | 131.8 |
| CD (P =0.05) | 0.6 | 1.5 | 5.7 | 3.6 | 2.6 | 2.0 | 1.7 |
| Kranti | 4.2 | 12.6 | 47.4 | 57.8 | 88.3 | 104.2 | 133.3 |
| RH 406 | 4.8 | 14.2 | 50.4 | 62.4 | 90.8 | 107.1 | 135.1 |
| RH 0749 | 5.6 | 15.6 | 55.4 | 65.7 | 93.3 | 108.6 | 137.7 |
| CD (P =0.05) | 3.8 | 1.4 | 2.3 | 2.8 | 1.2 | 0.5 | 0.8 |

*CD (P=0.05): Critical differences at 5 per cent level of significance
*Where $P_1$: Seedling emergence, $P_2$: Early vegetative phase (four leaf stage), $P_3$: First flower, $P_4$: 50 per cent flowering, $P_5$: Pod initiation, $P_6$: Seed development, $P_7$: Physiological maturity

0749 followed by RH 406 and Kranti. As increasing the temperature lowered the days to flowering and days to maturity thus shortening the seed formation period. A higher temperature leads to higher respiration rates, lower biomass production, and smaller and lighter grain therefore lower crop yield (Kumar *et al.*, 2010).

**Agro-meteorological indices:** GDD, HTU and PTU requirement were higher in 26th Oct. followed by 5th & 15th Nov. sown crop. But at $P_1$ and $P_2$ stages in 5th and 15th Nov. sown crop, there are fast consumptions of GDD, HTU and PTU because at that time there is increased in duration of vegetative phase in delayed sowing as compared to 26thOct. Subsequently in late sown crop, higher day and night temperature during reproductive phase leads to forced maturity and reduced the total GDD requirement. There is regular trend of increased consumptions of indices in earlier sown mustard. Among varieties, RH 0749 consumed significantly more GDD, HTU and PTU as compared to RH 406 and Kranti. Roy *et al.* (2005) observed that October sown crop recorded higher seed yield due to exposure to higher GDD. It was observed that 75 percent variation in biomass of mustard could be explained through GDD (Srivastava *et al.*, 2011).

The crop sown on 26th Oct. was most efficient in thermal and radiation utilization in comparison with sown crop on 5th & 15th Nov. In case of TUE at 90 DAS, canopy development was very good, then more interception of PAR (Photosynthetically active radiation) it produced more LAI (Leaf are index) that will lead to more biomass accumulation ultimately increased TUE at that growth intervals. At that interval, maximum in 5th Nov then followed by 15th Nov and 26th October later than they are continuous decreased. At 90

**Table 2.** Growing degree days, heliothermal units and photothermal units consumed by mustard varieties at various phenophases under different growing environments

| Treatment | $*P_1$ | $P_2$ | $P_3$ | $P_4$ | $P_5$ | $P_6$ | $P_7$ |
|---|---|---|---|---|---|---|---|
| Growing degree days (ºC day) | | | | | | | |
| 26th October | 76 | 205 | 605 | 758 | 896 | 1018 | 1472 |
| 5th November | 90 | 221 | 561 | 678 | 842 | 975 | 1325 |
| 15th November | 76 | 209 | 634 | 676 | 808 | 1031 | 1300 |
| CD (p=0.05) | 8.6 | 6.3 | 14.6 | 23.5 | 24.6 | 12.6 | 7.4 |
| Kranti | 69 | 192 | 570 | 653 | 824 | 979 | 1335 |
| RH 406 | 79 | 213 | 600 | 709 | 847 | 1013 | 1362 |
| RH 0749 | 95 | 231 | 629 | 749 | 875 | 1031 | 1401 |
| CD (p=0.05) | 8.6 | 13.0 | 19.6 | 28.6 | NS | 39.3 | 24.6 |
| Heliothermal units (˚C day hours) | | | | | | | |
| 26th October | 482 | 1327 | 4233 | 5147 | 5625 | 6097 | 8920 |
| 5th November | 600 | 1482 | 3851 | 4017 | 4948 | 5083 | 7977 |
| 15th November | 594 | 1676 | 3696 | 3539 | 4836 | 6016 | 8141 |
| CD (p=0.05) | 38.5 | 106.3 | 184.8 | 263.6 | 201.3 | 5.7 | 103.3 |
| Kranti | 490 | 1331 | 3683 | 4168 | 4993 | 5597 | 8122 |
| RH 406 | 561 | 1499 | 3802 | 4252 | 5136 | 5762 | 8309 |
| RH 0749 | 625 | 1655 | 4295 | 4283 | 5280 | 5835 | 8608 |
| CD (p=0.05) | 18.4 | 67.5 | 26.4 | 12.3 | 18.2 | NS | 12.6 |
| Photothermal unit (˚C day hours) | | | | | | | |
| 26th October | 843 | 2268 | 7003 | 8082 | 9574 | 10794 | 15936 |
| 5th November | 972 | 2306 | 5898 | 6412 | 8841 | 10277 | 14375 |
| 15th November | 748 | 2204 | 5785 | 6192 | 7325 | 11048 | 14282 |
| CD (p=0.05) | 42.3 | 1.4 | 81.2 | 59.4 | 32.1 | 3.2 | 4.6 |
| Kranti | 743 | 2026 | 5764 | 6655 | 8367 | 10380 | 14490 |
| RH 406 | 864 | 2267 | 6000 | 6937 | 8575 | 10761 | 14813 |
| RH 0749 | 954 | 2485 | 6922 | 7094 | 8799 | 10978 | 15289 |
| CD (p=0.05) | 28.4 | 12.5 | 23.5 | 5.3 | NS | NS | 2.8 |

*Where $P_1$: Seedling emergence, $P_2$: Early vegetative phase (four leaf stage), $P_3$: First flower, $P_4$: 50 % flowering, $P_5$: Pod initiation, $P_6$: Seed development, $P_7$: Physiological maturity.
* DAS −Days after sowing
*CD (P=0.05):Critical differences at 5 percent level of significance
* NS – Non significant

DAS, RH 0749 attained maximum RUE and TUE as compared to RH 406 and Kranti. Among varieties, RH 0749 recorded highest TUE and RUE at all stages followed by RH 406 and Kranti. TUE and RUE was higher in earlier sown crop due to maximum PAR absorption and dry matter production, both of which decreased subsequently with the delayed sowing.

## CONCLUSION

The thermal indices were found to vary significantly in three varieties of mustard under different growing environment. The performance of RH 0749was higher in

**Table 3.** Thermal and radiation use efficiency of mustard varieties during growth interval under different growing environments

| Treatment | 30 DAS* | 60 DAS | 90 DAS | 120 DAS | Maturity |
|---|---|---|---|---|---|
| Thermal use efficiency (g m$^{-2}$ ˚C day$^{-1}$) | | | | | |
| 26th October | 0.23 | 0.89 | 1.49 | 1.56 | 1.59 |
| 5th November | 0.11 | 0.69 | 1.51 | 1.49 | 1.46 |
| 15th November | 0.09 | 0.60 | 1.46 | 1.32 | 0.73 |
| CD (P =0.05) | 0.4 | NS | 0.1 | 1.2 | 0.3 |
| Kranti | 0.10 | 0.45 | 1.13 | 1.12 | 1.00 |
| RH 406 | 0.14 | 0.72 | 1.43 | 1.45 | 1.34 |
| RH 0749 | 0.23 | 1.23 | 2.00 | 1.68 | 1.40 |
| CD (P =0.05) | 0.3 | 0.5 | 0.2 | 0.1 | 1.1 |
| Radiation use efficiency (g MJ$^{-1}$) | | | | | |
| 26th October | 0.94 | 3.09 | 5.02 | 4.96 | 4.77 |
| 5th November | 0.42 | 2.19 | 4.62 | 4.46 | 4.25 |
| 15th November | 0.28 | 1.75 | 3.95 | 3.63 | 2.52 |
| CD (P =0.05) | 1.1 | 0.6 | 0.8 | NS | 0.1 |
| Kranti | 0.42 | 1.54 | 3.80 | 3.56 | 3.01 |
| RH 406 | 0.52 | 2.28 | 4.39 | 4.34 | 3.88 |
| RH 0749 | 0.74 | 3.58 | 5.43 | 4.64 | 4.87 |
| CD (P =0.05) | NS | 0.2 | 0.5 | 0.1 | 0.1 |

* DAS −Days after sowing
* NS – Non significant

26th October sown crop as compared to 5th and 15th November followed by RH 406 and Kranti at all phenological stages. The interaction between date of sowing and varieties are significant in phenological studies but in case of agrometeorological indices such as GDD, HTU and PTU, it was not significant in 50 per cent flowering and pod initiation. They showed better utilization of heat units and hence, suitable for cultivation in semi−arid region of Haryana under changing and prevailing climatic conditions of Gangetic plains.

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# Effect of Proline and Salicylic Acid on Physiological and Biochemical Parameters in Squash Melon (*Citrullus vulgaris* var. *fistulosus*) at Low Temperature

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**Abstract**: In the present investigation, the effect of seed treatments (Proline and Salicylic acid) on physiological and biochemical parameters of squash melon (*Citrullus vulgaris* var. *fistulosus* cv. S-48 seeds were studied at different temperatures (25°C and 20°C). Squash melon seeds were treated for 5 hours with different concentrations of proline (10 and 20mM) and salicylic acid (0.5 and 1mM ). After 12 days of germination, seedlings were used for analysis of physiological and biochemical parameters. The seed treatments increased the germination percentage, root length, shoot length, fresh weight, dry weight, vigour index I, vigour index II, electrolyte leakage, speed of germination, total soluble sugars and proteins  as compared to control at both the temperatures. Proline 20mM was more effective than proline 10mM. Similarly, salicylic acid 1mM was more effective than salicylic acid 0.5mM at both the temperatures 25°C and 20°C.

**Keywords**: Proline, Salicylic acid, Germination per cent, Speed of germination, Total soluble sugars, Total soluble proteins

At low temperature, poor seed germination is a major problem for the farmers who grow Squash melon (*Citrullus vulgaris* var. *fistulosus*) in the month of February. Low temperature results in late and reduced germination and the seedlings are more prone to pathogens (Demir and Ozokat, 2003). Low temperature after the sowing of many crops can lead to asynchronous seedling emergence. Chilling stress causes changes in membrane structure of plants (Xu *et al.,* 2008). Proline is a proteinogenic amino acid with an exceptional conformational rigidity and is essential for primary metabolism. There are several reports which indicate that abiotic stress conditions activate proline accumulation in the plant tissues (Hayat *et al.,* 2012; Slama *et al.,* 2014) which is important for the tolerance of certain adverse environmental conditions (Mattioli *et al.,* 2008; Miller *et al.,* 2009). Salicylic acid is a phenolic plant growth regulator which regenerates of physiological processes in plants. Salicylic acid (SA), induce chilling tolerance in plants (Sayyari, 2012) and is involved in the regulation of proline metabolism (Sakhabutinova *et al.,* 2003). Several reports indicate the role of salicylic acid in seed germination and plant growth and yield (Hussein *et al.,* 2007). SA improved drought and chilling stress tolerance in wheat (*Triticum aestivum* L.) by protecting the structure of plant cell under low temperature (Horvath *et al.,* 2007). Sayyari (2012) showed that SA application enhanced chilling tolerance of cucumber seedling during low temperature stress. Salicylic acid is reported to have a major role in germination process (Zhang *et al.,* 2003). With the above view the present investigation was planned to study the effect of proline and salicylic acid on various seed germination characteristics and biochemical parameters like soluble sugars and proteins in squash melon at two different temperatures.

## MATERIAL AND METHODS

The fresh seeds of S-48 of squash melon (*Citrullus vulgaris* var. *fistulosus)* were procured from department of vegetable science, PAU Ludhiana.  The seeds were soaked for 5 hours in the seed treatments. Twenty seeds replicated thrice were taken at random from each treatment and were kept on germination paper. The rolled towels were kept in the seed germinator and maintained constant temperature of 25 and 20°C and a relative humidity of 95 per cent. The number of normal seedlings were counted after 12 days and expressed as germination percentage (ISTA, 2008). For determining seedling length, fresh and dry weight five normal seedlings from each treatment were randomly selected for measuring the seedling length on 12 day of germination. Vigour indices (I & II) of seeds were calculated by Abdul Baki's Anderson (1973) method .Vigour Index I = Germination (%) x Seedling length (cm). Vigour Index II = Germination (%) x Seedling dry weight (g). The speed of germination was calculated as suggested by Maguire, 1962.

Speed of germination =    [n1/d1 + n2-n1/d2 +……nn-nn-1/dn ] = Σ n / d

Where n = number of seeds germinated and d = number

of days taken for germination

Estimation of electrolyte leakage ($\mu$S cm$^{-1}$ g$^{-1}$) was done by the method of Presley, 1958. Estimation of total soluble sugars (mg g$^{-1}$ FW) and proteins (mg g$^{-1}$ FW) was done by the method of Dubios *et al.* (1956) and Lowry *et al.* (1951) respectively.

## RESULTS AND DISCUSSION

**Percent germination:** The seeds had more percent germination at 25°C than at 20°C. Various seed treatments increased the percent germination over control at both the temperatures. Among all the concentration of proline, 20 mM had maximum germination percentage. All the concentration of salicylic acid increased the percent germination over control. SA 1 mM had maximum germination but germination was less at the higher concentration of SA 5 mM (Table 1). The results are in agreement with the earlier findings of Khan *et al.* (2011) in wheat seed. Exogenous proline increases germination and help in ameliorating salt stress in two rice cultivars (Deivanai *et al.,* 2011). Proline accumulation stimulated cellular activities and is also important for osmotic adjustment under abiotic stress condition and can be beneficial to stressed plants in increasing percent germination in rice (Hyun *et al.,* 2003) and in wheat (Talat, 2013). Espanany *et al.* (2016) reported that salicylic acid improves germination percentage, root and shoot length, dry weight of black cumin *(Nigella sativa)* in different cadmium levels and enhance tolerance to metal stress.

**Shoot length and root length:** Various seed treatments increased root and shoot length over control at both the temperatures at 25°C and 20°C (Table 1). The proline at 20 mM had maximum root and shoot length. All the concentration of salicylic acid increased the root and shoot length over control. SA 1 mM had maximum root and shoot length among different concentrations of SA but root and shoot length was decreased at the higher concentration of SA5 mM. Similar trend is observed in seedling length and foliar application of proline increases the root and shoot length, fresh and dry weight in wheat (Mahboob *et al.,* 2016). Ismail (2014) observed that proline increased the seedling length, fresh and dry weight in wheat caused by NaCl stress. Similar increase in seedling length by salicylic acid is observed by Rao *et al* (2012) in maize and by salicylic acid and proline in sweet pepper (*Capsicum annuum* L.) by Jasim *et al.,* 2012.

**Fresh weight (g) and Dry weight (g):** As shown in table 2 the squash melon seeds had more fresh and dry weight at 25°C as compared to 20°C. At 25°C, seed treatments increased fresh and dry weight in seedlings when seeds treated with proline and salicylic acid. Among all the concentrations of proline, proline 20 mM had maximum fresh and dry weight. SA 1 mM had maximum fresh and dry weight among different concentrations of SA but fresh and dry weight was minimum at the higher concentration of SA 5 mM. Our results agree with the findings of Al-Sahil (2016) who observed that exogenous application of salicylic acid in cucumber under salt stress increases percent germination, fresh and dry weight and seedling length. Pre-treatment with SA increases the seed germination, seedling growth i.e. coleoptile percentage, radicle length, coleoptile length, radicle number and fresh weight under stress conditions (Cavusoglu *et al.,* 2014; Gorni and Pascheco, 2015).

**Table 1.** Effect of proline and salicylic acid on percent germination, shoot length and root length at different temperatures in seeds of *Citrullus vulgaris* var. fistulosis)

| Treatments | Percent germination | | Shoot length (cm) | | Root length (cm) | |
|---|---|---|---|---|---|---|
| | 25°C | 20°C | 25°C | 20°C | 25°C | 20°C |
| Control | 85.00 | 80.00 | 2.07 | 1.72 | 3.17 | 2.08 |
| Hydration | 91.60 | 86.67 | 3.01 | 1.74 | 4.97 | 2.60 |
| Warm water | 92.78 | 87.00 | 3.04 | 1.86 | 6.57 | 2.12 |
| Proline 1 mM | 87.78 | 87.21 | 2.21 | 2.16 | 3.56 | 2.59 |
| Proline 5 mM | 90.89 | 87.78 | 2.50 | 2.18 | 3.89 | 2.90 |
| Proline 10 mM | 95.00 | 89.00 | 2.83 | 2.70 | 5.51 | 2.91 |
| Proline 20 mM | 96.19 | 90.00 | 3.50 | 2.75 | 6.28 | 3.47 |
| SA 0.1 mM | 86.04 | 81.67 | 2.82 | 2.00 | 5.73 | 2.70 |
| SA 0.5 mM | 91.67 | 86.12 | 3.35 | 2.13 | 6.59 | 2.94 |
| SA 1 mM | 93.33 | 90.67 | 4.49 | 2.85 | 6.61 | 3.52 |
| SA 5 mM | 86.00 | 81.00 | 2.45 | 1.73 | 4.85 | 2.16 |
| CD (p=0.05) | Treatment (A)=5.71, Temperature (B)=2.43, AB=NS | | Treatment (A)=0.454, Temperature (B)=0.193, AB=0.642 | | Treatment (A)=0.464, Temperature (B)=0.197, AB=0.656 | |

**Vigour Index I and Vigour Index II:** As shown in table 2 the seed treatments with proline and salicylic acid enhanced vigour index I and II of seeds of squash melon. The increase is attributed to the increase in germination percent, seedling length and dry weight by different seed treatments (Table 2). Similar trend was observed by Orzeszko and Podlaski (2003) as seed treatments increased seed respiration, vigour, percentage speed and synchrony of seedling in sugarbeet. Foliar application of salicylic acid increases plant height, number of tillers per plant, average leaf area and fresh and dry weight of shoot (Sabbour *et al.,* 2016). Hossain *et al.* (2015) observed that salicylic acid increases total dry weight, chickpea under salt stress.

**Speed of germination:** All the seed treatments increased the speed of germination at 25°C as well as 20°C (Table 3). Germination speed was more at 25°C than at 20°C. Seed treatments increased the speed of germination over control at both the temperatures. Among all the concentration of proline, proline 20 mM had maximum germination speed. All the concentration of salicylic acid increased the speed of germination over control. SA 1 mM showed maximum germination speed but germination speed was minimum at the higher concentration of SA 5 mM. Our results are in agreement with the earlier findings of Rehman *et al.* (2011) in cucumber as seed treatments increase the speed of germination.

**Electrolyte leakage:** As shown in table 3 the seeds had less electrolyte leakage at 25°C than at 20°C. The electrical conductivity was found to be lower in the seeds treated as compared to control. The decrease in electrical conductivity was more in proline 20 mM and salicylic acid 1 mM at both the temperatures. Similar decrease in electrolyte leakage by the

application of proline is observed in the melon cultivar (Yan *et al.,* 2011). Hayat and Ahmed (2007) found that pretreatment with salicylic acid helps in the reduction in the level of lipid peroxidation and leakage of electrolytes from plant tissues. Salicylic acid decreases the electrolyte leakage in sorghum under drought conditions (Arivalagan and Somasundaram, *2016*). Seed priming with salicylic acid increases the germination percentage and had higher vigour index in maize. It also decreases the electrical conductivity at low temperatures (Rehman *et al.,* 2015).

**Biochemical Parameters**

**Total Soluble Sugars:** The the sugar content of seedlings of squash melon increased due to seed treatments at 25°C and 20°C. The increase in sugar content was more in seeds treated with proline 20 mM and salicylic acid 1 mM (Table 3). The result agree with the observations of Jafar *et al.* (2011) in wheat. Shahid *et al.* (2014) investigated that in pea, proline treatment increased the sugar content. Liu *et al* (2016) found that exogenous SA treatment increases the sugar content in *Nitraria tangutorum* under salt stress. Salicylic acid seed soaking of okra increases the sugar content under salt stress in okra (Esan and Olaiya, 2016).

**Total soluble proteins:** The total soluble protein content of the seedling increased due to seed treatments increased the protein content at 25°C than at 20°C. Protein content was more at 25°C than at 20°C (Table 3). Among all the concentration of proline, proline 20 mM had maximum protein content whereas SA 1 mM had maximum protein content among its different concentrations. The higher concentration of SA (5 mM) recorded decreased protein content. Thus seed treatment with salicylic acid and proline

**Table 2.** Effect of proline and salicylic acid on fresh weight (g), dry weight (g) and vigour indices I and II at different temperatures in seedlings of *Citrullus vulgaris* var. fistulosis)

| Treatments | Fresh weight (g) | | Dry weight (g) | | Vigour index I | | Vigour index II | |
|---|---|---|---|---|---|---|---|---|
| | 25°C | 20°C | 25°C | 20°C | 25°C | 20°C | 25°C | 20°C |
| Control | 0.114 | 0.109 | 0.034 | 0.031 | 445.40 | 318.92 | 2.89 | 2.57 |
| Hydration | 0.125 | 0.114 | 0.036 | 0.032 | 731.58 | 345.00 | 3.33 | 2.77 |
| Warm water | 0.176 | 0.123 | 0.037 | 0.034 | 892.28 | 377.58 | 3.35 | 2.96 |
| Proline 1mM | 0.155 | 0.131 | 0.038 | 0.035 | 506.31 | 416.15 | 3.46 | 3.07 |
| Proline 5 mM | 0.156 | 0.141 | 0.039 | 0.036 | 581.06 | 448.10 | 3.54 | 3.15 |
| Proline 10 mM | 0.157 | 0.148 | 0.040 | 0.037 | 792.74 | 498.51 | 3.80 | 3.29 |
| Proline 20 mM | 0.222 | 0.171 | 0.041 | 0.038 | 941.04 | 590.60 | 3.97 | 3.60 |
| SA 0.1 mM | 0.163 | 0.129 | 0.042 | 0.039 | 735.88 | 384.83 | 3.58 | 3.20 |
| SA 0.5 mM | 0.193 | 0.148 | 0.043 | 0.040 | 920.30 | 436.92 | 3.91 | 3.44 |
| SA 1 mM | 0.205 | 0.174 | 0.046 | 0.041 | 1033.53 | 583.99 | 4.29 | 3.73 |
| SA 5 mM | 0.147 | 0.137 | 0.035 | 0.033 | 575.53 | 380.00 | 3.50 | 3.18 |
| CD (p=0.05) | Treatment (A)=0.013, Temperature (B)=0.0056, AB=0.018 | | Treatment (A)=0.0040, Temperature (B)=0.0017, AB=NS | | Treatments (A)=88.03, Temperature (B)=37.53, AB=124.50 | | Treatment (A)=0.350, Temperature (B)=0.149, AB=NS | |

**Table 3.** Effect of proline and salicylic acid on speed of germination from seedlings of squash melon and electrolyte leakage ($\mu S\ cm^{-1}\ g^{-1}$), total soluble sugars and proteins ($mg\ g^{-1}$ FW) at different temperatures in squash melon seeds (*Citrullus vulgaris var. fistulosus)* cv.S-48

| Treatments | Speed of germination | | Electrolyte leakage ($\mu S\ cm^{-1}g^{-1}$) | | Total soluble proteins ($mg\ g^{-1}$ FW) | | Total soluble sugars ($mg\ g^{-1}$ FW) | |
|---|---|---|---|---|---|---|---|---|
| | 25°C | 20°C | 25°C | 20°C | 25°C | 20°C | 25°C | 20°C |
| Control | 4.65 | 3.05 | 0.0047 | 0.0067 | 2.38 | 1.70 | 1.33 | 2.30 |
| Hydration | 4.75 | 3.58 | 0.0043 | 0.0057 | 2.51 | 2.00 | 1.81 | 2.50 |
| Warm water | 4.93 | 3.95 | 0.0027 | 0.0047 | 2.62 | 2.15 | 2.23 | 2.93 |
| Proline 1 mM | 5.05 | 4.31 | 0.0057 | 0.0070 | 2.71 | 2.44 | 2.43 | 3.12 |
| Proline 5 mM | 5.41 | 4.45 | 0.0041 | 0.0057 | 2.81 | 2.54 | 2.52 | 3.24 |
| Proline 10 mM | 5.93 | 4.73 | 0.0037 | 0.0046 | 3.25 | 2.83 | 3.02 | 3.32 |
| Proline 20 mM | 6.02 | 5.38 | 0.0033 | 0.0037 | 3.43 | 3.24 | 3.31 | 4.10 |
| SA 0.1 mM | 6.43 | 5.59 | 0.0037 | 0.0045 | 3.60 | 3.52 | 3.41 | 4.35 |
| SA 0.5 mM | 7.48 | 6.18 | 0.0032 | 0.0036 | 3.86 | 3.60 | 3.65 | 4.46 |
| SA 1 mM | 8.21 | 7.93 | 0.0023 | 0.0033 | 3.90 | 3.80 | 4.32 | 4.88 |
| SA 5 mM | 6.01 | 5.25 | 0.0036 | 0.0050 | 3.51 | 3.37 | 3.23 | 4.20 |
| CD (p=0.05) | Treatment (A)=0.027, Temperature (B)=0.011, AB=0.038 | | Treatment (A)=0.00084, Temperature (B)=0.00036, AB=NS | | Treatment (A)=0.068, Temperature (B)=0.029, AB=0.097 (for sugars) | | Treatment (A)=0.040, Temperature (B)=0.017, AB=0.056 | |

resulted in the stimulation of early seedling growth both at normal and low temperatures. The enhancement in the vigour parameters of seedling by SA and proline was associated with an increase in total soluble sugars and proteins. Similarly the simultaneous enhancement in seedling growth and biochemical parameters is observed in watermelon (Ram *et al.,* 2014). Proline enhances the formation of new proteins which improve the salinity tolerance in plants (Khedr *et al.,* 2003). It is concluded that the seed treatments by SA and proline can be useful in improving the seedling growth at low temperature.

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# Influence of Varying Dates of Shield Budding on Scion Growth and Insect-Pest Incidence in Kinnow Nursery under Lower Shiwaliks

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**Abstract**: The weather plays an important role in the vegetative growth of Kinnow in the lower Shiwalik. The success and growth of sapling was significantly affected by the weather parameters as the highest budding success (63.2 %) and survival (65.4%) was found on3rd week of March 2015 budding period. The best time for the successful budding with minimum time to sprout was achieved in the spring season in the 3rd week of March. The weekly average maximum temperature, total evaporation, total rainfall and relative humidity in open field condition during this period was 25.6˚C, 18.0 per cent,52.0 per cent and 70.0 per cent, respectively during this period. The least budding success (39.0 %) and survival (22.4 %) was on the 3rd week of September 2015. There was a significant and positive correlation of average maximum with the budding success (0.998), survival (0.997), citrus psyllid (0.923) and leaf miner (0.998) in the month of March. However, budding in the month of September the survival percent significant but negatively related with citrus psyllid infestation (0.982).

**Keywords**: Budding success, Correlation, Kinnow, Significant, Total evaporation

Kinnow is a large to medium, thorny evergreen fruit tree belonging to the family Rutaceae. Fruit plants can be reproduce by sexually or asexually. Sexual means of reproduction is rarely used in citrus plants. Asexual or vegetative method is for the mass reproduction of kinnow plants by either using part of two or more plants in a union or parts of the same plant. Kinnow fruit trees are usually propagated by vegetative means through budding or grafting. Budding is the method of choice for propagating Kinnow plants because it requires less skill than other types of methods of propagation. Commercially T‑budding method of propagation is used for multiplication in Kinnow. T− Budding in Kinnow is done during the spring and rainy season when the cambium is actively dividing. Budding in citrus usually performed in the spring or fall when the bark is easily separated from the stem wood. It should be timed just before spring season so that coming warm weather may help to ensure a good bud union with root stock. Simultaneously, it should not be so late in autumn season to avoid frost burn of the tender shoots of scion (Wajid *et al.,* 2007). In semi‑arid region of Punjab (Pakistan) the budding of sweet orange in the spring season gave the maximum budding success and survival (Salik *et al*., 2015). Success of bud in Kinnow depends on the several factors like temperature, relative humidity, moisture andplant water, growth stage of scion and rootstock, air, suitable time, techniques of uniting the cambium tissues of stock and scion genetic relationship

between the stock and the scion etc. (Hartmann *et al.*, 2007). Vegetative growth in citrus trees varies with the temperature and maximum between 25 and 31˚C Chaudhary and Ishfaq (2004). The commercial production of mandarin in Nepal was achieved best in budding between 16th to 31st January (Gautam *et al*., 2001). Budding success in the Kinnow was recorded higher under the climatic conditions of Allahabad in the month of November. In Nagpur mandarin highest budding success and survival percentage was recorded on the 15thof December under open field condition Bhusari and Jogdande (2012). Several studies were conducted in the past on Kinnow propagation at different times with commercial method of propagation at different agro‑ecological regions of Punjab. Intensive literature search did not reveal the best time of budding in Kinnow under Kandi region of Punjab. Therefore, the present study was designed to find out the best time of budding in Kinnow under the Kandi region of Punjab.

## MATERIAL AND METHODS

**Experiment site**: The experiment was conducted at Regional Research Station Ballowal Saunkhri Punjab Agricultural University Ludhiana during the 2013‑15. This centre is located in the agro‑climatic Zone‑I in the North− Eastern part of the Punjab, India (agro‑ecological sub‑region 9.1). The average annual rainfall of this region is about 988 mm, which is received during the southwestern monsoon

season and the winter season. The mean monthly minimum and maximum air temperature ranges from 2.6°C and 44.2°C during the year with their respective plateau in the month of January and May, respectively during three years.

**Plant material, experiment design and treatments**: The root stock Jatti Khatti (*Citrus jambhiri* L.) of same age and size were selected and budded with the scions at different weeks such as (2ⁿᵈ week of February), (3ʳᵈ week of February), $T_4$ (4ᵗʰ week of February), (2ⁿᵈ week of March), (3ʳᵈ week of March), (4ᵗʰ week of March), $T_{10}$ ( 2ⁿᵈ week of August), (3ʳᵈ week of August), $T_{12}$ (4ᵗʰ week of August), (2ⁿᵈ week of September), (3ʳᵈ week of September) and 4ᵗʰ week of September During the budding, the 500 rootstock plants of Jatti Khatti (*Citrus jambhiri* L.) were selected for the budding. The budding instruments viz; budding knife, scissors, transparent plastic for wrapping and sun fiber for tying the scion and stock were used. The scion wood was prepared by clipping off the leaves leaving petiole stubs 0.5 cm long intact. Bud wood, 10 cm in length, 9 to12 months old**,** rounded with white streaks, was detached from the scion trees and grafted on 18 month old stocks seedling at a point having a T shaped cut at a height 10–15 cm from the ground level (Williamson and Jackson, 1994) The bud was tied with sun fiber, polythene sheet wrapped over the graft and both ends were tied with sun fiber covering the scion stock union. The binding material and the top to the graft were removed when enough sprouting from the graft was observed. De–sprouting of the stock below the graft union was continued since the time of grafting. All the agronomic and cultural practices were kept constant and carried out regularly with all treatments.

**Plant growth characteristics**: The standard procedures were used for data collection and budding success, percent survival of the bud, leaf infestation twice a week after the treatment application.

## RESULTS AND DISCUSSION

Weather parameters had a great role in the vegetative propagation of Kinnow in the Kandi area of Punjab. Weekly mean maximum temperature ranged from 19.6–38.3°C and minimum temperature varied from 5.9–26.0°C; the weather was suitable for Kinnow budding. Weekly mean maximum temperature was recorded higher from the normal temperature in the month of February and lower in the month of March from year 2013 to 2015 (Fig. 1). However, maximum variation in maximum temperature (+3.6°C) was observed in the 4ᵗʰ week of March, 2015. Similar, trend was observed in the late rainy season (August–September). Weekly mean minimum temperature varies from 5.9 to 15.1°C in spring season (Fig. 2). The highest rainfall (140 mm) was received during second week of August 2014 (Fig. 3). Generally, more rains were received during the rainy season than the spring season. However, in the spring season more rains were received and lesser in the rainy season than that of normal rainfall during three consecutive years. In spring season maximum positive variation (+43.4 mm) in the rainfall was recorded in the 3ʳᵈ week of March 2015 while deficit (–82.7 mm) than the normal rainfall was recorded in the 1ˢᵗ week of August 2013 (Fig. 3). Relative humidity ranged between 46 and 87% in three consecutive years.In spring season more relative humidity (+36.0 %) was observed in the 4ᵗʰ week of February 2013 while in the rainy season it was lesser (–22.0 %) than the normal recorded in the 1ˢᵗ week of September 2015.
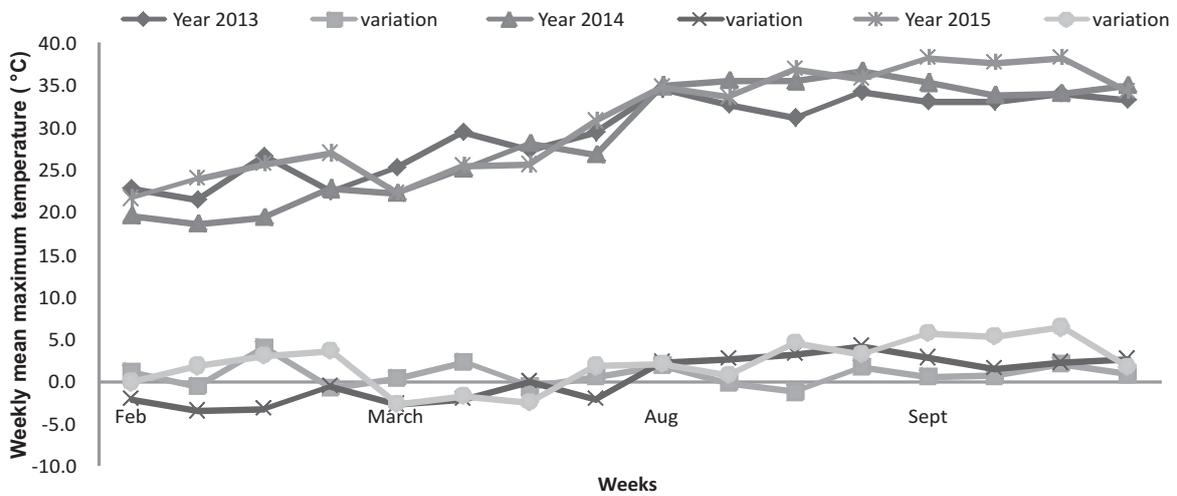


**Fig. 1**. Weekly mean maximum temperature and variation in spring and rainy season in lower foothills of Shiwalik

**Success and survival (%) of the bud:** The data recorded on survival percentage of bud showed that the scion budded during the spring season and the maximum budding success (63.2%) with survival (65.4 %) of bud, which was statistically similar with the scions, budded on 4th week of March2015 (61.4%). The minimum budding success (39.0 %) with survival of the grafts (22.4%) was observed by the scions budded during 3rd week of September 2015 (Table 1). The current study revealed that the most optimum time for budding for Kinnow is 3rd and fourth week of March in spring season under the lower Shiwaliks of Punjab. At these stages of bud took the minimum time for first sprout, maximum survived, attained the higher length of the graft and had more number of side branches. High relative humidity range 62 to 86% favoured the success of grafting compared to the low humidity range during the experimental period. Occurring of

comparatively high intensity rainfall had positive effect on the success of grafting as it increased the relative humidity in the atmosphere particularly during the spring season during 2013−15 showed the best results in the reported experiments. These results support the findings of previous studies which concluded that the temperature plays a significant role with respect to vegetative growth of citrus (Chaudhary and Ishfaq, 2004). Furthermore, the optimum grafting time is dependent on prevailing weather conditions, variety of the fruit plant, budding, grafting technique and the locality (Gandev, 2007).

**Leaf infestation**: the data presented in Table 1 revealed that leaf infestation was maximum recorded during the spring season while minimum was recorded in the late rainy season (August & September). The maximum leaf infestation of leaf miner (12.4 %) was observed in the 2nd week of March, 2015
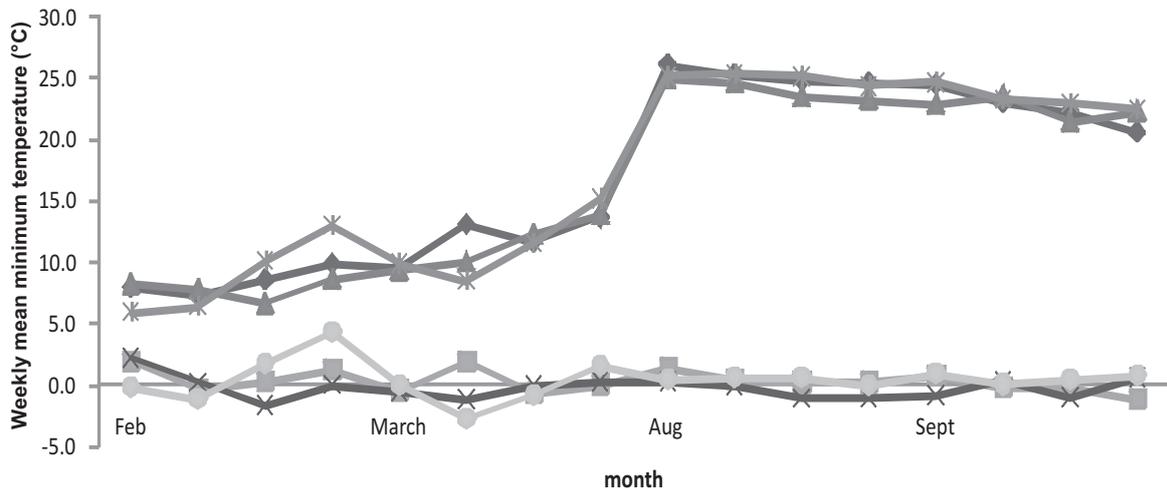


**Fig. 2**. Weekly mean minimum temperature and variation in spring and rainy season in lower foothills of Shiwalik
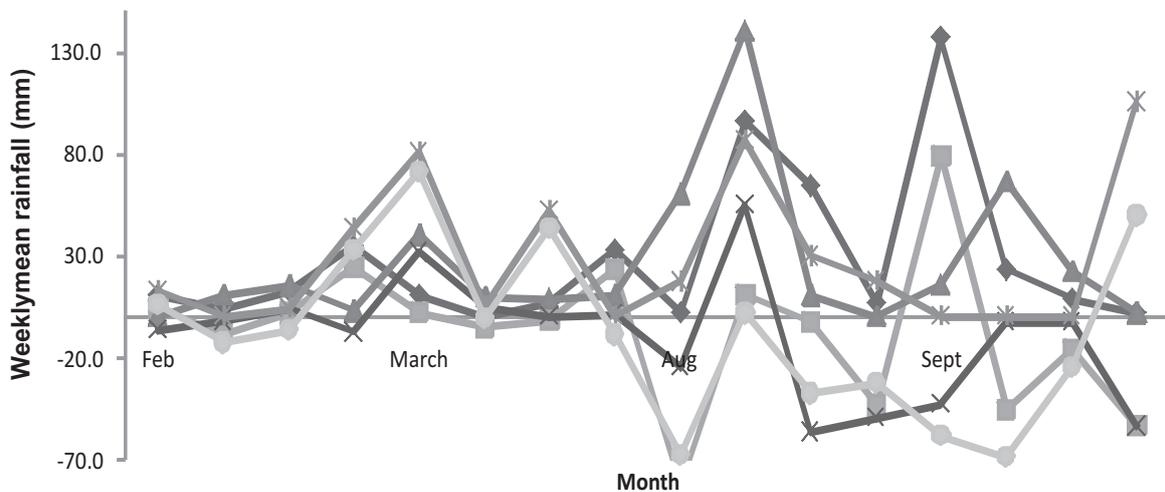


**Fig. 3.** Weekly mean rainfall and variation in spring and rainy season in lower foothills of Shiwalik

However, Severe incidence of citrus psylla was recorded in the 2nd week of March, 2013 (Table1). The severe infestation in the spring season may be due to the high humidity and sufficient moisture.

**Time taken for first sprout:** The results showed that the scions budded during spring season took significantly ($P<0.05$) the minimum times (17.7 days) for first sprout (Table 2). The data regarding time taken for first sprout indicated that scions budded during rainy season took maximum time

(*i.e.*, 28.8 days) for first sprout; however, it was statistically similar with the scions budded during 4th week of September 2015 (28.8 days). The sub-optimal weather conditions may results in maximum days for the first sprout in August & September during 2013–15. The favourable weather conditions during the spring season leads to lesser time to first sprout of bud in Kinnow.

**Length of graft:** The results showed that the treatments effects on length of graft was significance ($P>0.05$) among

**Table 1.** Influence of different dates of budding on budding success, survival and percent leaf infestation in Kinnow

| Budding time | Year (2013) | | | | Year (2014) | | | | Year (2015) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Budding success (%) | Survival (%) | Leaf infestation (%) | | Budding success (%) | Survival (%) | Leaf infestation (%) | | Budding success (%) | Survival (%) | Leaf infestation (%) | |
| | | | Citrus psylla | Leaf miner) | | | Citrus psylla | Leaf miner | | | Citrus psylla | Leaf miner |
| 2nd week of February | 49.0 | 42.3 | 2.3 | 7.8 | 45.8 | 39.0 | 2.1 | 7.9 | 54.5 | 51.5 | 2.4 | 8.4 |
| 3rd week of February | 42.5 | 38.0 | 2.9 | 9.0 | 52.2 | 49.1 | 2.6 | 10.4 | 57.4 | 61.4 | 2.0 | 8.0 |
| 4th week of February | 53.3 | 50.2 | 2.3 | 8.7 | 53.0 | 50.2 | 2.7 | 11.3 | 57.6 | 61.8 | 2.5 | 7.2 |
| 2nd week of March | 51.2 | 56.2 | 3.6 | 10.4 | 54.0 | 51.3 | 3.4 | 10.2 | 59.1 | 61.3 | 2.1 | 12.4 |
| 3rd week of March | 58.2 | 57.1 | 3.0 | 8.2 | 56.6 | 57.4 | 2.1 | 8.0 | 63.2 | 65.4 | 2.2 | 7.8 |
| 4th week of March | 59.2 | 56.2 | 3.0 | 7.1 | 55.3 | 56.2 | 2.0 | 7.2 | 61.4 | 64.1 | 2.9 | 7.4 |
| 2nd week of August | 51.5 | 49.0 | 0.1 | 0.1 | 52.3 | 52.3 | 0.3 | 1.7 | 48.3 | 43.0 | 0.8 | 2.1 |
| 3rd week of August | 52.7 | 52.1 | 0.3 | 0.3 | 53.1 | 50.1 | 0.1 | 1.2 | 48.1 | 44.2 | 0.6 | 2.4 |
| 4th week of August | 52.4 | 49.2 | 0.3 | 0.2 | 49.2 | 49.2 | 0.2 | 1.1 | 49.6 | 40.1 | 0.2 | 1.1 |
| 2nd week of September | 53.4 | 46.32 | 1.1 | 1.1 | 53.4 | 52.8 | 2.2 | 2.3 | 42.5 | 30.1 | 2.2 | 1.7 |
| 3rd week of September | 52.4 | 41.2 | 1.4 | 1.4 | 50.4 | 50.4 | 2.4 | 1.8 | 39.0 | 22.4 | 3.0 | 2.1 |
| 4th week of September | 52.2 | 40.1 | 1.3 | 1.4 | 50.0 | 50.0 | 2.3 | 1.9 | 39.2 | 23.3 | 3.1 | 2.3 |
| CD (p=0.05) | 1.2 | 1.8 | 0.4 | 0.2 | 1.2 | 3.1 | 0.5 | 1.3 | 1.0 | 1.3 | 0.2 | 0.8 |

**Table 2.** Influence of different budding dates on bud sprout and length in Kinnow

| Budding time | Year 2013 | | Year 2014 | | Year 2015 | |
|---|---|---|---|---|---|---|
| | Days for 1st sprout | Length of bud (cm) | Days for 1st sprout | Length of bud (cm) | Days for 1st sprout | Length of bud (cm) |
| 2nd week of February | 21.5 | 20.7 | 23.6 | 16.0 | 21.4 | 20.8 |
| 3rd week of February | 20.3 | 22.0 | 24.3 | 17.0 | 22.0 | 20.2 |
| 4th week of February | 22.0 | 21.0 | 23.7 | 17.4 | 19.8 | 22.3 |
| 2nd week of March | 18.1 | 23.0 | 21.3 | 20.6 | 21.0 | 20.0 |
| 3rd week of March | 19.1 | 22.6 | 18.0 | 22.8 | 17.7 | 24.1 |
| 4th week of March | 18.2 | 23.2 | 21.4 | 17.3 | 20.8 | 20.4 |
| 2nd week of August | 23.3 | 16.8 | 25.3 | 15.8 | 23.4 | 16.4 |
| 3rd week of August | 18.2 | 22.9 | 25.4 | 15.6 | 26.2 | 14.3 |
| 4th week of August | 24.2 | 16.4 | 26.0 | 14.5 | 25.6 | 15.6 |
| 2nd week of September | 22.2 | 17.3 | 22.0 | 17.5 | 27.5 | 12.4 |
| 3rd week of September | 24.1 | 16.9 | 24.3 | 16.5 | 28.8 | 11.9 |
| 4th week of September | 22.7 | 17.2 | 25.7 | 15.2 | 24.3 | 16.4 |
| CD (p=0.05) | 1.2 | 0.9 | 1.0 | 0.8 | 0.7 | 0.8 |

most ofthe treatments(Table 2). However, the maximum and significantly greater (P<0.05) length of bud (24.1 cm) was attained within two months by the scions budded during 3rd week of February 2015  while minimum length of graft (11.9 cm) was in (Table 2).

**Correlation studies:** The mean maximum temperature significantly and positively related with total evaporation (0.961) while significant but negatively (−0.974) with citrus psyllid infestation for budding in February (Table 3). The mean maximum temperature showed a significant and positive correlation with budding success, percent survival, citrus psyllid and percent leaf miner infestation in March budding time (Table 4). However, mean minimum temperature

correlated positively and significantly with total rainfall. Budding success positively and significantly related with survival percentage and leaf miner infestation (Table 4) budding during the March. There was a significant and positive relationship was observed between the budding survival percent and leaf miner in the month of March (Table 4). Mean minimum temperature was significantly and positively correlated with survival percentage for budding in the month of the September (Table 5). The mean relative humidity showed a positive and significant relationship with the percent leaf miner infestation. However, bud survival percent showed significant and negative correlation with citrus psyllid infestation with budding during above said period (Table 5).
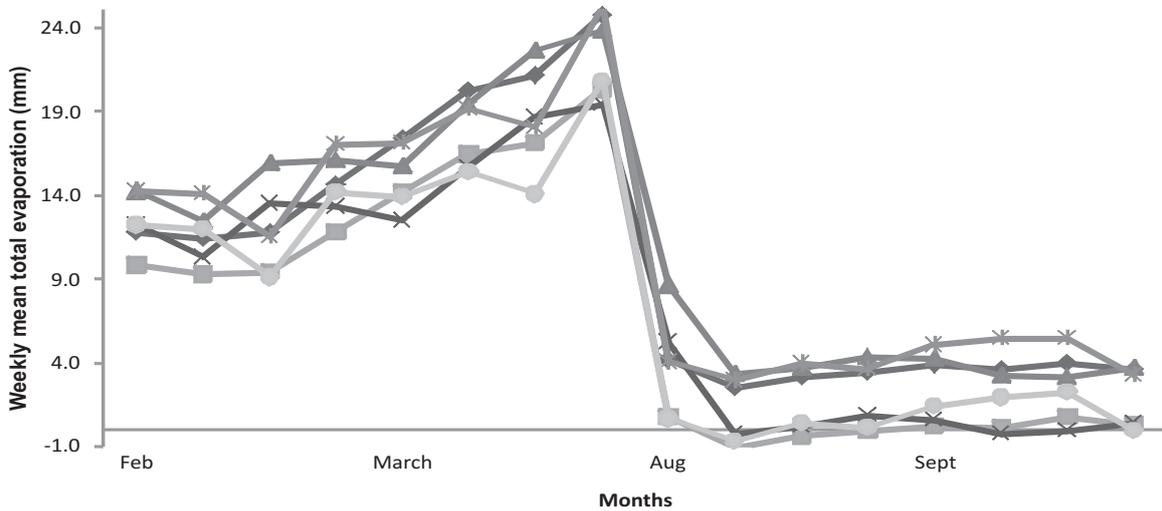


**Fig. 4.** Weekly mean total evaporation and variation in spring and rainy season in lower foothills of Shiwalik
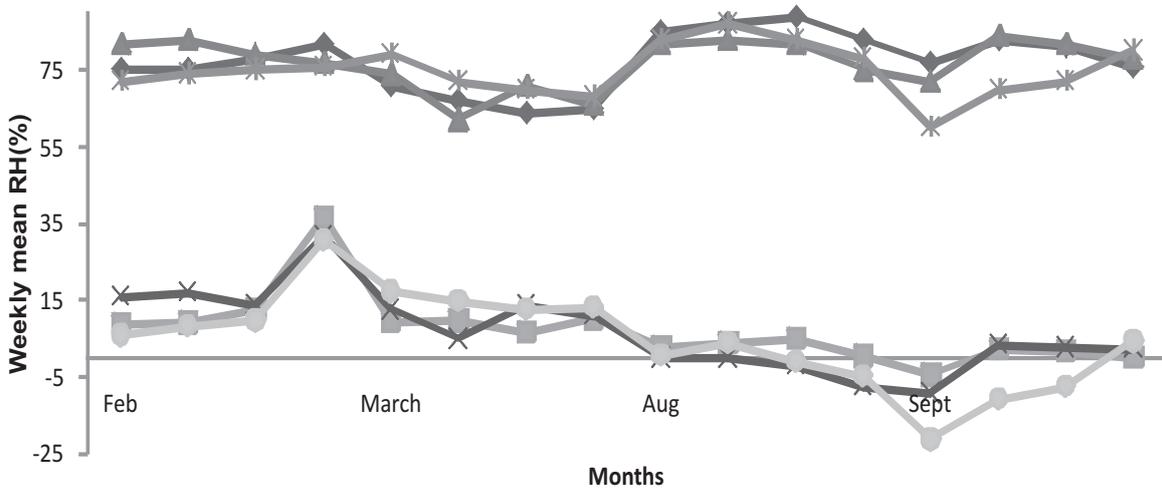


**Fig. 5.** Weekly mean relative humidity and variation in spring and rainy season in lower foothills of Shiwalik

**Table 3.** Relationship of climatic factors with vegetative growth and insect-pest incidence in the lower Shiwaliks of Punjab in February

|  | Mean maximum temperature | Mean minimum temperature | Total rainfall | Total evaporation | Mean RH | Budding success | Budding survival | Citrus psyllid | Leaf miner |
|---|---|---|---|---|---|---|---|---|---|
| Budding success | 0.998** | 0.838 | 0.732 | 0.594 | 0.681 | 1.000 | | | |
| Budding survival | 0.997** | 0.842 | 0.734 | 0.593 | 0.704 | 0.998** | 1.000 | | |
| Citrus psyllid | 0.923* | 0.852 | 0.789 | 0.711 | 0.517 | 0.936 | 0.924 | 1.000 | |
| Leaf miner | 0.998** | 0.882 | 0.784 | 0.650 | 0.751 | 0.994** | 0.997** | 0.923 | 1.000 |

**Table 4.** Relationship of climatic factors with vegetative growth and insect-pest incidence in the lower Shiwaliks of Punjab in March

|  | Mean maximum temperature | Mean minimum temperature | Total rainfall | Total evaporation | Mean RH | Budding success | Budding survival | Citrus psyllid | Leaf miner |
|---|---|---|---|---|---|---|---|---|---|
| Budding success | 0.916 | 0.807 | -0.666 | 0.836 | -0.894 | 1.000 | | | |
| Budding survival | 0.619 | 0.726 | -0.108 | 0.651 | -0.475 | 0.813 | 1.000 | | |
| Citrus psyllid | -0.435 | -0.612 | -0.121 | -0.504 | 0.263 | -0.660 | -0.974* | 1.000 | |
| Leaf miner | 0.315 | -0.092 | -0.723 | 0.042 | -0.578 | 0.453 | 0.025 | 0.131 | 1.000 |

**Table 5.** Relationship of climatic factors with vegetative growth and insect-pest incidence in the lower Shiwaliks of Punjab in September

|  | Mean maximum temperature | Mean minimum temperature | Total rainfall | Total evaporation | Mean RH | Budding success | Budding survival | Citrus psyllid | Leaf miner |
|---|---|---|---|---|---|---|---|---|---|
| Budding success | 0.183 | 0.714 | 0.228 | 0.545 | -0.028 | 1.000 | | | |
| Budding survival | 0.443 | 0.982* | 0.690 | 0.721 | -0.709 | 0.724 | 1.000 | | |
| Citrus psyllid | -0.273 | -0.935 | -0.742 | -0.597 | 0.623 | -0.774 | -0.982* | | |
| Leaf miner | -0.707 | -0.830 | -0.543 | -0.751 | 0.957* | -0.203 | -0.793 | 0.683 | 1.000 |

## CONCLUSION

Based on findings of this study it can be concluded that for first sprout, survival of the grafts, length of grafts and number of side branches within the first three months of budding can appear if the budding is performed during 3rd and 4th week of March, followed by those rootstocks budded during 3rd week of February.

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# Phenological Development and Agrometeorological Indices in Dual Purpose Barley (*Hordeum vulgare* L.) as Influenced by Planting Techniques and Cutting Practices in Indo-Gangetic Plains of India

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**Abstract**: Field experiment was conducted during *rabi* seasons of 2014–15 to study the phenology, accumulation of growing degree days (GDD), *p*hoto thermal Units (PTU), helio thermal unit (HTU), pheno thermal index (PTI), heat use efficiency (HUE) and performance of barley under planting techniques and cutting practices. The emergence under bidirectional and unidirectional planting techniques were at par, but significantly superior than broadcast. The regeneration of crop was significantly less days after cut in cut at 50 DAS compared to cut at 60 DAS. The uncut treatment took significantly less days taken for completion of crop in booting, heading and maturity under different phenological stage. The BC planting techniques accrued significantly higher Growing degree days, photo–thermal unit, helios thermal unit as compared to BD and UD planting techniques for emergence. The uncut treatment was significantly less GDD, PTU and HTU consuming of crop in booting, heading and maturity as compared to treatment cut at 50 and 60 DAS. The PTI was significantly higher in BD as compared to BC and UD planting techniques for emergence and jointing stage of crop.The PTI was significantly higher in cut at 50 DAS compared to cut at 60 DAS for regeneration stage of crop. The HUE at 30 DAS for biological yield was significantly higher in BD planting techniques followed by BC and UD planting techniques. HUE in uncut treatment was significantly higher than cut at 50 and 60 DAS.

**Keywords**: GDD, HTU, PTU, PTI, HUE & Phenology

Barley (*Hordeum vulgare* L.) can be grown in the wide range of climatic conditions, its productivity is largely depends on the prevailing weather conditions throughout the life cycle of the crop. Further, the different weather parameters affect growth and development of crop differently. Therefore, it was contemplated to have an insight on the effect of different weather parameters and thereby agro–meteorological indices for the production of barley. The agro meteorological indices have frequently been used as a weather based indices for assessing crop phenology growth and yield attributes. Therefore, all growth and development stages of crop may be estimated more accurately on the basis of GDD rather than calendar method (Warthington and Hatchinson, 2005). Heat unit concept is the agronomic application of temperature effect on plant, which has been employed to correlate phonological development in crop and to predict maturity dates (Pankaj *et al.*, 2014).The GDD is used to quantify effect of temperature and described the timing of different biological process (Kaur *et al.*, 2006 and Qiao–yan *et al.*, 2012.). Under North Indian condition, the maturity of barley hastened due to gradual rise in ambient temperature under delayed planting. Hence, it becomes imperative to have knowledge of exact duration of phenological stages in a particular crop–growing environment and their impact on yield of crop. Therefore, an experiment was planned to determine the phenological development and agrometeorological indices in barley as influenced by planting techniques and cutting practices in Indo–Gangetic plains of India.

## MATERIAL AND METHODS

The field experiment was conducted during the *rabi* season of 2014–15 at Punjab Agricultural University, Ludhiana (30º 54'N; 74º 48'E and 247 m above m. s. l.). The barley variety PL807 was on sown on November 5, 2014 with 22.5 cm row spacing and seed rate 87.5 kg seed ha$^{-1}$. In factorial randomized block design with three planting techniques viz. unidirectional (UD), bidirectional (BD), broadcast (BC) and three cutting practices *viz.* uncut (UN$_C$), cut at 50 DAS (C$_{50}$ DAS) and cut at 60 DAS (C$_{60}$ DAS) with four replications. The daily meteorological data was collected from School of Climate Change and Agricultural Meteorology, Punjab Agricultural University, Ludhiana, Punjab. Different agrometeorological indices were calculated on daily basis and accumulated during different phenological stages *viz.* sowing to emergence, tillering,

jointing, booting, regeneration, heading and maturity of the crop using following formulae.

Growing degree days (GDD) = $\sum_{i=1}^{n}\left\{\left(\frac{T\max+T\min}{2}\right)-Tb\right\}$ ;'

$T_b$ = 4.4 °C *(Pankaj et al,,* 2014.)

Photo thermal Units (PTU) = $\sum_{i=1}^{n}$ GDD × Day length

Heliothermal unit (HTU) = $\sum_{i=1}^{n}$ GDD × Actual sunshine hours

PTI = Accumulated GDD/ Number of days between two phenological stages.

Heat use efficiency (HUE) was computed for dry matter accumulation at 30, 60, 90, 120 days after sowing and at the harvest as well as for grain yield, straw yield, biological yield and forage yield at different cuts separately. HUE (Kg ha$^{-1}$ °C days) is the dry matter produced per unit degree day consumption which was calculated as –

HUE (Kg ha$^{-1}$ °C days) = above ground dry matter (kg ha$^{-1}$) / Accumulated GDD.

### RESULTS AND DISCUSSION

**Phenological development:** Days taken for completion of crop emergence under BD and UD planting techniques were at par, but significantly superior than broadcast (Table 1). The days taken to attain different phenophases like tillering, jointing, booting, heading and maturity under planting techniques were non-significant. The cutting practices were non-significant in emergence, tillering, jointing. The uncut treatment has taken significantly less days for completion of crop in booting, heading and maturity as compared to cut at 50 and 60 DAS.

**Growing Degree Days (GDD):** The BC planting techniques consumed significantly higher degree days compared to BD and UD planting techniques for emergence (Table 2). The degree days in tillering, jointing, booting, heading and maturity under planting techniques were non-significant. The cutting treatments were non-significant in emergence, tillering and jointing. The uncut treatment consumed significantly less degree days booting, heading and maturity as compared to cut at 50 and 60 DAS.

**Pheno Thermal Index (PTI):** The BD and UD planting techniques were significantly at par, but significantly superior

**Table 1.** Effect of different planting techniques and cutting practices on phenological stage (DAS) of barley cv. PL807, *Rabi* 2014–15

| Treatment | Emergence | Tillering | Jointing | Booting | Heading | Maturity |
|---|---|---|---|---|---|---|
| **Planting techniques** | | | | | | |
| Unidirectional | 7 | 27 | 49 | 98 | 105 | 164 |
| Bidirectional | 7 | 27 | 49 | 98 | 105 | 164 |
| Broadcast | 9 | 27 | 49 | 98 | 105 | 164 |
| CD (p=0.05) | 0.4 | NS | NS | NS | NS | NS |
| **Cutting practices** | | | | | | |
| Un-cut | 8 | 27 | 49 | 88 | 97 | 161 |
| Cut at 50 DAS | 8 | 27 | 49 | 98 | 102 | 164 |
| Cut at 60 DAS | 8 | 27 | 50 | 106 | 117 | 167 |
| CD (p=0.05) | NS | NS | NS | 0.31 | 0.60 | 1.23 |

**Table 2.** Effect of different planting techniques and cutting practices on growing degree days (GDD) (°C days) of different phenological stage of barley cv. PL807, *Rabi* 2014–15

| Treatment | Emergence | Tillering | Jointing | Booting | Heading | Maturity |
|---|---|---|---|---|---|---|
| **Planting techniques** | | | | | | |
| Unidirectional (UD) | 98.28 | 356.92 | 646.21 | 1278.06 | 1376.06 | 2145.08 |
| Bidirectional (BD) | 96.94 | 358.67 | 647.87 | 1280.06 | 1379.01 | 2146.54 |
| Broadcast | 111.69 | 355.61 | 647.03 | 1279.01 | 1377.00 | 2142.18 |
| CD (p=0.05) | 5.91 | NS | NS | NS | NS | NS |
| **Cutting practices** | | | | | | |
| Un-cut | 101.79 | 356.48 | 645.34 | 1155.15 | 1264.80 | 2105.38 |
| Cut at 50 DAS | 102.32 | 358.23 | 647.26 | 1286.05 | 1337.03 | 2142.39 |
| Cut at 60 DAS | 102.79 | 356.48 | 648.52 | 1392.99 | 1526.60 | 2186.03 |
| CD (p=0.05) | NS | NS | NS | 4.02 | 7.90 | 16.07 |

to BC planting techniques for emergence and jointing of growth stages (Table 3). The photo thermal index was significantly higher in BC as compared to BD and UD planting techniques for tillering stage of crop. The pheno thermal index was non-significant in jointing, booting, heading and maturity under different planting techniques. Similarly cutting practices were non-significant in emergence, tillering and jointing. The pheno thermal index was significantly higher in uncut treatment compared to cut at 50 DAS and cut at 60 DAS for booting stage. It was significantly higher in cut at 60 DAS compared to uncut and cut at 50 DAS for heading and maturity.

**Photo Thermal Units (PTU):** The BC planting techniques significantly higher photo thermal units compared to BD and UD planting techniques for emergence (Table 4). The photo thermal units in tillering, jointing, booting, heading and maturity under planting techniques were non-significant. The cutting practices were non-significant in emergence, tillering, jointing. The uncut treatment significantly less photo thermal

units in booting, heading and maturity as compared to cut at 50 and 60 DAS. These results are in line with the finding of Pandey *et al*, 2013.

**Helio Thermal Units (HTU):** The BC planting techniques significantly higher helio thermal units compared to BD and UD planting techniques for emergence (Table 5). The helio thermal units in tillering, jointing, booting, heading and maturity stage of crop under planting techniques were non-significant. The cutting treatments were non-significant in emergence, tillering and jointing growth stages of crop. The uncut treatment significantly less helio thermal units in booting, heading and maturity as compared to cut at 50 and 60 DAS.

**Heat Use Efficiency (HUE):** Heat use efficiency recorded at 30 DAS to biological yield was significantly higher in BD planting techniques followed by BC and UD planting techniques (Table 6). Heat use efficiency in uncut treatment was significantly higher than cut at 50 and 60 DAS. The forage cut stage of the crop was non-significant. Similar

**Table 3.** Effect of different planting techniques and cutting practices on pheno thermal index (PTI) of different phenological stage of barley cv. Pl807, *Rabi* 2014−15

| Treatment | Emergence | Tillering | Jointing | Booting | Heading | Maturity |
|---|---|---|---|---|---|---|
| **Planting techniques** | | | | | | |
| Unidirectional | 13.05 | 17.96 | 21.95 | 26.76 | 223.84 | 36.98 |
| Bidirectional | 13.25 | 17.85 | 22.77 | 26.77 | 211.49 | 36.94 |
| Broadcast | 12.41 | 19.06 | 20.87 | 26.75 | 236.97 | 37.08 |
| CD (p=0.05) | 0.57 | 0.51 | 0.37 | NS | NS | NS |
| **Cutting practices** | | | | | | |
| Un-cut | 12.96 | 18.26 | 21.69 | 29.62 | 152.24 | 32.80 |
| Cut at 50 DAS | 13.04 | 18.35 | 21.67 | 26.25 | 322.49 | 34.87 |
| Cut at 60 DAS | 12.71 | 18.26 | 21.44 | 24.44 | 149.57 | 43.43 |
| CD (p=0.05) | NS | NS | NS | 0.09 | 11.68 | 0.54 |

**Table 4.** Effect of different planting techniques and cutting practices on photo thermal unit (PTU) (Degree-days hour) of different phenological stage of barley cv. PL807, Rabi 2014−15

| Treatment | Emergence | Tillering | Jointing | Booting | Heading | Maturity |
|---|---|---|---|---|---|---|
| **Planting techniques** | | | | | | |
| Unidirectional | 1072.86 | 3896.41 | 7054.49 | 13952.28 | 15022.12 | 23417.34 |
| Bidirectional | 1058.25 | 3915.46 | 7072.59 | 13953.38 | 15023.12 | 23433.22 |
| Broadcast | 1219.25 | 3838.12 | 7063.55 | 13952.29 | 15024.67 | 23385.59 |
| CD (p=0.05) | 64.53 | NS | NS | NS | NS | NS |
| **Cutting practices** | | | | | | |
| Un-cut | 1111.29 | 3891.64 | 7044.97 | 12610.45 | 13807.47 | 22983.88 |
| Cut at 50 DAS | 1117.00 | 3910.69 | 7065.93 | 14039.45 | 14595.96 | 23387.96 |
| Cut at 60 DAS | 1122.08 | 3891.64 | 7079.74 | 15206.94 | 16665.47 | 23864.30 |
| CD (p=0.05) | NS | NS | NS | 43.96 | 87.06 | 175.08 |

**Table 5.** Effect of different planting techniques and cutting practices on helio thermal units (HTU) *(*Degree-days hour) of different phenological stage of barley cv. PL807, Rabi 2014–15

| Treatment | Emergence | Tillering | Jointing | Booting | Heading | Maturity |
|---|---|---|---|---|---|---|
| **Planting techniques** | | | | | | |
| Unidirectional | 594.77 | 2160.08 | 3910.86 | 7734.84 | 8327.94 | 12982.07 |
| Bidirectional | 586.67 | 2170.64 | 3920.89 | 7738.29 | 8327.94 | 12990.87 |
| Broadcast | 675.93 | 2152.16 | 3915.87 | 7736.21 | 8329.35 | 12964.46 |
| CD (p=0.05) | 35.77 | NS | NS | NS | NS | NS |
| **Cutting practices** | | | | | | |
| Un-cut | 616.07 | 2157.44 | 3905.58 | 6990.96 | 7654.56 | 12741.77 |
| Cut at 50 DAS | 619.24 | 2168.00 | 3917.20 | 7783.16 | 8091.69 | 12965.78 |
| Cut at 60 DAS | 622.06 | 2157.44 | 3924.85 | 8430.39 | 9238.98 | 13229.85 |
| CD (p=0.05) | NS | NS | NS | 24.37 | 47.88 | 97.91 |

**Table 6.** Effect of different planting techniques and cutting practices on heat use efficiency (HUE) (Kg/ha/ °C days) of different phenological stage of barley cv. PL807, *Rabi* 2014–15

| Treatment | DM 30DAS | DM 60DAS | DM 90DAS | DM 120DAS | DM at harvest | Grain yield | Straw yield | Biological yield | Forage cut stage |
|---|---|---|---|---|---|---|---|---|---|
| **Planting techniques** | | | | | | | | | |
| Unidirectional | 0.14 | 0.80 | 1.09 | 4.53 | 5.14 | 1.87 | 3.29 | 5.16 | 0.93 |
| Bidirectional | 0.15 | 0.84 | 1.23 | 5.04 | 5.64 | 2.08 | 3.74 | 5.82 | 0.97 |
| Broadcast | 0.14 | 0.80 | 1.10 | 4.56 | 5.20 | 1.90 | 3.34 | 5.22 | 0.93 |
| CD (p=0.05) | 0.007 | 0.03 | 0.04 | 0.19 | 0.20 | 0.10 | 0.13 | 0.22 | NS |
| **Cutting practices** | | | | | | | | | |
| Un-cut | 0.15 | 1.03 | 1.98 | 5.63 | 5.69 | 2.12 | 3.60 | 5.71 | – |
| Cut at 50 DAS | 0.14 | 0.41 | 1.27 | 4.96 | 5.44 | 1.99 | 3.46 | 5.45 | 0.97 |
| Cut at 60 DAS | 0.14 | 0.99 | 1.17 | 3.55 | 5.85 | 1.75 | 3.29 | 5.03 | 0.93 |
| CD (p=0.05) | 0.007 | 0.03 | 0.04 | 0.19 | 0.20 | 0.10 | 0.13 | 0.22 | NS |

results were reported by Pankaj *et al*, 2014.

**Regeneration of crop:** Regeneration of phonological development was significantly less days after cut in cut at 50 DAS compared to cut at 60 DAS (Table 7). The regeneration consumed significantly higher GDD in case of crop cut at 60 DAS (151.23) as compared the crop cut at 50 DAS (66.15). Regeneration of PTI was non-significant. The pheno thermal index was significantly higher in cut at 50 DAS compared to cut at 60 DAS for regeneration stage of crop. The helio thermal units in regeneration stage of crop under planting techniques were non-significant. The cutting treatments in regeneration of crop required significantly less helio thermal units cut at 50 DAS compared to cut at 60 DAS.

**Table 7.** The regeneration of barley after fodder cut under different heat units

| Treatment | Phenological stage (DAS) | GDD | PTI | PTU | HTU |
|---|---|---|---|---|---|
| **Planting techniques** | | | | | |
| Unidirectional | 8 | 72.46 | 13.06 | 791.00 | 438.53 |
| Bidirectional | 8 | 73.81 | 13.10 | 993.00 | 439.86 |
| Broadcast | 8 | 72.78 | 13.03 | 791.00 | 438.50 |
| CD (p=0.05) | NS | NS | NS | NS | NS |
| **Cutting practices** | | | | | |
| Un-cut | - | - | - | - | - |
| Cut at 50 DAS | 5 | 66.15 | 13.23 | 722.12 | 400.33 |
| Cut at 60 DAS | 12 | 151.23 | 12.97 | 1650.97 | 1650.05 |
| CD (p=0.05) | 0.4 | NS | NS | 23.28 | 12.90 |

## CONCLUSION

The pheno-thermal index and heat use efficiency recorded under bidirectional planting technique followed by broadcast and unidirectional planting techniques. The broadcast planting techniques accrued significantly higher growing degree days, photo thermal units and helio thermal *units* as compared to bidirectional and unidirectional planting techniques. Under cutting practices pheno-thermal index and heat use efficiency in uncut treatment were significantly higher than cut at 50 and 60 DAS. The uncut treatment was reduced significantly less growing degree days, *photo*

thermal units and helio thermal units consuming of crop as compared to cut at 50 and 60 DAS.

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# Yield, Quality and Economics of different Sweet Corn (*Zea mays* L. Var. *Saccharata*) Cultivars at different Planting and Harvesting Dates under Shiwalik Foothills of J&K

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*Abstract*: A field experiment was conducted at Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu by extending planting time of crop from March-June during 2013 to study yield attributes, yield, quality and economics of sweet corn (*Zea mays* L.Var. *Saccharata*) cultivars 'Misthi', 'Sugar-75' and 'Gold star' under six planting dates (29 March, 15 April, 30 April, 15 May, 30 May and 19 June). Cultivar 'Misthi' recorded higher value of plants/m$^2$, cobs/plant, cob weight (g), fresh cob and stover yield, number of grains/row, number of grains/cob, grain and stover yield except for 1000 grain weight over 'Sugar-75' and 'Gold star' both at harvest and physiological maturity. Planting on April 15, March 29 and April 30 recorded statistically similar but significantly highest fresh cob weight, fresh cob and stover yield, grains/cob, 1000-grain weight, grain and stover yield at harvest maturity, at physiological maturity than the crop sown on 15$^{th}$ May and onwards. Cultivar 'Misthi' recorded higher starch (63.68 %), net returns (Rs.157068/ha) and B:C ratio 4.45 at harvest, whereas at physiological maturity it registered 66.38 %, Rs. 156702/ha and 4.25 values for starch, net returns and B:C ratio. Planting on April 15 recorded highest net returns (Rs. 166603/ha and 169020/ha) and B: C ratio (4.15 and 4.05) at harvest and physiological maturity, respectively. For getting more yield and profit at physiological maturity and harvesting cultivar 'Misthi' should be planted at April 15.

**Keywords**: Sweet corn cultivar, Planting dates

The J&K state is one of the major maize growing states in the country and has a significant role in the economy as it forms the staple diet of majority of the people. It is mostly grown in *kharif* season in the state with an effective crop growth period. Sweet corn (*Zea mays* L. Var. *Saccharata*) is one amongst the seven different maize types and it differs from other corns in terms of its genetic constitution rather than its systematic or taxonomic characterization. The kernels of sweet corn are translucent, having thinner pericarp and more or less wrinkled at maturity (Pradeep *et al*., 2005). Besides their genetic makeup, sweetness of these varieties is also governed by their management practices and stages of harvest i.e. harvest maturity and physiological maturity. Climatic variability has direct impact on the quantity and quality of sweet corn. To deal with the impact of climatic change, the potential adaptation strategies are identified changing planting dates, crop diversification, integrated farming system *etc*. Crops respond differently to different planting dates and sweet corn due to its short time growth could be planted in several sowing dates. In this study, different planting dates and cultivars were studied to access their yield, quality parameters and economics under irrigated sub-tropics of Jammu region.

## MATERIAL AND METHODS

A field experiment was conducted at Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Main campus Chatha, by extending planting time of the crop from March-June during 2013 on sandy clay loam texture soil, low in organic C and available N but medium in available P and K with pH 7.31. The experiment consisted of three sweet corn cultivars *viz*. 'Misthi', 'Sugar-75' and 'Gold star' and six planting dates 29 March, 15 April, 30 April, 15 May, 30 May and 19 June was laid out in split plot design having three replications. The cultivars and planting dates were allocated in main and sub plots, respectively. The crop was sown at specified row to row distance of 60 cm and plant to plant distance of 20 cm. The recommended doses of N (120 kg), $P_2O_5$ (60 kg) and $K_2O$ (40 kg) were applied in the form of urea, diammonium phosphate and muriate of potash, respectively. Half of the dose of N and whole amount of P and K was applied as basal and the remaining half dose of nitrogen was applied in two equal splits at knee high and pre-tasseling stages. The yield attributes, *viz*. plants m$^{-2}$, number of cobs plant$^{-1}$, number of grain rows cob$^{-1}$, number of grains row$^{-1}$, number of grains cob$^{-1}$ were worked out from randomly selected cobs at both the stages. The fresh cob and fresh

stover yield, grain and stover yield, protein and starch per cent at harvest and at physiological maturity were estimated by using the standard methodologies for analysis and formulae. The economics was worked out at the prevailing market rates of inputs and produce.

## RESULTS AND DISCUSSION

**Yield attributes and yield:** Cultivar 'Misthi' being statistically at par in plant m$^{-2}$ with 'Gold star' recorded not only maximum but also significantly higher number of plants m$^{-2}$ from cultivar 'Sugar–75' (Table 1). Number of cobs plant$^{-1}$ was significantly influenced by different cultivars. Among the cultivars, maximum number of cobs plant$^{-1}$ was recorded by cultivar 'Misthi' which was statistically at par with 'Sugar–75' however, significantly superior to 'Gold star' also produced statistically equal cobs/plant with 'Sugar–75'. Varietal variation in the yield attributing characters of different cultivars may be regarded as genetic character of the cultivars. Planting date 15 May recorded significantly highest number of plants m$^{-2}$ but statistically at par with 15 April, 30 April and 30 May plantings and all these planting dates recorded their significant superiority over 29 March and 19 June planting dates which in turn also differed significantly from one another in this parameter. Planting date 29 March and 15 April each produced maximum number of cobs plant$^{-1}$ and were statistically at par with 30 April planting. Earlier sowings produced significantly higher number of cobs/plant as compared to later sowings of 15 May, 30 May and 19 June, which remained statistically alike with each other bearing 1 cob plant$^{-1}$. It might be due to vigorous plant growth owing to

favourable weather conditions prevailing during the planting dates. Thus resulting in adequate supply of photosynthates for the formation of cobs, number of grains/cob and increased 1000-grain weight. These results are in line with Lashkari *et al.* (2011) and Azadbakht *et al.* (2012). Non–significant effect of different cultivars was observed on fresh cob weight of sweet corn, though some numerical variations have been observed among them. Lowest fresh cob weight was for 'Gold star' cultivar while the highest fresh cob weight was for 'Misthi' followed by 'Sugar–75'. Fresh cob and stover yield of sweet corn was significantly influenced by type of cultivar grown (Table 1). Cultivar 'Misthi' recorded maximum but statistically higher fresh cob yield as compared to 'Sugar–75' and 'Gold star' cultivars which recorded statistically comparable yields with themselves but these were less to the tune of 29.52 and 33.06 per cent from cultivar 'Mishti'. Cultivar 'Misthi' produced highest fresh stover yield which was statistically at par with fresh Stover yield realized for 'Sugar–75'while cultivar 'Gold star' recorded significantly lower fresh stover yield which might be due to cumulative effect of superior attributing characters in the cultivar 'Mishti'. Khan *et al.,* 2009 also observed the similar findings. The per cent increase in stover yield for cultivars 'Misthi' and 'Sugar–75' over 'Gold star' was 29.6 and 28.9 per cent, respectively.

Sweet corn sown on 15 April produced cobs with maximum fresh weight (g) followed by statistically similar fresh cob weight realized under 29 March and 30 April planting dates and all these dates recorded their significant superiority in this parameter over all the other sowing dates. 15 and 30 May planting dates being statistically at par in fresh

**Table 1**. Effect of cultivars and planting times on yield attributes and yields of sweet corn

| Treatments | At harvest maturity | | | | | At physiological maturity | | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. of plants m$^{-2}$ | No. of cobs plant$^{-1}$ | Fresh cob weight (g) | Fresh cob yield (t$^{-1}$ ha) | Fresh stover yield (t$^{-1}$ ha) | No. of grains cob$^{-1}$ | 1000 grain weight (g) | Grain yield (t$^{-1}$ ha) | Stover yield (t$^{-1}$ ha) |
| **Cultivars** | | | | | | | | | |
| Misthi | 6.67 | 1.14 | 117.91 | 9.16 | 11.13 | 258.63 | 113.98 | 2.20 | 6.82 |
| Sugar–75 | 5.57 | 1.13 | 110.95 | 7.07 | 11.08 | 230.88 | 135.91 | 1.74 | 5.74 |
| Gold star | 6.11 | 1.05 | 108.63 | 6.89 | 8.59 | 189.98 | 137.00 | 1.57 | 5.43 |
| CD (p=0.05) | 0.68 | 0.08 | NS | 1.10 | 0.64 | 12.52 | 9.06 | 0.37 | NS |
| **Planting times** | | | | | | | | | |
| March 29$^{th}$ | 5.78 | 1.22 | 131.38 | 9.17 | 11.65 | 245.07 | 138.28 | 2.31 | 7.26 |
| April 15$^{th}$ | 6.78 | 1.22 | 133.86 | 10.89 | 11.84 | 245.94 | 140.52 | 2.39 | 7.65 |
| April 30$^{th}$ | 6.57 | 1.20 | 123.99 | 9.55 | 11.44 | 241.47 | 137.00 | 2.23 | 7.01 |
| May 15$^{th}$ | 6.89 | 1.00 | 103.98 | 7.01 | 9.65 | 224.08 | 127.75 | 1.81 | 5.68 |
| May 30$^{th}$ | 6.57 | 1.00 | 101.43 | 6.50 | 9.54 | 220.21 | 122.79 | 1.72 | 5.46 |
| June 19$^{th}$ | 4.11 | 1.00 | 80.33 | 3.12 | 7.50 | 182.22 | 107.40 | 0.56 | 2.92 |
| CD (p=0.05) | 0.56 | 0.12 | 19.33 | 1.87 | 1.01 | 15.13 | 8.98 | 0.41 | 1.24 |

cob weights were regarded more significantly superior than 19 June sown crop which produced fresh cobs of lowest weight. Planting dates also significantly affected the fresh cob yield of sweet corn (Table 1). The crop planted on 15 April recorded highest fresh cob yield which was statistically at par with 29 March and 30 April planting dates. 15 and 30 May planting dates were also statistically at par with each other with fresh cob yield of 7.016 t ha$^{-1}$ and 6.507 t ha$^{-1}$ but significantly superior than 19 June planting date. Among the planting dates, the maximum fresh stover yield was obtained with 15 April planting which was statistically at par with 29 March and 30 April planting dates with fresh stover yield. All these three planting dates (15 May, 30 May and 19 June) produced significantly higher fresh stover yield over all the other planting dates, wherein 15 and 30 May plantings produced not only statistically similar yields but significantly higher than 19 June planting which recorded significantly lower yields. The variation in different factors may be because of the fact that 15 April sown crop got sufficient time for proper growth under suitable climatic conditions in comparison to late sowings and also early planting in spring causes encountering of grain formation and filling stages with long days and maximum energy needed to photosynthesis results in higher yields in comparison to late sowing (June 19).

Cultivar 'Misthi' recorded significantly higher number of grains per cob followed by 'Sugar‑75' and 'Gold star' in the descending order. Cultivars significantly influenced the 1000 grain weight of sweet corn. 'Gold star' produced heavier grains with 1000 grain weight at par with 'Sugar‑75' while as

cultivar 'Misthi' was rated as significantly inferior cultivar in this parameter. Cultivar 'Misthi' recorded significantly highest grain yield which was significantly superior to cultivars 'Sugar‑75' and 'Gold star'. Between 'Sugar‑75' and 'Gold star', though cultivar 'Sugar‑75' recorded higher grain yield than 'Gold star' but both of these cultivars were observed to be statistically similar recording grain yields. The reduction in yield with June 19 planting time might be due to reduction of yield attributing characters, pollination and fertilization disturbance. Similarly, reproductive phase also shortened for June 19 sown crops. Statistically similar results in grain and stover yield with first three dates of sowings might be due to almost similar conditions of temperature and optimum soil moisture during initial stages of growth (Oktem *et al*., 2004).

**Quality:** Non‑significant differences were recorded among different cultivars and planting dates with respect of protein content in grains (Table 2). The per cent starch content in grains at harvest and physiological maturity revealed that the highest per cent starch content was recorded in cultivar 'Misthi' which in turn was statistically at par with 'Sugar‑75', whereas 'Gold star' was significantly inferior in this quality parameter at harvest and physiological maturity. The higher starch content in 'Misthi' and 'Sugar‑75' might be due to the higher starch content present in their grains which is exclusively dependent and correlated with the genetic constitution of the cultivars. Similar findings in respect of higher starch content in different sweet corn cultivar were also reported by Erdal *et al*. (2011). Among the planting dates, 19 June planted crop recorded higher protein content in the grains at harvest and physiological maturity stages, respectively. It was followed by sweet corn sown on 30 May,

**Table 2.** Effect of cultivars and planting times on quality and economics of sweet corn

| Treatments | At harvest maturity | | | | | | At physiological maturity | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Protein (%) | Starch (%) | Cost of cultivation (Rs ha$^{-1}$) | Gross returns (Rs ha$^{-1}$) | Net returns (Rs ha$^{-1}$) | B:C ratio | Protein (%) | Starch (%) | Cost of cultivation (Rs ha$^{-1}$) | Gross returns (Rs ha$^{-1}$) | Net returns (Rs ha$^{-1}$) | B:C Ratio |
| **Cultivars** | | | | | | | | | | | | |
| Misthi | 9.92 | 63.68 | 35299 | 192367 | 157068 | 4.45 | 9.33 | 66.38 | 36874 | 193577 | 156702 | 4.25 |
| Sugar ‑75 | 10.41 | 63.61 | 39079 | 165648 | 126569 | 3.24 | 9.83 | 66.35 | 40654 | 154033 | 113379 | 2.79 |
| Gold star | 10.57 | 57.69 | 44119 | 157648 | 113528 | 2.57 | 9.99 | 60.35 | 45694 | 139629 | 93935 | 2.06 |
| CD (p=0.05) | NS | 0.76 | – | – | – | – | NS | 0.60 | – | – | – | – |
| **Planting times** | | | | | | | | | | | | |
| March 29$^{th}$ | 10.22 | 63.43 | 40496 | 181248 | 140751 | 3.48 | 9.63 | 65.78 | 42071 | 203327 | 161255 | 3.83 |
| April 15$^{th}$ | 9.87 | 63.36 | 40181 | 206785 | 166603 | 4.15 | 9.29 | 65.41 | 41756 | 210777 | 169020 | 4.05 |
| April 30$^{th}$ | 10.31 | 63.35 | 39866 | 198720 | 158853 | 3.98 | 9.73 | 65.46 | 41441 | 196253 | 154812 | 3.74 |
| May 15$^{th}$ | 10.36 | 61.30 | 39236 | 171582 | 132345 | 3.37 | 9.78 | 64.02 | 40811 | 159290 | 118478 | 2.90 |
| May 30$^{th}$ | 10.39 | 61.28 | 38921 | 164513 | 125591 | 3.23 | 9.81 | 63.68 | 40811 | 151985 | 111173 | 2.72 |
| June 19$^{th}$ | 10.63 | 57.22 | 38291 | 108480 | 70188 | 1.83 | 10.05 | 61.82 | 39866 | 52846 | 12979 | 0.33 |
| CD (p=0.05) | NS | 0.61 | – | – | – | – | NS | 0.71 | – | – | – | – |

15 May, 30 April, 29 March and 15 April, respectively. Significant effect of planting dates was also observed for per cent starch content in grains at harvest and physiological maturity. Significantly highest starch per cent content was in 29 March sowing being statistically comparable with 15 and 30 April plantings at harvest and physiological maturity, whereas significantly lowest starch content was in the grains both at harvest and physiological maturity in 19 June planting. The 15 and 30 May plantings also recorded statistically similar per cent starch content. Starch content was maximum with 15 April planting date and was significantly lowest for 19 June. This might be due to high starch content with 15 April planting date which acquired longer vegetation period and resulted in more intense carbohydrate accumulation. Also, starch content was less in the grains at harvest maturity because of mutant recessive sugary–1 gene (su–1) that retards the conversion of sugar into starch in the immature grains. These results are in accordance with the findings of Reddy *et al*. (2012).

**Economic returns:** Cultivar 'Misthi' provided higher gross returns followed by 'Sugar –75' and 'Gold star'. At physiological maturity, the highest gross return with net returns and benefit cost ratio was for cultivar 'Misthi' followed by 'Sugar–75'. At harvest maturity both these parameters showed significant variations among the cultivars due to significant difference in number of cobs and fresh stover yields and cost of seed of the sweet corn. Whereas at physiological maturity the significant differences in net returns and benefit cost ratio were due to variation in grain and stover yield. Thus, among the different hybrids 'Misthi' proved more remunerative followed by 'Sugar–75' and 'Gold star'. The highest net returns and benefit cost ratio for 'Misthi' at harvest and physiological maturity was ultimately the reflection of highest cob number, fresh stover yield, grain yield and stover yield of this cultivar. Similar results were also reported by Williams (2008). The highest gross returns, net returns and benefit cost ratio was in 15 April sown crop followed by–30 April, 29 March, 15 May, 30 May and 19 June and 15 April. At physiological maturity the planting date the lowest gross returns with net returns and benefit cost ratio was 19 June whereas highest for 15 April followed by 29 March, 30 April, 15 May and 30 May with gross returns and benefit for per rupee invested in the descending order, respectively. Among the different planting dates it was

economical to invest on 15 April planting date of sweet corn followed by 30 April and Marchat harvest and physiological maturity as compared to15 May, 30 May and 19 June planting dates (Table 2). This variation might be due to higher yields resulting higher net returns achieved from these treatments due to the variation in fresh cob and stover yield, grain and stover yield besides cost of irrigation incurred at different planting dates. Thus, among the six planting dates 15 April planting date is the optimum date of sowing for sweet corn cultivation.

On the basis of the results of present investigation it can safely be concluded that among the three sweet corn cultivars and six planting dates, sweet corn cultivar 'Mishti' when planted on 15th, April proved to be the more viable combination under irrigated sub–tropics of Shiwalik foothills.

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# Hill Reaction Activity and Starch Content in Wheat Seedlings under Heat Stress as Affected by Trehalose Application

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**Abstract**: Hill reaction activity and starch contentwere evaluated under heat stress (35±2˚c and 40±2˚c) and interactive effect of heat stress and trehalose in six wheat (*Triticum aestivum* L.) genotypes (HD 2967, PBW 175, C 306, PBW 343, PBW 621 and PBW 590). Trehalose at concentration of 1 mm and 1.5 mm was applied at 7 days after sowing followed by heat stress of 35˚c (moderate) and 40˚c (severe) on 8 days after sowing for 4 and 8 hours.In six selected wheat genotypes, the Hill reaction activity showed decline with increase in temperature stress. The level of starch content decreased to some extent when heat stress (35˚C and 40˚C) was given for duration of 4 and 8 hours. Severe heat stress (8 hours) more adversely affected these mentioned parameters. Trehalose application ameliorated the adverse effect of heat stress to certain extent. The application of Trehalose @ 1.5 mm concentration was more effective as compared with1mM concentration.

**Keywords**: Heat stress, *Triticum aestivum* L., Trehalose, Hill reaction activity, starch

India, the second largest producer of wheat (*Triticum aestivum* L.) in the world, has made rapid progress in food grain production during second half of the 20th century (Dhillon *et al.,* 2010). Though, Punjab is one of the smallest states of India representing 1.6 per cent of its geographical area and 2.6 percent of cropped area, with a total land area of only 0.33% of the world yet it contributes to 1% of rice and 2% of the wheat in the total world production vis-à-vis 42% rice and 55% wheat production in the country.

Transitory or constantly high temperatures cause an array of morpho-anatomical, physiological and biochemical changes in wheat and other plants, which affect plant growth and development. Heat stress drastically reduces both yield and quality of wheat (Wardlaw *et al.,* 2002; Altenbach *et al.,* 2003; Dupont *et al.,* 2006). Trehalose is a soluble, non-reducing disaccharide of glucose. Three isomers exist: α, α -trehalose, α, β-trehalose and β, β-trehalose. Of these, only α, α-trehalose (1-O- (α -Dglucopyranosyl) α -glucopyranoside) is found in biological material. It is present in a large variety of organisms and can serve as reserve of carbohydrate and as a protectant in response to different stress conditions. Trehalose is known to protect membranes and macromolecules. Its accumulation has been implicated in allowing plants to tolerate stress, including heat-shock. Though, this role has been largely replaced by sucrose, trehalose does protect against desiccation in certain specialized resurrection plants. Therefore, a primary aim of present study is to determine whether trehalose helps to sustain the hill reaction activity and starch content of wheat seedlings when exposed to heat stress conditions.

## MATERIAL AND METHODS

**Plant material:** Six genotypes of wheat (*Triticum aestivum* L.) viz. HD 2967, C306, PBW 621, PBW 590, PBW 343 and PBW 175 were obtained from Department of Plant Breeding and Genetics (PAU) and used for studies pertaining hill reaction activity and starch content under control and different heat stress (35±2˚C and 40±2˚C) conditions. Seeds were surface sterilized with 0.1 per cent mercury chloride for 2-3 min. to avoid any fungal infection during seed germination. Petri dishes were sterilized in oven at 100˚C for 1 hour. Ordinary blotting papers were used in Petri dishes and were autoclaved before use. Twenty seeds were sown in each Petri-dish lined with circular blotting paper and incubated at 25±2˚C temperature. On seventh DAS trehalose (1 mm and 1.5 mm) application was given followed by heat stress by incubating at 35˚C and 40˚C, for 4 and 8 hrs. Controlled Petri-dishes were placed in an incubator in which temperature was maintained at 25˚C.

**Hill reaction activity:** Hill reaction activity was estimated by method as given by Cherry (1973).Leaf samples (100mg) were taken and gently ground in 5ml extraction medium (0.067 m Phosphate buffer, pH 7.5 containing 0.35 m sucrose). During extraction the temperature was maintained at 0-4˚C.Potassium ferricyanide solution was prepared by dissolving sodium chloride (1.02 g) and potassium ferricyanide (13 mg) in phosphate buffer (25 ml). The reaction was started by mixing 0.5 ml of supernatant from above

extract with 2.5 ml of ferricyanide solution. The tubes were kept in light (approx.5000 lux) for 10 min. and another similar set of experiment was kept in dark. The reaction was stopped by adding 20% TCA (0.3 ml). The absorbance was recorded at 420 nm in spectrophotometer. Hill reaction activity was expressed as decrease in absorbance $mg^{-1} Chl\, h^{-1}$.

**Starch:** Starch was estimated by method as given by McCready *et al.* (1958). Fresh tissue sample was homogenized within 80 per cent ethanol. Centrifuged and retained the residue, washed with 80 per cent ethanol 4–5 times to remove all traces of soluble sugars. To confirm it tested last wash for sugars with anthrone reagent. When result was negative, added 5ml of DW and 6.5 ml of 52 per cent perchloric acid. Kept at 0˚C for 20 min, centrifuged and retained the extract. Repeated same step 3–4 times and diluted to the desired volume. Took 0.5 ml of diluted extract in a test tube added 4.5 ml of DW and 10 ml of cold anthrone−sulphuric acid reagent (200 mg of anthrone in 100 ml of cold 95 per cent $H_2SO_4$ stored at 0˚C) in an ice bath. Heated for 8 min at 100˚C, cooled to room temperature and read absorbance at 630 nm.

### RESULTS AND DISCUSSION

**Hill reaction activity in wheat seedlings:** PBW621 followed by C306 had higher hill reaction activity under controlled conditions (Table 1). The application of trehalose increased the hill reaction activity in all selected genotypes. Moderate heat stress of 35˚C for 4 hours significantly reduced the hill reaction in all genotypes. Genotype PBW621 had higher and PBW175 had lower hill reaction activity as compared to other genotypes. Trehalose application significantly improved the hill reaction activity in all presently studied genotypes and trehalose @ 1.5 mm had more promotary effect as compared to 1mM concentration of trehalose. Under severe heat stress of 40˚C for 4 hours all the genotypes further significantly showed the reduction in hill reaction and both trehalose applications significantly increased the hill reaction in all genotypes. Under moderate heat stress for 8 hours all genotypes showed decline in hill reaction as compared to moderate stress of 4 hours. Genotype PBW621 followed by HD2967 had maximum hill reaction activity as these were lesser affected by adverse effect of moderate and severe heat stress for 8 hours. All the genotypes performed better along the application of trehalose both under control as well as under moderate and severe heat stress conditions. Trehalose at the concentration of 1 mm increased hill reaction in all the genotypes, but the concentration of 1.5 mm showed better results to increase in hill reaction activity. It can be concluded that trehalose pre−treatment, probably

**Table 1.** Treatments given to six wheat genotypes during experiment

| Treatments | Temperatures + Trehalose applications |
|---|---|
| T1 | 25˚C normal temperature |
| T2 | 25˚C + Trehalose 1mM |
| T3 | 25˚C + Trehalose 1.5mM |
| T4 | 35˚C (moderate stress) |
| T5 | 35˚C + Trehalose 1mM |
| T6 | 35˚C + Trehalose 1.5mM |
| T7 | 40˚C (Severe stress) |
| T8 | 40˚C + Trehalose 1mM |
| T9 | 40˚C + Trehalose1.5mM |

stabilized the photosynthetic apparatus against heat−induced photo inhibition by accelerating the recovery of the photo system 2 protein complex. (Wang *et al.,* 2010). With an increase in temperature, the degree of the hill reaction inhibition increased and that of its recovery decreased. Zhao *et al.,* 2007 recorded that under drought stress, hill reaction activity was reduced by 26.6 and 36.1 % in leaves of HF 9703 and SN 215953 plants, respectively, suggesting that an injured PS 2 function was involved in the reduction of photosynthesis. The photosynthesis is the least recovering process; it can be irreversibly damaged while other thermolabile functions of the cell can be restored (Sharkova, 2001).

**Starch content of wheat seedlings:** Under control conditions maximum content of starch was recorded in C306 and PBW621 and lesser in PBW590 and PBW175 genotypes (Table 3 and 4). The level of starch content decreased when heat stress (35˚ and 40˚C) was given for duration of 4 and 8 hours. Genotype C 306 and PBW 621 had more starch content even when moderate heat stress was given for 8 hours duration. On imposition of severe heat stress (40˚C for 8 hours) PBW 621 and C 306 genotypes contained higher starch content as compared to other genotypes whereas starch content was lesser in PBW590 and PBW175 under severe heat stress. In all the genotypes, the starch content increased with the application of trehalose. The trehalose @ 1.5 mm had more ameliorative effect as compared with 1mM trehalose. As temperature rises, the duration of starch biosynthesis and deposition is reduced and reductions in starch content account for most of the reduction in grain dry matter at temperatures above 18–22˚C (Spiertz *et al.,* 2006). The activity of invertase reduced with the application of trehalose because the trehalose accumulation promotes sucrose and starch biosynthesis by inhibiting the activity of sucrose degrading enzymes like acid invertase and sucrose synthase (Ahmed *et al.,* 2013).

**Table 2.** Hill reaction (mg chlorophyll$^{-1}$ hr$^{-1}$) in wheat genotypes under moderate (35°C) and severe (40°C) heat stress as affected by trehalose application

| Treatments/Genotypes | HD 2967 | PBW 175 | C306 | PBW 343 | PBW 621 | PBW 590 |
|---|---|---|---|---|---|---|
| **4 hours** | | | | | | |
| T1 | 0.876 | 0.469 | 0.893 | 0.850 | 0.941 | 0.695 |
| T2 | 0.876 | 0.472 | 0.894 | 0.851 | 0.942 | 0.696 |
| T3 | 0.878 | 0.496 | 0.896 | 0.854 | 0.946 | 0.698 |
| T4 | 0.670 | 0.367 | 0.788 | 0.746 | 0.840 | 0.593 |
| T5 | 0.671 | 0.368 | 0.783 | 0.747 | 0.846 | 0.594 |
| T6 | 0.679 | 0.382 | 0.789 | 0.749 | 0.848 | 0.596 |
| T7 | 0.462 | 0.262 | 0.568 | 0.536 | 0.632 | 0.488 |
| T8 | 0.468 | 0.268 | 0.569 | 0.536 | 0.636 | 0.489 |
| T9 | 0.486 | 0.274 | 0.588 | 0.566 | 0.638 | 0.496 |
| CD (p=0.05) | V=0.0.003, T=0.0.004, V×T=0.011 | | | | | |
| **8 hours** | | | | | | |
| T1 | 0.876 | 0.466 | 0.896 | 0.856 | 0.946 | 0.696 |
| T2 | 0.877 | 0.466 | 0.897 | 0.857 | 0.946 | 0.697 |
| T3 | 0.879 | 0.469 | 0.899 | 0.859 | 0.947 | 0.699 |
| T4 | 0.761 | 0.353 | 0.678 | 0.636 | 0.640 | 0.590 |
| T5 | 0.768 | 0.361 | 0.683 | 0.646 | 0.646 | 0.596 |
| T6 | 0.769 | 0.366 | 0.691 | 0.556 | 0.676 | 0.598 |
| T7 | 0.428 | 0.252 | 0.526 | 0.506 | 0.561 | 0.481 |
| T8 | 0.436 | 0.255 | 0.548 | 0.516 | 0.726 | 0.483 |
| T9 | 0.466 | 0.258 | 0.556 | 0.536 | 0.706 | 0.496 |
| CD (p=0.05) | V=0.191, T=0.233, V×T=0.573 | | | | | |

**Table 3.** Starch (mg gm$^{-1}$ fresh weight) content in wheat genotypes under moderate (35°C) and severe (40°C) heat stress as affected by trehalose application

| Treatments/Genotypes | Starch (mg gm$^{-1}$ fresh weight) content | | | | | |
|---|---|---|---|---|---|---|
| | HD 2967 | PBW 175 | C 306 | PBW 343 | PBW 621 | PBW 590 |
| T1 | 1.32 | 1.05 | 1.46 | 1.31 | 1.45 | 1.08 |
| T2 | 1.32 | 1.05 | 1.46 | 1.32 | 1.45 | 1.08 |
| T3 | 1.33 | 1.06 | 1.47 | 1.32 | 1.46 | 1.09 |
| T4 | 1.28 | 1.03 | 1.42 | 1.29 | 1.41 | 1.04 |
| T5 | 1.28 | 1.03 | 1.43 | 1.29 | 1.41 | 1.04 |
| T6 | 1.29 | 1.04 | 1.44 | 1.30 | 1.42 | 1.05 |
| T7 | 1.23 | 1.01 | 1.36 | 1.26 | 1.36 | 1.01 |
| T8 | 1.23 | 1.01 | 1.37 | 1.27 | 1.36 | 1.02 |
| T9 | 1.24 | 1.02 | 1.37 | 1.28 | 1.37 | 1.03 |
| CD (p=0.05) | V=0.0587, T=NS, V×T=0.176 | | | | | |

Present study that concluded, exogenously applied trehalose to wheat seedlings has considerable potential to maintain the hill reaction activity and starch content in all the studied wheat seedlings growing under various heat stress (moderate and severe) conditions.

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**Table 4.** Starch (mg gm$^{-1}$ fresh weight) content in wheat genotypes under moderate (35°C) and severe (40°C) heat stress of 8 hours as affected by trehalose application

| Treatments/Genotypes | Starch (mg gm$^{-1}$ fresh weight) content | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | HD 2967 | PBW 175 | C 306 | PBW 343 | PBW 621 | PBW 590 |
| T1 | 1.31 | 1.05 | 1.43 | 1.30 | 1.42 | 1.08 |
| T2 | 1.32 | 1.06 | 1.44 | 1.31 | 1.44 | 1.08 |
| T3 | 1.33 | 1.07 | 1.45 | 1.32 | 1.46 | 1.09 |
| T4 | 1.24 | 1.01 | 1.40 | 1.23 | 1.41 | 1.03 |
| T5 | 1.25 | 1.02 | 1.41 | 1.24 | 1.42 | 1.04 |
| T6 | 1.26 | 1.02 | 1.42 | 1.25 | 1.43 | 1.05 |
| T7 | 1.20 | 1.00 | 1.32 | 1.21 | 1.35 | 1.00 |
| T8 | 1.22 | 1.01 | 1.32 | 1.23 | 1.36 | 1.01 |
| T9 | 1.23 | 1.01 | 1.33 | 1.26 | 1.37 | 1.02 |
| CD (p=0.05) | V=0.048, T=NS, V×T=0.144 | | | | | |

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# Diet Composition of Barn Owl, *Tyto alba* as Determinant for its Potential as Biocontrol Agent of Rodent Pests in Punjab, India

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**Abstract**: The present study was conducted to record the diet composition of Barn Owl, *Tyto alba* through regurgitated pellet analysis as determinant for its potential as biocontrol agent of rodent pests in Punjab, India. Analysis of 390 regurgitated pellets of Barn Owl collected from six different locations revealed their average weight, length, breadth and thickness to be 4.7 g, 3.9 cm, 2.7 cm and 2.0 cm, respectively. Remains of total 843 prey individuals ,includes 44.1,43.9 and 11.4 per cent rats, mice, and shrews. Diet of Barn Owl consisted only of vertebrates, 99.6 per cent of which were small mammals and 88 per cent were rodents alone. Based on skeletal remains, average number of rats and mice consumed per pellet of Barn Owl were 1.1 and 2.3, respectively with maximum capacity of consuming 4 rats and 9 mice per night. Rodent species consumed by Barn Owl were the lesser bandicoot rat, *Bandicota bengalensis;* Indian gerbil, *Tatera indica;* the soft furred field rat, *Millardia meltada*; Indian bush rat, *Golunda ellioti* and field mouse, *Mus booduga*. Total active rodent burrows were highest at the radius of 1001–2000m from the nesting or roosting sites of Barn Owl with highest number of burrows of *B. bengalensis* at all the three locations. Study suggests the use of Barn Owl in biological control of rodents as one of the components in integrated pest management.

**Keywords**: Barn Owl, Biological control, Diet composition, Regurgitated pellet analysis, Rodents

Rodents are one of the most important vertebrate pests causing economical, environmental, social and health problems (Singla *et al.,* 2016). The use of rodenticides is an effective remedy against overwhelming rodent population (Witmer and Eisemann; 2007, Baldwin *et al.,* 2013). Due to the development of rodenticide resistance and environmental health hazards, there is a need to develop some environment friendly and sustainable methods (Weeden *et al.,* 2006). Biological control using natural predators has been suggested for pest management in recent years. This method can be used as part of an integrated management approach for rodent pest species. Barn Owls (*Tyto alba*) (Order: *Strigiformes,* Family: *Tytonidae)* are cavity nesting birds which have adapted well to trees, redundant buildings and other shelters in rodent rich environments. They are nocturnal hunters and feed almost exclusively on small animals, especially rats, mice, shrews and occasionally bats, birds, reptiles, amphibians and insects (Mushtaq-ul-Hassan *et al.,* 2004; Leonardi and Arte, 2006) and can switch between prey species according to their availability (Tores *et al.,* 2005). The high mobility of these predators permits a quick response to spatially scattered rodent populations (Sekercioglu, 2006). Owls generally swallow their prey whole or in large pieces and the indigestible pieces (hair, bones, exoskeleton etc.) are regurgitated in the form of a pellet. The Barn Owl regularly drops at least one pellet daily from a roosting perch after hunting. The pellets are compact and slow to disintegrate. Diet composition studies of owls can provide the foundation for additional investigations, besides documenting the existence of certain prey species within the owl's range, its capability to take such prey and relative abundance of prey species in the owl's diet. In particular, these studies may be used in the process of evaluating the use of owls as biological control agents.

The use of Barn Owls for the control of rodents has been implemented in many regions of the world (Meyrom *et al*., 2009; Motro, 2011). In India, research on Barn Owls has mainly been conducted in southern parts in relation to their prey composition, feeding behaviour and utilization of man-made nest boxes (Neelanarayanan & Kanakasabai, 2003; Neelanarayanan, 2007). There is no information about their occurrence and diet composition from northern parts of India. The present study is the first of its kind from Punjab state of India (situated in northern part) recording diet composition of Barn Owl, *T. alba* as determined through regurgitated pellet analysis along with some information on rodent pest density in its home range.

## MATERIAL AND METHODS

**Study area**: A survey was carried out of different trees, buildings, electric poles, crop fields, other perches etc. in the campus area of Punjab Agricultural University (PAU), Ludhiana and different villages in districts Ludhiana,

Jalandhar and Fatehgarh Sahib of Punjab (India) to find out the roosting and nesting sites of Barn Owl, *T. alba*. A total of six sites were identified based on the presence of owls and their regurgitated pellets (Table 1). Regurgitated pellets found at all the six sites were collected in polythene bags and brought to the laboratory. Some of the pellets which had disintegrated in the form of powder with bones lying separately on the ground were not collected.

**Pellet analysis:** In the laboratory, regurgitated pellets were kept at 60°C in a hot air oven for 24 hour to kill the associated insects and any other infectious agent. These infection free pellets were then used for analysis. All the pellets were first weighed and then their morphometric measurements i.e. length (cm), breadth (cm) and thickness (cm) were taken. To record the prey composition of the owl, each pellet was first soaked in 8% sodium hydroxide solution for about 2 hour as described by Neelanarayanan *et al.* (1998). This solution assisted in easy separation of the osteous remains (skulls and other bones) from other contents like hair, debris etc. The contents were then sieved to separate all the prey remains from the dust and soil particles. To completely separate the prey remains from these unwanted components, a number of washings were given. Then the prey remains were put on filter paper and dried in oven for 24 hour at 60 °C. After complete drying, based on the structure of different skeltal parts, the prey animals were identified.

**Prey identification**: The identification of different prey items in the diet of Barn Owl was made by using the keys developed by Neelanarayanan *et al.* (1998). Different prey items were identified mainly on the basis of lower jaws. In the absence of lower jaws, other bones like skull, limb bones, pectoral and pelvic girdles and astragalus-calcanium (in case of frog) were useful for identification and quantification of vertebrate prey items. Identification of small mammals was also done using bone sorting chart (Devon, 1989). Different species of rats, mice and shrews consumed by the Barn Owl were identified based on characteristic features of their skull as described by Talmale and Pradhan (2009). Depending upon the number of skulls or lower jaws or fore and hind limb bones found in each pellet, number of particular type of prey individual consumed per pellet was determined. One set of lower jaws (left and right) or one skull or one pair of fore and hind limb bones were counted as remains of one prey item. Based upon the data, percentage of different prey items in the diet of Barn Owl was determined. The mean number of prey items consumed per pellet was also calculated. From typical skull characteristics of different species of rats, mice and shrew, the individual species were also identified later on.

To study rodent fauna around the roosting and nesting sites of Barn Owl and role of Barn Owl in regulating rodent population, three locations i.e. village Mannewal, district Ludhiana; village Nangal Fateh Khan, district Jalandhar and village Naraingarh, district Fatehgarh Sahib were selected. All of these locations were surrounded by crop fields in addition to buildings, houses etc. The crops grown were wheat, rice, bajra, mustard, spinach, potato, peas, carrot, beans, maize, sugarcane etc. and the trees grown were poplar, eucalyptus, dek, peepal, mulberry etc. Burrows of different rodent species were identified based on their characteristic burrow entrances (Singla and Babbar, 2012). Active rodent burrows of all the species within a radius of upto 100, 101–500, 501–1000 and 1001–2000m from the nesting and roosting sites of Barn Owls were counted at each of the above three locations.

## RESULTS AND DISCUSSION

A total of 390 Barn Owl pellets were collected from all the six locations (Table 1). In the new orchard area of PAU campus, the pellets (4) were found only once, so this may not be the permanent roosting site of Barn Owls. From the semi–open store at village Mannewal, the pellets (73) were collected thrice. The store was full of wheat straw prior to

**Table 1.** Different locations selected for study and the pellets collected

| Location | Location | Habitat type | Total pellets | Months of collection |
|---|---|---|---|---|
| I | PAU campus, Ludhiana | New orchard area near tube well | 4 | August, 2014 |
| II | Village Mannewal, District Ludhiana | Semi open store of wheat straw | 73 | January, 2014 (40 pellets), March, 2014 (24 pellets) and December, 2014 (9 pellets) |
| III | Village Ladhowal, District Ludhiana | Indoor seed store of Ladhowal Seed Farm | 21 | March, 2014 |
| IV | Village Ladhowal, District Ludhiana | Crop field area of Ladhowal Seed Farm | 5 | December, 2014 |
| V | Village Naraingarh, District Fatahgarh Sahib | Tress in the vicinity of crop field area of Naraingarh Seed | 12 | First week of January, 2015 (6 pellets) and last week of anuary, 2014 (6 pellets) |
| VI | Village NangalFateh Khan, District Jalandhar | Old deserted building in crop area | 275 | September, 2014 (185 pellets) and February, 2015 (90 pellets) |

March, 2014 when there was seen one pair of Barn Owls. However, in March, 2014, when whole of the wheat straw was lifted by the farmer, though pellets (24) were found but the owls had changed their roosting/nesting site. During August, 2014, when the store was again filled with wheat straw, one Barn Owl was seen with three young ones suggesting April− May to be the breeding season of Barn Owls. The owls were at the same site in the month of December, 2014 also. From the indoor store of Ladhowal Seed Farm of PAU (village Ladhowal), pellets (21) could be collected only once in the month of March, 2014 when there was seen a pair of Barn Owls inside the store. After March, 2014, the owls might have shifted their roosting site. Since the semi open store at village Mannewal and indoor store at Ladhowal Seed Farm were not much far away from each other (at a distance of 2–3 km), it can be speculated that the pair of Barn Owls seen prior to March, 2014 in the semi open store at village Mannewal might had temporarily shifted to indoor store at Ladhowal Seed Farm due to removal of wheat straw and later on the same pair again shifted back to the store at village Mannewal when it was full of wheat straw. The owls were seen going out of the indoor store through broken panes of ventilators when disturbed.

In the crop field area of Ladhowal Seed Farm also pellets (5) were found only once in the month of December, 2014. Perching/roosting sites of Barn Owls were found in the adjacent dense forest area. Owls visited the crop fields only temporarily. Large number of rodent burrows was also seen in these crop fields. At Naraingarh Seed Farm of PAU (village Naraingarh), there was seen a pair of Barn Owls having their roosting sites on the trees near the crop field area. Owls were seen in the crop fields at day time and pellets (12) were also in the crop fields. In the deserted building at village Nangal Fateh Khan, there was found a pair of Barn Owls with one young one. The building was situated in between the crop field area. The large number of pellets (185) collected in September, 2014 were found piled up in the form of a heap as the building was not visited by any one for long before that. Further, in a period of five months from September, 2014 to February, 2015, a total of 90 pellets were collected.

The pellets were compact and brownish black in color (Fig. 1). The size of pellets varied from small to large depending upon the kind and number of prey consumed. The average weight of a pellet was 4.9g. The morphometric measurements of all the pellets collected revealed average length 3.9cm, average breadth 2.7cm and average thickness 2.0cm. All the pellets collected from different locations were found to contain skeltal remains. Remains of total 843 prey items were in 390 pellets of Barn Owl. These were of 372 rats (44.1%), 370 mice (43.9%), 96 shrews (11.4%), 2 bats (0.2%), 1 bird (0.1%) and 2 frogs (0.2%). In total, rat bones were in 313 pellets, mouse bones in 150 pellets followed by shrew (79), bat (2), bird (1) and frog bones (1). Per pellet analysis of prey composition revealed on average, remains of 1.2 rats, 2.5 mice, 1.2 shrews, 1 each of bat, bird and frog per pellet of the Barn Owl. The maximum remains of rats and mice per pellet of Barn Owl were found to be 4 and 9, respectively indicating the capacity of the Barn Owl in consuming these prey types per night (Table 2).

Different bones found were that of skull, lower jaws, ear capsules and other dislocated bones of skull, breast bones, ribs, shoulder bones, bones of fore limbs, hip bones, bones of



**Fig. 1.** Different sized pellets of Barn Owl collected from different locations

**Table 2.** Number of prey items per pellet of Barn Owl at all the six locations

| Location | Mean number of prey items per pellet (Range) | | | | | |
|---|---|---|---|---|---|---|
| | Rat | Mouse | Shrew | Bat | Bird | Frog |
| I | 1.2 (1–2) | – | – | – | – | – |
| II | 1.0 (0–1) | 2.4 (0–6) | 1.1 (0–2) | – | – | 1.0 (0–1) |
| III | 1.1(0–2) | 1.2 (0–2) | 1.0 (0–1) | 1.0 (0–1) | – | |
| IV | 1.2 (0–2) | 1.7 (0–3) | 1.0(0–1) | – | – | – |
| V | 1.0 (0–1) | 3.7 (0–6) | 1.0 (0–1) | – | – | |
| VI | 1.2 (0–4) | 2.5 (0–9) | 1.3 (0–2) | 1.0 (0–1) | 1.0 (0–1) | 1.0 (0–1) |

hind limbs, hand/foot bones, patella, vertebrae and some unidentified bones (Fig. 2). Only humerus, radius and femur of bat; humerus and radius of bird and humerus and tibia−fibula of frog were found in the pellets of Barn Owl. From typical skull characteristics of different species of rats, mice and shrews (Figs. 3 & 4), the individual species consumed by the Barn Owls were identified to be the lesser bandicoot rat, *Bandicota bengalensis;* Indian gerbil, *Tatera indica;* the soft furred field rat, *Millardia meltada*; Indian bush rat, *Golunda ellioti* and field mouse, *Mus booduga*. Only one species of shrew (Asian house shrew, *Suncus murinus*) was found consumed by the Barn Owl. The species of bat, bird and frog found in the pellets could not, however, be identified.

Rodent fauna as identified based on characteristic burrow entrances around the nesting or roosting sites of Barn Owls at three locations consist of *B. bengalensis*, *T. indica, M. meltada* and *M. booduga*. The burrows of *G. ellioti* could not be located as it digs small shallow burrows on the ground and are usually covered with nests. Total mean number of active burrows at three selected locations was 16.1 with maximum of 18.3 in the radius of upto100 m, 24.1 with maximum of 32.7 in radius of 100−500 m, 28.7 with maximum of 46.0 in radius of 500−1000m and 48.7 with maximum of 79.7 in radius of 1000−2000 m. Though the average burrow count at all the three locations was more in the radius of 1000m−2000m around the nesting or roosting site but the difference in burrow count among the four radii was statistically non−significant (Table 3). The results thus
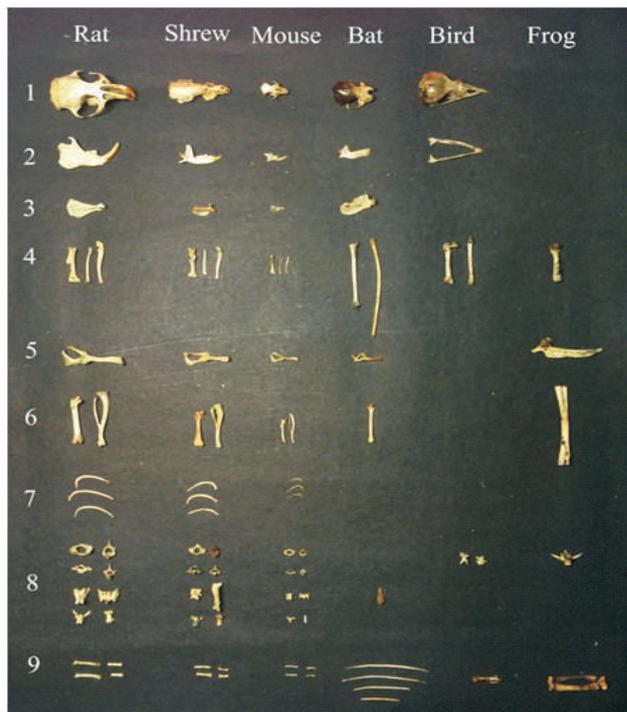


1−Skull, 2−Lower jaw, 3−Shoulder blade, 4−Fore limbs: Humerus, Radius, Ulna, 5−Hip bone, 6−Hind limbs: Femur, Tibia−Fibula, 7−Ribs, 8−Vertebrae: Atlas, Axis, Cervical, Thoracic, Lumbar, Sacral and Caudal (Rat, Shrew and Mouse), Sacrum (Bat), Typical cervical, Free thoracic (Bird), Typical (Frog), 9−Hand/Foot bones (Rat, Shrew, Mouse), Hand bones (Bat and Bird), Foot bones (Frog)

**Fig. 2.** The bones of different prey items found in pellets of Barn Owl

**Table 3.** Rodent density near the nesting or roosting sites of Barn Owls

| Location | Rodent species | Average no. of active rodent burrows at different radii from the nesting or roosting sites of Barn Owls | | | |
|---|---|---|---|---|---|
| | | Up to 100 m | 101−500 m | 501−1000 m | 1001−2000m |
| Village Mannewal | *Bandicota bengalensis* | 11.7±3.5 | 22.3±4.0 | 31.0±2.4 | 50.0±14.4 |
| | *Tatera indica* | 0 | 0 | 1.3±1.1 | 2.3±1.9 |
| | *Millardia meltada* | 2.0±1.2 | 2.7±1.1 | 6.0±1.2 | 12.3±5.2 |
| | *Mus* spp. | 4.7±2.4 | 7.7±2.0 | 7.7±3.3 | 15.0±1.7 |
| | Total | 18.3±6.4 | 32.7±6.0 | 46.0±9.8 | 79.7±18.2 |
| Village Nagal Fateh Khan | *Bandicota bengalensis* | 7.5±1.1 | 6. 5±1.1 | 7.5±1.8 | 6.5±0.3 |
| | *Tatera indica* | 1.5±1.1 | 1.0±0.7 | 4.0±0.0 | 5.5±1.8 |
| | *Millardia meltada* | 1.5±1.1 | 3.0 ±0.7 | 2.0±1.4 | 4.0±2.1 |
| | *Mus* spp. | 2.0±1.4 | 2.5±0.3 | 3.5±1.1 | 7.5±3.2 |
| | Total | 12.5±4.6 | 13.0±1.4 | 17.0±4.2 | 23.5±7.4 |
| Village Narayangarh | *Bandicota bengalensis* | 7.5±0.3 | 12.5±0.3 | 13.0±2.8 | 19.0±2.1 |
| | *Tatera indica* | 0 | 2.5±1.8 | 2.0±1.4 | 4.0±2.8 |
| | *Millardia meltada* | 0 | 0 | 0 | 3.5±2.5 |
| | *Mus* spp. | 10.0±0.7 | 11.5±4.6 | 8.0±0.7 | 16.5±9.5 |
| | Total | 17.5±1.1 | 26.5± 6.7 | 23.0±4.9 | 43.0±17.0 |
| | Total Mean | 16.1±1.5 | 24.1±4.7 | 28.7±7.2 | 48.7±13.4 |

Values are mean±SE
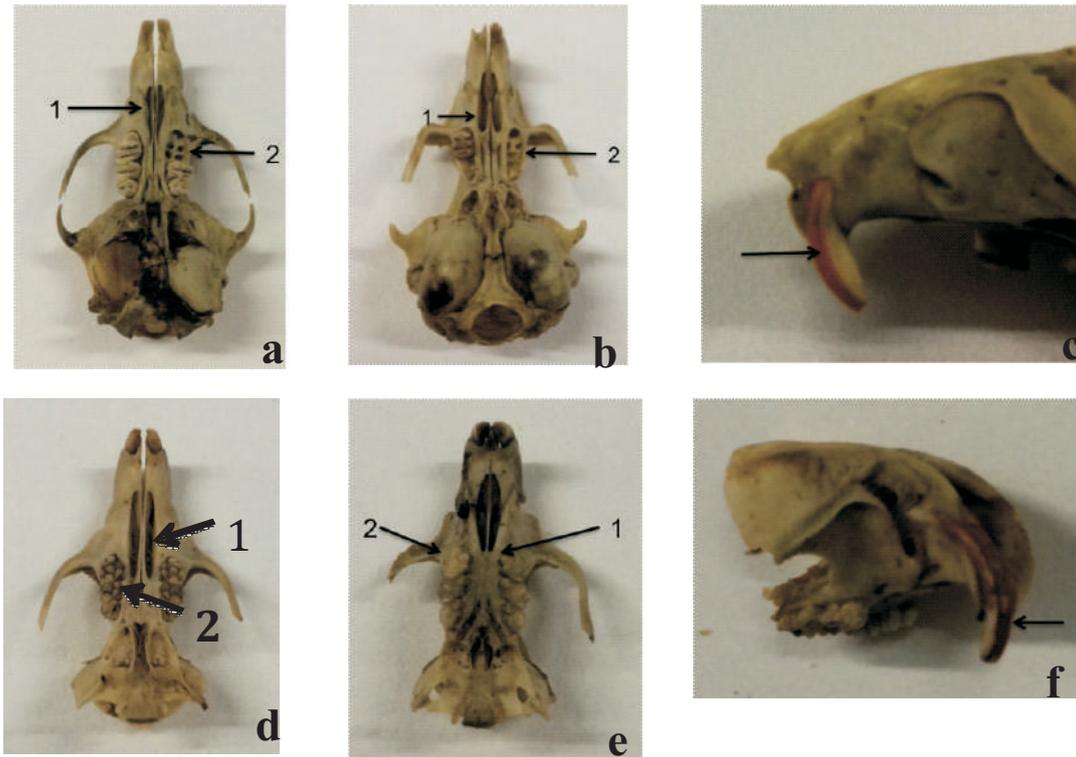
**Fig. 3.** Skull material of rats and gerbils found in Barn Owl pellets. 3a). Ventral view of skull of *lesser bandicoot rat, Bandicota bengalensis*, arrow (1) shows anterior palatal foramina long, narrower at posterior end and extending posteriorly upto first lamina of first upper molar tooth and arrow (2) shows position of first upper molar tooth. 3b). Ventral view of skull of *Indian gerbil, Tatera indica,* arrow (1) shows anterior palatal foramina extending posteriorly upto the molars and arrow (2) shows upper molar root sockets, 3c). Lateral view of skull of *T. indica,* arrow shows grooved incisors. 3d). Ventral view of skull of soft furred field rat, *Millardia meltada, a*rrow (1) shows anterior palatal foramina long, extending between maxillary tooth rows and arrow (2) shows upper second molar with t3 cusp. 3e). Ventral view of skull of *Indian bush rat, Golunda ellioti*, arrow (1) shows anterior palatal foramina just touching the first molars and arrow (2) shows heavily cuspidate (Grape-shaped) molar teeth, 3f). Lateral view of skull of *G. ellioti*, arrow shows grooved incisors

indicate that Barn Owls can predate upon rodent pests up to the radius of minimum 1000m from the nesting or roosting site.

Including predation in ecologically based rodent management actions might be a valuable strategy to achieve long term rodent pest population suppression. The high mobility of avian predators permits a quick response to spatially scattered rodent populations (Sekercioglu, 2006). In the present study, rats were found to be the major contributors of Barn Owls' diet followed by mice and shrews. Bats among small mammals, birds, and frogs among amphibians constituted the minor components of the diet. Investigations on the diet of the Barn Owl in different geographical regions of the world have also found small mammals (such as rats, mice and shrews) to be the main components of the diet of Barn Owl (Mushtaq-ul-Hassan *et al.,* 2004; Leonardi and Arte, 2006; Mahmood-ul-Hassan *et al.,* 2007; Nadeem *et al.,* 2012). Smal (1990) reported 99.4% of the diet of the Barn Owls consisting almost exclusively of

rats. The data on number of prey items per pellet obtained in present study corroborates the findings of others (Neelanarayanan, 2007; Nadeem *et al.,* 2012; Patki *et al.,*2014). The remains of rodent species found in the pellets of Barn Owl during present study are the ones which form major rodent pest complex of agriculture in Punjab state (Singla and Babbar, 2010). *S. murinus* is also found in large numbers in crop fields. During present study also, the burrows of all of these rodent species were found near the nesting and roosting sites of Barn Owl. The presence of these preys in the diet of Barn Owls indicates that they have the potential to control these rodent pests in crop fields.

The size of the pellets depends upon the composition of the diet and the size as well as the nutritive value of the prey taken. During present study there was found a great variation in the size of the regurgitated pellets of Barn Owl. The morphometric measurements of pellets collected during present study were almost similar to those reported by
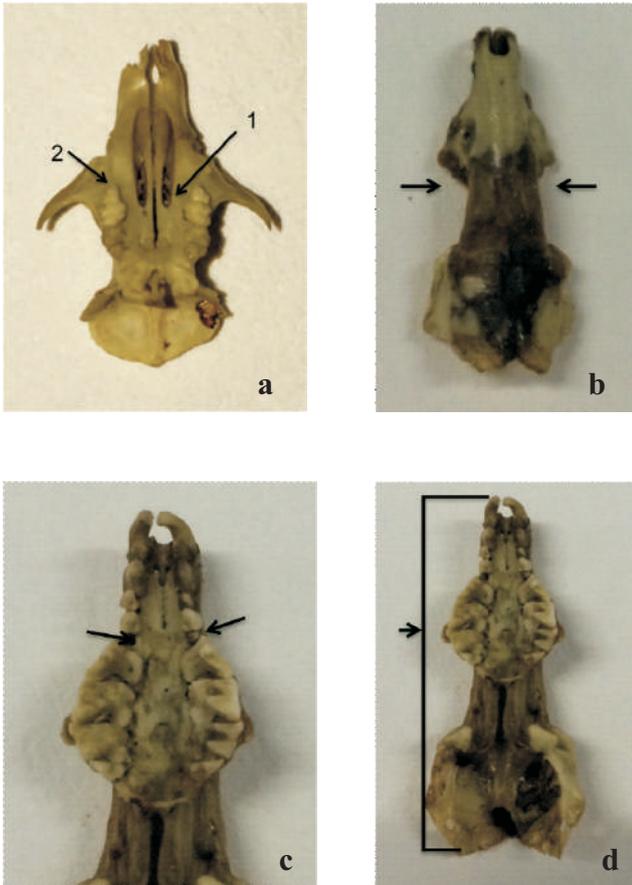
**Fig. 4.** Skull material of mouse and shrew found in Barn Owl pellets. 4a). Ventral view of skull of field mouse, *Mus booduga*, arrow (1) anterior palatal foramin along, extending posteriorly between maxillary tooth rows and arrow (2) anterior accessory cusp on first upper molar tooth. 4b). Dorsal view of skull of Asian house shrew, *Suncus murinus,* arrow shows absence of zygoma, 4c). Ventral view of skull of *S. murinus*, arrow shows presence of minute fourth upper unicuspid premolar tooth, and 4d).Ventral view of skull of *S. murinus*, showing condylobasal length

Alvarez-Castaneda *et al.* (2004) and Nadeem *et al.* (2012). Research on impact of avian predation on rodent pest populations in agriculture is limited (Singleton and Petch, 1994). Without investigating the relationship between avian predator density and prey rodent pest density, it would be difficult to make assertive conclusions as to the actual impact of avian predators on rodent populations. During present study rodent density in the form of rodent burrows was estimated in the area surrounding the nesting sites of Barn Owls. Results revealed highest total rodent density in the area situated at a radius of 1001-2000 m from the nesting or roosting site. The number of active burrows of *B. bengalensis* were highest than other rodent pest species at all the three locations.

Attracting avian predators can be useful in the biological control of rodent pests (Paz *et al.,* 2013). In agricultural areas with rodent pest problems, Barn Owl numbers can be increased by providing suitable nest boxes, which they can occupy and use for breeding. Once in place, the owls may hugely increase the levels of predation pressure on the nocturnal rodent population, and reduce the rodent pest factor in the area by consuming large numbers of rats and mice. However, it is crucial to determine avian predator population sizes prior to erection of nest boxes at a particular site. Also there is a need of continuous monitoring after the initial occupancy of nest boxes as the avian predators may disperse when prey species are unable to maintain their densities. Continued and long term investigation is thus needed to determine stable predator populations and pest population sizes (Wood and Fee, 2003).

## CONCLUSION

The potential of Barn Owls as biocontrol agents of rodent pests can be included as one of the components in integrated rodent pest management. The Barn Owl population is, however, scanty in Punjab state may be due to habitat destruction. Steps should be taken not only to protect and conserve Barn Owls, but also to increase their population by way of installing artificial nest boxes and T-shaped perching poles so as to attract them to the crop fields.

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# Prevalence of Papaya Mealy Bug, *Paracoccus marginatus* Williams and Granara de Willink in Consistency with Natal and Non-Natal Host Plants

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**Abstract**: The investigation was aimed at observing the battle between, *P. marginatus* and its natal (papaya) and non‑natal host plants (cotton, tapioca, mulberry, brinjal and hibiscus) *vis-a vis* major nutrients *viz.*, Nitrogen (N), Phosphorous (P) and Potassium (K) and antioxidant enzymes *viz.*, peroxidase, catalase, polyphenol oxidase (PPO) and phenylalanine lyase (PAL) content. The parameters were studied in both healthy and infested host plant leaves. The quantity of major nutrients (N,P, K) was drastically reduced and that of the antioxidant enzymes were enhanced due to *P. marginatus* infestation. The variation in host plants modulation of increase and reduction in antioxidants and major nutrients due to infestation showed the '*arms race*' between pest and host plants.

**Keywords**: Papaya mealy bug, *Paracoccus marginatus*, Nutrients, Antioxidant enzymes, Arms race

Papaya mealybug (PMB), *Paracoccus marginatus* Williams and Granara de Willink is a serious polyphagous pest infesting several host plants. The intimate reaction between a pest species and its host species may result in specific adaptations towards each other. Survival of the pest depends on the suitability of the host, while survival of the host is warranted by developing unsuitability for attack. Such an adaptive interaction between phytophagous insects and the plant hosts can be characterized as "arms race" (Mello and Silva‑Filho, 2002). Thus, due to the pest attack, nutritive value and the resistance enzymes present in host plants will be changed to compensate their loss, as tolerance or resistance mechanism. The nutritive value of a host plant for insects feeding on them appears to play an important role in determining the susceptibility of the plant to insect attack (Goggin *et al.*, 2010). Reports indicate that plants have different mechanisms of resistance as several enzymes respond differently to aphid *Rhopalosiphum padi* infestation (Chaman *et al.*, 2001; Ni *et al.,* 2001). Oxidative enzymes, such as polyphenol oxidase, lipoxygenase, and peroxidase are known to be positively correlated with induced defenses to herbivory and pathogen attack (Thaler *et al.*, 2001). Hence, the present investigation‑depicts the 'arms race' between the host plants and the phytophagous insects *vis-a vis* P. *marginatus* with natal and non‑natal host plants by studying the major nutrients and anti‑oxidant enzymes.

## MATERIAL AND METHODS

The research work was carried out at TamilNadu Agricultural University, Coimbatore India. The different host plants viz., papaya, tapioca, mulberry, brinjal, cotton and hibiscus were pot cultured. The *uniform stage of both healthy and mealybug infested leaves were taken for the nutrient analysis.*

**Estimation of nutrients:** Total nitrogen was estimated using the Foss‑Analyser Digestion. One gram of the leaf samples from both healthy and mealybug infested host plants was weighed and the total nitrogen was estimated as per the method of Kjeldahl (1883). Total phosphorus was estimated using the method by Emmert (1930). The intensity was measured at 410 nm in spectrophotometer and potassium was estimated using Kuroyanagi *et al*. (1964) method. Concentration of potassium in the solution was measured using flame photometer.

**Assay of enzymes:** The selected host plants mentioned earlier with respect to resistance were used for the assay of enzymes and studies. The leaf samples were drawn from each healthy and mealybug infested host plants of the same age and analysed for antioxidant enzymes. Leaf samples were weighed and analysed for the catalase activity by the method followed by Chanceand Oshino (1973) and peroxidase (po) by Hammerschmidt *et al*. (1982). The polyphenol oxidase (PPO) was assayed using the modified method of Mayer *et al*. (1965) in both healthy and mealybug infested host plant leaves. Phenylalanine ammonia lyase (PAL) was estimated using the method given by Valifard *et al*. (2015).

## RESULTS AND DISCUSSION

**Nutrient contents in the leaves of host plants of *Paracoccus marginatus*:** The nutrient contents were more in the healthy leaves than in mealybug infested leaves (Table 1). The nitrogen content varied in the host plants from 1.10 to 1.53 % in the healthy leaves and 0.97 to 1.09 % in the mealybug infested leaves. The highest percentage of nitrogen was on papaya followed by cotton in healthy leaves. In all the host plants, there was a significant reduction in the nitrogen content due to mealybug injury. Among the infested leaves, the maximum reduction of nitrogen was in papaya (28.76 %) and minimum in Hibiscus (10.57 %). Among the healthy plants, tapioca (0.62 %) recorded the highest amount of phosphorous and it was followed by Hibiscus. The mealybug infested plants showed a significant reduction in the potassium content being significantly higher in healthy host leaves as compared to the mealybug infested host leaves. The per cent reduction was the lowest in tapioca (8.68 %) and highest in cotton (27.56 %). The outbreak of mealy bug has been recorded on more than 154 plant species, however, the level of infestation remained variable among different plant species. This may be due to the difference in the quality and quantity of macro and micronutrients, volatile compounds, secondary metabolites, phenology, tissue hardness and defense mechanisms (Shahid *et al*., 2013). Host plant nutrients not only affect the growth and development of plant species but also alter the quality of the food source of herbivorous insect pest (Goncalves-Alvim *et al*., 2004).

It is obvious from the data contained in the table that the PO, CAT, PPO and PAL enzyme activities showed a general trend of increase in the infested leaves. Among the host plants tested, tapioca recorded the highest and papaya recorded the lowest PO (0.89 and 0.36), CAT (21.79 and 10.02) PPO (0.75 and 0.47) and PAL (3.26 and 2.73) activity. The mean enzymatic activity of PO, CAT, PPO and PAL in infested tapioca and papaya leaves was 2.07 and 0.45, 21.79 and 12.68, 2.01 and 0.71 and 4.43 and 3.09, respectively in

their activity. The enzyme activity was significantly higher in the mealybug infested plants than healthy plants. The per cent activity was ranged from 20.00 to 57.00 in peroxidase, 33.80 to 62.69 for PPO, 20.98 to 37.59 for CAT and 11.65 to 26.41 for PAL in the current study.

In the present study, the activity of PPO and peroxidase was more or less same in the host plants. The plants with low infestation of mealybugs exhibited greater activities of PPO in their infested leaves than the uninfested leaves. The results are in line with Felton *et al*. (1994) who concluded that herbivory by phloem-feeding three cornered alfalfa hoppers caused increases in the activity of several oxidative enzymes including, lipoxygenases, peroxidase, ascorbate oxidase and polyphenol oxidase on soybean plants.

The present study revealed that, the host plants showed the varying level of increase in enzyme activity. This was supported by Ni *et al*. (2001), who found *Diuarphis noxia* (Mordvilko) feeding elicited greater increase of peroxidase specific activity on resistant Halt Wheat, while the feeding of same insect on barley caused nine folds, increase of peroixidase in the resistant 'Morex' barley. Furthermore, *D. noxia* feeding did not elicit any differential changes in catalase and polyphenol oxidase activity.

Highest peroxidase activity might affect the biology and development of the pest on the host plant and leads to resistance of the plants. A negative effect of peroxidase activity on insect growth was demonstrated by Duffey and Felton (1991). The glandular trichomes of plants contain polyphenol oxidase and has higher peroxidase activity which contributes to oxidative reaction and results in polymerization of phenolic constituents, which in turn act as a physical barrier preventing from feeding and immobilization of insects (Traw and Dawson, 2002). The reasons discussed with above literatures might be the cause for increase of PO activity after infestation by mealybug in the current study. Spence *et al*. (2007) found that the incidence of *T. urticae* and flower thrips, *Frankliniella occidentalis (Pergande)* on cotton increased the activity of peroxidase, but the peroxidase

**Table 1.** Major nutrient contents in theleaves of host plants of *Paracoccus marginatus*

| Host plants | Nitrogen (%) | | | Phosphorous (%) | | | Potassium (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Healthy | Infested | Reduction (%) | Healthy | Infested | Reduction (%) | Healthy | Infested | Reduction (%) |
| Papaya | 1.53[a] | 1.09[b] | 28.76[a] | 0.32[f] | 0.21[f] | 34.38[a] | 3.28[a] | 2.54[ab] | 22.56[b] |
| Cotton | 1.46[b] | 1.14[a] | 21.92[b] | 0.36[e] | 0.27[e] | 25.00[b] | 3.12[b] | 2.26[e] | 27.56[a] |
| Tapioca | 1.10[e] | 0.97[c] | 11.82[e] | 0.62[a] | 0.59[a] | 4.84[f] | 2.65[e] | 2.42[cd] | 8.68[f] |
| Mulberry | 1.30[c] | 1.09[b] | 16.15[c] | 0.43[d] | 0.35[d] | 18.60[c] | 3.04[bc] | 2.50b[c] | 17.76[c] |
| Brinjal | 1.28[c] | 1.12[ab] | 12.50[d] | 0.49[c] | 0.42[c] | 14.29[d] | 2.98[c] | 2.61[a] | 12.42[e] |
| Hibiscus | 1.23[d] | 1.10[b] | 10.57[f] | 0.56[b] | 0.51[b] | 8.93[e] | 2.79[d] | 2.35[d] | 15.77[d] |

Means followed by the same alphabets are not significantly different at 5 % level by DMRT

**Table 2.** Anti-oxidant enzymes activities in the leaves of host plants of *Paracoccus marginatus*

| Host plants | Peroxidase changes in OD value$^{-1}$ min g$^{-1}$ of fresh leaves | | | Polyphenol oxidase changes in OD value$^{-1}$ min g$^{-1}$ of fresh leaves | | | Catalase (µg of H$_2$O$_2$ g$^{-1}$) | | | Phenylalanine ammonia lyase (nmol trans-cinnamic acid$^{-1}$ min g$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Healthy | Infested | Per cent | Healthy | Infested | Per cent | Healthy | Infested | Per cent | Healthy | Infested | Per cent |
| Papaya | 0.36$^f$ | 0.45$^f$ | 20.00$^f$ | 0.47$^e$ | 0.71$^e$ | 33.80$^d$ | 10.02$^e$ | 12.68$^f$ | 20.98$^e$ | 2.73$^e$ | 3.09$^f$ | 11.65$^f$ |
| Cotton | 0.42$^e$ | 0.58$^e$ | 27.59$^e$ | 0.54$^d$ | 0.99$^d$ | 45.45$^c$ | 11.25$^d$ | 15.52$^e$ | 27.51$^d$ | 2.84$^d$ | 3.32$^e$ | 14.46$^e$ |
| Tapioca | 0.89$^a$ | 2.07$^a$ | 57.00$^a$ | 0.75$^a$ | 2.01$^a$ | 62.69$^a$ | 13.60$^a$ | 21.79$^a$ | 37.59$^a$ | 3.26$^a$ | 4.43$^a$ | 26.41$^a$ |
| Mulberry | 0.57$^d$ | 0.99$^d$ | 42.42$^d$ | 0.55$^d$ | 1.02$^d$ | 46.08$^c$ | 11.75$^c$ | 17.17$^d$ | 31.57$^c$ | 2.98$^c$ | 3.58$^d$ | 16.76$^d$ |
| Brinjal | 0.71$^c$ | 1.33$^c$ | 46.62$^c$ | 0.6$^c$ | 1.39$^c$ | 56.83$^b$ | 12.84$^b$ | 20.04$^c$ | 35.93$^b$ | 3.08$^{bc}$ | 3.79$^c$ | 18.73$^c$ |
| Hibiscus | 0.78$^b$ | 1.65$^b$ | 52.73$^b$ | 0.68$^b$ | 1.81$^b$ | 62.43$^a$ | 13.23$^{ab}$ | 20.96$^b$ | 36.88$^{ab}$ | 3.12$^b$ | 4.15$^b$ | 24.82$^b$ |

***Note:*** Means followed by the same alphabets are not significantly different at 5 % level by DMRT

activity of mite damaged plants was higher than that of thrips damaged plants.

The association of PPO activity with host plant resistance to insects has been reported in many plants including tomato, potato, coffee and poplar (Duffey and Felton, 1991; Wang and Constabel, 2004). Polyphenols function as phenol oxidase in higher plants and the PPO oxidized compounds have been associated with antifeedant and antioxidant property. The enhanced activities of polyphenol oxidase and polyphenol peroxidase enzymes may increase the scavenging capacity for free oxygen species. The antifeedant and antioxidant property might be the reason for tapioca and hibiscus having low preference to papaya mealybug.

The higher amounts of total phenols and ortho-dihydroxy phenols in the host plants were accompanied by increased activities of PPO and peroxidases, resulting in more oxidation of phenolic substances to form more toxic quinines and other oxidative products and these oxidative products might be the key to combat the mealybugs in plants with low infestation (tapioca and hibiscus) and marginal infestation (mulberry and brinjal). On the other hand, lower amount of phenols and lower enzyme activities in plants with higher infestation failed to produce toxic quinines and other oxidative product to that extent as found in low and marginally infested hosts that quoted earlier.

The catalase activity in the current study was higher in all the host plants when compared to other enzymes. The results have conflict with report of Janaki (2010), who found the reduction in CAT activity in brinjal leaves when infested with *P.marginatus.* This was supported by Heng-Moss *et al.* (2004) who reported a loss in CAT activity in response to feeding by *B. occiduus* on susceptible buffalograss varieties. Zhu-Salzman *et al.* (2004) speculated that the reduced CAT activity could help the plant to maintain high H$_2$O$_2$ levels thus causing damage to the insect midgut.

In present study the activation of PAL increased after mealybug infestation. Murugan (2003), noticed increase in PAL activity after infestation of *Liriomyza trifoli* (Burgess) and *Bemisia tabaci* in *Pseudomonas fluorescens* treated plants. Activation of PAL and subsequent increase in phenol contents were general responses associated with resistance mechanism in plants (Ghosal *et al.,* 2004). The final product of such enzymatic activities would be anti-nutritive because they cannot be efficiently digested and assimilated by insects (Constabel and Ryan, 1998). Tapioca had the highest level of PAL activity among the host plants and PAL was higher in the mealybug infested plants compared to uninfested plants. Phenyl alanine ammonia lyase is the key enzyme of phenyl propanoid metabolism in higher plants, which catalyzes the conversion of phenyl alanine to trans-cinnamic acid, which supplies the precursors for flavanoids, lignin and phytoalexins (Hahlbrock and Scheel, 1989).

These reports were strongly supported by findings that, PAL plays an important role in the biosynthesis of various defense chemicals in phenyl propanoid metabolism (Daayf *et al.,* 1997). PAL activity could be induced during plant-pathogen and plant-pest interactions (Harish, 2005). The timing and expression patterns of the defense mechanisms are important for the reduced attack and expression of pest and pathogen respectively. Higher level expression of defense related proteins and timely accumulation of chemicals at the infection and feeding site certainly prevents the entry of pathogen and reduces the attack by insect in rice seedlings treated with mixture *Pseudomonas* and *Beauveria* bio-formulations (Karthiba, 2008). From the current study, it was significantly confirmed that the nutritive value of the host plants played an important role in determining the susceptibility and tolerability of plants to pest attack *vis-à-vis* 'arms race'.

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# Distribution and Prevalence of Bacterial Blight of Pomegranate in Southern Rajasthan

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**Abstract:** Field survey was carried out in the four major pomegranate growing districts of Southern Rajasthan during *Kharif* 2013 and 2014 to study the prevalence of pomegranate bacterial blight. This disease was very severe in Udaipur district, but least in Bundi and Chittorgarh. Ten different isolates of bacterial pathogen were obtained from the highly infected plant materials collected from different locations during the survey programme. Initially the pathogenic isolates were identified as *Xanthomonas axonopodis* pv. *punicae* and later on, their identity was confirmed by pathogenicity test. Five different inoculation methods were adapted to proof their pathogenicity. Among them, the carborundum abrasion method was superior at different incubation period. On the basis of pathogenicity test, isolates were categorized into highly, moderately and less aggressive nature. Highly aggressive isolates were mostly isolated form heavy disease prone locations of Udaipur district. This will help to correlate the disease prevalence of pomegranate bacterial blight with the distribution of pathogenic isolates in different pomegranate ecosystem.

**Keywords**: Aggressiveness, Bacterial blight, Disease incidence, Pomegranate, and *Xanthomonas*

India is the largest pomegranate producing country in the world followed by Iran and it is commercially cultivated in Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu. From last decade, pomegranate cultivation becomes very popular to the poor farmers of Rajasthan, especially in the districts like Udaipur, Bhilwara, Chittorgarh, Bundi, Jalore, Jodhpur, Jhalawar and Barmer (Anonymous, 2014). But with the introduction of crop and intensification in cropping system, pomegranate is facing many biotic and abiotic stresses which are responsible for significant yield loss. Among, many pests and diseases, bacterial blight caused by *Xanthomonas axonopodis* pv. *punicae*, is a major constraint in most of pomegranate producing states of India and causes around 30‒50 per cent losses (Raghuwanshi *et al.,* 2013). Sometimes it may extend up to 60‒80 per cent in unmanaged orchards under favourable environmental and epidemic condition (Chand and Kishun, 1991; Mondal and Singh, 2008). The disease appeared in epiphytotic form in pomegranate growing states of South‒West India including Maharashtra (Dhandar *et al.,* 2004), Karnataka and Andhra Pradesh (Benagi and Ravikumar, 2011; Subramanyam, 2011) and also Himachal Pradesh (Khosla *et al.,* 2009). Now this disease is rapidly spreading to the new pomegranate growing areas of Rajasthan, Gujarat, Haryana, and Uttar Pradesh due to bulk movement of planting materials of most popular and bacterial blight susceptible cultivar, Bhagwa (Kesar) from Maharashtra (Mondal *et al.,* 2012). In Rajasthan, this disease was first recorded in Hanumangarh district in 2009 (Anonymous, 2010). Gradually, it becomes an emerging and severe problem in Southern Rajasthan. The present study was carried out to reveal the distribution and prevalence of pomegranate bacterial blight in different pomegranate growing districts of Southern Rajasthan, and to characterise the pathogenic nature of disease inicitant.

## MATERIAL AND METHODS

**Field survey:** Field survey was carried out during *Kharif* 2013 and 2014. Eleven different orchards of major pomegranate growing districts, i.e., Udaipur, Bhilwara, Chittorgarh and Bundi were selected where pomegranate cultivars *viz.,* Bhagwa (Kesar), Ganesh and Mirdula were grown. Infected plants were diagnosed on the basis of typical symptoms of bacterial blight, like yellow water soaked lesions at early stages and corky, dark oily spots at later stages of infection (Table 1). The disease incidence and disease intensity on leaves and fruits were recorded in every orchard. The disease severity was noted based on 0‒5 disease rating scale (Raju *et al*., 2011). Per cent disease index on leaves and fruits (Wheeler, 1969) were calculated*.*

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of individual ratings}}{\text{...}} \times 100$$

**Collection of disease specimen:** The diseased plant parts were collected separately from the individual orchard, labelled and brought to the laboratory for the further

investigation. To confirm the presence of bacterium, ooze test was performed from different plant parts of pomegranate. The severely infected plant parts collected from different location during survey were used for isolation of bacterial pathogen.

**Isolation and purification of pathogen:** The bacterium was isolated from the infected leaves, small twigs and fruits of pomegranate plants collected during survey. These tissues were washed in tap water, exposed to air for drying adherent moisture, and then cut into small pieces with sterilized razor blades and finally disinfected with 0.1% HgCl$_2$ solution for about 1–1½ minute followed by washing thrice with sterilized water to remove traces of HgCl$_2$. They were macerated with sterilized blade in a sterilized glass slide containing few drops of sterilized distilled water in order to allow the bacterium to diffuse out. A loop-full of suspension was then streaked on nutrient agar medium with the help of sterilized bacteriological needle under aseptic condition and incubated at 28±2°C for 24–72 hrs. After 2–3 days, incubated plates were observed for the presence of typical pale yellow, glistening colonies which were transferred to the nutrient agar medium with sucrose slants and cultures were maintained on YDCA medium containing slant for further studies. In this way, ten different isolates, designated as Xap–01 to Xap–10 were obtained as the representative isolate for each location.

**Pathogenicity test:** To prove pathogenicity of ten isolate, each isolate was inoculated on healthy pomegranate seedlings cv. Bhagwa (Kesar) grown in pot. Five different inoculation methods *viz*., (i) spray inoculation by hand automizer, (ii) smear inoculation, (iii) carborundum abrasion method,(iv)multi-needle pricking method, and (v) injection infiltration were used. Adequate moisture was filled in order to maintain humidity. The plants were kept in the humid chamber for about 2 to 3 days before inoculation. Inoculum was prepared by gentle scrapping of 48 hrs old bacterial culture growth which was developed on YDCA medium. The culture was harvested in 10 ml sterilized distilled water and further diluted by serial dilution method to a concentration of 1 x 10$^8$ c.f.u. ml$^{-1}$ and used immediately. The plants were kept for incubation in moist chamber. A set of control was maintained for each set which were sprayed with distilled water only. Warm and humid conditions were maintained in the chamber by spraying sterilized water daily in the morning and at evening time. The bacterium was reisolated on nutrient agar medium from artificially inoculated leaves of pomegranate plants showing typical symptoms of blight disease and compared with the mother culture of the test bacterium.

## RESULTS AND DISCUSSION

During the survey in *Kharif* 2013 and 2014, the disease severity pattern was similar and maximum disease was recorded at Horticulture farm, RCA followed by Kankarwa, and Mainar of Udaipur district on leaves and fruits, whereas, the minimum disease was recorded at KVK, Bundi followed by KVK, Chittorgarh on leaves and fruits in the both years (Table 2). During *Kharif* 2013, this disease was more severe as compared to *Kharif* 2014. This year wise differential disease response is due to the influence of environmental factors. The mean per cent incidence varied from 58.33 to 81.66 on leaves and 61.66 to 86.66 on fruits during 2013, similar trend was observed in 2014.The per cent disease index varied from 49.60 to 67.60 on leaves; and 51.60 to 70.80 on fruits in 2013. Similarly the disease index was 46.20 to 64.40 on leaves and 48.80 to 68.80 per cent on fruits. Among the districts, the disease was very severe in Udaipur, especially in orchards of RCA (Udaipur city), Kankarwa, Mainar, Bargaon and at Bhimpura of Bhilwara district, where Bhagwa (Kesar) is most preferred cultivar. On Bhagwa (Kesar) cultivar, the highest disease intensity (PDI) was recorded at Horticulture farm, RCA with 66.00% on foliage and 69.80% on fruits infection, respectively, followed by orchard at Kanakarwa and Bargaon. The disease was appeared comparatively in less severe form on other cultivars *viz*., Ganesh and Mirdula. On cultivar Ganesh 61.60% on foliage and 66.60% on fruits infection was observed at Mainar and that of 53.40% on foliage and 58.50% on fruits infection at Rundera of Udaipur district. It clearly indicates higher susceptibility of Bhagwa (Kesar) cultivar. The intensive cultivation of this susceptible cultivar

**Table 1.** Disease rating scale of bacterial blight of pomegranate

| Grade | Per cent infection | | Symptoms |
|---|---|---|---|
| | Leaves | Fruits | |
| 0 | 0.00 | 0.00 | No disease |
| 2 | >1–10 | >1–10 | Spots coalesced and spots enlarged on leaf lamina and fruits |
| 3 | >10–20 | >10–20 | Leaves turned yellow and lesions on stem/ branches |
| 4 | >20–40 | >20–40 | Leaf infection, twigs and branches with brown black enlarged water soaked lesions brown black spots on fruits |
| 5 | >40–100 | >40–70 | Defoliation of leaves, girdling and cracking of nodes, fruit pericarp turned brown black, cracking of fruit and fruit drop |

supports wider adaptation, survivability, dissemination of bacterial blight pathogens. In comparison to Udaipur district, disease intensity of bacterial blight is quite lower in Bhilwara, Chittorgarh, and Bundi. The disease severity reaches upto 61.60 PDI on foliage and 63.50 PDI on fruits in Bhimpura of Bhilwara district, and gradually reduced at Banera and Menghros. The low disease intensity was at KVK, Bundi and at KVK, Chittorgarh. This finding indicates the distribution and prevalence of pomegranate bacterial blight in southern Rajasthan. Within Southern Rajasthan, Udaipur district is supposed to be the main epic-centre for introduction and initial establishment of this pathogen, and gradually spread to other adjoining districts like Bhilwara, Chittorgarh, and Bundi.

Numerous surveys have been carried out to study the distribution and prevalence of bacterial blight of pomegranate in India (Mulla *et al.,* 2008; Benagi and Ravikumar, 2011). Among them, survey of pomegranate bacterial blight in Karnataka (Ravikumar *et al.,* 2004) and

**Table 2.** Distribution and prevalence of pomegranate bacterial blight in different districts of Southern Rajasthan during *Kharif* 2013 and 2014

| Districts | Locations | Cultivar | Mean per cent incidence (PI) | | | | | | Mean per cent disease index (PDI) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Foliage | | | Fruits | | | Foliage | | | Fruits | | |
| | | | 2013 | 2014 | Pooled | 2013 | 2014 | Pooled | 2013 | 2014 | Pooled | 2013 | 2014 | Pooled |
| Udaipur | RCA, Udaipur city | Bhagwa | 81.6 | 78.3 | 80.0 | 86.6 | 83.3 | 85.0 | 67.6 | 64.4 | 66.0 | 70.8 | 68.8 | 69.8 |
| | Kankarwa | Bhagwa | 76.6 | 73.3 | 75.0 | 81.6 | 75.0 | 78.3 | 65.8 | 62.8 | 64.3 | 69.6 | 66.6 | 68.1 |
| | Mainar | Ganesh | 71.6 | 68.3 | 70.0 | 76.6 | 70.0 | 73.3 | 63.6 | 59.6 | 61.6 | 67.8 | 65.4 | 66.6 |
| | Bargaon | Bhagwa | 75.0 | 71.6 | 73.3 | 80.0 | 73.3 | 76.6 | 61.2 | 57.4 | 59.3 | 64.8 | 61.8 | 63.3 |
| | Mannakhera | Mirdula | 68.3 | 63.3 | 65.8 | 71.6 | 66.6 | 69.1 | 53.6 | 50.2 | 51.9 | 58.4 | 55.2 | 56.8 |
| | Rundera | Ganesh | 60.0 | 56.6 | 58.3 | 65.0 | 58.3 | 61.6 | 55.2 | 51.6 | 53.4 | 60.6 | 56.4 | 58.5 |
| Bhilwara | Bhimpura | Bhagwa | 73.3 | 70.0 | 71.6 | 75.0 | 71.6 | 73.3 | 63.4 | 59.8 | 61.6 | 65.4 | 61.6 | 63.5 |
| | Banera | Mirdula | 68.3 | 63.3 | 65.8 | 70.0 | 65.0 | 67.5 | 55.6 | 51.6 | 53.6 | 60.8 | 55.4 | 58.1 |
| | Menghros | Bhagwa | 63.3 | 60.0 | 61.6 | 68.3 | 61.6 | 65.0 | 50.4 | 46.8 | 48.6 | 56.2 | 53.6 | 54.9 |
| Chittorgarh | KVK | Bhagwa | 61.6 | 58.3 | 60.0 | 66.6 | 60.0 | 63.3 | 51.6 | 47.8 | 49.7 | 53.6 | 50.2 | 51.9 |
| Bundi | KVK | Bhagwa | 58.3 | 55.0 | 56.6 | 61.6 | 56.6 | 59.1 | 49.6 | 46.2 | 47.9 | 51.6 | 48.8 | 50.2 |
| CD (p=0.05) | | | 4.6 | 4.0 | 3.0 | 4.9 | 4.3 | 3.2 | 3.9 | 4.3 | 2.8 | 4.2 | 3.9 | 2.8 |
| CV% | | | 5.2 | 4.8 | 5.0 | 5.3 | 4.9 | 5.1 | 5.2 | 6.1 | 5.7 | 5.3 | 5.2 | 5.3 |

**Table 3.** Pathogenic variability among different isolates of test bacterium *Xanthomonas axonopodis* pv. *punicae* by using carborundum abrasion inoculation method

| Aggressiveness | Location | GPS Data | | Isolates | Incubation period (days) for development of water soaked symptoms on leaves | Time required for yellowing of leaves (days) | Time required for development of dark brown to blackening symptoms (days) | Time required for drooping of infected leaves (days) |
|---|---|---|---|---|---|---|---|---|
| | | Latitude | Longitude | | | | | |
| Highly aggressiveness | RCA | 24°34'51.6"N | 73°42'07.8"E | Xap-01 | 04 | 06 | 09 | 14 |
| | Mainar | 24°36'07.3"N | 74°06'32.0"E | Xap-03 | 04 | 06 | 09 | 14 |
| | Bargaon | 24°38'18.2"N | 73°40'43.9"E | Xap-04 | 04 | 06 | 09 | 14 |
| Moderately aggressiveness | Mannakhera | 24°33'47.8"N | 73°44'10.7"E | Xap-05 | 06 | 08 | 11 | 16 |
| | Menghros | 25°29'52.8"N | 74°40'42.8"E | Xap-08 | 06 | 08 | 11 | 16 |
| | KVK, Chittorgarh | 24°50'15.1"N | 74°34'29.1"E | Xap-09 | 06 | 08 | 11 | 16 |
| | KVK, Bundi | 25°26'32.5"N | 75°40'76.2"E | Xap-10 | 06 | 08 | 11 | 16 |
| Less aggressiveness | Kankarwa | 24°34'44.4"N | 73°40'58.3"E | Xap-02 | 08 | 10 | 13 | 18 |
| | Bhimpura | 25°43'09.3"N | 74°57'33.7"E | Xap-06 | 08 | 10 | 13 | 18 |
| | Banera | 25°29'45.8"N | 74°41'33.8"E | Xap-07 | 08 | 10 | 13 | 18 |

Maharashtra (Raghuwanshi *et al.,* 2013) signify the influence of environment and cultivar response on disease prevalence. These previous study supports the increasing prevalence and wider distribution of this disease within intensified cropping system of pomegranate. Pathogenic variability among different pathogenic isolates clearly indicates that isolates *viz*., Xap-01, Xap-03 and Xap-04 were highly aggressive in nature. Whereas, isolates *viz*., Xap-05, Xap–08, Xap-09 and Xap–10 were moderately aggressive while the remaining isolates *viz*., Xap-02, Xap-06 and Xap-07 were less aggressive when tested on susceptible host(cv. Bhagwa) (Table 3). All the highly aggressive isolates were obtained from Udaipur district. In response to favourable environment and susceptible host, the aggressive nature of this pathogen helps to cause havoc in this locality. It also supports their rapid adaptation and longer survivability. From the present investigation, some valuable information on the distribution and prevalence of pomegranate bacterial blight was generated. It reveals the magnitude of the problem that is gradually emerging in a dangerous form. This will help us to formulate the suitable management strategies to prevent spread and dissemination of this pathogen.

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# Bioefficacy of Plant Extracts and Biocontrol Agents against Some Plant Pathogenic Fungi

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**Abstract**: The present study was undertaken to assess the *in vitro* antifungal activity of methanol, ethanol, petroleum ether and aqueous extracts of five plants viz., *Ranunculus muricatus* L.*, Vitex negundo* L.*, Murraya koenigii* (L.) Sprengel, *Melia azedarach* L. and *Eupatorium* L. and five bioagents viz., *Trichoderma koningii* (DMA-8 and JMA-11), *T. harzianum* (JMA-4 and SMA-5) and *T. viride* (H$_3$) were evaluated for their antifungal properties against seven fungal phytopathogens namely *Sclerotium rolfsii, Fusarium solani* f.sp. *pisi, F. oxysporum* f.sp. *pisi, Sclerotinia sclerotiorum, Rhizoctonia solani, Phytophthora nicotianae* var *nicotianae* and *Colletotrichum capsici*. Among plant extracts, aqueous extract of *V. negundo* showed significant antifungal activity against *S. rolfsii* (91.3%) and *C. capsici*. which was followed by *M. azedarach* against *P. nicotianae* var *nicotianae* (92.8%) and *Eupatorium* against *P. nicotianae* var *nicotianae* (93.5%) and *C. capsici* (93.2%). Among bioagents, *T. harzianum* JMA-4 (82.8%), *T. koningii* DMA-8 (82.6%) and *T. viride* (H$_3$) (80.6%) showed significantly higher mycelial growth inhibition of *C. capsici*. Percent mycelial inhibition of *F. oxysporum* f. sp. *pisi* was found 79.3 and 79.1 percent in case of *T. koningii* DMA-8 and *T. viride* (H$_3$) respectively.

**Keywords**: Antifungal activity, Aqueous and organic extracts, Bioagents, Fungal pathogens

Crop production is hampered by many biotic stresses such as fungi, bacteria, viruses and nematodes. Most of the plant diseases are caused by fungal pathogens which result in huge economic losses. Since fungi can cause disasters on the crops, the metabolites of many fungi may have adverse or stimulatory effects on plants such as suppression of seed germination, malformation, and retardation of seedling growth. The fungi also could cause other various symptoms such as vascular wilt, yellows, corm rot, root rot, and damping-off. For the control of diseases, many synthetic fungicides have been found effective. However, indiscriminate use of fungicides has created serious environmental problems and affects human health (Guhukar, 2012). The effectiveness of synthetic fungicides has been reduced by the frequent development of resistance by the pathogens. Hence there is a great demand for safer, alternative and effective ecofriendly management strategy. In this regard, greater attention is towards the use of bioagents and plant extracts and their products for managing the fungi in a sustainable manner (Han *et al*., 2005). Higher plants are a treasure house of phytochemicals, which serve as valuable drugs that have helped to combat several fatal diseases the worldover (Zahid *et al., 2012*). A number of plant extracts have been demonstrated to control pathogenic fungi (Hao *et. al.,* 2010; Roopa *et. al.,* 2014). Some of *Trichoderma* species had been identified as potential biological control agents of plant pathogenic fungi on many crops (Adebesin *et. al.,* 2005). *Trichoderma harzianum* Rifai, *Trichoderma viride* Pers.ex.Gray, *Trichoderma koningii* Oudem are important antagonists of plant pathogens (Ganie *et al.,* 2013). These bioagents were used for the control of foliar, soil-borne and post harvest diseases in various crops in the field, greenhouses and in storage. Exploitation of plant metabolites and antagonists in crop protection and prevention of biodeterioration caused by fungi appear to be promising. So, keeping these facts in view, the present study was planned to evaluate the antifungal properties of leaf extracts of botanicals namely *Ranunculus muricatus* L.*, Vitex negundo* L.*, Murraya koenigii* (L.) Sprengel*, Melia azedarach* L. and *Eupatorium* L. and five *Trichoderma* strains against plant pathogenic fungi *viz; Sclerotium rolfsii* Sacc.*, Fusarium solani (Mart.) Appel.* And Wollenw, *F. oxysporum* (Schlect.) emend. Synd. and Hans.*, Sclerotinia sclerotiorum* (Lib.) de Bary*, Colletotrichum capsici* (Synd.) Butler and Bisby, *Phytophthora nicotianae* Breda de Haan and *Rhizoctonia solani* Kuhn.

## MATERIAL AND METHODS

**Fungal agents used:** Pure culture of *S. rolfsii, F. solani* f.sp. *pisi, F. oxysporum* f.sp. *pisi, S. sclerotiorum, R. solani, C. capsici* and *P. nicotianae* var *nicotianae* infecting cauliflower, pea, cabbage, tomato, capsicum respectively, were obtained

from Department of Plant Pathology, CSKHPKV, Palampur, India.

**Collection and preservation of botanicals:** Five botanicals namely *Ranunculus muricatus, Vitex negundo, Murraya koenigii, Melia azedarach* and *Eupatorium* species were used in the present study. Leaves of all the botanicals were collected from the surroundings of Palampur. The leaves were oven dried by spreading them on the shelves of hot air oven over two to three layered blotting sheets at $50^{\circ}C$ for 5 to 6 hours for two to three days. After drying, the respective plant material was ground in a blender to obtain fine dry powder. Sufficient powdery biomass of all the botanicals was stored in paper bags (Tassel bags) at room temperature for further use.

**Preparation of extracts:**

**Aqueous plant extract:** Fifty gram fine powder of each botanical was soaked overnight in 100 ml of sterilized distilled water (1:2 w/v). Next day, the extract obtained was filtered through a double layer of muslin cloth and twice through Whatman No. 1 filter paper to get clear filtrate. This was considered as standard aqueous extract.

**Solvent plant extract:** Fifty gram fine powder of each botanical was soaked overnight in 100 ml of organic solvents *viz.,* methanol, ethanol and petroleum ether in 500 ml conical flasks. The flasks were covered with aluminium foil to avoid evaporation of the solvent. The extract obtained as mentioned above. The organic extracts obtained were heated on water bath at $40-50^{\circ}C$ for 10–15 minutes to evaporate the solvents. The crude extracts thus obtained after evaporation with 100 percent concentration were further diluted to 50 percent by adding dimethyle sulphoxide (DMSO). This solution thus obtained regarded as standard organic extract. All organic and aqueous plant extracts were filtered with filter syringes (0.22 µ pore size) under aseptic conditions and stored in sterilized flasks (250 ml) in refrigerator for further use.

**Antifungal bioassay of plant extracts:** The aqueous and solvent extracts of botanicals were evaluated for their antifungal activity against the test pathogens by following poisoned food technique (Nene and Thapliyal, 1993). Double strength sterilized PDA medium was amended with equal quantity of plant extracts at different concentrations ranging from 10 to 100 percent and aseptically poured in sterilized plates. Medium mixed with equal quantities of distilled sterilized water without any treatment served as control. Seven days old mycelial bits (5 mm) were placed in the centre of plates and plates were incubated at $25\pm1^{\circ}C$. Each treatment was replicated four times. The experiment was laid out in a completely randomized design. When control plates were covered with mycelial growth of pathogens, percent inhibition was determined by following I = (C–T/C)*100 [I = Percent inhibition of mycelium, C = Growth of mycelium in control (mm), T = Growth of mycelium in treatment (mm)].

**Evaluation of bioagents:** Antagonistic activity of locally available bioagents *viz. T. koningii* (DMA–8 and JMA–11), *T. harzianum* (JMA–4 and SMA–5) and *T. viride* (Hyderabad isolate H$_3$) were evaluated against the test pathogens*,* on potato dextrose agar medium using dual culture technique (Utkhede and Rahe, 1983). Five millimeters discs of actively growing seven day old cultures of test pathogen as well as biocontrol agents were taken with the help of a cork borer. Two discs, one each of pathogen and biocontrol agent, were placed equidistantly (60 mm) apart in each 90 mm Petriplate containing PDA under aseptic conditions. The plates containing PDA medium inoculated with pathogen alone served as control. The plates were incubated at $25 \pm 1^{\circ}C$. The radial growth of the bioagents and the pathogens from the centre of disc towards the centre of the plate was recorded after the control plates were completely covered by pathogens. Each treatment was replicated four times. Observations on the growth of biocontrol agent and pathogen were recorded after 10 days of incubation and percent mycelial inhibition was calculated.

## RESULTS AND DISCUSSION

**Evaluation of aqueous plant extract:** The aqueous extract of *V. negundo* was found more effective against all the test pathogens followed by *M. azedarach* and *Eupatorium* sp. Plant extracts showed maximum mycelial inhibition when used in higher concentrations (80 and 100%). Increase in inhibition was observed with increase in concentration of extract. The aqueous extract of *V. negundo* was most effective against *S. rolfsii* followed by *C. capsici*, *P. nicotianae*, *F. oxysporum* f.sp. *pisi* and *F. solani* f.sp. *pisi*. *M. koenigii* caused maximum inhibition (90.7%) of *F. solani* f.sp. *pisi* followed by *P. nicotianae, F. oxysporum* f. sp. *pisi* and *C. capsici*. Its aqueous extract was not effective against *S. sclerotiorum*. Whereas, *M. azedarach* was found strongly fungitoxic to *P. nicotianae* followed by *C. capsici* and *F. oxysporum* f. sp. *pisi*. It was least inhibitory to *S. rolfsii* (56.9%) and *S. sclerotiorum* (54.9%). *Eupatorium* sp. showed more than 93 percent inhibition in mycelial growth of *P. nicotianae* and *C. capsici* (Table 1). All the extracts were statistically at par and differ significantly among themselves in inhibiting the mycelial growth of pathogens.

**Evaluation of solvent plant extract :** The solvent extracts of plant were less effective in inhibiting the mycelial growth of fungal pathogens than aqueous plant extracts. Among three solvent extracts evaluated for fungitoxicity, methanol extract of all botanicals was found most effective against test

pathogens followed by ethanol and petroleum ether extract (Table 2, 3 and 4). Extracts of *M. koenigii, V. negundo* and *R. muricatus* in methanol, ethanol and petroleum ether showed maximum inhibition of *F. solani* f.sp. *pisi* when used at highest concentration followed by *P. nicotianae* and *C. capsici*. In case of solvent extract of *Eupatorium* sp., methanol extract showed maximum mycelial growth inhibition against *F. solani* f. sp. *pisi* followed by ethanol and petroleum ether extract against *C. capsici*. Extract of *M. azedarach* in all the solvents was least effective against test pathogens with mycelial inhibition ranged between 68.0–81.3 per cent. The tested concentrations were at par in inhibiting the mycelial growth of

pathogens and solvent extracts were differ significantly among themselves against tested pathogens.

*In vitro* evaluation revealed that aqueous extracts at various concentrations were significantly effective in inhibiting the mycelial growth of test pathogens than solvent extracts. The antifungal substances contained in the extracts were fungistatic at lower concentrations, while becoming fungicidal at higher concentrations of the extracts. Several workers observed the antifungal activities of different botanicals against *F. oxysporum* and *Alternaria solani, P. capsici, F. oxysporum, F. moniliformae* and *Aspergillus niger, Rhizoctonia solani* and *Colletotrichum falcatum* (Bhattarai

**Table 1.** Effect of aqueous extract of various botanicals on mycelial growth inhibition of fungal pathogens

| Botanical* | Percent inhibition of mycelial growth over control | | | | | | | | | | | | | |
| | S. rolfsii | | F. solani | | F. oxysporum | | S. sclerotiorum | | C. capsici | | P.nicotianae | | R. solani | |
| | a** | b | a | b | a | b | a | b | a | b | a | b | a | b |
| *Eupatorium sp.* | 55.6 | 64.8 | 88.2 | 89.3 | 76.4 | 78.5 | 47.0 | 53.2 | 91.0 | 93.2 | 90.3 | 93.6 | 64.5 | 68.0 |
| *Murraya koenigii* | 78.2 | 80.5 | 88.9 | 90.7 | 87.3 | 89.1 | 0.0 | 16.4 | 87.5 | 89.0 | 87.1 | 89.3 | 80.2 | 82.2 |
| *Vitex negundo* | 89.7 | 91.3 | 79.3 | 81.0 | 86.6 | 88.9 | 75.0 | 77.5 | 89.6 | 90.5 | 87.4 | 88.9 | 69.1 | 71.2 |
| *Ranunculus muricatus* | 49.4 | 61.0 | 71.6 | 74.2 | 72.7 | 77.0 | 25.1 | 36.3 | 77.2 | 80.1 | 49.4 | 57.4 | 31.5 | 45.7 |
| *Melia azedarach* | 51.9 | 57.0 | 84.8 | 86.1 | 85.1 | 87.2 | 49.0 | 54.9 | 84.5 | 87.4 | 91.7 | 92.8 | 78.2 | 80.9 |
| CD (p=0.05) | 0.5 | 0.4 | 0.3 | 0.2 | 0.2 | 0.4 | 0.4 | 0.5 | 0.3 | 0.2 | 0.4 | 0.3 | 0.3 | 0.4 |

*Lower concentrations ranging from 10–70% were found least effective against test pathogens, therefore effective concentrations were presented in results
** a= Concentration of aqueous plant extract (80%), b= Concentration of aqueous plant extract (100%)

**Table 2.** Effect of methanol extract of various botanicals on mycelial growth inhibition of fungal pathogens

| Botanical* | Percent inhibition of mycelial growth over control | | | | | | | | | | | | | |
| | S. rolfsii | | F. solani | | F. oxysporum | | S. sclerotiorum | | C. capsici | | P.nicotianae | | R. solani | |
| | a** | b | a | b | a | b | a | b | a | b | a | b | a | b |
| *Eupatorium* sp. | 56.7 | 69.1 | 76.2 | 82.1 | 65.2 | 71.9 | 60.2 | 70.5 | 71.3 | 81.3 | 69.5 | 79.1 | 53.1 | 62.0 |
| *Murraya koenigii* | 50.4 | 67.7 | 74.1 | 83.1 | 66.9 | 73.1 | 58.6 | 69.0 | 76.1 | 79.9 | 71.2 | 80.1 | 67.1 | 75.4 |
| *Vitex negundo* | 49.0 | 60.3 | 79.9 | 84.2 | 68.9 | 76.2 | 57.9 | 71.2 | 64.6 | 74.2 | 69.0 | 82.3 | 48.4 | 64.4 |
| *Ranunculus muricatus* | 56.3 | 78.7 | 80.2 | 86.2 | 70.1 | 78.2 | 65.8 | 75.4 | 75.3 | 82.2 | 75.2 | 84.1 | 65.3 | 77.2 |
| *Melia azedarach* | 54.9 | 71.2 | 70.1 | 73.1 | 67.8 | 77.1 | 63.5 | 68.0 | 67.9 | 77.7 | 70.9 | 81.2 | 56.7 | 73.2 |
| CD (p=0.05) | 0.7 | 0.5 | 0.4 | 0.2 | 0.6 | 0.3 | 0.6 | NS | 0.4 | 0.3 | 0.6 | 0.4 | 0.4 | 0.3 |

*Lower concentrations ranging from 10–70% were found least effective against test pathogens, therefore effective concentrations were presented in results
** a= Concentration of aqueous plant extract (80%), b= Concentration of aqueous plant extract (100%)

**Table 3.** Effect of ethanol extract of various botanicals on mycelial growth inhibition of fungal pathogens

| Botanical* | Percent inhibition of mycelial growth over control | | | | | | | | | | | | | |
| | S. rolfsii | | F. solani | | F. oxysporum | | S. sclerotiorum | | C. capsici | | P.nicotianae | | R. solani | |
| | a** | b | a | b | a | b | a | b | a | b | a | b | a | b |
| *Eupatorium* sp. | 57.9 | 69.3 | 77.0 | 81.2 | 66.0 | 73.1 | 61.7 | 72.0 | 72.5 | 82.0 | 70.1 | 80.3 | 57.0 | 63.1 |
| *Murraya koenigii* | 51.0 | 68.1 | 75.1 | 84.0 | 67.5 | 75.4 | 57.9 | 70.1 | 77.6 | 79.0 | 74.1 | 81.7 | 68.9 | 77.1 |
| *Vitex negundo* | 51.2 | 61.2 | 78.6 | 85.1 | 65.1 | 77.1 | 53.4 | 68.5 | 65.4 | 75.3 | 69.0 | 81.5 | 67.5 | 70.2 |
| *Ranunculus muricatus* | 60.0 | 76.0 | 79.8 | 84.3 | 70.4 | 80.1 | 64.3 | 77.8 | 76.1 | 83.0 | 75.7 | 84.3 | 67.0 | 76.2 |
| *Melia azedarach* | 57.9 | 74.9 | 69.2 | 72.1 | 67.0 | 79.5 | 65.1 | 70.8 | 68.8 | 78.9 | 71.2 | 80.0 | 65.9 | 71.2 |
| CD (p=0.05) | 0.3 | 0.4 | 0.2 | 0.2 | 0.1 | 0.2 | 0.3 | 0.2 | 0.3 | 0.3 | 0.4 | 0.4 | 0.6 | 0.3 |

*Lower concentrations ranging from 10–70% were found least effective against test pathogens, therefore effective concentrations were presented in results
** a= Concentration of aqueous plant extract (80%), b= Concentration of aqueous plant extract (100%)

and Shrestha, 2009; Prince and Prabakaran, 2011) while studying effects of botanicals in *vitro.* The findings are in close conformity with Pandey (2015) who have reported that leaf extract of *Azadirachta indica, Emblica officinalis* and *Pongamiag labra* @ 0.5 per cent concentration was most effective against *Magnaporthe oryzae*. The inhibition of mycelial growth of pathogen may be attributed to the presence and detrimental effects of allelochemicals on cell division, cell elongation and nutrient uptake (Blake, 1985). The presence of antibiotic constituent in the form of phenolic, resinous, gummy and non‑volatile substances in different botanicals is reported by Skinner (1955) and these compounds have attributed such antimicrobial properties. The presence of saponin, phenolic and flavonoid content are also considered responsible for such inhibition (Rinez *et al.*, 2013). Such compounds being biodegradable and selective in their toxicity are considered valuable in controlling some plant diseases (Singh and Dwivedi, 1987).

Also, the results of this conceptual study clearly reflect that organic extracts of botanicals has ability to inhibit the mycelial growth of fungal species. In fact alcoholic extracts were found most inhibitory than petroleum ether extract. The findings in the present investigation are corrobated by Nagarsekar *et al*. (2010) who have studied the antibacterial activity of ethanol and petroleum ether extracts of *V. negundo* against *Bacillus subtilis* and *Staphylococcus aureus*. The fungicidal property of *V. negundo* may be attributed to the presence of polyphenolic compounds, flavones glycoside 4, 5 and essential oils (α-pinene, camphene and citral) (Aswar *et al.*, 2009). The results were further confirmed by Khuntia and Sunder (2011) who have reported the antifungal and antibacterial properties of petroleum ether and alcoholic extracts of *M. koenigii* against *Aspergillus niger* and *Helminthosporium solani* and their fungitoxic activity might be due to the presence of alkaloids, steroids, flavonoids and triterperoid, benzoic acid, β-sitosterol (Jabeen *et al.*, 2011). The extract of Eupatorium triplinerve in petroleum ether,

chloroform and ethyl acetate was found to contain D‑glucoside and ceramide 2 which exhibited fungitoxicity against Alternaria alternate, Colletotrichumcorchori, Fusarium equisetiand Macrophominaphaseolina (Devi *et. al.*, 2007). The phytochemicals viz. glycosides, flavonoids, saponins and ranuncoside has been isolated from aqueous and alcoholic extracts of Ranunculus sceleratus which has antibacterial properties (Hussain *et al.*, 2011).

**Evaluation of efficacy of fungal bioagents:** Percent mycelial inhibition of test pathogens as affected by bioagents *viz., Trichoderma harzianum* (JMA‑4 and SMA‑5), *T. viride* (H₃) and *T. koningii* (DMA‑8 and JMA‑11) are presented in Figure 1. Results indicate that *T. harzianum* JMA‑4 caused maximum mycelial growth inhibition (82.8%) of *C. capsici* followed by *F. oxysporum* f.sp. *pisi* (76.5%), *R. solani* (73.9%), *S. rolfsii* (70.7%) and *F. solani* f.sp. *pisi* (70.1%). *T. harzianum* SMA‑5 exhibited 79.3 percent inhibition in mycelial growth of *C. capsici*. *T. viride* (H₃) showed maximum mycelial growth inhibition of *C. capsici* followed by *F. oxysporum* f.sp. *pisi*. *T. koningii* DMA‑8 showed 82.6 percent inhibition of *C. capsici* followed by *F. oxysporum* f.sp. *pisi*. Whereas *T. koningii* JMA‑11 was found most effective against *C. capsici* with 78.0 percent mycelial inhibition. Out of five bioagents, *T. harzianum* JMA‑4 was more efficient followed by *T. koningii* DMA‑8 and *T. viride* (H₃) in inhibiting the mycelial growth of fungal pathogens*.*

*These findings are in conformity with Rudresh et al. (2005) who have noticed the growth inhibition of Rhizoctonia solani and F. oxysporum by T. harzianum. Earlier rapid growth and antagonism of T. harzianum against Sclerotinia sclerotiorum have been reported by many workers (Amin et al., 2010; Roopa et. al., 2014).* Rahman *et. al.* (2015) also observed the antifungal activity of *T. viride* against *Alternaria porri* causing purple blotch in bunch onion. The growth inhibition in presence of *Trichoderma* species could be attributed to all the three modes of antagonism *in vitro viz.,* competition, antibiosis and mycoparasitism and due to its

**Table 4.** Effect of petroleum ether extract of various botanicals on mycelial growth inhibition of fungal pathogens

| Botanical* | Percent inhibition of mycelial growth over control | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. rolfsii* | | *F. solani* | | *F. oxysporum* | | *S. sclerotiorum* | | *C. capsici* | | *P. nicotianae* | | *R. solani* | |
| | a** | b | a | b | a | b | a | b | a | b | a | b | a | b |
| *Eupatorium* sp. | 49.0 | 63.5 | 73.5 | 80.5 | 69.1 | 78.1 | 59.0 | 70.1 | 76.2 | 82.3 | 72.0 | 79.6 | 48.9 | 60.1 |
| *Murraya koenigii* | 50.1 | 68.0 | 72.0 | 83.0 | 63.2 | 77.5 | 64.0 | 71.2 | 72.6 | 80.1 | 76.3 | 80.0 | 65.0 | 74.8 |
| *Vitex negundo* | 53.0 | 64.4 | 75.5 | 85.5 | 71.5 | 80.9 | 54.9 | 69.3 | 71.2 | 79.4 | 69.9 | 79.5 | 62.9 | 72.3 |
| *Ranunculus muricatus* | 62.0 | 77.0 | 75.8 | 86.0 | 73.7 | 82.0 | 66.7 | 78.0 | 77.7 | 84.0 | 72.6 | 82.1 | 67.0 | 77.2 |
| *Melia azedarach* | 56.8 | 74.0 | 70.1 | 80.2 | 68.8 | 79.2 | 67.4 | 74.4 | 74.0 | 80.2 | 76.3 | 81.3 | 64.6 | 76.0 |
| CD (p=0.05) | 0.3 | 0.3 | 0.3 | 0.4 | 0.2 | 0.2 | 0.3 | 0.2 | 0.4 | 0.3 | 0.4 | 0.3 | 0.3 | 0.2 |

*Lower concentrations ranging from 10‑70% were found least effective against test pathogens, therefore effective concentrations were presented in results
** a= Concentration of aqueous plant extract (80%), b= Concentration of aqueous plant extract (100%)

*Ashlesha and Y.S. Paul*
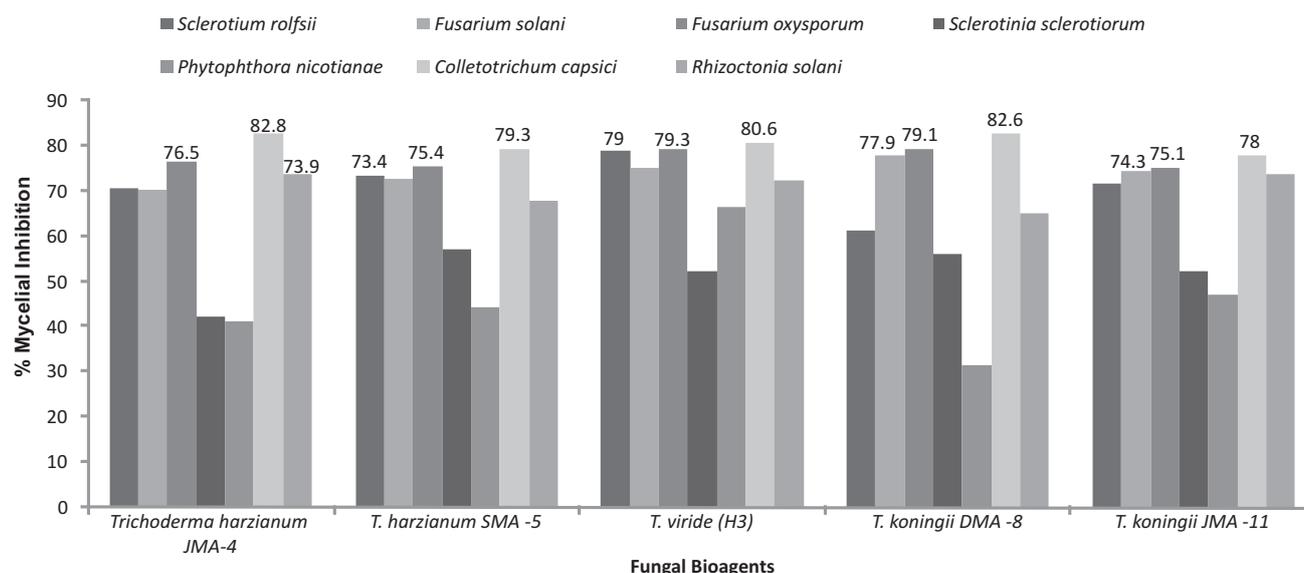


**Fig. 1.** Antifungal efficacy of fungal bioagents against plant pathogens

fast growing nature, rapid sporulation, secretion of gliotoxin and cell wall lytic enzymes such as chitinase, endochitinase and β-1,3-glucanase (Shalini and Kotasthane, 2007).

Thus the aqueous extracts of botanicals possess significant antifungal activity against test pathogens than extracts in different solvent systems. Among organic extracts, alcoholic extracts of botanicals were found most effective. Further studies aimed at isolation and purification of bioactive compounds of these botanicals. The spectrum of activity observed in the study that alcoholic and aqueous extracts of plants and bioagents could be a possible source to obtain new and effective products against various fungal plant pathogens.

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# Technology Gaps Analysis and Prominence of Blossom Midge, *Contarinia maculipennis* Felt of Jasmine (*Jasminum sambac* l.) In Tamil Nadu

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**Abstract**: Investigations were conducted in major jasmine growing districts of Tamil Nadu, for assessing the distribution, infestation level and the relative importance of blossom midge of jasmine, *Contarinia maculipennis*. The midge incidence was more in Madurai (53.27 %) followed by Tiruneveli and Tuticorin districts. Seventy six per cent respondents were aware of blossom midge as a pest, but only sixteen per cent respondents ranked as the most important pest. The technological gap indices (TGI) were high in adopting right frequency in spraying of chemicals (91 %), non-planting of alternate hosts around main cropped area (89.00%) and usage of biological control agents (86 %). Regular raking of soil to destroy pupa, which is an effective management strategy of blossom midge recorded TGI of 85 %. A majority of respondents (47.05 %) had high level of technological gap, 35.47 and 17.64 % respondents had medium and low level technology gap. Therefore, awareness should be created among jasmine growers for the use of environmentally safe methods against blossom midge as well as other pests of jasmine.

**Keywords**: Jasmine, Blossom midge, Biological control, Management

Jasmine is cultivated in an area of more than 8,000 ha with an annual production of flowers worth Rs. 80–100 million in India. Tamil Nadu is the leading producer of jasmine in the country with an annual production of 77, 247 tonnes from an area of 9,360 ha (Prakash and Muniandi, 2014). The production of Jasmine is affected by various factors, among which, insect pests are the most devasting factor. Major pests affecting jasmine are jasmine budworm (*Hendecasis duplifascialus* Hampson), blossom midge (*Contarinia maculipennis* Felt), galleryworm (*Elasmopalpus jasminophagus* Hampson), leaf webworm (*Nausinea geometralis* Guenee), leaf roller, (*Glyphodes unionalis* Hubner), and red spider mite (*Tetranychus urticae* Koch.). Of these, budworm and blossom midge pose heavy damage as they directly attack the economic part, the buds.

Blossom midge, *Contarinia maculipennis* Felt (Cecidomyiidae, Diptera) has attained the status of a major pest causing severe economic loss by reducing the marketable quality of the flowers. Indiscriminate and injudicious uses of conventional insecticides for managing this pest have been causing different environmental hazards including resurgence, resistance and residue problems (Nalini and Kumar, 2016). Therefore, it is now an urgent need to use safe but effective, biodegradable pesticides with no or less toxic effects on beneficials. As the farmers are end users and the final decision-makers for the adoption of any

technology, it is essential to know their knowledge about the pests and practices recommended for pest management. However, least attention has been paid to assess the farmer's perception. Hence, considering the economic losses caused by this pest, this study was undertaken to assess the incidence of blossom midge in major jasmine growing districts of Tamil Nadu and to assess farmer's perception on the awareness about this pest and its prominence over other key pests of jasmine.

## MATERIAL AND METHODS

Investigations were conducted during 2015–2016 in ten major jasmine growing districts (Fig.1) for assessing the distribution of blossom midge of jasmine in Tamil Nadu. The study areas were selected based on the extent of cultivation of the crop. From each selected village, 10 jasmine growing farmers were randomly selected and the data was collected by means of a structured questionnaire prepared in local language administered via personal interviews (Pinyupa *et al.,* 2009). Technological Gap Index (TGI) was computed using the formula (Sakthivel *et al.,* 2012).

$$\text{Technological Gap Index (TGI)} = \frac{R - A}{R} \times 100$$

Where,   R = Recommended practice
       A = Adopted practice
On account of a wide range of technological gap, the
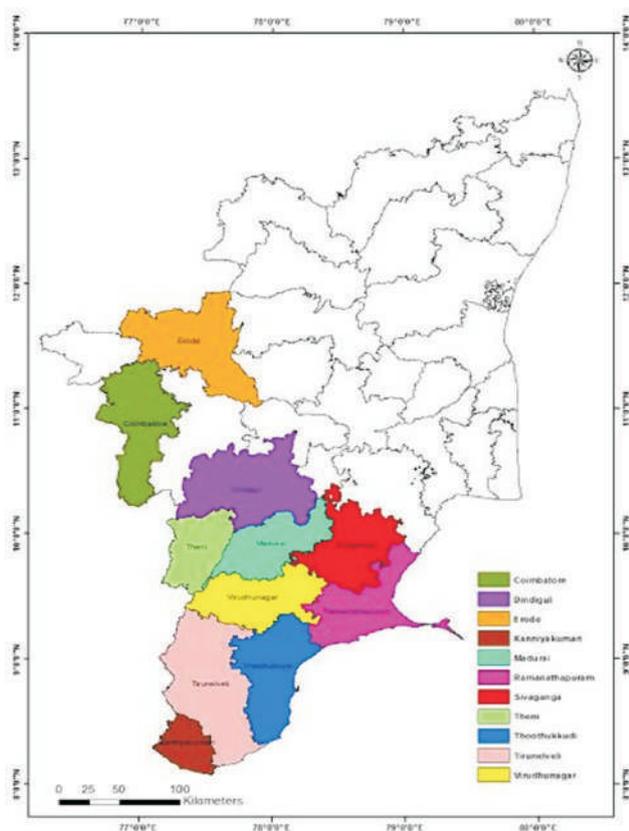
**Fig. 1.** Map showing the study site

**Table 1.** Frequency distribution and incidence of blossom midge of jasmine, *C. maculipennis* by jasmine growers

(n=100)

| Districts | No. of pink discoloured buds/plant | % Blossom midge incidence |
|---|---|---|
| Kanyakumari | 8.6 [c] (2.93) | 27.18 [ef] (5.15) |
| Tirunelveli | 14.8 [b] (3.84) | 43.19 [b] (6.26) |
| Tuticorin | 12.3 [b] (3.50) | 37.15 [bc] (5.73) |
| Virdhunagar | 7.1 [cd] (2.66) | 30.68 [de] (5.06) |
| Madurai | 19.1 [a] (4.37) | 53.27 [a] (6.99) |
| Ramanathapuram | 14.3 [b] (3.78) | 33.62 [cd] (5.53) |
| Theni | 7.2 [cd] (2.68) | 23.54 [fg] (4.51) |
| Dindugal | 8.9 [c] (2.98) | 21.31 [g] (4.76) |
| Erode | 5.3 [e] (2.30) | 19.42 [g] (4.35) |
| Coimbatore | 6.7 [de] (2.58) | 26.64 [ef] (4.92) |
| CD (p=0.05) | 0.2680 | 0.5309 |

Figures in parentheses are square root transformed values.
In a column, means followed by common alphabet (s) is / are not significantly different by LSD at P=0.05.

Jasmine growers were categorized as 'High' for those having TGI of 70 and above, 'Medium' and 'Low' having TGI between 40 and 70 and below 40 respectively.

The total number of buds in a plant and the pink discoloured buds were counted and the per cent incidence was worked out. The data on blossom midge incidence was transformed to x+0.5 and analyzed by randomized block design. The treatment mean values of the experiment were compared using Latin Square Distribution (LSD).

### RESULTS AND DISCUSSION

**Incidence of blossom midge of jasmine:** The incidence of blossom midge of jasmine was observed in all the ten districts of Tamil Nadu. However, the incidence was maximum in Madurai district, followed by Tirunelveli, Tuticorin, and Ramanathapuram districts which were at par. The hot weather in the region is likely to pose heavy incidence of midges. The lowest per cent incidence was recorded in Erode, Dindugal and Theni district, which were statistically on par (Table 1). Kanyakumari district and Coimbatore districts witnessed medium incidence of 26.64 per cent and 27.18 per cent. Thus, it is inferred that districts belonging to same weather belts recorded analogous trend of incidence of

blossom midge. Similar trend was noticed in the number of pink discoloured buds/plant in Madurai district recording the maximum infested buds per plant followed by Tirunelveli and Tuticorin districts, which were statistically at par. Hence, it is concluded that blossom midge density was high in Southern districts of Tamil Nadu and the hot weather in the region might be the probable reason for the pest buildup (Prasad and Logiswaran, 1997).

**Relative importance of blossom midge of jasmine and perception among jasmine growers:** Majority of respondents (76%) knew about blossom midge of Jasmine, but only 16 per cent ranked as the most important pest (Fig. 2). The respondents who ranked jasmine budworm as the most important pest were 94 per cent, whereas only 9 per cent perceived leaf webworm and 7 per cent recorded two spotted mite as a major problem. Nearly half of the respondents (64%) felt that the incidence of blossom midge is severe in May–August, medium (21%) in February to April, while the September–November was 15 per cent only (Fig. 3). Similar results were reported by Vanitha (2001) in Southern districts of Tamil Nadu.

**Technology Gap indices (TGI) on management practices of blossom midge among jasmine growers:** Jasmine blossom midge is an emerging major pest of jasmine. Cultural control measures like pruning of bushes (91%) and field sanitation (86%) were followed by majority of Jasmine growers, thus recorded lower TGI. This is due to the ease of the practice, which can be done while doing day to day field works. The important cultural practice of collection and

destruction of fallen and discoloured buds and flowers, which leads to removal of the pupae found inside the discoloured infested buds and flowers were not followed by most jasmine growers, recording a TGI of 68 per cent. Most growers are unaware of the biology of the pest that they pupate in the dried and fallen discoloured buds and flowers, indicating the lack of extension education programmes. The TGI was found high in other cultural/mechanical practices *viz.,* setting traps, regular weeding and raking of soil to expose pupa, as these operations are labor intensive, thus not commonly followed by the growers.

Regarding the usage of bio-control agents, growers were less educative and only an average of 10 per cent respondents use *Bt, Metarhizium anisopliae* drenching and *Chrysoperla* eggs with a TGI of 86 per cent. The main reason was the lack of awareness about biological control, biocontrol agents and their unavailability. Knowledge about use of botanical pesticides *ie.,* neem seed kernel extract or Azhadirachtin 5 % (5 ml per litre ) at bimonthly intervals and their usuage were adopted by 48 per cent of growers, lessening the tehnology gap. Raising of intercrops/border

crops and their role in attracting beneficials to naturally check the pest by providing pollen and nectar to the natural enemies and in turn getting additional income were un aware to the jasmine growers with a widened technology gap of 87 per cent.

In case of chemical control, comparatively medium TGI was observed, which is due to the ease of application and availability of chemicals. Jasmine growers chiefly rely on synthetic chemicals due to the quick recovery of pink discolouration and lack of awareness about environmentally safe bio control strategies. Regarding the frequency of application of chemicals, there is a long technology gap (91 per cent), as the lack of patience and swift reaction by the growers on finding even a pink bud by spraying indiscriminately without any proper interval. Farmers who send flowers to export market are spraying once in two, three and four days to minimize the appearance of pink buds mainly caused by blossom midge, in order to avoid their products from being rejected for export.

**Distribution of respondents:** Majority of the respondents (51%) belonged to the high technological gap category,

**Table 2.** Technological gap at jasmine grower's level in adopting recommended management practices for blossom midge, *C. maculipennis* of jasmine

(n=100)

| Particulars of practices | Respondents (%) | Technological gap (%) |
|---|---|---|
| **Cultural/mechanical practices** | | |
| Field sanitation with good drainage facilities | 86.00 | 14.00 |
| Collect and destroy fallen and discoloured buds and flowers | 18.00 | 82.00 |
| Setting of light traps | 27.00 | 73.00 |
| Setting of yellow sticky traps | 21.00 | 79.00 |
| Regular weeding | 41.00 | 59.00 |
| Regular tilling or raking of soil to destroy pupae | 15.00 | 85.00 |
| Non planting of alternate hosts around main cropped area | 11.00 | 89.00 |
| Pruning the bushes in winter | 91.00 | 9.00 |
| Planting inter/border crops | 13.00 | 87.00 |
| **Biological control** | | |
| Spray/ drenching of *Metarhizium anisopliae* @ 1x $10^8$ spores g$^{-1}$ | 10.00 | 90.00 |
| Spray *Bacillus thuringiensis* @ 2g/litre | 14.00 | 86.00 |
| Use of *Chrysoperla* eggs | 8.00 | 92.00 |
| Spray neem seed kernel extract or Azhadirachtin 5 % (5 ml per litre ) at bimonthly intervals | 48.00 | 52.00 |
| **Chemical control** | | |
| Thiocloprid  (Alanto® 240SC) @1 ml/litre | 47.00 | 53.00 |
| Profenophos (Curacron®50 EC) @ 2 ml/lit | 51.00 | 49.00 |
| Basal application of  carbofuran3G@30kg/hectare | 43.00 | 57.00 |
| Drenching the soil surface with chlorpyrifos 20EC @ 5ml/lit | 31.00 | 69.00 |
| Right  frequency of application of chemical pesticides | 9.00 | 91.00 |
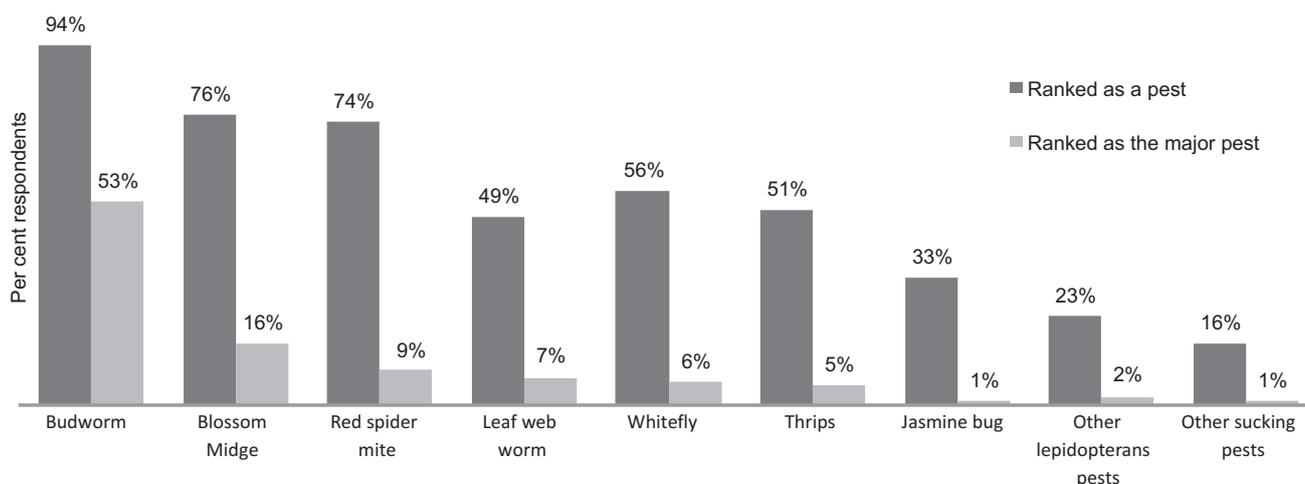
**Fig. 2.** Frequency distribution and ranking of Jasmine pests by jasmine farmers

whereas, 30 per cent under medium technology gap category. Only 19 per cent of the respondents were found in low level of technology gap. The adoption gap analysis clearly indicates that among the various practices recommended for the management of blossom midge of jasmine like application of chemicals and few cultural/mechanical practices with less complexity were more feasible and adopted. Several constraints *viz.,* unavailability of labors for carrying out cultural practices, lack of awareness about the use of bio-pesticides etc. leads to widening of technology gap among the usuage of environmentally safe pest management strategies. More or less similar findings were reported by Verma *et al.* (2003) and Singh *et al.* (2007).

It is therefore suggested that extension agencies should intensify their efforts to organize extension educational programs like trainings, demonstrations and field days to motivate the farmers to accept and adopt the IPM practices. In the extension programs, a special emphasis should be given to promote eco-friendly bio-control methods against blossom midge as well as other pests of jasmine by conducting skilled demonstrations and specialized participatory trainings.

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# Waterfowls Diversity of Morna Reservoir

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**Abstract**: Two years' study on waterfowl diversity revealed that the number and diversity of water fowls more than remaining group of birds (Grebes, Wader, Herons, Coot, Terns, Kingfishers, Storks, Ibis, Cormorant and Crane). The numbers of aquatic birds excluding waterfowls were negligible. This observation indicated that present water body was suitable feeding ground for water fowls. The availability of planktons and aquatic macrophytes helped to divert water fowls in present water body. But the present water body is under great pressure due to enhanced use of water for irrigation, washing of domestic cattle, washing of cloths, utensils and hunting of water birds by local peoples.

**Keywords**: Diversity, Morna reservoir, Water fowls

Water birds are commonly grouped into several categories based on their behaviors, Diving birds, Waterfowl, Wading birds, Shore birds, Gulls and Terns. Water fowls are majority ducks like Brahminy ducks, Pin Tails, Common teals, Shovellers, Common pochards, Spot bill ducks, etc. Water fowls feed at the centre of water during morning and evening on algae, insects, crustaceans, molluscan and fishes. Some water fowls are herbivores and some are carnivorous. In India several researchers have studied water birds such as Singh and Roy (1990), Sanjay (1993), Hosetti *et al*. (2001).

In South-East Maharashtra especially Sangli District large number of lakes, ponds and river Krishna and their tributaries are source of water. Majority ponds are manmade and lakes are constructed for irrigation purposes. The water remains throughout years only in the Krishna River, few ponds and lakes. Manmade ponds are expired; few lakes are dried from October to May get dried. In last few years, frequent drought situation in Sangli district have resulted in Morna reservoir. But the present water body is under great pressure due to much use of water for irrigation, washing of domestic cattle, washing of cloths, utensils and hunting of water birds by local peoples, therefore present work was under taken to observe the status of birds, their habitat and make awareness among peoples for conservation.

## MATERIAL AND METHODS

The Morna reservoir is an irrigation project on river Morna in Shirala Tehsil. River Morna is a tributary of river Warna in Krishna Basin. This reservoir is historical, ethno-cultural, and religious and irrigation important. It lies one Km West of Shirala. The reservoir occupies an area of about 85.5Km$^2$ and has an irregular octagonal outline. Actual length is 1015m. Height 31.2m and nature is shallow and deep with original Catchment area of 85.5 sq.km. The capacity of lake at flood reserve level is 20-74 cu.m. The capacity of live storage is 16-53 Mcu.m. The capacity of dead storage is 4-21 Mcu.m.

Line transect method is suitable for estimating density and abundance which involves moving along a fixed rout (transect) and recording the bird species seen and heard on both sides of transects. The bird count were carried out in the morning and evening. A binocular (Olympus) was used to confirm the identification of the birds. The identification of birds were done with help of Ali, Grimmettbook. The photography work was done with help of Canon (D) camera.

## RESULTS AND DISCUSSION

During present investigations 25 species of aquatic birds belonging to 4 orders (Ciconiformes, Anseriformes, Gruiformes and Charadriformes) and 11 families (Podicipedidae, Phalacrocoracidae, Ardeidae, Anatidae, Gruidae, Rallidae, Alcedinidae, Decelonidae, Ciconidae, Chariidae and Laridae). Figure 1 shows that family Anatidae was dominant than remaining families. The species number of order Ciconiformes was more than order Anseriformes but the population of order Ciconiformes was less in winter. Single species of Grebe, two species of Cormorant, three species of Herons, two species of Egrets, single species of Crane, Coot, Tern and Ibis. Three species of Storks and six species of Ducks were observed in winter during present investigation (Table 1). Out of 25 species, 5 were migratory, 12 resident migratory and 8 resident. The aquatic birds observed in the present investigations were grouped into Grebes, Cormorants, Herons, Egrets, Ducks, Cranes, Coots, Waders, Terns, King Fisher, Stork and Ibis.

Water fowls were dominant in the present water body which may be due to shallow nature of lake, full of water and

**Table 1.** Showing water birds groups, Scientific name and Status of water birds of Mornareservoir(2014 to 2016)

| Group | Common Name | Sc. Name | Order | Family | Bird Count | | Status |
|---|---|---|---|---|---|---|---|
| | | | | | 2014−15 | 2015−16 | |
| Grebe | Little Grebe | *Tachybaptus ruficollis* (Pallas) | Ciconiformes | Podicipedidae | 07 | 10 | R.M. |
| Carmorant | Greater Carmorant | *Phalacrocorax fuscicollis* | " | Phalacrocoracidae | 02 | 04 | R.M. |
| " | Little Carmorant | *Phalacrocorax niger* | " | " | 03 | 05 | R.M. |
| Herons | Indian pond Heron | *Ardeola gratii (Sykes)* | " | Ardeidae | 04 | 05 | R. |
| " | Purple Heron | *Ardea Pupurea (Linnaeus)* | " | " | 05 | 07 | R.M. |
| " | Night Heron | *Nycticorax nycticorax* | " | " | 05 | 04 | R. |
| Egrets | Little egret | *Egretta gorzetta* | " | " | 10 | 25 | R. |
| " | Large egret | *Ardea alba* | " | " | 15 | 20 | R.M. |
| Ducks | Brahminy duck | *Tadorna ferruginea* | Anseriformes | Anatidae | 02 | 04 | R.M. |
| " | Pin tail | *Anas acuta* | " | " | 15 | 20 | M. |
| " | Common teal | *Anas crecca* | " | " | 12 | 25 | M |
| " | Shoveller | *Anas clypeata* | " | " | 10 | 15 | M. |
| " | Common pochard | *Aythya ferina* | " | " | 03 | 05 | M. |
| | Spot bill | *Anas poecilorhyncha* | | | 20 | 30 | R.M. |
| Crane | Demoiselle crane | *Anthropoides virgo* | Gruiformes | Gruidae | 05 | 07 | M. |
| Coot | Common coot | *Fulica atra* | " | Rallidae | 200 | 245 | R.M. |
| Waders | Black winged stilt | *Himantopus himantopus* | Charadriformes | Chariidae | 05 | 07 | R. |
| " | Little ringed plover | *Charadrius dubius* | " | " | 05 | 10 | R.M. |
| Tern | Common tern | *Sterna hirundo* | " | Laridae | 02 | 05 | R.M. |
| Kingfisher | Small blue kingfisher | *Alcedo atthis* | " | Alcedinidae | 05 | 07 | R. M. |
| " | White breasted kingfisher | *Halcyon smyrnensis* | " | Decelonidae | 10 | 15 | R. |
| Stork | Painted stork | *Mycteria leucocephala* | Ciconiformes | Ciconidae | 05 | 10 | R.M. |
| " | White necked stork | *Ciconia episcopus* | " | " | 03 | 05 | R. |
| " | Open bill stork | *Anastomus oscitans* | Ciconiformes | Ciconidae | 06 | 08 | R. |
| Ibis | Black ibis | *Pseudibis Papillosa* | " | " | 03 | 03 | R. |

(R−Residence, RM.−residence migratory, M−migratory)
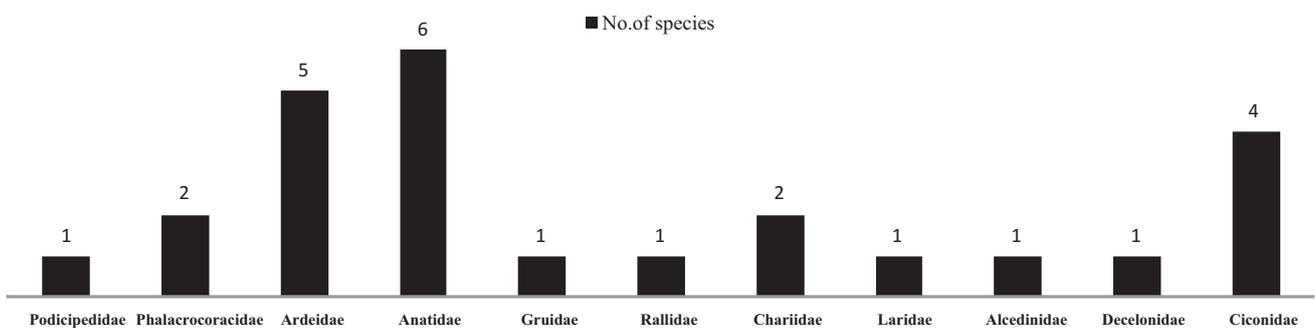


**Fig.1.** Graph showing dominant family of water birds (2014−2016)

**Spot bill duck**



**Black winged Stilt**



**Shoveller**



**Brahminy duck**



**Common Coot**



**River Tern**



**Demoiscelle crane**

**Fig.1.** Map of India showing study area
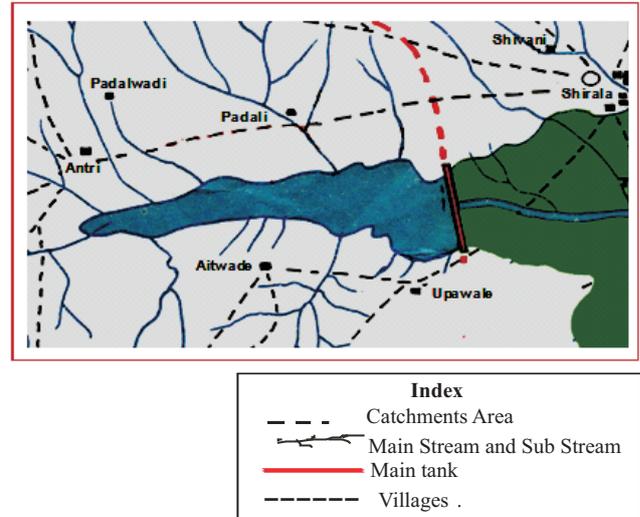


**Fig. 2.** Morna reservoir Dist. Sanli (M.S.) India

bulk of algae and zooplankton (Uttangi, 2001). Saikia (2005), reported that family Anatidae was dominant in winter at Assam Wetland. The population of species of order Ciconiformes was less and limited presence of Cormorant may be due to less polluted water body. In present investigation two species of Cormorant were observed. According to Dzika (2003), the population of Cormorants increased with progressing eutrophication and according to Dirksen *et al.* (1995). Cormorants can also be used in biological water management in relation to eutrophication. The Demoiselle Cranes was also observed. The population of Coot was remarkable due to large number of algae like Cyanophyceae, Bacillariophyceae, Chlorophyceae etc. Some species of wader were observed which is common occurrence in lake, Wetlands and marshy places. The regular occurrences of Kingfisher in the present water body indicated availability of plenty of fishes. Single species of Black Ibis was observed during present investigation. The regular occurrence of terns indicates that this water body is in good condition. The present water body is under great pressure and anthropogenic pressure affected the habitat of water birds.

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# Morphological Markers to Identify *Ceratophyllum demersum* L. and *C. muricatum* Cham.

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**Abstract:** *Ceratophyllum,* commonly known as coontail *is widely distributed genus of family* Ceratophyllaceae. It is the only genus of the family with a number of species of *rootless submerged freshwater macrophytes*. There are numerous species described in literature but the two species are most common (*Ceratophyllum demersum* L. and *C. muricatum* Cham). Both species are markedly distinct in their fruit morphology. Nevertheless, fruits are not always available in the field. But there is hardly any sharp distinguishing features available based on vegetative characters. Vegetative morphological distinguished characters are described to identify these two species of genus *Ceratophyllum*. A simple microscopic examination of bristle pair on leaf tip and angle of spine with leaf axis along the margin are two important characters that can be used as morphological markers to differentiate the two species of *Ceratophyllum* and in classification of these two species.

**Keywords:** Ceratophyllum, Classification, Bristle pair, Spines, Morphological markers

*Ceratophyllum* is the only genus of widely distributed family Ceratophyllaceae comprising of many submerged rootless species (Zuang 2013). Being a cosmopolitan genus, species exhibit huge morphological variations as a result a number of species have been described in it. *C. demersum* and *C. submersum* were described by Linnaeus. *C. muricatum* was described by Chamisso in 1829 whereas *C. echinatum* by Gray in 1837. Though, Lowden (1978) finally reduced all species of this genus to three species *viz*., *C. demersum* L., *C. submersum* L., and *C. muricatum* Chamisso (= *C. echinatum* Gray). A new species *C. tanaiticum* has been reported from the flora of Hungary (Csiky *et al*. 2010). In India, *Ceratophyllum* occurs throughout the country in abundance and three species have been recorded at different times. Hooker (1885) described *C. muricatum* Cham from India. Sehgal and Mohan Ram (1981) have reported that *C. echinatum Gray* is a new world species not previously recorded from India but the fact was ignored that it is synonymous to *C. muricatum Cham (Chamisso 1829; Gray 1837)*. Ramaprabhu (1964) and Datta and Roy (1983) have reported *C. submersum* L. from upper Gangetic plain. However, only *C. demersum* is recognized in India. There has been no attempt to identify the species properly, and any population of *Ceratophyllum* recognized by its dichotomously forked whorled leaves is readily labeled as *C. demersum*. Extensive field observations in the state of Rajasthan and neighboring areas revealed that *C. muricatum* (= *C. ehinatum*) is most commonly found in freshwater bodies (Kulshreshtha 1982; Kulshreshtha and Gopal, 1983) than *C. demersum*. *C. muricatum* was collected from Jaipur,

Bharatpur, Sawai Madhopur, Udaipur (Rajasthan) and Delhi while *C. demersum* was found only in Jaipur (downstream area of Jamwa Ramgarh). Presently *C. demersum* has been disappeared from Ramgarh reservoir which is completely dried today. There has been rather greater emphasis on the spines on seeds as a character for distinction between species and floral characters have not been studied in detail, creating all the confusion about its systematic. The objective of this paper is to clear the mist around the identity of *Ceratophyllum* in India, and to record distinguishing morphological and anatomical features in detail.

## MATERIAL AND METHODS

*C. demersum* L and *C. muricatum* both species are found in Rajasthan (Kulshreshtha, 1982). A survey was made in several water bodies in Jaipur district. Ten samples (30 cm length) of *C. demersum* were collected from Jamwa Ramgarh reservoir and its downstream areas whereas, ten samples of *C. muricatum* was collected in polybags from downstream areas of highly polluted water body near Mansagar reservoir. All samples were collected in the morning hours. Five surveys of both water bodies were made from July to November. Vegetative parts and male flowers were collected in the month of September whereas female flowers in October. Mature fruits of both the species were collected in the last week of November. The plant materials (leaves, stems, male flowers, female flowers and fruits) were selected 5 cm from shoot apex, cleaned thoroughly with water and fixed in formalin acetic acid (FAA). It was stored in 70% ethanol. Ten samples of each plant parts

of each species were used for block preparation. Thin sections were taken by using rotary microtome, stained, and observed under calibrated light microscope and the measurements of leaves were taken by a pre-calibrated ocular micrometer. Various morphological parameters viz., number, colour, size of leaves, spines, dichotomy of leaves, stem characteristics, number of anthers, size and colour of male flowers, color of female flower spines present on them etc. were observed in the field. However, micro techniques were followed for anatomical characteristics of leaves, stem, male and female flowers and fruits (Johansen, 1940).

## RESULTS AND DISCUSSION

A survey of several water bodies around Jaipur revealed that the two species occur in different habitats. *C. muricatum* is abundant in the water bodies generally polluted with domestic sewage (Kulshrestha, 1981, 1982; Gopal, 1990) while *C. demersum* is restricted to comparatively less polluted water bodies. It does not tolerate turbidity and is found only in small pockets of clear shallow water bodies. *C. muricatum* on the other hand, is able to grow in deep turbid water bodies by remaining near the surface. The habit of *C. demersum*is anchored submerged while *C. muricatum* is floating submerged (Kulshrestha, 1982).

**Morphology and Anatomy:** The mature plant of *Ceratophyllum* is rootless and is up to 1 m in length in undisturbed habitats. It consists of a long green cylindrical stem with whorls of leaves. The length of internodes varies from 10.0 mm to 35.0 mm in a 30 cm sample size. A long pointed bristle pair is present at tip of the leaf (Metcalfe and Chalk, 1950) whose base is broad convergent (Fig. 3 a) in *C. demersum* and narrow and divergent in *C. muricatum* (Fig. 3b). The angles of spines measured by protector in 10 samples are 60˚ and 30˚ with the leaf axis along the margin in

*C. demersum* and *C. muricatum*, respectively measured using protector (Fig. 3d and f*)*. Spines are conspicuous in the former species while they are inconspicuous in the latter. The air spaces in the leaves are more prominent and can be seen with unaided eye in *C. muricatum*. However, these are inconspicuous in *C. demersum*. White, small, truncate, apiculate (with tanniferous oil glands) anthers are present in *C. muricatum* whereas light purple, large, bicuspidate (with tanniferous oil glands) anthers are present in *C. demersum*. There is a difference in the color and size of style. They are magenta, 3–3.8 mm long in the former species whereas white, 0.8–1.0 mm long in the latter species. Mature fruit of *C. demersum* is dull green colored in fresh and dried specimen, almost triangular compressed biconvex with three spines, two basal lateral obtuse spines and one dorsal persistent style. The surface is smooth with entire margin (Fig. 8). The fresh fruit of *C. muricatum* is magenta colored which turns grayish black in dried fruit material. It is deep biconvex, oval with granular surface with winged and toothed margins. Besides two acute basal spines, few more lateral marginal spines (4–8) are also present in *C. muricatum* which form the characteristics features of the species. The length of lateral marginal spines is variable which gives sometimes the appearance of wavy winged margin only. Morphological (vegetative as well as floral) differences between two species of *Ceratophyllum* are summarized in Table (1 & 2).Thus two species of the genus *Ceratophyllum* presently reported from India can be distinguished in the following way:

**Key to the species**

1a. Leaf forked twice dichotomously, 4–6 leaf segments in number; Spines at 60˚ angle with the leaf axis along the margin, a long pointed bristle pair with broad convergent base at tip of each leaf, staminate flower, large, 3–5 mm, stamens purple, 15–20, anthers bicuspidate; style white,

**Table 1.** Morphological (vegetative) difference between two species of *Ceratophyllum*

| Character | Differences between *C. demersum* and | Species *C. muricatum* |
|---|---|---|
| **Leaf** | | |
| Color | Dark bottle green | Parrot green |
| Length | 17–20 mm | 22 to 30 mm |
| Order of dichotomy | Twice forked with 4–6 ultimate leaf segments, never more than 6 | Thrice forked with leaf segments 8–10 |
| Bristle pair on leaf tip | Base of the bristle teeth is broad and convergent | Base of bristle teeth is narrow |
| Spines | Very conspicuous, spines are at 60˚ with the leaf axis along the margin (Fig. 3, d). | Spines are at 30˚ angle with the leaf axis along the margin (Fig.3 e) |
| **Stem** | | |
| Color | Dark green pink at tip and nodes | Parrot green throughout the length |
| Thickness | Solid | Hollow |
| Cortex | 2–3 mm | Less than 2 mm |
| Air lacunae | 14–16 celled | 3–4 celled thick |

**Fig. 1.** *Ceratophyllum muricatum* Cham. (left) and *C. demersum* L. (right)

0.8–1.0 mm long, fruit dull green compressed, triangular, margin entire, spines, dorsal 2 forming an obtuse angle, persistent, lateral marginal spines absent.

                    −*C. demersum*

1b. Leaf forked thrice dichotomously, 8–10 leaf segments in number; Spines are at 30˚ angle with the leaf axis along the margin, a long pointed bristle pair with broad convergent base, staminate flower small, less than 2 mm,

stamens white, 4–10, anthers truncate; style magenta, 3–3.8 mm long fruit marginal spines 3–7.

                    −*C. muricatum*

**Germination of the seed:** *C. demersum* orms only 20% seeds in comparison to *C. muricatum*. The seedlings of *C. demersum* have never been seen in the field whereas latter produces large number of seeds which germinate after the first rains in May–June. Seedlings of *C. muricatum* have simple lobed first plumule leaves. *C. demersum* and *C. muricatum* are widely distributed throughout the world among other species (Linnaeus 1753, 1763; Chamisso 1829). These species have been classified mainly on the basis of leaf dichotomy and the absence or presence of spines on the fruits. According to Sehgal and Mohan Ram (1981) a characteristic features of stamens in both the populations is that there is no distinction between filament and anther. They have also noted that the apex bears a float which is also tipped with a tanniferous emergence and lateral teeth in both the populations. Longitudinal section of the anther also shows the presence of bristle pair on the upper surface of the anther of *C. demersum* (Fig. 7b). *C. echinatum* exhibited variation in first epicotyl leaf and possessed forked leaves at all the nodes of plumule whereas *C. demersum* has simple leaves at first node of plumule (Les, 1985). In the past, the species of *Ceratophyllum*

**Table 2.** Morphological (reproductive) differences between two species of *Ceratophyllum*

| Character | *C. demersum* | Species *C. muricatum* |
|---|---|---|
| **Staminate flower** | | |
| Color | Light–purple | White |
| Size | 1–2 mm | Less than 1 mm |
| Perianth | Base of bristle pair on perianth tip is broad and convergent | Base of bristle pair on perianth tip is narrow and divergent. |
| Anther | 15–20 sub sessile | 4–10 |
| Color | Light–purple | White |
| Size | 1–2 mm | Less than 1 mm |
| Shape | Elliptical, bicuspidate bristles absent | Oblong, apiculate, truncate |
| **Pistillate flower** | | |
| Color | White | Magenta |
| **Ovary** | | |
| Length | 3–4 mm | 2–3 mm |
| Color | White | Magenta |
| Length | 8–10 mm | 3–4 mm |
| **Fruit** | | |
| Color | Dull green | Magenta turns, grayish black in dried material |
| Surface | Smooth | Tubercle |
| Number, position of spines | I apical (long), 2 basal lateral spines (long) forming an obtuse angle | 1 apical (short) 2 basal lateral spines (short) forming an acute angle and of varied length |
| Margin | Entire | winged or toothed |

were formed on the basis of fruit spines which later proved to be highly variable character and needs to be verified in fresh specimens (Hooker 1885, Lowden 1978) and therefore it is a non-reliable character for the classification of the genus.



**Fig. 2.** Leaf of *Ceratophyllum muricatum* Cham. (Left) and *C. demersum* L. (right)
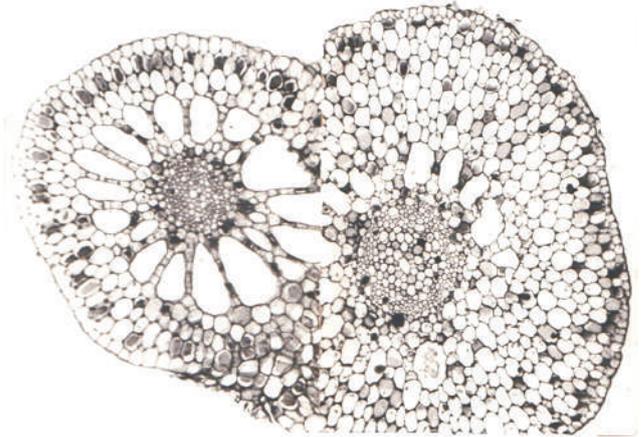


**Fig. 4.** T.S. of stem of *C. muricatum* and *C. demersum*. Large air cavities are seen in the former species
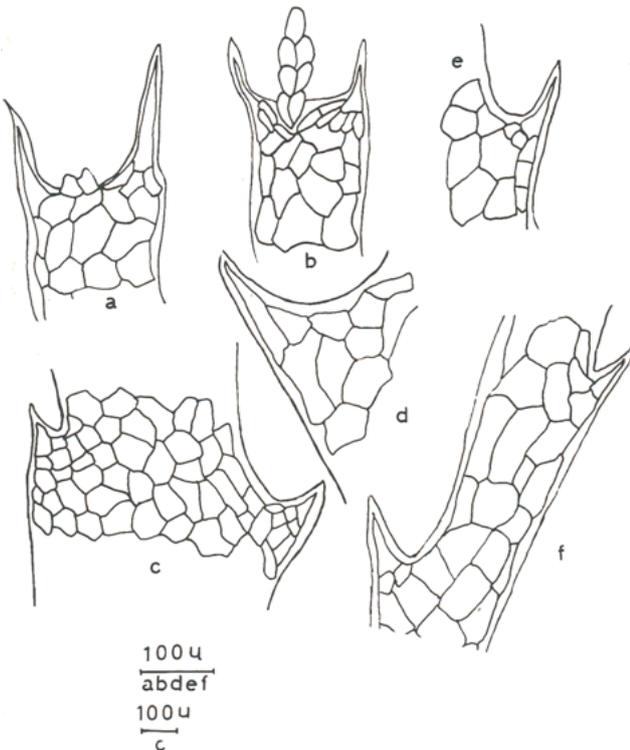


**Fig. 3.** Tip of *Ceratophyllum demersum L.* with broad convergent (a) and *C. muricatum* (b) 60˚ angle with broad base along the leaf axis at the margin in C. *demersum* (c &d) 30˚ angle with narrow base along the leaf axis at the margin in C*. muricatum* (e & f)



**Fig. 5.** Small male flowers of *C. muricatum* with 4–10 anthers and large *C. demersum* with 15-20 anthers
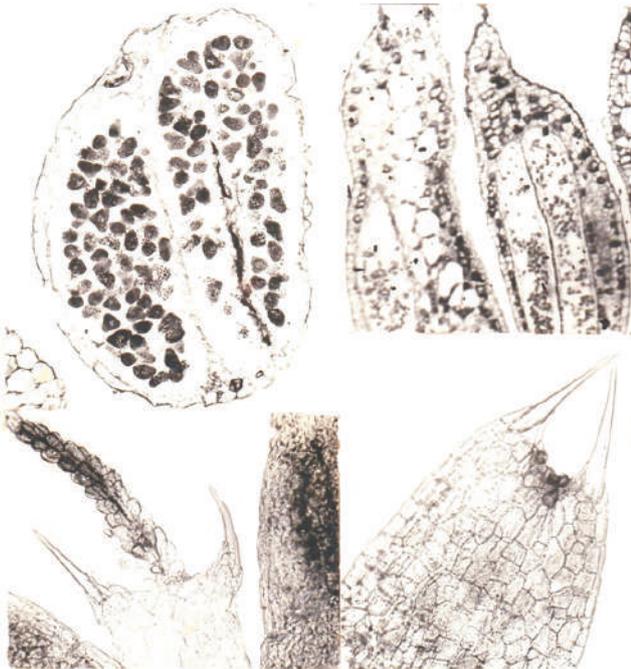
**Fig. 6.** Apiculate anthers of *C. muricatum* (top, left)Base of bristle pair on perianth tip is broad and convergent (bottom, left) Bicuspidate anthers of *C. demersum* (top, right)Base of bristle pair on perianth tip is narrow and divergent (bottom, right)



**Fig. 7.** Fruit of *C. demersum* showing three spines of equal lengths (top, left) Fruits of *C. muricatum* showing variation in length of lateral and marginal spines



**Fig. 8.** Microtome section of fruit of *Ceratophyllum demersum* (left) and C. *muricatum* (right)

The classification of the genus *Ceratophyllum* is based on the variable spinal character of the fruit whose persistence in the dried specimen is doubtful and has resulted in a number of species, a few of which were later found to be synonymous with each other. Sehgal and Mohan Ram (1981) reported that the anthers in both the species are apiculate with tanniferous emergence bearing a pair of basal teeth. However, in this study bicuspidate anthers were observed only in *C. demersum* and not in *C. muricatum*. This is in conformity with the observations of Wight (1853) and Hooker (1885). The broad angles of spines (teeth) of leaves of *C. demersum* make them hard and conspicuous. Color of male and female flowers of both species are also entirely different and enough to identify the species. The apical portions of leaves of both species under microscope show clear cut demarcation in their morphological features on the basis of which correct identification can be done. With these differences in vegetative as well as floral characters there is a need to identify each of the available specimens correctly to know its distribution on the globe.

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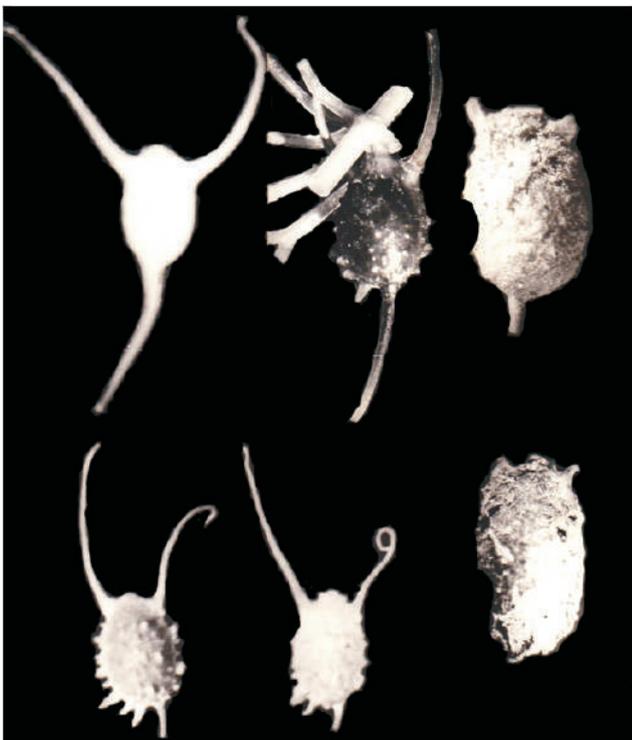
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# Effect of High Pressure Processing and Thermal Treatments on Physical, Chemical and Microbiological Characteristics of Silver Pomfret (*Pampus Argenteus*) Fillets

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**Abstract**: This study was undertaken to assess the quality of fresh silver Pomfret *(Pampus Argenteus)* fillets during thermal treatment at 60, 70, 80 and 90°C and high pressure processing (HPP) at 200, 300, 400 and 500 MPa for 5, 10 and 15 minutes atan ambient temperature (25±2°C). The thermal treatment increased the pH, drip loss, imparted cooked appearance to the muscle and resulted in improved texture. Thermal treatment and HPP significantly reduced the microbial counts. HPP above 200 MPa resulted in firmer muscles with higher hardness. Moreover, elevated temperature and pressures increased the hardness of Pomfret fillets. HPP results in less change in colour as compared to thermal treatment. Thermal treatment of 80°C for 5 minutes and HPP of 400 MPa for 5 minutes was most effective in preserving the quality and the extending the shelf life of fresh silver Pomfret.

**Keywords**: High pressure processing, Microbial analysis, Silver Pomfret fillet, Thermal treatment

Silver Pomfrets (*Pampus argenteus*) are perciform fishes found along the coast of China from the Bohai Sea to the South China Sea and in Bay of Bengal, Arabian Gulf, and the Northern Sea. Pomfret contributes to one of the main groups of table fish consumed in India and fetch the highest price. The shelf life and quality of Pomfret fish should be preserved from bacterial contamination due to the fact that the temperature during the smoking of it never exceeds 28°C. Therefore, thermal treatment is the existing method to achieve microbial stability and safety. Although this technology is effective, economical and readily available, in many cases it has undesirable effects on food quality. The high temperature may cause quality deterioration, such as degradation in colour and texture and rendering the products less attractive to most consumers. The changes in nutritional quality in terms of colour, texture and microbial quality occur during thermal processing. High pressure processing (HPP) is a novel technology, which represents a non-thermal preservation technique for various food materials including sea foods. In this technology, the pressure is transmitted instantaneously and uniformly throughout a system regardless of the shape of packaging or volume of product. HPP can also be used by the food industry to create new product textures since it induces modifications on food functional properties. HPP treatments have also the ability to inactivate spoilage and pathogenic microorganisms, thus extending food shelf life.The effects of HPP on various food materials have been studied by several authors (Yagiz et al., 2007; Huang et al., 2011; Kaur et al., 2012; Chouhan et al., 2015). Fish freshness is traditionally judged with chemical index such as pH. Colour is one of the main organoleptic characteristics used to establish the quality and acceptability of food products. Most fish species, like fillet fish has a dark pink/red colour and contains a considerable amount of myoglobin similar to red meats. The texture is one of the important quality parameters affecting the consumer acceptability of a food item. Further, the various parameters analyzed were hardness, cohesiveness, gumminess, springiness, and chewiness. Hardness is probably the most critical texture attribute in meat or seafood products and can be defined as a property, which depends on connective tissues consisting of mainly collagen and myofibrils, myosin and actin. Trimethylamine (TMA) and Total Volatile Basic Nitrogen (TVB-N) are reported to be good indicators of freshness in case of fishes

The total microbial count is an important criterion for evaluation of fresh and frozen seafood products. Landed fish harbors a large number of microorganisms on its body. The most commonly used method for testing the raw material bacterial quality is the total viable count. Total aerobic

bacterial counts had been used by many investigators to follow the deterioration of fish flesh and shellfish flesh. Therefore, this study was undertaken to analyze the quality of Silver Pomfret Fillets fish in terms of various physical (moisture content, colour, drip loss, and texture), chemical (pH, TVB-N, and TMA) and microbiological (total plate count) parameters during thermal (60, 70, 80 and 90 ˚C) and HPP (200, 300, 400 and 500 Ma) for 5, 10 and 15 minutes.

## MATERIAL AND METHODS

**Raw Material and Sample Preparation:** Fresh Silver Pomfret fishes were collected directly from local market of Kharagpur in West Bengal, India. The fish specimens were brought in chilled condition (4 ˚C) to the IIT Kharagpur laboratory and were stored in the freezer at -25 ˚C over night and then dressed and washed with chilled water and filleted the next morning. The fillets were put in Low Density Polyethylene bags and vacuum packed and frozen stored at -25 ˚C until processing. Thermal processing has mainly two dimensional processes such as time and temperature are two process variables. Packed fillets were thermally treated in an autoclave with the water bath at 60, 70, 80 and 90 ˚C for 5, 10 and 15 minutes and samples were stored at 4 ˚C until the complete analysis of micro-biological and physicochemical parameters. HPP was carried out in laboratory mode with high pressure equipment (Model, S-IL-100-250-09-W; Make: Stansted Fluid Power, UK). Fillets were treated at pressure levels of 200, 300, 400, and 500 MPa for 5, 10 and 15 minutes at ambient temperature (25±2 ˚C) using a ramp rate of 300 MPa/min and decompression time of 15 seconds. After processing, samples were stored at 4 ˚C for 30 days in refrigerator and analysis for microbiological, chemical and physical parameters was done.

**Determination of Microbiological and Physicochemical Analysis**

**Moisture content:** It is determined by 5 grams of samples (fresh silver Pomfret fillets) in a moisture box at temperature of 105 ˚C for 24 hours.

$$\text{Moisture Content } (\%, \text{w. b.}) = \frac{w_1 - w_2}{w_1} \times 100 \qquad (1)$$

$w_1$ = weight of sample (g) and $w_2$ = weight of dry sample (g).

**pH:** The pH is determined using a standard portable pH meter (Model, CL 46+; Make: Toshcon Industries Pvt. Ltd.). A 5 gram fish fillet sample was thoroughly pasted in the mortar by pestle and mixed with 25 mL of distilled water for 30 seconds.

**Colour measurement:** Colour of Pomfret fillets after removing from polyethylene bags were measured using Portable Colorimeter (Model: Spectro-guide 45/0 gloss; Make: BYK Gardner, Germany) in a reflection mode. Colour was expressed in L*(brightness), a*(+a, red; -a, green), and b* (+b, yellow; -b, blue), Colour difference *ΔE**, denotes the square root of $(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})$.

**Drip loss:** The weight of raw sample ($m_1$) and treated sample ($m_2$) was recorded to calculate drip loss. Samples were then packed in vacuum sealed pouches. The samples after treatment were removed and blotted with tissue paper to remove the surface drip and were weighed. The percentage of cooking loss is calculated as:

$$\text{Drip Loss } (\%) = \frac{m_1 - m_2}{m_1} \times 100 \qquad (2)$$

**Texture Profile Measurement:** Texture parameters were measured with a texture analyzer TA.XT2 (Stable Micro Systems, Surrey, UK) equipped with a 25 kg load cell. Before analysis, raw and heated samples were kept for 1 hour at room temperature. Pomfret fillet was compressed by 30% of its original height using a cylindrical probe of 6 mm diameter(SMSP/6) with speed of 2 mm/s. The various parameters represent the texture of food product was hardness (g), cohesiveness, gumminess (g), springiness, and chewiness (Kaur et al., 2009). The hardness is defined as the force necessary to attain a given deformation (30% deformation); cohesiveness, defined as the extent to which material could be deformed without rupturing; gumminess (g) = hardness * cohesiveness; springiness, the extent to which a material returns to its original condition after the deformation force is removed and calculated; chewiness (gumminess * springiness) is defined as the energy required to disintegrate material to a state ready for swallowing.

**TVB-N and TMA Analysis:** TVB-N and TMA were determined by Conway micro-diffusion method as proposed by Kaur et al. (2012). The results were expressed in mg/100 g of sample. A sample of 5 g fillet was taken and it was homogenized with 10 mL of 20% trichloroacetic acid and filtered using Whatman filter paper no. 1 and make up the volume to 50 mL in the volumetric flask. 1 mL of sample and 1 mL of saturated potassium carbonate was pipetted into the outer ring of micro diffusion unit and 2 mL of a methyl red and bromocresol green indicator in boric acid solution in its inner chamber. The unit was covered and sealed using silica grease and kept it in an incubator for 90 min at 37 ˚C, then after the inner ring solution is turned to green colour, and it is titrated with 0.02 N sulphuric acids until pink colour attained. The average of three titration volume was taken for calculating the TVB-N value.TMA of Pomfret fillet was determined in the same way as TVB-N determination, but prior to the addition of potassium carbonate, 1 mL of formalin was added to the extract to react with ammonia and thus

allow only the TMA to diffuse over the unit.

$$\text{TVB (mg per 100 g sample)} = \frac{\text{mL of titrant x 0.014x Normality of titrant}}{\text{weight of sample (g)}} \text{ x 100} \quad (3)$$

**Microbial quality:** Analysis of total microbial quality was done according to the method of plate count (Chouhan et al. 2015). 10 g sample was cut from fillet sample and macerated with 90 mL (N Saline) diluents, i.e. 1 part of the sample with 9 parts of diluents. This gives 10 times dilution of the sample. For further dilution, 1 mL from $10^{-1}$ dilution mixed with 9 mL of the diluents ($10^{-2}$ dilution). This type of dilution called *serial decimal dilution*. 1 mL of suitable dilution was spread plate on tryptone glucose agar and keep in an incubator at 37 °C for 36-48 hours. Results were expressed as the log of a colony forming unit per gram (cfu $g^{-1}$) of the sample.

**Optimization:** The optimization was done in Design Expert–version 7.0.0 (Stat-Ease, Minneapolis, USA) software. The software was used to generate optimum processing conditions and to predict the corresponding responses as well (Table 1 and 2). Independent process variables considered were temperature (60-90 °C at the processing time of 5-15 min) for thermal processing and pressure level (200 to 500 MPa at the processing time of 5-15 min) for HPP. Dependent process variables are total plate count (maximum inactivation), colour values (minimal changes), and hardness (minimal changes). The optimized thermal treatment condition obtained on the basis of maximum microbial inactivation and minimal changes in colour and minimal changes in hardness was 80 °C for 5 min (Table 3). Similarly, for optimization of processing conditions for HPP, twelve experiments were carried out. Based on the similar criteria as for thermal treatment, for HPP optimized condition obtained was 400 MPa for 5 min (Table 4).

**Table 1.** Numerical optimization of thermally treated process parameters

| Variable | Goal | Lower limit | Upper limit | Lower weight | Upper weight | Importance |
|---|---|---|---|---|---|---|
| Temperature (°C) | It is in range | 60 | 90 | 1 | 1 | 3 |
| Time (min) | It is in range | 5 | 15 | 1 | 1 | 3 |
| Colour difference | Minimize | 7.68 | 12.02 | 1 | 1 | 2 |
| Microbial count (log10 cfu $g^{-1}$) | Minimize | 2.69 | 5.03 | 1 | 1 | 3 |
| Hardness (g) | Minimize | 222 | 275 | 1 | 1 | 1 |

**Table 2.** Numerical optimization of HP process parameters

| Constraint | Goal | Lower limit | Upper limit | Lower weight | Upper weight | Importance |
|---|---|---|---|---|---|---|
| Pressure (MPa) | It is in range | 200 | 500 | 1 | 1 | 3 |
| Dwell time (min) | It is in range | 5 | 15 | 1 | 1 | 3 |
| Colour difference | Minimize | 6.12 | 8.21 | 1 | 1 | 2 |
| Microbial count (log10 cfu $g^{-1}$) | Minimize | 0.78 | 4.1 | 1 | 1 | 3 |
| Hardness (g) | Minimize | 314 | 605 | 1 | 1 | 1 |

**Table 3.** Optimized solutions for thermally treated process parameters

| Temperature (°C) | Time (min) | Colour difference | Microbial count (log10cfu $g^{-1}$) | Hardness (g) | Desirability |
|---|---|---|---|---|---|
| 80 | 5 | 10 | 3.88 | 360 | 0.476 |
| 90 | 5 | 10.48 | 3.3 | 424.5 | 0.465 |
| 70 | 15 | 10.5 | 3.8 | 375 | 0.438 |
| 80 | 10 | 10.6 | 3.6 | 420 | 0.418 |
| 60 | 15 | 10 | 4.3 | 279 | 0.415 |
| 70 | 10 | 10.1 | 4.2 | 350 | 0.403 |
| 70 | 5 | 9 | 4.5 | 290 | 0.400 |
| 90 | 10 | 11.5 | 2.9 | 445 | 0.330 |
| 60 | 10 | 9.2 | 4.7 | 260 | 0.317 |
| 80 | 15 | 11.4 | 3.2 | 450 | 0.314 |
| 60 | 5 | 8 | 5 | 230 | 0.110 |
| 90 | 15 | 12 | 2.7 | 470 | 0.086 |

## RESULTS AND DISCUSSION

### Effect of Thermal Treatment and HPP on Quality of Silver Pomfret Fillet

### Physical Parameters

**Moisture Content:** On thermal treatment in the water bath, the heating caused moisture to decrease 80.2–77% by weight with increased temperature and processing time (Fig. 1a). Bell et al.(2001) reported that the loss of water during cooking occurred as a result of changes in both myofibrillar and collagen muscle proteins and thermal denaturation of muscle proteins is the primary mechanism leading to the moisture loss. The moisture content of Pomfret fillet decreased after HPP as compared to control sample (Fig. 1b). High pressure samples presented a small reduction in moisture content immediately after pressure treatment, most probably due to protein conformational changes induced by the high pressure (500 MPa). Similar results have been reported by Ramirez–Suarez and Morrissey (2006) for albacore tuna *(Thunnsalalunga)* minced muscle pressurized at 275 and 310 MPa for 2, 4 and 6 min.

**Colour:** The colour changes and change of $L*$, a*, b* and $\Delta E*$ with heating time. During heating, the muscle colour underwent a two-phase change: a rapid whitening phase followed by a slow browning phase (Fig. 2a). A higher L* value indicates a lighter colour, which is desirable in order to ensure that the meat products will have high consumer acceptance (Resurreccio'n, 2003). Pomfret fillet samples both control and pressure treated at different pressure levels and holding times (200, 300, 400 and 500 MPa for 5, 10 and 15 min) turned as the red/pink colour immediately after HPP. The pressure treated Pomfret fillets generally lost their transparency and their colour became lighter. L* values of all samples pressure treated above 200 MPa for l5 min, increased with an increase in pressure level and duration of pressure treatment (Fig. 2b). Chevalier et al. 2001 also reported that high pressure induces modifications in the appearance of fish muscle which generally became whiter when a pressure level of more than equal to 100 MPa was

**Table 4.** Optimized solutions for HPP

| Pressure (MPa) | Dwell Time (min) | Colour difference | Microbial count (log10cfu g$^{-1}$) | Hardness (g) | Desirability |
|---|---|---|---|---|---|
| 400 | 5 | 6.7 | 2.015 | 435.5 | 0.634 |
| 300 | 10 | 6.8 | 2.605 | 402 | 0.576 |
| 400 | 10 | 7.1 | 1.8 | 470.5 | 0.566 |
| 300 | 5 | 6.5 | 3.05 | 380 | 0.540 |
| 300 | 15 | 7.3 | 2.255 | 430 | 0.537 |
| 500 | 5 | 7.4 | 1.35 | 517.5 | 0.489 |
| 400 | 15 | 7.5 | 1.6 | 500 | 0.483 |
| 200 | 15 | 6.9 | 3.5 | 400 | 0.388 |
| 200 | 10 | 6.6 | 3.7 | 352 | 0.370 |
| 500 | 10 | 7.8 | 0.99 | 559.5 | 0.349 |
| 200 | 5 | 6.37 | 4 | 321.5 | 0.223 |
| 500 | 15 | 8.2 | 0.8 | 601 | 0.063 |
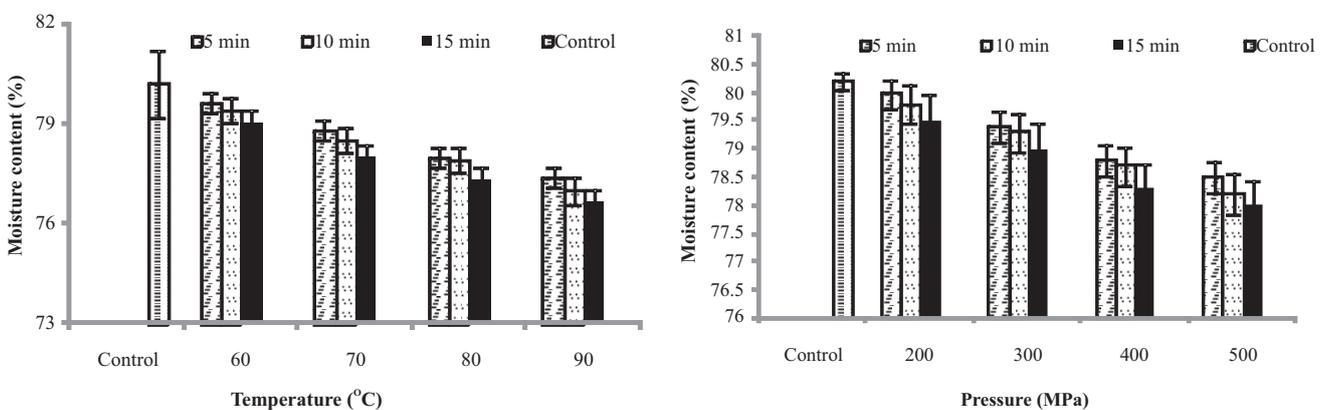


**Fig. 1(a-b)**. Effect of thermal treatment and pressure on moisture content of Pomfret Fillets

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applied. a* values, normally used as an index of visual redness, which decreased as pressure increased (Fig. 2b). The b* values increased with pressure and holding time for the sample treated at 200, 300, 400 and 500 MPa for 5, 10, 15 min and above (Fig. 2b). It is concluded that the HPP treated sample showed increased L* and b* values and decreased a* values as compared to an untreated sample. Similar results were obtained by Kaur et al. (2012) in black tiger shrimp.

*Drip Loss:* The changes in drip loss of Pomfret fillet affected by different processing conditions. The thermal treatment increased drip loss in all samples immediately after treatment and the maximum drip loss were obtained in the sample treated at 90 °C for 15 minutes (Fig. 3). A similar finding had been reported by another researcher of drip loss with the increased processing temperature (Kaur et al., 2009).Cooking induces denaturation of myosin and shrinkage of myofibrillar, sarcoplasmic proteins, and solubilization of the connective tissue, which decreases the

water holding capacity of the meat (Garcia–Segovia et al., 2007).The changes in drip loss of Pomfret samples affected by different pressure treatments and holding time. Comparing the drip loss in control samples and pressure treated samples, it can be concluded that HPP increased the drip loss in all samples immediately after pressurization. Samples treated at 200, 300, 400 and 500 MPa for holding time 5, 10 and 15 min.Kaur et al. (2009) also suggested that drip loss in pressure treated samples could be one of the reasons behind the increased in the lightness of samples.

*Texture Profile Analysis:* The temperature had a small effect on springiness and cohesiveness of the fish fillets. Treated sample showed higher values of gumminess and chewiness as compared to control sample. This changes may be due to the denaturation of collagen fibers occurred around 58–65 °C (Fig. 4a). It has been reported that protein denaturation rate increased about 600–fold for every 10 °C in temperatures and subsequently more rapid textural
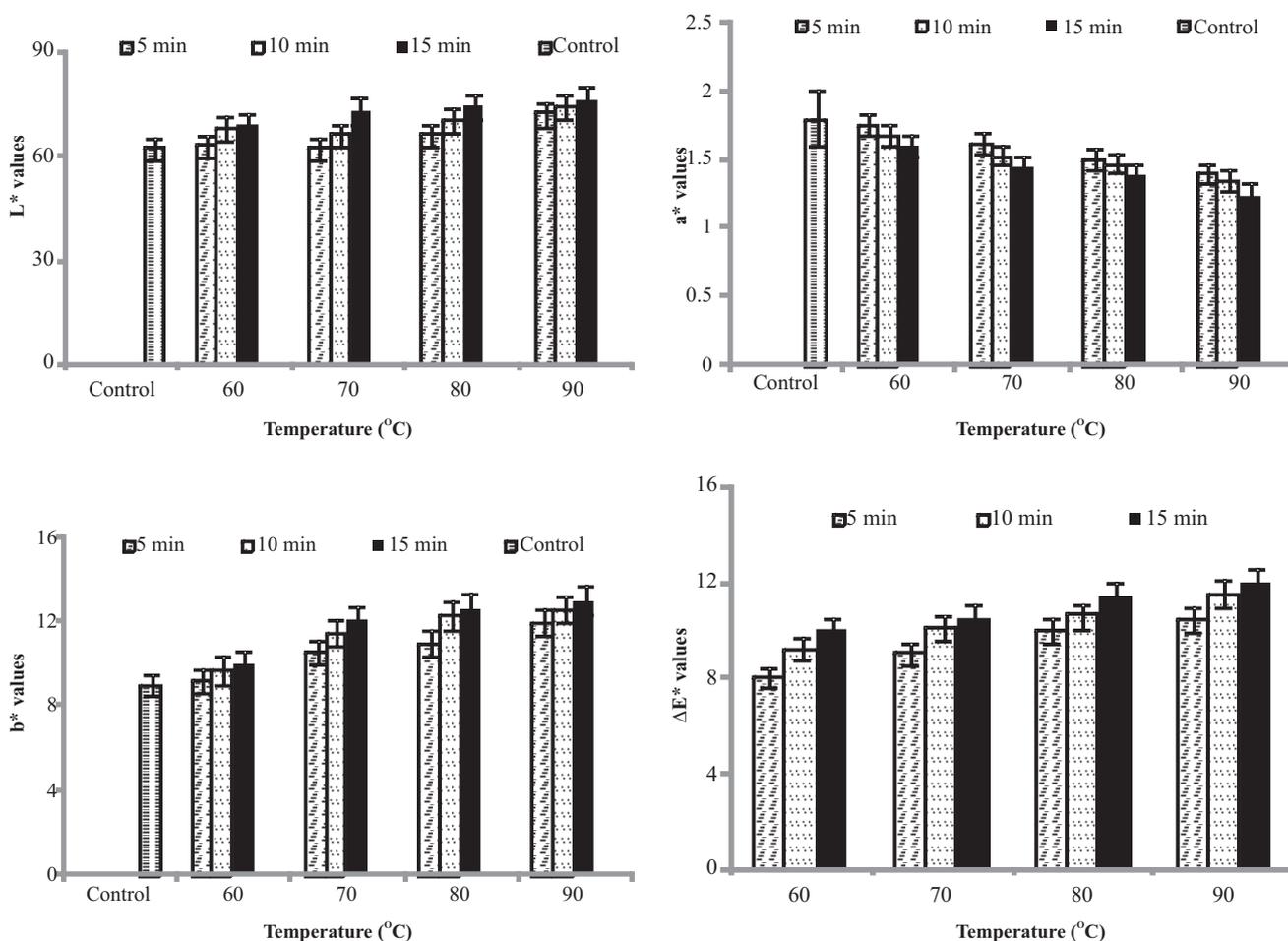


**Fig. 2a.** Effect of thermal treatment on L*, a*, b* and $\Delta E^*$ values of Pomfret Fillet

changes. The pressure increased the hardness of all samples in all applied conditions, and higher pressure levels and holding times resulted in harder muscle tissue. Typical results of springiness changes were also similar to the hardness changes. Pressure treatment 200 MPa and its above increased the springiness in fish tissue (Fig. 4b).These results are in agreement with the findings of Kaur et al. (2012) who studied texture profile changes of black tiger shrimp treated at 100 to 435MPa.

**Chemical Parameters**

**pH:** The pH of pomfret fillet showed the slight increase as compared to control after thermal processing, both with the temperature and processing time (Fig. 5a). The change in pH value of meat during heating was likely a result of the
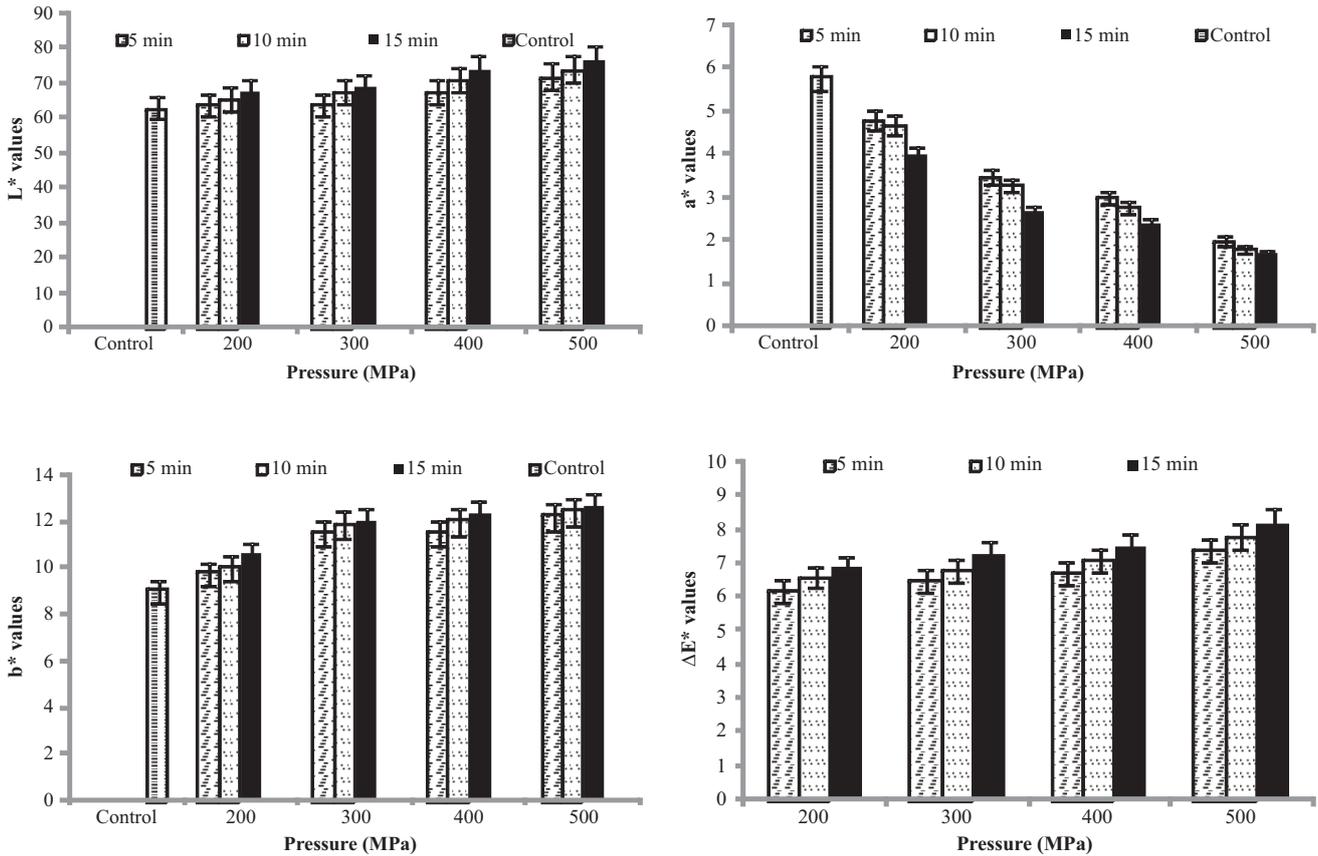


**Fig. 2b.** Effect of HPP on L*, a*, b* and $\Delta E^*$ values of Pomfret Fillet
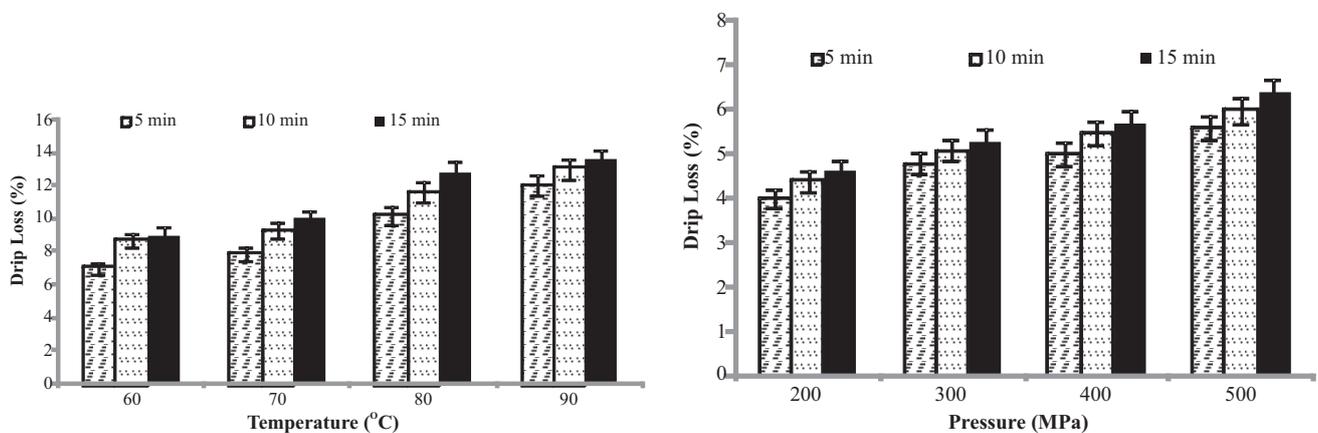


**Fig. 3.** Effect of thermal treatment and HPP on drip loss of Pomfret Fillet

dynamic balance of acid‑base groups at the surface of sarcoplasmic proteins (Huang et al., 2011). The pH values above 6.53 were attained in samples pressure treated at 200 MPa for 5 min (Fig. 5b). Generally, in aerobically stored refrigerated fish, the increase in pH is due to TMA reduction by spoilage bacteria and autolysis to TMA, which follows by conversion of TMA to other basic volatiles and further decomposition.

**TVB‑N and TMA:** TVB‑N and TMA‑N are used as a biochemical index to determine keeping quality and shelf life of fresh fish and seafood. TVB‑N and TMA‑N were determined according to the Conway micro‑diffusion method as proposed by Chouhan et al. (2015). Triplicate analyses were performed on all samples and the results were expressed in mg‑N/100 g of sample. The thermal treatment at 90 °C for 15 min holding time had the highest impact in reducing both the TVB‑N and TMA values as compared to control and other temperatures (60 °C, 70 °C, and 80 °C). The
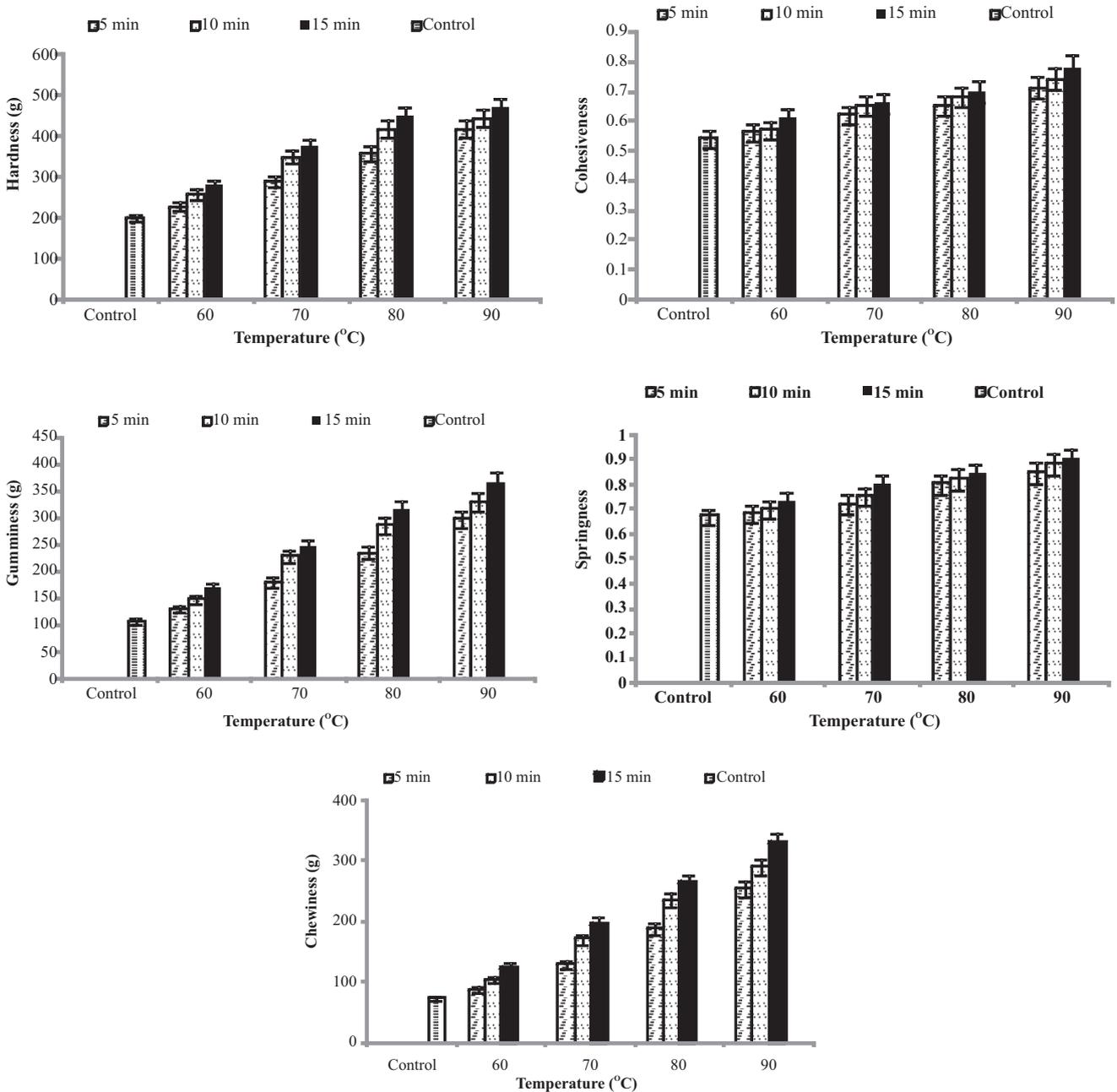


**Fig. 4a.** Effect of thermal treatment on texture profiles of Pomfret Fillet

gradual decrease in volatile bases measured as TVB‑N or as individual amines (TMA) has been observed by comparing the control sample and the final canned product (Fig. 6a). The rate of TVB‑N increase in the control (untreated) sample was more pronounced than in the pressure‑treated samples. A limit of 30 mg/100 g of muscle tissue has been considered as the upper limit above which seafood products are considered

stale. Within this limit, fish is considered normal and fresh and above this limit up to 40 mg/100 g fish is unsuitable for human consumption. The pressure treatment at 220 MPa for 30 min and 15 min holding times had the highest impact in reducing the TVB values, while the HPP treatment at 150 MPa for 30 min and 15 min holding times had the least effect in reducing TVB‑N values (Fig. 6b). Overall, from the TVB‑N perspective
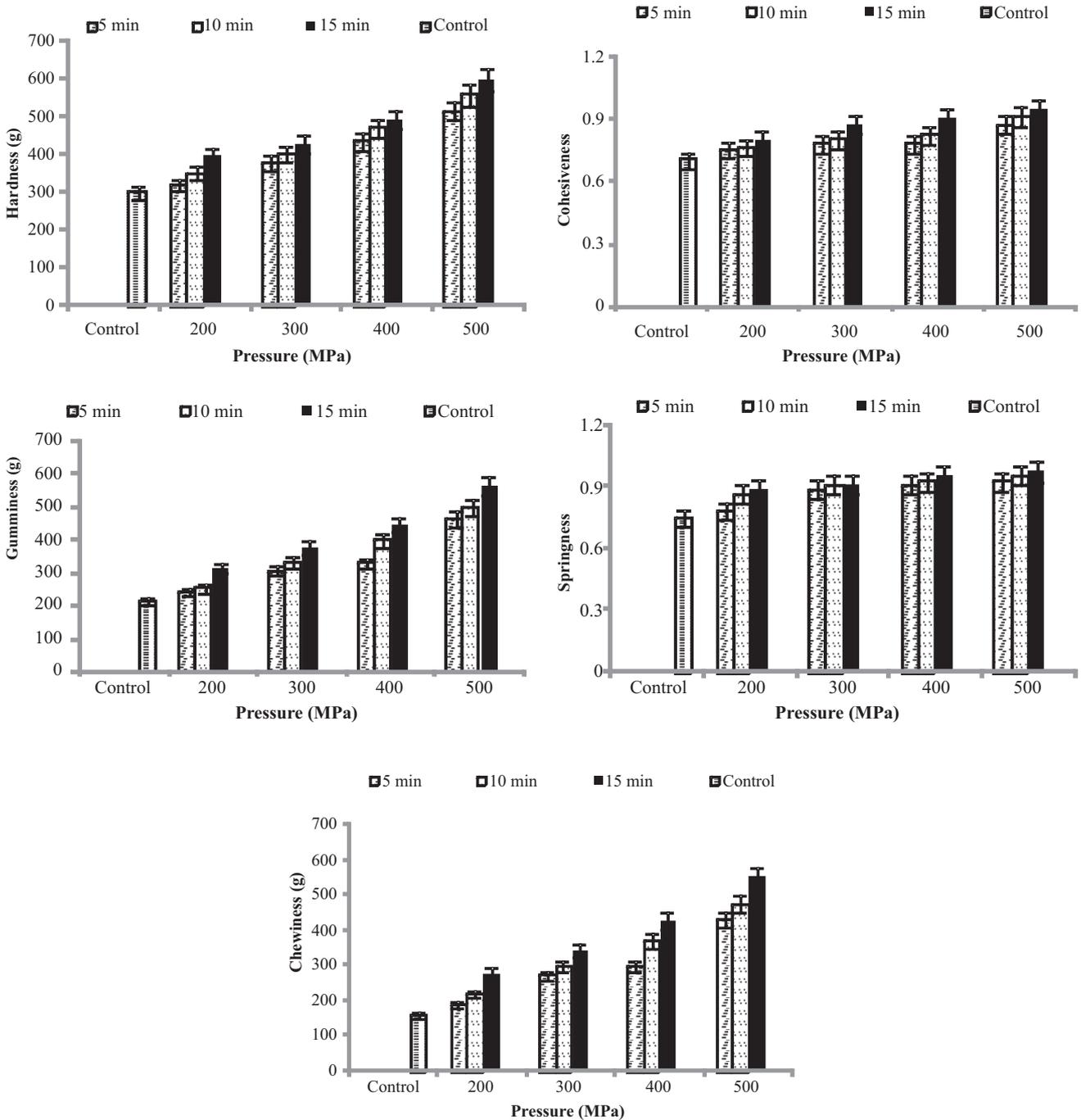


**Fig. 4b.** Effect of HPP on texture profiles of Pomfret Fillet

results indicated that the HPP of fillet fish could have a great impact in reducing TVB-N produced in muscle tissue resulting perhaps, from partial inhibition of bacterial growth involved in TMA reduction. However, these results also suggest that none of the applied pressure conditions did completely inhibit the TMA reductase enzyme activity in tuna fish tissue.

**Microbiological Parameter**

**Total Plate Count:** According to the International Commission of Microbiological Standards for Foods, the maximum acceptable microbial limit in fresh fish is 7 log10 cfu/g. HPP and thermal treatment reduce the microbial load of Pomfret fillet (Fig. 7). The inactivation of microbial load varied with the pressure, temperature and holding time.
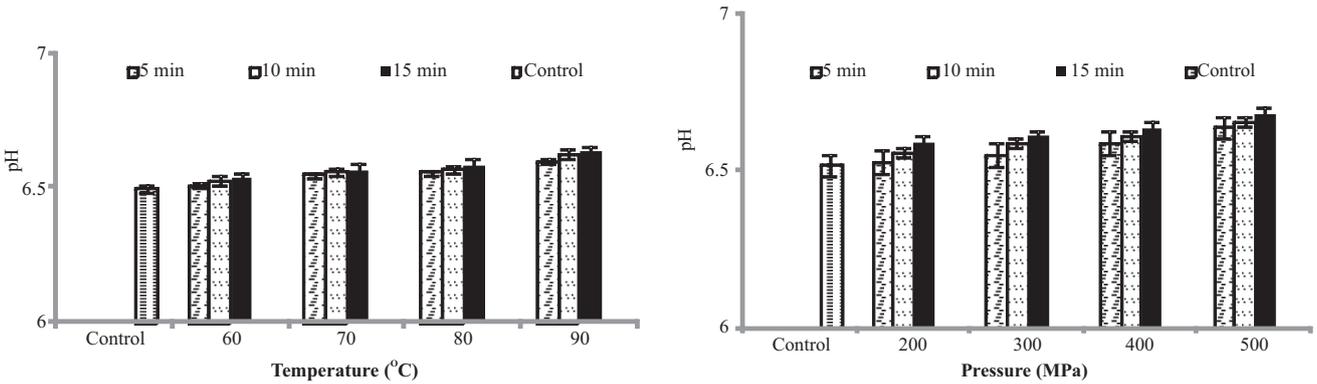


**Fig. 5(a-b).** Effect of thermal treatment and pressure on pH of Pomfret Fillet
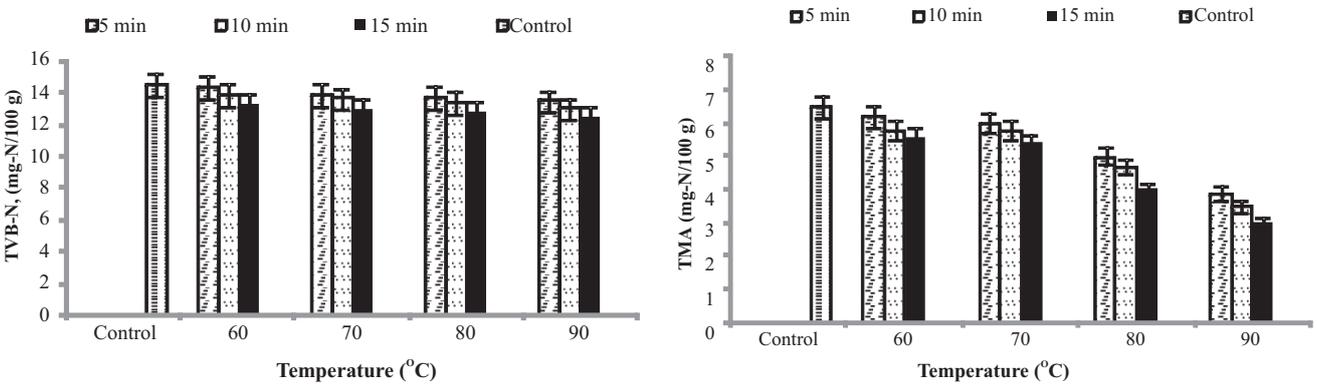


**Fig. 6a.** Effect of thermal treatment on TVB-N and TMA-N of Pomfret Fillet
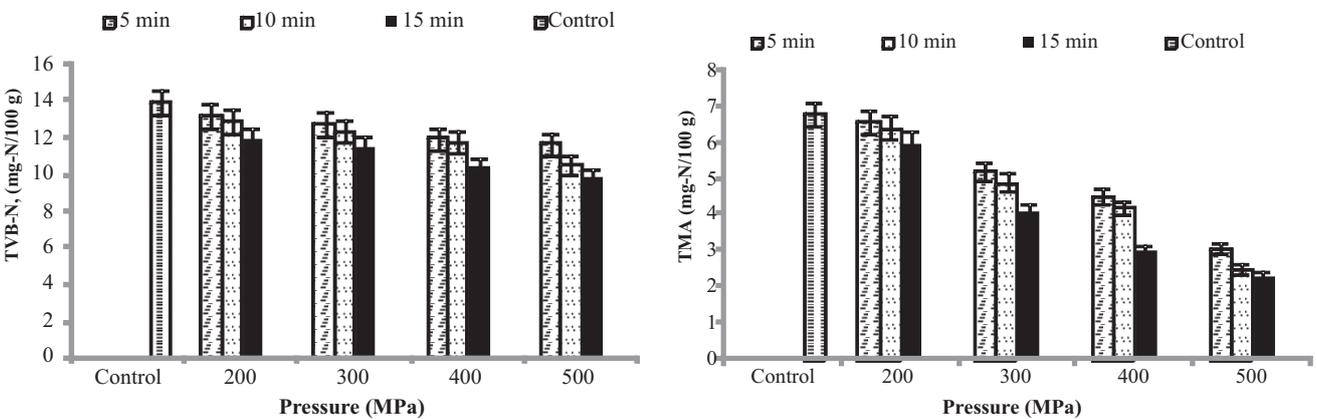


**Fig. 6b.** Effect of HPP on TVB-N and TMA-N of Pomfret Fillet
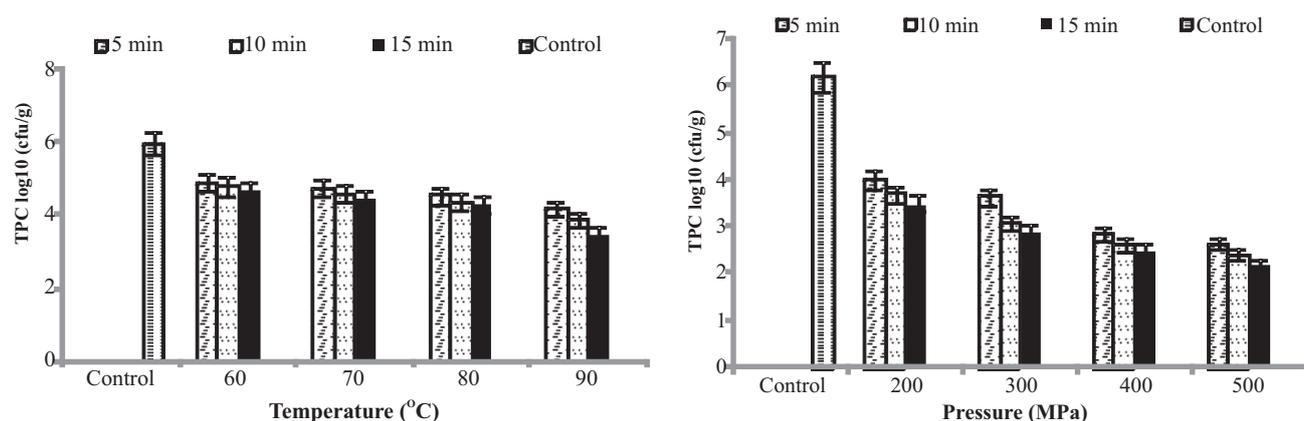
**Fig. 7.** Effect of thermal treatment and HPP on TPC of Pomfret Fillet

Higher pressure and holding time is more effective to microorganism than lower pressure and time. Similarly, higher temperature and holding time is more effective to microorganism than lower temperature and time. Among all the samples, samples treated at the 500 MPa for 15 min and 90 °C for 15 min had demonstrated higher reduction value (4.0 log10 cfu/g) of microbiological count as compared to other pressure treatment.

Both thermal treatment and HPP significantly reduced microbial growth; however, this reduction was much higher in high pressure processed samples as compared to thermal treatment. Pressure treatment of 200, 300, 400 and 500 MPa had a smaller effect on colour as compared to thermal treatment. Overall, these results prove the usefulness of HPP of Pomfret fillets is potentially a more suitable technology than heat treatment, causing less negative effects on the quality attributes and resulting in significantly better quality than untreated samples.

**CONCLUSION**

Pomfret fillets were subjected to thermal treatment of 60–90 °C and HPP of 200–500 MPa for 5–15 min under ambient temperature (25±2 °C). All samples were analyzed for microbiological (total plate count), physical (moisture content, drip loss, colour and texture profiles) and biochemical (TVB-N, TMA, and pH) characteristics. The results revealed that both the treatments had a similar effect on the texture of Pomfret fillets but pressure treatment caused fewer modifications in colour as compared to thermal treatment. Moreover, high pressure treated samples showed increased hardness and higher microbial inactivation as compared to thermal ones. Overall results suggested that the HPP proved to be better technology for processing of Pomfret fillets as compared to thermal treatment.

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# Biometric Characteristics of Pacific White Shrimp, *Litopenaeus vannamei* (Boone, 1931) Cultured in the Salt Affected Area of District Fazilka (Punjab), India

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**Abstract**: Biometric characteristics of brackish water shrimp, *Litopenaeus vannamei* (Boone, 1931), cultured in the salt affected area of district Fazilka, Punjab (India), were observed to find out relationship between length and weight of different body segments. Similarity matrix among different body segments was highest between telson and carapace shell weight without muscle (0.991), while least relationship in between abdomen weight with shell and carapace shell without muscle (0.917). The regression coefficient ($r^2$) for body weight and total length was observed, abdomen weight and abdomen length, carapace weight (with tissue) and carapace width, and carapace shell weight (without tissue) in relation to carapace width was 0.881, 0.941, 0.369 and 0.092, respectively. The value of linear relationship indicates a substantial degree of growth w.r.t. tested geographical location; hence there is significant scope of its culture in salt affected areas of Punjab.

**Keywords**: *Litopenaeus vannamei*, biometric characteristic, linear relationship, Bray−Curtis matrix

Shrimp industry is an important pillar of aquaculture sector, it provides much needed protein, employment, income and livelihood support to many people in the world. India is one of the major shrimp producing country, using locally as well as exotic species in both salt and brackish waters. In 2001−2002, shrimp industry faced severe disease outbreaks that affected the country's production government started searching for an alternative shrimp species suitable for farming and ended with the specific pathogen free (SPF) *Litopenaeus vannamei* (whiteleg shrimp or Pacific white shrimp) (Pramod and Salim, 2012). In 2009, *L. vannamei* has been accepted for its commercial culture in the country and subsequently, permission has been accorded for the establishment of hatcheries to produce its SPF seed. Very soon whiteleg shrimp gained wide popularity as a potential alternative species and expanded very fast, due to its wide range of salinity tolerance, disease resistance; compared to other cultivable shrimps, growth and market demand (Karuppasamy, 2013). To meet out the demand of shrimp for domestic as well as for export, shrimp industry is exploring new possibilities to enhance its production. Expanding its cultivation area through bringing new areas under cultivation is one of the positive approaches. The whiteleg shrimp is native to the Eastern Pacific coast from Sonora, Mexico in the North, through Central and South America as far South as Tumbes in Peru (FAO, 2006). Acclimatization of non−native animal may bring the genetic as well as changes in morphometric characteristics and reflects variations in growth of different body segments. Morphometric characteristic expresses the wellbeing of an animal under culture conditions. Change in morphometric characteristics and relationship between different body segments, is an indicator of unhealthy growth of animal. The present study was conducted to investigate the suitability of inland saline water of salt affected areas of Punjab and to study morphometric characteristics of *L. vannamei* to establish baseline data for the genetic trait and can be used to establish its breeding programme.

## MATERIAL AND METHODS

Post larvae of *L. vannamei* were reared under extensive culture conditions in manmade ponds in salt affected water logged area in district Fazilka (Punjab) for 105 days. Stocking was done @ 5 post larvae $m^{-3}$ and were fed with commercial shrimp diet. Water quality parameters like temperature, pH, dissolved oxygen (DO), salinity, total alkalinity and total hardness of the pond water were measured as per standard methods (APHA, 2005). The harvested shrimp were brought at College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, Punjab under iced condition and were analysed formorphometric traits like total length, cephalothorax length, carapace width, abdomen length, telson length, major chelate leg length, minor chelate leg length using measuring scale. Gravimetric traits were measured by taking body weight, carapace weight with integrated muscle and shell, carapace shell weight

without integrated muscle, abdomen weight with and without shell, and telson weight by taking the weight of whole and respective segments, using digital balance model: Mattler Toledo ME103E. Total 21 specimens were taken to study the morphometric characteristics of whiteleg shrimp. Data collected for morphometric traits were statistically analyzed for average values, standard deviation, Bray's Curtis matrix for the regression value and Linear relationship using one way ANOVA by SPSS 16.00 software.

### RESULTS AND DISCUSSION

Being an aquatic animal, water quality puts significant impact on the physiology, metabolic activities, health, growth, survival and reproductive biology. Fitness and suitability of pond water was evaluated on water quality parameters such as temperature, dissolve oxygen (DO), pH, salinity, total alkalinity and total hardness (Table 1). No abrupt change in the water quality parameters of pond water was observed during culture period. Mascareno and Jimenez (2013) recovered the satisfactory growth of *L. vannamei* with water temperature 22.5 to 28.3°C, salinity 35 to 36 ppt, pH 8.2 to 8.4 and DO concentrations 4.8–7.1 mg$^{-1}$, MPEDA (2017) suggested the water quality parameters under best management practices for whiteleg shrimp that water temperature should be within 28–32°C, where as in present study temperature was recorded in between 18.2–34.6°C and such variations may be due to geographical location of the pond where environmental temperature fluctuations are quite wide in range. Dissolved oxygen, pH, and total alkalinity were well within the suggested range, while salinity was little low. After 105 days of rearing period, *L. vannamei* average length and body weight recorded 134 mm and 14.29g, respectively with corresponding $r^2$ value of 0.881 (Table 2 and 3). Suriya *et al.* (2016) reported the harvest size of whiteleg shrimp 20.0–34.0 g after 120 culture days in shrimp farms at Vellar estuary, Cuddalore district, Tamil Nadu. In present investigation, average size of harvested shrimp was comparatively low 14.29 g, which may be due to short culture days and different agro-climatic conditions. Average cephalothorax length (mm) was 47.81, carapace width 14.18. The carapace weight with integrated muscle and shell was 4.68 g, while abdomen length was 66.5 mm with average abdomen weight with shell 8.67 g. The major chelate leg, minor chelate leg and telson length measured as 40.00, 23.45 and 24.09 mm, respectively. The length–weight relationship play a significant role in establishing the relationship between the two variables, enables conversion of one variable to describe growth in natural and artificial culture conditions (Abohweyere and Williams, 2008; Deekae and Abowee, 2010) and to examine possible differences among different stocks of the same species (King, 2007). Among body

**Table 1.** Water quality parameters of pond water during culture period

| Parameters | Average value | Range | Ideal range* |
|---|---|---|---|
| Water temperature (°C) | 24.5 | 18.2–34.6 | 28–32 |
| Dissolved oxygen (mg$^{-1}$) | 5.28 | 4.80–5.60 | 4–6 |
| pH | 8.32 | 7.44–8.76 | 7.5–8.3 |
| Salinity (ppt) | 7.50 | 4.00–10.00 | 10–25 |
| Total alkalinity (CaCO$_3$ mg$^{-1}$) | 146 | 128–152 | 100–200 |
| Total hardness (CaCO$_3$ mg$^{-1}$) | 2152 | 1540–2740 | – |

* MPEDA, 2017

**Table 2.** Study of morphometric traits of cultured *L. vannamei* (Mean ± SD)

| Morphometric traits | Average values (mm) |
|---|---|
| Total length | 134.00±7.04 |
| Cephalothorax length | 47.81±2.96 |
| Abdominal length | 66.54±3.20 |
| Telson length | 24.09±2.02 |
| Carapace width | 14.18±1.07 |
| Major chelate leg length | 40.00±1.67 |
| Minor chelate leg length | 23.45±3.83 |

**Table 3.** Study of gravimetric traits of cultured *L. vannamei* (Mean ± SD)

| Gravimetric traits | Average values (g) |
|---|---|
| Body weight | 14.29±2.13 |
| Carapace weight with integrated muscle and shell | 4.68±0.79 |
| Carapace shell weight without integrated muscle | 0.30±0.05 |
| Abdomen weight with shell | 8.67±1.32 |
| Abdomen weight without shell | 7.53±1.11 |
| Telson weight | 0.45±0.07 |

measurements of shrimps, important parameters are carapace length, body length and total length and their respective weight. In general, shrimp farmers are more concerned about the biomass of their produce, which they record only in terms of body weight, whereas biologists and researchers prefer morphometric measurements (Primavera, 1998). Success of breeding programme depends on three major factors i.e., total length, body weight, ovary weight and fecundity (Arthi *et al.*, 2013). Gautam *et al.* (2014) reported $r^2$ value for the length and weight of 0.999 for male and 0.998 for female brood stock of *L. vannamei* in different hatcheries of Tamil Nadu. In present investigation, value of $r^2$ is comparatively less which indicates the body of organism is highly variable due to different environmental and culture practices.

The Bray–Curtis matrix was prepared to find out biometric

relationship with single body segment with body segments (Table 4). Through Bray-Curtis matrix, highest similarity matrix recorded between telson and carapace shell weight without muscle (0.991), while least relationship in between abdomen weight with shell and carapace shell without muscle (0.917). Length and weight is the most common

morphometric characteristics used to find outgrowth with respect to environmental conditions, which is measured using length-weight relationship. Okpala and Bono (2015) compared the biometric indices of *L. vannamei* by Spearman's rank correlation matrix and found positive correlation ( 0.429) in between standard length and total
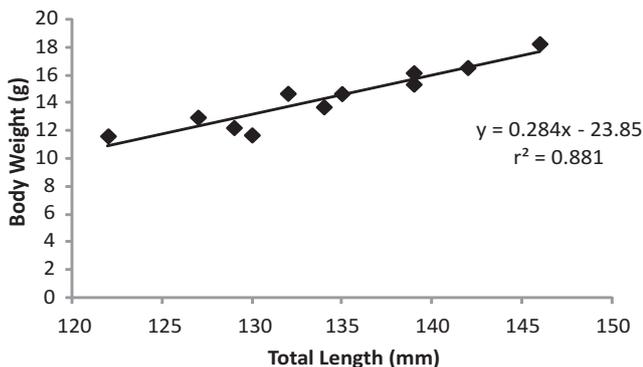


**Fig. 1.** Linear relationship between total length and body weight of *L. vannamei*



**Fig. 2.** Linear relationship between abdomen length and abdomen weight of *L. vannamei*



**Fig. 3.** Linear relationship between Carapace width and Carapce weight with Muscle and Shell of *L. vannamei*
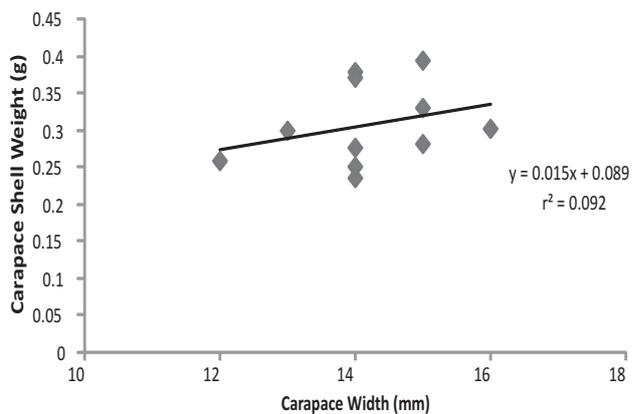


**Fig. 4.** Linear relationship between Carapace width and Carapace shell weight of *L. vannamei*

**Table 4.** Bray-Curtis Matrix of different body segments of *L. vannamei*, reared in inland saline water

| Traits | TL | AL | TEL | CW | BW | AWS | TEW | CWMS | CSW |
|---|---|---|---|---|---|---|---|---|---|
| TL | 1.000 | | | | | | | | |
| AL | 0.976 | 1.000 | | | | | | | |
| TEL | 0.972 | 0.986 | 1.000 | | | | | | |
| CW | 0.988 | 0.968 | 0.961 | 1.000 | | | | | |
| BW | 0.986 | 0.986 | 0.982 | 0.979 | 1.000 | | | | |
| AWS | 0.951 | 0.975 | 0.975 | 0.944 | 0.961 | 1.000 | | | |
| TEW | 0.971 | 0.947 | 0.944 | 0.979 | 0.961 | 0.922 | 1.000 | | |
| CWMS | 0.988 | 0.968 | 0.964 | 0.989 | 0.981 | 0.943 | 0.979 | 1.000 | |
| CSW | 0.966 | 0.942 | 0.939 | 0.974 | 0.956 | 0.917 | 0.991 | 0.974 | 1.000 |

* TL−Total Length, AL−Abdomen Length, TEL−Telson Length, CW−Carapace Width, BW−Body Weight, AWS−Abdomen Weight With Shell, TEW−Telson Weight, CWMS−Carapace Weight With Muscle and Shell, CSW−Carapace Shell Weight Without Muscle

weight, while negative correlation (−0.794) in between standard length and condition factor.

## CONCLUSION

Under the present study, survival and growth of *L. vannamei* obtained under the agro climatic conditions of salt affected areas of Punjab gives an indication that whiteleg shrimp can be one of the potential species for its culture in such areas. The water quality parameters w.r.t. tested geographical location is suitable for this species and its culture can be further expand to increase the income of farmers, provided timely availability of quality seed, feed and following best management practices. The genetic traits recorded can be used as a baseline data to establish its breeding programme.

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# Evaluation of Aquaculture Units Established in Salt Affected Areas of District Sri Muktsar Sahib, Punjab

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**Abstract**: Field study was undertaken in six salt affected villages (Tappa Khera, Ratta Khera, Fatehpur Mania, Ratta Tibba, Ghagga and Channu) in district Sri Muktsar Sahib to evaluate the status of aquaculture units with respect to water quality and suitability for rearing freshwater/ brackish water species. The water samples from 29 aquaculture units in different villages were collected and analysed for different physico-chemical parameters viz., pH, salinity, electric conductivity, total hardness, total alkalinity, sodium, potassium, calcium, magnesium, sulphate and chloride. Among the total aquaculture units surveyed, salinity range of 6 (20.68%), 12 (41.37%), 9 (31.03%) and 2 (6.89%) units was 1–2, 3-4, 5 and >5 ppt, respectively and all the units, except one (9 ppt), were being used for rearing freshwater carps under semi intensive polyculture system. The present study revealed that 62% of the aquaculture units with salinity upto 4 ppt can be used for rearing freshwater carps without much management with respect to maintaining salinity <5 ppt. However, salinity of aquaculture units with salinity 5 ppt is likely to increase over a period of time, with special reference to high evaporation rates during summers, which is detrimental for growth and survival of carps.

Salt affected soils are an important ecological entity in the landscape of any arid and semi-arid region of north India, as it has converted a large area of fertile lands into non-productive waste lands.. In India nearly 9.38 million hectare (m ha) area is occupied by salt affected soils, out of which 5.50 m ha are saline (including coastal areas) and 3.88 m ha are alkaline (IAB, 2000). Out of the total 5.0 m ha geographical area of Punjab, ground water quality varies from good (59%) to marginal (22%) and poor (19%). The poor waters are saline (22%), sodic (54%) and saline-sodic (24%) (Central Soil Salinity Research Institute, Karnal).Underground water in many areas of this region is neither fit for agriculture nor for industrial use or human consumption due to salinity. Such inland salt affected waterlogged areas, where agriculture has very less or no scope and water is abundantly available, aquaculture is the most suitable option. Notable efforts have been made in the past to reclaim and utilize these lands through aquaculture, with special reference to brackish water fin fish/shellfish species like mullets, milk fish, pearl spot, sea bass, white legged shrimp, tiger prawn etc. (Jain *et al.,* 2007; Pathak *et al.,* 2013; Dhawan *et al.,* 2016). Although these brackish water species hold great potential for aquaculture development in inland saline waters, but it could not be commercialized due to non-availability of seed in the northern states. In the recent past significant work has been carried out by Guru Angad Dev Veterinary and Animal

Sciences University (GADVASU), Ludhiana to develop aquaculture technologies for inland salt affected waterlogged waste lands of Punjab, with special reference to rearing freshwater carps in low saline water, which has been successfully demonstrated and is also being replicated in affected districts of the State. Freshwater carps (catla, rohu, mrigal, commom carp and grass carp) were reared successfully in inland saline water under a semi-intensive poly culture system, with an average productivity of 2.48 t ha$^{-1}$ yr$^{-1}$ at stocking density of 10,000 fingerlings ha$^{-1}$, which was later enhanced to 3.40 and 4.75 t ha$^{-1}$ yr$^{-1}$ through stocking density enhancement (15,000 and 20,000 fingerlings ha$^{-1}$, respectively) and species combination selection (Dhawan *et al.,* 2009, 2010, 2016; Ansal *et al.*, 2013, 2016).

Salinity is a significant abiotic factor in aquaculture, which determines level of osmoregulation stress in an aquatic organism with significant effects on its physiology, besides altering the water quality, which leads to poor growth and mortality if exposed beyond salinity tolerance (species specific) levels (Gholampoor *et al.,* 2011). Fresh water carps are stenohaline species and grow well in hypo-osmotic environment (fresh water). Salinity variations induce osmoregulatory stress in these species, with growth hormone playing the significant role in sustaining hypo-osmoregulatory functions (Deane and Woo, 2008). Many aquaculture units have been developed in salt affected

waterlogged under productive or unproductive waste lands in district Sri Muktsar Sahib and district Fazilka during the last 2 years. Hence, success of rearing freshwater carps in inland salt affected areas largely depends on the factor that salinity of the ponds remains 5 ppt, because salinity >5 ppt is detrimental for their growth and survival in saline water (Dhawan *et al.,* 2010; Ansal *et al.,* 2013; Islam *et al.,* 2014). Hence, present study was undertaken to evaluate suitability of aquaculture units established in salt affected areas of district Shri Muktsar Sahib for rearing freshwater/brackish water species with respect to salinity and water quality.

## MATERIAL AND METHODS

For the present study, six salt affected villages (Tappa Khera, Ratta Khera, Fatehpur Mania, Ratta Tibba, Ghagga and Channu) in district Sri Muktsar Sahib (Fig. 1), where aquaculture units were established in the recent past, were selected. The water samples (in triplicate) were collected (in sterilized plastic bottles)from these aquaculture units during the dry months (November to July) and transported to College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) for analysis of physico−chemical parameters including pH, salinity, electric conductivity (EC), total hardness (TH), total alkalinity (TA) and concentration of salts [sodium ($Na^+$), potassium ($K^+$), calcium ($Ca^{+2}$), magnesium ($Mg^{+2}$), sulphate ($SO_4^{-2}$) and chloride ($Cl^{-1}$)] as per standard methods of APHA (1991). The pH, EC and salinity were measured using pH meter (LI, 127, ELICO), digital conductivity meter (EC-3, COM-80, HM Digital), and digital salinity meter (ELICO), respectively. Standard titration methods were used for estimation of TA, TH, $Ca^{+2}$ and $Mg^{+2}$ concentration, while $Na^+$ and $K^+$ concentrations were estimated by flame emission

photometric method. The $Cl^{-1}$ and $SO_4^{-2}$ concentrations were analysed using 'Argentometeric' and 'Turbidimetric' methods, respectively.

## RESULTS AND DISCUSSION

Among six salt affected villages surveyed, aquaculture units in five villages (Tappa Khera, Fatehpur Mania, Ghagga, Channu and Ratta Tibba), were used for rearing freshwater carps under semi−intensive poly−culture system, while in village Ratta Khera, both freshwater carps culture (semi−intensive poly−culture) and brackish water shrimp culture (intensive monoculture system) were undertaken. In villages Tappa Khera, Ratta Khera, Fatehpur Mania, Ghagga and Ratta Tibba, all the aquaculture units were established in last 2−3 years, while in village Channu the aquaculture units were established during the last 4-8 years. Maximum number of aquaculture units was found in village Ratta Khera followed by Ghagga, Tappa Khera, Fatehpur Mania, Ratta Tibba and Channu. All the farmers were adding canal water (freshwater) in their aquaculture units for maintaining low salinity level for rearing fresh water carps like catla, rohu, mrigal, common carp and grass carp.

The water quality of aquaculture units in different villages, with respect to different physico−chemical parameters (pH, salinity, EC, TA, TH, $Na^+$, $K^+$, $Ca^{+2}$, $Mg^{+2}$, $SO_4^{-2}$ and $Cl^{-1}$), is presented in Tables 1-6. Among the total aquaculture units (29) surveyed during the present study, salinity range of 6 (20.68%), 12 (41.37%), 9 (31.03%) and 2 (6.89%) units was 1−2, 3-4, 5 and >5 ppt, respectively and all the units except one (9 ppt), were being used for rearing freshwater carps under semi intensive polyculture system. Water salinity in 18 aquaculture units (62%) was < 5 ppt (1−4 ppt), which was maintained by addition of canal water (fresh water) and/or



(Source: www.veethi.com)
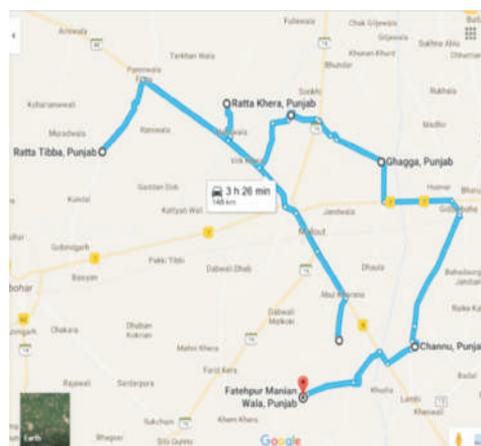
(Source: Google Earth)

**Villages Co-ordinates**

Tappa Khera
30.1038ºN, 74.5331ºE

Ratta Khera
30.29633ºN, 74.4306ºE

Fatehpur Mania
30.06220ºN, 74.4953ºE

Ghagga
30.2363ºN, 74.57956ºE

Channu
30.610ºN, 74.3516ºE

Ratta Tibba
30.1453ºN, 74.1617ºE

**Fig. 1.** Geographic location of District Sri Muktsar Sahib and road map of the selected villages in the district

periodic exchange with low salinity underground water (1−4ppt). The pH (7.27-9.12) in all aquaculture units was favourable for aquaculture (Parmeshwaran *et al.,* 1971, Jhingran, 1991). The EC and TH of water increased in accordance with the salinity and also varied significantly (p 0.05) at same salinity levels, which can be attributed to differences with respect to ionic composition and their respective ability to conduct electric current. Significant differences were also recorded with respect to TA of water in different aquaculture units, but no specific changes with respect to salinity were observed. Although, at higher salinity levels, the overall salt concentration ($Na^+$, $K^+$, $Ca^{+2}$ and $Mg^{+2}$, $Cl^{-1}$ and $SO_4^{-2}$) was significantly high (p  0.05), but the ionic composition  of water, in terms of $Na^+$, $K^+$, $Ca^{+2}$ and $Mg^{+2}$, did not show any specific variation trend with respect to salinity. However, significant ionic composition differences (p  0.05) were recorded among aquaculture units with different/same salinity levels, even within the same village. It can be attributed to differences in the management practices, with respect to water source, salinity management, liming, manuring, feeding, probiotics and application of chemicals (prophylaxis/disinfection, mineral supplements, zeolite, oxidants etc.), followed at farmer's level.  In all the ponds, concentration of $Cl^{-1}$ was higher than $SO_4^{-2}$.

Fish, in, general do not have an internal salt concentration that closely match the water in which they swim. In freshwater fish, internal salt concentration varies between 8.5 to 10.4 ppt, which is hypertonic to (higher than) the surrounding water (0−0.5 ppt). Hence, freshwater fish continually gain water from the environment and lose salts in the urine and through diffusion at the gills. The stenohaline fresh water fish can tolerate and grow in saline water if its internal salt concentration remains hypertonic to the culture medium. Once the internal salt concentration in the fresh water fish becomes hypotonic to the external medium, it will experiences reverse osmoregulatory

**Table 1.** Water quality of aquaculture units in village Tappa Khera

| Unit | Salinity ppt | pH | EC mScm$^{-1}$ | TH CaCO$_3$ mgl$^{-1}$ | TA CaCO$_3$ mgl$^-$ | Na$^+$ mgl$^{-1}$ | K$^+$ mgl$^{-1}$ | Ca$^{+2}$ mgl$^{-1}$ | Mg$^{+2}$ mgl$^{-1}$ | SO$_4^{-2}$ mgl$^{-1}$ | Cl$^{-1}$ mgl$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 4.0 | 8.35$^{ab}$ | 12.41$^{ab}$ | 1160$^a$ | 384$^a$ | 379$^d$ | 43$^a$ | 315$^a$ | 205$^a$ | 86$^a$ | 950$^a$ |
| II | 5.0 | 8.15$^b$ | 10.85$^c$ | 880$^c$ | 344$^b$ | 409$^c$ | 36$^b$ | 231$^d$ | 158$^{bc}$ | 79$^a$ | 800$^d$ |
| III | 5.0 | 8.30$^{ab}$ | 11.85$^b$ | 780$^e$ | 316$^c$ | 433$^b$ | 42$^a$ | 105$^e$ | 164$^b$ | 86$^a$ | 837$^c$ |
| IV | 5.0 | 8.40$^{ab}$ | 10.90$^c$ | 840$^d$ | 340$^b$ | 373$^d$ | 37$^b$ | 273$^b$ | 138$^c$ | 86$^a$ | 750$^e$ |
| V | 5.0 | 8.46$^a$ | 13.08$^a$ | 1080$^b$ | 344$^b$ | 451$^a$ | 41$^a$ | 252$^c$ | 201$^a$ | 86$^a$ | 910$^b$ |

Values with different superscript in a column differs significantly (p  0.05)

**Table 2.** Water quality of aquaculture in village Ratta Khera

| Unit | Salinity ppt | pH | EC mScm$^{-1}$ | TH CaCO$_3$ mgl$^{-1}$ | TA CaCO$_3$ mgl$^-$ | Na$^+$ mgl$^{-1}$ | K$^+$ mgl$^{-1}$ | Ca$^{+2}$ mgl$^{-1}$ | Mg$^{+2}$ mgl$^{-1}$ | SO$_4^{-2}$ mgl$^{-1}$ | Cl$^{-1}$ mgl$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 3.0 | 8.04$^e$ | 4.11$^f$ | 900$^f$ | 176$^e$ | 163$^d$ | 50$^d$ | 210$^c$ | 163$^e$ | 62$^c$ | 426$^f$ |
| II | 5.0 | 7.86$^e$ | 12.34$^d$ | 1460$^a$ | 200$^d$ | 388$^c$ | 60$^c$ | 179$^d$ | 311$^a$ | 92$^a$ | 1205$^b$ |
| III | 2.0 | 8.31$^d$ | 3.37$^i$ | 920$^e$ | 344$^a$ | 148$^e$ | 44$^{de}$ | 620$^a$ | 73$^g$ | 52$^d$ | 190$^h$ |
| IV | 5.0 | 8.31$^d$ | 13.90$^b$ | 1040$^c$ | 180$^e$ | 86$^g$ | 37$^{ef}$ | 105$^f$ | 227$^c$ | 94$^a$ | 90$^i$ |
| V | 3.0 | 7.27$^f$ | 3.52$^h$ | 860$^g$ | 316$^c$ | 98$^f$ | 14$^g$ | 84$^g$ | 189$^d$ | 96$^a$ | 275$^g$ |
| VI | 9.0 | 8.83$^b$ | 22.10$^a$ | 1450$^a$ | 156$^f$ | 4403$^a$ | 114$^a$ | 263$^b$ | 281$^b$ | 86$^b$ | 2899$^a$ |
| VII | 4.0 | 8.57$^c$ | 9.90$^e$ | 1130$^b$ | 212$^d$ | 545$^b$ | 72$^b$ | 168$^e$ | 225$^c$ | 94$^a$ | 650$^d$ |
| VIII | 3.0 | 9.12$^a$ | 3.73$^g$ | 740$^h$ | 312$^c$ | 163$^d$ | 49$^d$ | 158$^e$ | 145$^f$ | 64$^c$ | 550$^e$ |
| IX | 5.0 | 8.91$^b$ | 12.95$^c$ | 1004$^d$ | 333$^b$ | 85$^g$ | 36$^f$ | 211$^c$ | 192$^d$ | 94$^a$ | 1125$^c$ |

Values with different superscript in a column differs significantly (p  0.05)

**Table 3.** Water quality of aquaculture units in village Fatehpur Mania

| Unit | Salinity ppt | pH | EC mS cm$^{-1}$ | TH CaCO$_3$ m gl$^{-1}$ | TA CaCO$_3$ m gl$^{-1}$ | Na$^+$ m gl$^{-1}$ | K$^+$ m gl$^{-1}$ | Ca$^{+2}$ m gl$^{-1}$ | Mg$^{+2}$ m gl$^{-1}$ | SO$_4^{-2}$ m gl$^{-1}$ | Cl$^{-1}$ m gl$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 3.0 | 8.4$^b$ | 6.67$^c$ | 480$^c$ | 220$^c$ | 234$^c$ | 89$^b$ | 189$^c$ | 71$^b$ | 89$^a$ | 345$^d$ |
| II | 4.0 | 8.8$^a$ | 9.49$^b$ | 600$^b$ | 420$^b$ | 267$^b$ | 64$^c$ | 357$^b$ | 59$^c$ | 64$^b$ | 402$^b$ |
| III | 3.0 | 8.5$^b$ | 4.94$^d$ | 440$^d$ | 120$^d$ | 196$^d$ | 73$^c$ | 168$^d$ | 66$^{bc}$ | 43$^c$ | 385$^c$ |
| IV | 6.0 | 8.1$^c$ | 14.03$^a$ | 1860$^a$ | 520$^a$ | 313$^a$ | 357$^a$ | 416$^a$ | 351$^a$ | 57$^b$ | 1190$^a$ |

Values with different superscript in a column differs significantly (p  0.05)

conditions forcing the fish to make unwanted exhaustive physiological adjustments to survive under high salinity (hypertonic) conditions, which affects its growth and may also lead to mortality once the salinity levels crosses the tolerance level (species specific) in terms of osmoregulatory stress (Mustafayev and Mekhtiev, 2008). Significant decline in flesh moisture content (dehydrating effect) and food intake (De‑Boeck *et al.,* 2000; Kumar *et al.,* 2016) has been reported in fresh water fish exposed to high salinity waters, leading to poor growth and (or) mortality due to undesired physiological changes. In fresh water fish, salinity stress also lowers liver glycogen levels due to high rate of glycogenolysis activity to meet high energy demand for making osmoregulatory adjustments, ultimately restricting glucose supply to the brain (De‑Boeck *et al.,* 2000).

Salinity tolerance and growth response of fresh water carps in inland saline water has been found to be species specific (Dhawan *et al.,* 2009, 2010; Ansal *et al.,* 2013; Islam *et al.,* 2014). Although, growth performance of freshwater carps decreases with increase in salinity (salt concentration), but fresh water carps like catla, rohu, mrigal, common carp have been reared successfully up to 5 ppt salinity in salt affected degraded land in Village Shajrana (District Fazilka), with an average productivity of up to 4.75 tonnes (t) $ha^{-1} yr^{-1}$ through suitable technological interventions with respect to stocking density, species combination, water quality management and integrated approaches (Dhawan *et al.,* 2010, 2016; Ansal *et al.,* 2013).

Inland saline water resources in arid and semi‑arid areas are vulnerable to wide range salinity fluctuations due to evaporation loss and high rate of precipitation during different seasons**.** Aquaculture units with salinity range 1‑2 ppt are most suitable for freshwater carp culture. Further, aquaculture units with salinity range 3‑4 ppt can also be used for rearing fresh water craps without much management with respect to maintaining salinity levels 5 ppt. Although, aquaculture units in village Tappa Khera (4‑5 ppt), Ghagga (1‑4 ppt), Channu (1‑5 ppt) and Ratta Tibba (2‑5 ppt) are suitable for rearing freshwater carps, but rearing of freshwater carps in ponds with 4‑5 ppt salinity is not expected to be sustainable, as salinity in these ponds is expected to increase above 5 ppt over a period of time due to evaporation during summers, which will need to be managed either through water exchange or dilution with freshwater. Further, in village Ratta Khera (2‑9 ppt) and Fatehpur Mania (3‑6 ppt), it is recommended to take up freshwater carp culture in aquaculture units with salinity < 5 ppt and brackish water species in units with salinity >5 ppt. In aquaculture units with salinity 5 ppt, freshwater carps can be reared only after

**Table 4.** Water quality of aquaculture units in village Ghagga

| Unit | Salinity ppt | pH | EC mS $cm^{-1}$ | TH $CaCO_3$ mg $l^{-1}$ | TA $CaCO_3$ mg $l^{-1}$ | $Na^+$ mg $l^{-1}$ | $K^+$ mg $l^{-1}$ | $Ca^{+2}$ mg $l^{-1}$ | $Mg^{+2}$ mg $l^{-1}$ | $SO_4^{-2}$ mg $l^{-1}$ | $Cl^{-1}$ mg $l^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 3.0 | 8.85[b] | 7.97[b] | 620[c] | 363[d] | 118[b] | 14[b] | 126[d] | 120[a] | 65[ab] | 444[b] |
| II | 2.0 | 8.23[c] | 6.28[d] | 570[e] | 344[e] | 78[c] | 10[b] | 147[c] | 100[b] | 54[bc] | 380[c] |
| III | 2.0 | 8.02[d] | 6.28[d] | 603[d] | 508[a] | 78[c] | 9[b] | 252[a] | 85[c] | 52[c] | 351[d] |
| IV | 3.0 | 8.25[c] | 7.35[c] | 640[b] | 380[c] | 108[b] | 11[b] | 231[b] | 100[b] | 64[ab] | 567[a] |
| V | 1.0 | 7.97[e] | 3.14[e] | 308[f] | 220[f] | 33[d] | 8[b] | 34[e] | 75[c] | 35[d] | 324[e] |
| VI | 4.0 | 8.91[a] | 8.14[a] | 680[a] | 464[b] | 202[a] | 40[a] | 252[a] | 104[b] | 72[a] | 401[b] |

Values with different superscript in a column differs significantly (p 0.05)

**Table 5.** Water quality of aquaculture units in village Channu

| Unit | Salinity ppt | pH | EC mS $cm^{-1}$ | TH $CaCO_3$ mg $l^{-1}$ | TA $CaCO_3$ mg $l^{-1}$ | $Na^+$ mg $l^{-1}$ | $K^+$ mg $l^{-1}$ | $Ca^{+2}$ mg $l^{-1}$ | $Mg^{+2}$ mg $l^{-1}$ | $SO_4^{-2}$ mg $l^{-1}$ | $Cl^{-1}$ mg $l^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 1.0 | 8.40[a] | 3.34[b] | 462[b] | 273[b] | 42[b] | 5[b] | 92[b] | 91[b] | 35[b] | 67[b] |
| II | 5.0 | 7.83[b] | 13.10[a] | 703[a] | 559[a] | 128[a] | 34[a] | 148[a] | 133[a] | 62[a] | 176[a] |

Values with different superscript in a column differs significantly (p 0.05)

**Table 6.** Water quality of aquaculture units in village Ratta Tibba

| Unit | Salinity ppt | pH | EC mS $cm^{-1}$ | TH $CaCO_3$ mg $l^{-1}$ | TA $CaCO_3$ mg $l^{-1}$ | $Na^+$ mg $l^{-1}$ | $K^+$ mg $l^{-1}$ | $Ca^{+2}$ mg $l^{-1}$ | $Mg^{+2}$ mg $l^{-1}$ | $SO_4^{-2}$ mg $l^{-1}$ | $Cl^{-1}$ mg $l^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 2.0 | 7.97[a] | 3.37[c] | 340[c] | 120[c] | 41[c] | 12[b] | 105[c] | 57[c] | 31[c] | 362[b] |
| II | 5.0 | 8.10[a] | 6.12[a] | 620[a] | 223[a] | 72[a] | 25[a] | 158[a] | 112[a] | 55[a] | 516[a] |
| III | 4.0 | 8.05[a] | 4.86[b] | 425[b] | 172[b] | 63[b] | 18[ab] | 126[b] | 73[b] | 40[b] | 400[b] |

Values with different superscript in a column differs significantly (p 0.05)

dilution with freshwater, but it is not recommended as an economically and ecologically viable approach on long term basis for areas already affected by dual problem of underground salinity and water logging. Addition of canal water or fresh water from any other source may further aggravate the problem of waterlogging in the region. Problems of water logging and drainage of water is a serious matter of concern, which may lead to social conflict with respect to land use and degradation.

## CONCLUSION

Due to non-availability of seed of brackish water species, farmers are rearing fresh water carps in aquaculture units established in salt affected areas of Punjab. However, sustainability of fresh water crap culture in inland saline water ponds depends on the factor that salinity is maintained below 5ppt. Although in the present study, salinity of 62% (18) of the aquaculture units (1-4 ppt) evaluated in the 6 villages of district Shri Muktsar Sahib, were found suitable for fresh water crap culture, but productivity of fresh water carps in 31% (09) of the aquaculture units (5ppt) is not expected to remain sustainable on long term basis. Further, culture of brackish water finfish/shellfish species shall be taken up in aquaculture units with salinity levels > 5ppt.

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# Trends of Fish Marketing Strategy and Trade in Punjab-A Survey

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*Abstract:* Fish market survey of six major markets of Punjab (Ludhiana, Amritsar, Taran taran, Jalandhar, Kapurthala and Patiala) was conducted over a duration of 3years (January, 2014 to December, 2016). The survey was designed to have a snap-shot of the prevailing domestic fish markets of Punjab, with the objectives to record the trends of fish catch, sector wise contribution of different fish species, market structure, major marketing channels, constrains of fish venders etc. The survey was conducted at seasonal (summer, monsoon and winter) interval basis. Total 52 fish species were recorded from fish markets under capture and culture sector within the State as well as fish imported from far off states. Cyprinidae family (9 species) contributed 65 % (weight basis) of market share (both from culture and capture fisheries) and among them, *Labeo rohita* ,*Catla catla* and *Cyprinus carpio* contributed significantly. Fish species available in markets from capture fisheries were harvested mainly from Harike wetland, Nangal and Pong dams. 26 fish species (25.0% of market share on weight basis) were recorded exclusively from the capture fisheries and among them Siluridae−*Wallago attu* (9.90 %) followed by Bagiridae−*Aorichthys seenghala* and *Rita rita* (8.43 %), Channidae− *Channa marulius* and *C. striatus* (8.30%) contributed significantly. *Pangasiodon hypothalamus* from Andhra Pradesh had highest consumer demand among native population. Among six district survey, maximum number of fish species recorded in Ludhiana fish market (41 species) followed by Taran taran (40 species). Some of the major constrains like waste disposal, unregulated fish supply in the market and lack of good quality storage facilities faced by the fish vendors are need to be addressed for further development in this sector.

*Keywords*: Fish market, Culture fisheries, Capture fisheries, Carps, Waste disposal

Fish being highly perishable commodity almost entirely depends on an efficient marketing system. Fisheries marketing comprise all the activities and agencies conducting them, involved in the movement of fish or fish products from the farm or industries to the final consumers or end users (Flowra, 2012). Fish marketing involves the channel of communication between the producers and consumers which passes through a number of intermediaries. Major problems in fish marketing includes high perish ability and bulkiness of material, poor transportation facilities, high heterogeneity in size and weight among species, high cost of storage and transportation, low demand elasticity and high price spread (Ravindranath, 2008). Devadasan (2005) stated post-harvest loss of resources is an area of major concern in fisheries sector and emphasized on the efficient marketing channels to minimize these losses. Kumar *et al.* (2008) surveyed domestic fish marketing trend in India with special emphasis on changing structure, conduct, performance and policies. Sathiadhas *et al.* (2012) analyzed the fish marketing trend in coastal states of India and found that marketing of marine fish is done through more than 1400 landing centres spread along the Indian coast and nearby wholesale and retail markets. In few cases, the exportable varieties go directly to the export market; however in inland fishery sector fish marketing is

entirely different. Vrutti (2008) reported that inland fish market in India has evolved on its own and there has been limited intervention by Government to strengthen the inland fish marketing. In few instances, Government has constructed market yards in wholesale markets. Being a surplus freshwater fish in state it creates pressure on its business pattern. In India the fishery sector accounts for 0.92 percent of total GDP and 5.58 percent of the Agriculture sector's GDP at current price for the year 2013−14 (GOI, 2016). In India, fish is consumed by 56% population (@ 14.44 kg/capita/yr.) and provide livelihood as well as employment to over 14 million people (75% engaged in Inland fisheries and 25% in marine fisheries). Punjab is a land locked state thus fish production is strictly restricted from inland sectors only. Fish produced in individual and community ponds and harvested from natural resources like rivers (Sutlej, Beas, Ravi and Ghaggar), canals, small reservoirs/ wetlands are marketed fresh condition (Jerath *et al.*, 2014). Fresh water and marine fish from far off states like Andhra Pradesh, West Bengal, Gujrat, Maharashtra and Karnataka in iced condition also have a huge demand among consumers. The present survey was designed to have a snap-shot of the prevailing domestic fish markets in six different districts of Punjab. Availability of different fish species, sector wise contribution of different fish species, market structure, major marketing

channels have been estimated. Recommendations to improve fish markets in Punjab have also been discussed.

## MATERIAL AND METHODS

The survey was conducted in six districts (Ludhiana, Amritsar, Taran taran, Jalandhar, Kapurthala and Patiala) (Fig. 1) of Punjab over a duration of 3years (January, 2014 to December, 2016) at seasonal (summer, monsoon and winter) intervals basis. The data were collected through survey of fish markets by direct observations and queries/ interviews with fish venders, wholesalers, retailers, fish society members and consumers. Information was collected as per the availability of different fish and shellfish species, demand and supply of fish produced within Punjab and procured from other states, prices, marketing channels, marketing costs and marketing margins to estimate price spread for the selected fish species, using average and percentage analyses. Collected data were analyzed by SPSSv16 and MS–Excel statistical package.



**Fig. 1.** Different districts of Punjab surveyed

## RESULTS AND DISCUSSION

Taxonomic identification and categorization of 52 fish species from different fish markets and its seasonal abundance are depicted in Tables 1 and 2. Family wise Principal Component Analysis (PCA) ordination of fish recorded from different markets revealed that Cyprinidae contributed maximum variability in the first Principal Component (PC) and considered as dominant group and controlling factor in overall market share. Second PC component revealed family Siluridae, Bagridae,

Channidaeand Notopteridae had close association in fish catch composition with significant contribution in market share (Fig. 2).
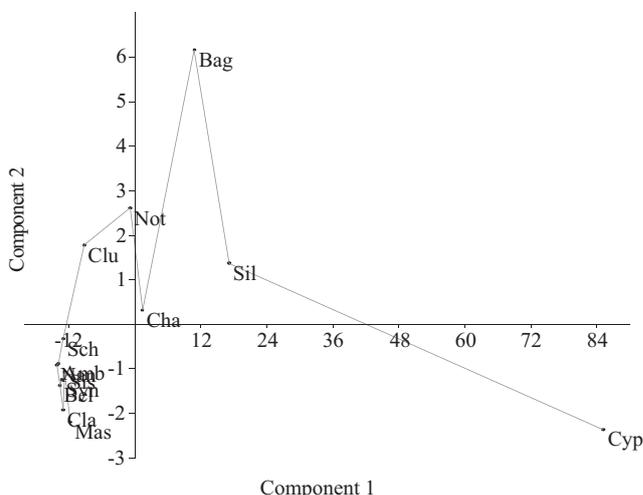


Cyp–Cyprinidae, Bag–Bagridae, Sil–Siluridae, Sch–Schilbeidae, Sis–Sisoridae, Cla–Clariidae, Bel–Belonidae, Syn– Synbrachidae, Amb–Ambassidae, Nan–Nandidae, Cha – Channidae, Mas–Mastacemblidae, Clu–Clupidae and Not– Notopteridae

**Fig. 2.** Family wise Principal Component Analysis (PCA) ordination of fish recorded from different markets

**Marketing Trend of Fish from Culture and Capture Fisheries:** In Punjab, under culture fisheries, fish produced in individual and community ponds are marketed in live and ice preserved condition. Family Cyprinidae contributes 65% (weight basis) of market share, mainly contributed by 9 fish species (*Labeo rohita*, *Catla catla*, *Cirrihinus mrigala*, *Cyprinus carpio*, *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix L. bata*, *L. calbasu* and *H. nobilis*) both from culture and capture sector (Fig. 3).

**Marketing Trend of Fish, Exclusively From Capture Fishery:** Fish species available in markets exclusively from capture fisheries, within the State, mainly harvested from Harike wetland (31˚13'N and 75˚12'E), Nangal (31˚ 23' N and 76˚ 22' E) and Pong dams(31˚97' N and 75˚94' E) (70–75 %). Rest of the capture fisheries (25–30 %) generally harvested from rivers. Out of the 26 fish species were recorded exclusively from the capture fisheries *Catla catla, Labeo rohita*, *Cirrihinus mrigala, Cyprinus carpio communis*, *L. calbasu,* of Cyprinidae family; *Rita rita* of Bagridae family; *Wallago attu* of Siluridae family; *Channa marulius and C. striatus* of Channidae family; *Notopterus notopterus and N. chitala* of Notopteridae family were found in all the seasons (Table 2). The maximum share of different fish species from capture sector was of Siluridae (9.90 %) (*Wallago attu*) followed by Bagiridae (8.43 %) (*Aorichthys seenghala* and

**Table 1.** Taxonomic identification of finfish and shellfish species recorded from different fish markets

Superclass: Gnathostomata, Class: Actinopterygii, Subclass: Neopterygii, Division: Teleostei

Order : Cypriniformes

      Family: Cyprinidae

*Catla catla* (Hamilton‒Buchanan)

*Cirrihinus mrigala* (Hamilton‒Buchanan)

*Cyprinus carpio* communis (Linnaeus)

*Labeo bata* (Hamilton‒Buchanan)

*Labeo calbasu* (Hamilton‒Buchanan)

*Labeo dero* (Hamilton‒Buchanan)

*Labeo dyocheilus* (McClelland)

*Labeo gonius* (Hamilton‒Buchanan)

*Labeo rohita* (Hamilton‒Buchanan)

*Hypophthalmichthys molitrix* (Valenciennes, 1844)

*Hypophthalmichthys nobilis* ( Richardson, 1845)

*Osteobrama cotio cotio* (Hamilton‒Buchanan)

*Puntius sarana* (Hamilton‒Buchanan)

*Puntius ticto* (Hamilton‒Buchanan)

*Salmostoma phulo* (Hamilton‒Buchanan)

*Amblypharyngodon mola* (Hamilton‒Buchanan)

*Esomus danricus* (Hamilton‒Buchanan)


Order : Siluriformes

      Family: Bagridae

*Aorichthys aor* (Hamilton‒Buchanan)

*Aorichthys seenghala* (Sykes)

*Mystus bleekari* (Day)

*Mystus cavasius* (Hamilton‒Buchanan)

*Mystus vittatus* (Bloch)

*Rita rita* (Hamilton‒Buchanan)

      Family: Siluridae

*Ompok bimaculatus* (Bloch)

*Ompok pabda* (Hamilton‒Buchanan)

*Wallago attu* (Bloch & Schneider, 1801)

      Family: Schilbeidae

*Clupisoma garua* (Hamilton‒Buchanan)

      Family: Sisoridae

*Bagarius bagarius* (Sykes)

      Family: Clariidae

*Clarius gariepinus* (Linnaeus)

      Family: Heteropneustidae

*Heteropneustes fossilis* (Bloch)

      Family:  Pangasiidae

*Pangasiodon hypothalamus*(Sauvage, 1878)

Order : Cyprinodontiformes

      Family: Belonidae

*Xenentodon cancila* (Hamilton‒Buchanan)

      Family: Synbrachidae

*Monopterus cuchia* (Amphinous)

Order : Perciformes

      Family: Ambassidae

*Chanda nama* (Hamilton‒Buchanan)

      Family: Nandidae

*Nandus nandus* (Hamilton‒Buchanan)

      Family: Gobiidae

*Boleophthalmus pectinirostris* (Linnaeus)

      Family:Cichlidae

*Oreochromis mossambicus* (Peters, 1852)

      Family: Stromateidae

*Pampus argentus* (Euphrasen, 1788)

      Family: Scombridae

*Scomberomorus guttatus* (Bloch & Schneider, 1801)

      Family: Channidae

*Channa marulius* (Hamilton‒Buchanan)

*Channa striatus* (Bloch)

*Channa punctate* (Bloch)

Order: Synbranchiformes

      Family: Mastacemblidae

*Macrognathus pancalus* (Hamilton‒Buchanan)

*Mastacembelus armatus* (Lacepede)

Order: Clupeiformes

      Family: Clupidae

*Gadusia chapra*(Hamilton‒Buchanan)

*Tenualosa ilisha* (Hamilton‒Buchanan)

*Sardinella longiceps* (Valenciennes)

Order: Osteoglossiformes

      Family: Notopteridae

*Notopterus notopterus* (Pallas, 1769)

*Notopterus chitala* (Hamilton‒Buchanan)

Order:Characiformes

      Family: Serrasalmidae

*Piaractus brachypomus* (Cuvier, 1818)

Phylum : Arthropoda, Subphylum: Crustacea, Class: Malacostraca,

      Family: Palaemonidae

*Macrobrachium rosenbergii* (De Man, 1879)

      Family: Portunidae

*Scylla serrata* (Forsskål, 1775)

**Fig. 3.** Share of different fish species under family Cyprinidae (% weight basis) from both capture and culture sector in fish markets of Punjab

*Rita rita*), Channidae (8.30%) (*Channa marulius* and *C. striatus*) and Notopteridae (3.98 %) (*Notopterus notopterus and N. chitala*) contributed significantly Fig. 4.

**Marketing Trend**

**Low Value/ Forage Species:** Eight fish species (*Xenentodon cancila*, *Chanda nama*, *Colisa fasciatus*, *Puntius sarana*, *P. ticto*, *Esomus danricus*, *Oreochromis mossambicus*, *Boleophthalmus pectinirostris*) were recorded from different markets with low consumer demand, hence low market value. These species are generally enter into the market as a by−catch of other targeted species from capture as well as culture fisheries sector within the State.
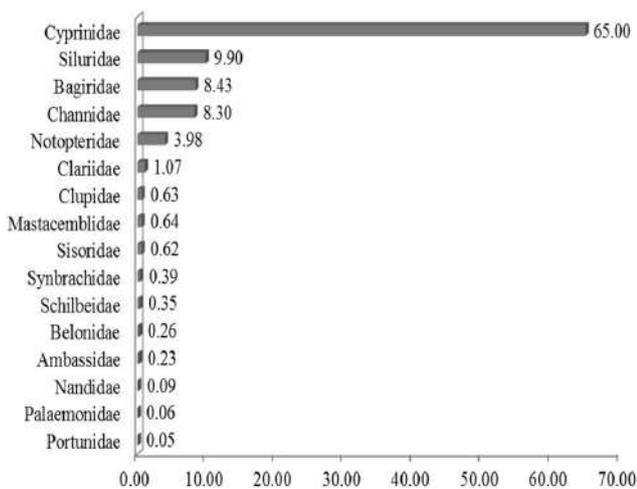


**Fig. 4.** Share of different families (% weight basis) from both capture and culture fisheries in fish markets of Punjab

**Shellfish Harvested Within the State:** Two shellfish species mainly *Macrobrachium rosenbergii* and *Scylla serrata* were recorded in Ludhiana fish market during winter imported from other states.

**Fish Procured From Outside State:** Six fish species i.e. *Pangasiodon hypothalamus* (Andhra Pradesh), *Pampus argentus* (Gujrat, Maharashtra), *Sardinella longiceps* (Karnataka, Maharashtra), Red belly pacu *Piaractus brachypomus* (Andhra Pradesh), *Tenualosa ilisha* (West Bengal) and *Scomberomorus guttatus* (Karnataka) imported from other states have higher consumer demand in Punjab and among these *Pangasiodon hypothalamus* has the highest demand. Fish from different states are distributed via Delhi and transported to different neighboring states including Punjab via road in large trucks. Fish are transported in thermocol boxes and individual box holds 40‑45 kg of fish with ice packing at the ratio of ice: fish = 1:8.

**Fish Markets in Punjab:** Species wise maximum number of fishery item trade (41) recorded in Ludhiana, followed by Taran taran (Harike) (40), Kapurthala (26), Jalandhar (18), Patiala (17) Amritsar (15). Details regarding quantum, trade pattern and contribution of different sectors including its import and species diversity are given in table 3.

Species wise *Labeo rohita*, *Catla catla* and *Cirrihinus mrigala* were recorded in all markets during all seasons (Table 2). *Cyprinus carpio*, *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix* and *H. nobilis* were recorded in Ludhiana, Taran taran and Jalandhar fish markets during all seasons. Fish from capture fisheries were highest in Taran taran market followed by Ludhiana. *Channa marulius* harvested from capture fisheries recorded in all markets during all seasons. *Pangasiodon hypothalamus* from Andhra Pradesh was recorded in all the markets except Taran taran.

**Sector Wise Contribution in Fish Market (Weight Basis):** Present survey recorded a lion share from inland culture and capture fishery items within the State contributed about 90% of market share. About 10% of marketed fish were from freshwater and marine fisheries resources from other states (2.0 ‑3.0 % contribution from marine fisheries)

**Marketing Channels**

Fish passes through several intermediaries from the landing center or fish pond to the consumer. The intermediaries are involved in providing services of head loading, processing, preservation, packing and transporting and these activities result in cost addition at every stage of marketing (Bishnoi, 2005). The key intermediaries in fish marketing are: auctioneer, wholesaler, retailer and the vendor. In Taran taran fish market where fish are marketed exclusively from capture sector whereas, in rest of the fish markets fish from capture as well as culture sectors are

**Table 2.** Seasonal abundance of fish species from different markets of Punjab

| Name of species | Ludhiana (Cap + Cul) | | | Taran taran (Cap) | | | Jalandhar (Cap + Cul) | | | Kapurthala (Cap + Cul) | | | Amritsar (Cap + Cul) | | | Patiala (Cap + Cul) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | W | S | M | W | S | M | W | S | M | W | S | M | W | S | M | W | S | M |
| **Fish from culture and capture fishery within the state** | | | | | | | | | | | | | | | | | | |
| *Labeo rohita* | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| *Catla catla* | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| *Cirrihinus mrigala* | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | – | * | * |
| *Cyprinus carpio* | * | * | * | * | * | * | * | * | * | * | – | – | * | * | * | – | * | * |
| *Ctenopharyngodon idella,* | * | * | * | * | * | * | * | * | * | * | – | – | – | – | – | * | * | – |
| *Hypophthalmichthys nobilis* | * | * | * | * | * | * | * | * | * | * | – | – | – | – | – | * | * | * |
| *Hypophthalmichthys molitrix* | * | * | * | * | * | * | * | * | * | * | – | – | – | – | – | * | * | – |
| *Labeo bata* | * | * | * | – | * | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Labeo calbasu* | – | – | – | * | * | * | – | – | – | – | – | – | – | – | – | * | * | – |
| **Fish from capture fishery within the state** | | | | | | | | | | | | | | | | | | |
| *Notopterus notopterus* | * | – | – | * | * | * | – | – | – | * | * | – | – | – | – | – | – | – |
| *Notopterus chitala* | – | * | – | * | * | * | – | – | – | – | – | – | * | – | – | – | – | – |
| *Labeo dero* | – | – | – | * | * | * | – | – | – | * | * | * | – | – | – | – | – | – |
| *Labeo gonius* | – | – | – | * | * | * | – | – | – | * | * | * | – | – | – | – | – | – |
| *Labeo dyocheilus* | – | – | – | – | * | * | – | – | – | – | – | – | – | – | – | – | – | – |
| *Osteobrama cotio cotio* | – | – | – | – | * | * | – | – | – | – | – | – | – | – | – | – | – | – |
| *Aorichthys aor* | * | – | – | * | * | * | – | – | – | – | – | – | * | * | – | – | – | – |
| *Mystus seenghala* | * | * | * | * | * | * | – | – | – | – | – | – | * | * | * | * | * | * |
| *Mystus bleekari* | – | – | – | * | * | * | – | – | – | – | – | – | – | – | – | – | – | – |
| *Mystus cavasius* | * | * | – | * | * | * | – | – | – | – | – | – | – | – | – | – | – | – |
| *Mystus vittatus* | * | * | * | * | * | * | – | – | – | * | * | – | – | – | – | – | – | – |
| *Rita rita* | * | * | * | * | * | * | * | * | * | * | * | – | – | * | * | * | * | – |
| *Ompok bimaculatus* | * | * | * | * | * | * | – | – | – | – | – | – | – | – | – | – | – | – |
| *Ompok pabda* | * | * | * | * | * | * | – | – | – | * | * | * | – | – | – | – | – | – |
| *Wallago attu* | * | * | * | * | * | * | * | – | * | * | – | * | * | * | * | * | * | * |
| *Bagarius bagarius* | * | * | * | * | * | * | * | – | * | * | – | * | * | * | * | – | – | – |
| *Clarias gariepinus* | * | * | * | – | – | – | – | – | – | * | – | * | – | – | – | * | * | * |
| *Heteropneustes fossilis* | * | – | * | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Channa marulius* | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| *Channa punctatus* | * | * | * | * | * | – | – | * | – | – | * | * | – | – | – | * | – | – |
| *Channa striatus* | * | * | * | * | * | * | * | * | * | * | * | * | – | – | – | – | – | – |
| *Macrognathus pancalus* | – | – | – | * | * | * | – | – | – | – | – | – | – | – | – | – | – | – |
| *Mastacembelus armatus* | – | – | – | * | * | * | – | – | – | – | – | – | – | – | – | – | – | – |
| *Gudusia chapra* | * | – | – | – | * | – | – | – | – | – | – | – | – | – | – | – | – | |
| *Nandus nandus* | – | – | – | – | * | – | – | – | – | – | – | – | – | – | – | – | – | |
| *Clupisoma garua* | – | – | – | – | * | * | – | – | – | – | – | – | – | – | – | – | – | – |
| *Salmostoma phulo* | – | * | * | – | * | * | – | * | * | – | – | – | – | – | – | – | – | – |
| *Xenentodon cancila*** | * | * | – | * | * | * | – | – | – | * | * | * | – | – | – | – | – | – |
| *Chanda nama*** | * | * | – | * | * | * | – | – | – | * | * | * | – | – | – | – | – | – |
| *Colisa fasciatus*** | * | * | * | * | * | * | – | – | – | * | * | * | – | – | – | – | – | – |
| *Puntius sarana *** | * | * | * | * | * | * | – | – | – | * | * | * | – | – | – | * | * | * |
| *Puntius ticto*** | * | * | * | * | * | * | – | – | – | * | * | * | – | – | – | * | * | * |

**Cont...**

| Species | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Esomus danricus*** | * | * | – | – | * | – | – | – | – | * | * | * | – | – | – | – | – | – |
| *Boleophthalmus pectinirostris*** | – | * | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Oreochromis mossambicus*** | * | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Macrobrachium rosenbergii#* | * | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Scylla serrata#* | * | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| **Fish outside from state** | | | | | | | | | | | | | | | | | | |
| *Pangasiodon hypothalamus* (Andhra Pradesh) | * | * | * | – | – | – | * | * | * | * | * | * | * | * | * | * | * | * |
| *Pampus argentus* (Guj, Maharashtra) | * | – | – | – | – | – | * | – | – | – | – | – | * | – | – | – | – | – |
| *Sardinella longiceps* (Maharashtra,) | * | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Piaractus brachypomus* (Red belly pacu) | * | – | – | – | – | – | – | – | – | – | – | – | – | – | – | * | * | * |
| *Tenualosa ilisha* (West Bengal via Delhi) | * | – | – | – | – | – | – | – | – | – | – | * | – | – | – | – | – | – |
| *Scomberomorus guttatus* (Karnataka via Delhi) | * | – | – | – | – | – | * | – | – | – | – | – | * | – | – | – | – | – |

* Present, −Absent, W−Winter, S−Summer and M−Monsoon, Cap−Capture fisheries, Cul−Culture fisheries, ** Low value fish, # Shellfish species

**Table 3.** Status of different fish markets in Punjab

| Components | Markets | | | | | |
|---|---|---|---|---|---|---|
| | Ludhiana | Amritsar | Taran Taran | Jalandhar | Kapurthala | Patiala |
| Total fish sale (ton/day) | 3.0–10.0 | 0.35–3.5.0 | 0.5–3.0 | 2.0–5.0 | 1.5–2.0 | 0.50–3.0 |
| Share of capture fish (%) | 25.0–30.0 | 65.0–70.0 | 100 % | 30−35.0 | 35.0–40.0 | 35.0–40.0 |
| Share of culture fish (%) | 60.0–65.0 | 25.0 –30.0 | 0.0% | 25.0 –30.0 | 65.0–70.0 | 50.0–55.0 |
| Imported fish (Other states) (%) | 15.0–20.0 | 10.0–15.0 | 0.0 % | 35.0–40.0 | 8.0–10.0 | 10.0–15.0 |
| Major fish | Carps | Sole (*Channa striatus*) | Carps | Pangas | Carps | Carps |
| Total species in trade (Number) | 41 | 15 | 40 | 18 | 26 | 17 |

marketed. The biggest challenge in documenting intermediaries in fish marketing is their multifunctional performances. Economically sound fish venders/ fish auctioneer directly purchase fish from fish farms in early morning in live condition and same are transported by small trucks or jeeps in fish markets (particularly in Ludhiana) for further auctioning to the small venders. Diagrammatically the marketing channels are represented as follows in Fig. 5.

**Constrains Faced by Fish Venders in Markets**
**Unregulated Fish Supply in The Market:** Survey revealed that there was no control over the amount of fish that should be marketed/ auctioned per day. As a result over supply of fish sometimes drastically reduce the selling price.
**Lack of Proper Storage Facilities:** Fish venders generally use thermocol boxes where 40–45 kg of fish can be stored using crushed ice as refrigerant maintaining the ratio of fish:
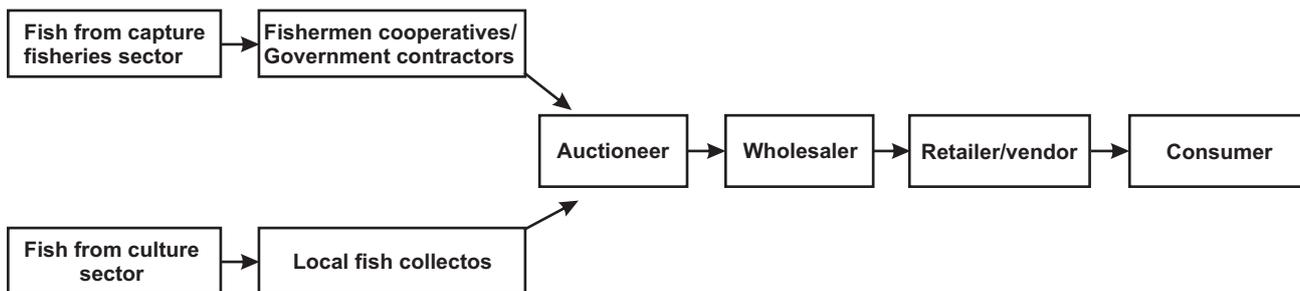


**Fig. 5.** Fish marketing channels in Punjab

ice = 8: 1. The ice blcok is purchased from the adjacent ice factories to the fish market @ Rs 1–2.5/kg. Lack of cold storage facilities in fish markets have been observed during the survey.

## CONCLUSIONS

Survey revealed that inland culture and capture fisheries a lion share from within the State contributed about 90% of market share. About 10% of marketed fish were recorded from freshwater and marine fisheries resources from other states. Total 52 fish species were recorded under capture and culture sector within the State as well as fish imported from far off states. 26 fish species were recorded exclusively from the capture fisheries. Cyprinidae family contributed highest market share. Fish species available in markets from capture fisheries were harvested mainly from Harike wetland, Nangal and Pong dams. Maximum number of fish species was recorded in Ludhiana fish market followed by Taran taran. The key intermediaries in fish marketing were auctioneer, wholesaler, retailer/ vendor. Some of the major constrains like lack of cold chain and waste disposal facilities are needed to be addressed for further development in this sector. Marketing channels can be simplified so that the fish produced in farm or harvested from natural sources can directly be reached to the consumers without intervention of number of intermediaries.

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# Natural Regeneration of *Quercus glauca* Thunberg Forests and its Relation with Site Conditions

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**Abstract**: This study was conducted to assess the relationship between site conditions and natural regeneration of six *Quercus glauca* bearing forests of northwest Himalaya. The results showed that regeneration and stocking percent of the study sites was high although *Quercus glauca* had a very low regeneration and stocking percents. In addition to stand characteristics and human pressure, site condition were found to be linked with lack of *Quercus glauca* regeneration. Generally, undisturbed sites had better soil fertility, soil moisture and low solar influx to the forest floor. Natural regeneration of *Quercus glauca* was recorded higher in moist and better nutrient rich sites, whereas, natural regeneration decreased with increase in soil pH, available phosphorus and solar influx. Available potassium had no correlation with regeneration of the species. Soil moisture and solar influx had higher $R^2$ values, which can better explain variation in natural regeneration among the sites. Therefore, regulation of soil moisture, available nutrients and light conditions can facilitate natural regeneration of *Quercus glauca*.

**Keywords**: Organic matter layer, Soil moisture, Soil nutrients, Soil pH, Solar influx

The economical and ecological benefit of oak forests is strongly linked with agriculture. However, sever anthropogenic influence imposed on these forests coupled with lack of regeneration in its natural habitat threatened these forests. Regeneration of oak species was reported to be problematic usually resulting in poor germination (Ibanez *et al.*, 2015).Various physical and biotic factors including soil moisture/drought (Orwa *et al.,* 2009), soil nutrient (Royse *et al.*, 2010; Major *et al.*, 2013), light conditions (Rodriguez *et al.*, 2008), competition (Jensen, 2011), disturbance (Anitha *et al.* 2009, Pokhriyal *et al.*, 2012) and acorn damage (Mancilla‑Leyton *et al.,* 2012, Yoko‑o and Tokeshi, 2012) were reported to affect natural regeneration of oaks. Shrestha (2003) reported poor natural regeneration of *Quercus semecarpifolia* due to heavy and indiscriminate annual lopping, litter collection, overgrazing, forest fire, available light, and herbaceous cover of the forest site. These factors have a direct influence in modifying site conditions altering seed bed receptivity. Controlled fire also reported to modify forest floor, open canopy gaps, reduce competitors and break acorn dormancy so that it enhanced natural regeneration of oak species (Wang *et al.,* 2005; Royse *et al.,* 2010). Major *et al.* (2013) and Hutchinson *et al.* (2005) reported the importance of periodic selective harvesting together with controlled fire for successful regeneration of oak species. Considering the above inter‑linkage between various site factors, it becomes vital to investigate how site conditions like soil pH, moisture, nutrients and light conditions vary in *Quercus glauca* forests and assess their implications on the regeneration of the species.

## MATERIAL AND METHODS

The study had been conducted in six *Quercus glauca* dominating forests of shilli, Oachghat, Deothal, Narag, Chakla and Tikri at Himachal Pradesh, India (Table 1). Three plots of 20m x 20m were randomly allocated in each stand from which natural regeneration was assessed in 5 quadratic sub‑plots of 2m x 2m distributed in the four corners and center of these plots. Number of recruits (current year seedlings), unestablished regenerations (seedling other than recruits and their height less than 2 m) and established regeneration (seedlings with a height of 2m or more) of different tree species were counted and their heights was measured in each sub‑plot. The data collected was analyzed using the method given by Chako (1965). Site conditions including soil properties, organic matter layer, and available light on a forest floor were accounted. Three composite samples were taken at a depth of 0‑15cm and 15‑30cm to assess soil parameters. Soil moisture content was assessed based on dry weight basis. Soil pH, organic carbon, available N, P, K were assessed based on standard methods. Organic matter layer was measured with the help of measuring scale at three randomly selected spots along

**Table 1.** General details of the study sites

| Forest area | Forest range | Latitude | Longitude | Altitude | Forest status | Access |
|---|---|---|---|---|---|---|
| Shilli | Solan | 30°54.563N | 77°07.172E | 1467 | Protected | Restricted |
| Oachghat | Solan | 30°52.055N | 77°07.432E | 1220 | Private | Open (to owner) |
| Deothal | Narag | 30°49.767N | 77°09.538E | 1052 | Community | Open |
| Narag | Narag | 30°49.385N | 77°10.251E | 1127 | Protected | Restricted (loose) |
| Chakla | Sarhan | 30°48.352N | 77°10.581E | 1165 | Community | Open |
| Tikri | Sarhan | 30°48.024N | 77°09.894E | 1096 | Community | Open |

the slop. Solar influx was measured by using lux meter at the center of the plot.

## RESULTS AND DISCUSSION

**Regeneration status of the study sites:** A total of 22 species were recorded in the regeneration spectrum (Table 2) among which *Punica granatum, Pyrus pashia* and *Quercus glauca* were recorded at all the study sites, while majority of other species had restricted occurrence. In general, natural regeneration (of all species) was the highest (229.2 %) at Shilli followed by Tikri, Deothal, Oachghat, Narag and Chakla. However, natural regeneration of *Quercus glauca* was quite poor. The number of tree species regenerated as recruits and unestablished regenerations were much higher than the established ones. This suggested that other factors are responsible for regeneration failure of most tree species than seed supply or regeneration behaviors.

The highest number of recruits, unestablished and established regenerations were recorded at Shilli (Table 2). Establishment stocking per cent and regeneration per cent were also the highest. The recruits, unestablished and established regenerations of the site were dominated by *Cryptolepis buchanani, Myrica esculenta* and *Pyrus pashia,* respectively. These species are known for their shade tolerance and forming close association with *Quercus* and *Pinus* species (Hussain *et al.,* 2008; Tayung and Saikia; 2003, Bhatt *et al.,* 2000). This site was also better protected, especially from grazing, browsing and also had the lowest (7.9%) lopping intensity (Amare and Bhardwaj, 2016). In contrast to their dominance, the regeneration status of *Quercus glauca* and *Quercus leucotrichophora* was moderate. The result partially supports regeneration problem of the genus as also reported by other workers in the past *(*McCreary, 2009; Shrestha, 2003*).*

The recruit and unstablished regeneration of the forest at Ochghat was dominated by *Quercus glauca* and *Punica granatum, respectively*. Established regeneration was also high for *Ailanthus excelsa*, *Bauhinia variegata* and *Quercus glauca*. However, the first two species had no recruits and unestablished regenerations that may be due to shading

effect. Although, it has the highest number of recruits, the regeneration performance *Quercus glauca* was much similar to Shilli site*.* Higher density of trees at Shilli and Oachghat may not have effect on recruits of *Quercus* species, however light was an essential requirement for further growth and establishment of the species (Welander and Ottosson, 2000).

Deothal was the most disturbed site that had a direct effect on stand structure, species composition and regeneration of the site. The lowest tree density and tree species richness was recorded at this site and its effect was reflected on the regeneration. Few number of tree species accounted for their regeneration. The lowest number of recruits was observed at this site, while its established regenerations were occupied by only two tree species i.e. *Punica granatum* and *Quercus glauca*. Both the low number of recruits and the absence of established regeneration for most species were a result of shrub competition. Highest density of shrubs was recorded at this site and the density of herbs was also high. Although shrubs may protect oak seedlings from browsing, their aboveground competition effect after three years of regeneration is considerable (Jensen, 2011).

The highest number of recruits, unestablished and established regenerations at Narag was attributed to *Quercus glauca.* The highest regeneration and stocking per cent was also accounted by *Quercus glauca*. The site was characterized by its low tree density, high crown shading, high organic matter layer, high soil moisture, organic carbon and available nitrogen contents. The site was also protected forest that entertains low intensity of grazing. Thus, it can be presumed that these site conditions may play an important role for the prolific regeneration of *Quercus glauca.* Fernandez (2008) reported the importance of available soil nutrients in the forest to predict site quality and its productivity. Welander and Ottosson (2000) also reported the role of light, water and nutrients on seedling growth of *Quercus robur.* Whereas, Royse *et al.* (2010) and Major *et al.* (2013) reported that high litter depth and soil fertility leads to high seedling mortality and less natural regeneration of oak species. On the other hand, Shrestha (2003) reported that

**Table 2.** Natural regeneration status of the study sites

| Forest Site | Species | Recruits (N ha$^{-1}$) | Unestablished regeneration (N ha$^{-1}$) | Established regeneration (N ha$^{-1}$) | Establishment stocking (%) | Regeneration (%) |
|---|---|---|---|---|---|---|
| Shilli | *Ailanthus excelsa* | 0 | 500 | 0 | 0 | 5 |
| | *Albizia* spp. | 1000 | 500 | 500 | 5 | 10 |
| | *Celtis australis* | 2333 | 1167 | 1000 | 10 | 21.7 |
| | *Cryptolepis buchanani* | 4000 | 0 | 0 | 0 | 0 |
| | *Machilus duthiei* | 500 | 0 | 500 | 5 | 5 |
| | *Myrica esculenta* | 2833 | 3833 | 1667 | 16.7 | 55 |
| | *Pistacia integerrima* | 1000 | 0 | 0 | 0 | 0 |
| | *Punica granatum* | 1000 | 0 | 0 | 0 | 0 |
| | *Pyrus pashia* | 2000 | 2667 | 1833 | 18.3 | 45 |
| | *Quercus glauca* | 1167 | 1167 | 1000 | 10 | 21.7 |
| | *Quercus leucotrichophora* | 1250 | 1667 | 1000 | 10 | 26.7 |
| | *Triadica sebifera* | 0 | 1000 | 0 | 0 | 10 |
| | *Zanthoxylum alatum* | 1000 | 1667 | 1250 | 12.5 | 29.2 |
| | Total | 18083 | 14167 | 8750 | 87.5 | 229.2 |
| Oachghat | *Acacia* spp. | 0 | 500 | 0 | 0 | 5 |
| | *Acer oblongum* | 0 | 500 | 500 | 5 | 10 |
| | *Ailanthus excelsa* | 0 | 0 | 1000 | 10 | 10 |
| | *Bauhinia variegata* | 0 | 0 | 1000 | 10 | 10 |
| | *Celtis australis* | 1500 | 1000 | 500 | 5 | 15 |
| | *Cryptolepis buchanani* | 2000 | 0 | 0 | 0 | 0 |
| | *Pistacia integerrima* | 1000 | 1000 | 0 | 0 | 10 |
| | *Punica granatum* | 1250 | 2750 | 0 | 0 | 27.5 |
| | *Pyruspashia* | 3000 | 0 | 0 | 0 | 0 |
| | *Quercus glauca* | 6750 | 1167 | 1000 | 10 | 21.7 |
| | Total | 15500 | 6917 | 4000 | 40 | 109.2 |
| Deothal | *Celtis australis* | 1500 | 1000 | 0 | 0 | 10 |
| | *Melia azedarach* | 0 | 500 | 0 | 0 | 5 |
| | *Punica granatum* | 1000 | 1500 | 3500 | 35 | 50 |
| | *Pyrus pashia* | 0 | 3500 | 0 | 0 | 35 |
| | *Quercus glauca* | 1500 | 1250 | 667 | 6.7 | 19.2 |
| | *Zanthoxylum alatum* | 1250 | 1500 | 0 | 0 | 15 |
| | Total | 5250 | 9250 | 4167 | 41.7 | 134.2 |
| Narag | *Ailanthus excelsa* | 2000 | 0 | 0 | 0 | 0 |
| | *Celtis australis* | 1000 | 500 | 0 | 0 | 5 |
| | *Ficus palmata* | 1500 | 0 | 0 | 0 | 0 |
| | *Machilus duthiei* | 0 | 500 | 0 | 0 | 5 |
| | *Myrica esculenta* | 750 | 500 | 500 | 5 | 10 |
| | *Punica granatum* | 0 | 1500 | 1000 | 10 | 25 |
| | *Pyrus pashia* | 1000 | 1500 | 0 | 0 | 15 |
| | *Quercus glauca* | 2167 | 2833 | 1167 | 11.7 | 40 |
| | Total | 8417 | 7333 | 2667 | 26.7 | 100 |
| Chakla | *Acer oblongum* | 750 | 750 | 500 | 0 | 12.5 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *Glochidion* spp. | 1500 | 0 | 0 | 0 | 0 |
| | *Machilus duthiei* | 1000 | 1000 | 750 | 7.5 | 17.5 |
| | *Olea paniculata* | 0 | 1000 | 0 | 0 | 10 |
| | *Punica granatum* | 1500 | 2000 | 0 | 0 | 20 |
| | *Pyrus pashia* | 1000 | 0 | 0 | 0 | 0 |
| | *Quercus glauca* | 500 | 500 | 667 | 6.7 | 11.7 |
| | Total | 6250 | 5250 | 1917 | 14.2 | 71.7 |
| Tikri | *Ailanthus excelsa* | 1000 | 1500 | 0 | 0 | 15 |
| | *Celtis australis* | 0 | 1000 | 0 | 0 | 10 |
| | *Ficus sarmentosa* | 1500 | 0 | 0 | 0 | 0 |
| | *Machilus duthiei* | 1500 | 1000 | 0 | 0 | 10 |
| | *Myrica esculenta* | 0 | 0 | 500 | 5 | 5 |
| | *Punica granatum* | 0 | 0 | 1000 | 10 | 10 |
| | *Pyrus pashia* | 1167 | 1500 | 0 | 0 | 15 |
| | *Quercus glauca* | 0 | 1667 | 1250 | 12.5 | 29.2 |
| | *Toona ciliata* | 500 | 500 | 3000 | 30 | 35 |
| | *Zanthoxylum alatum* | 1500 | 2000 | 0 | 0 | 20 |
| | Total | 7167 | 9167 | 5750 | 57.5 | 149.2 |

**Table 3.** Site characteristics of *Quercus glauca* forests of the study sites

| Treatment | Soil pH | Moisture content (%) | Organic carbon (%) | Available nitrogen (kg ha$^{-1}$) | Available phosphorus (kg ha$^{-1}$) | Available potassium (kg ha$^{-1}$) | Organic matter layer (cm) | Solar influx (%) |
|---|---|---|---|---|---|---|---|---|
| **Study site** | | | | | | | | |
| Shilli | 5.67 | 35.0 | 1.38 | 313.1 | 29.5 | 219.3 | 1.34 | 16.3 |
| Oachghat | 5.82 | 30.9 | 1.36 | 312.0 | 19.8 | 231.5 | 1.43 | 21.6 |
| Deothal | 6.44 | 29.5 | 1.32 | 254.5 | 30.2 | 224.5 | 0.74 | 21.0 |
| Narag | 4.86 | 39.5 | 1.46 | 344.5 | 27.2 | 229.1 | 1.49 | 12.4 |
| Chakla | 6.00 | 26.8 | 1.29 | 266.8 | 25.7 | 222.1 | 0.63 | 22.1 |
| Tikri | 5.46 | 36.0 | 1.44 | 312.0 | 27. 8 | 221.3 | 0.76 | 17.3 |
| CD (p=0.05) | 0.10 | NS | 0.11 | NS | NS | 6.41 | 0.41 | NS |
| **Soil depth** | | | | | | | | |
| 0–15 cm | 5.70 | 34.1 | 1.75 | 339.9 | 32.0 | 253.3 | – | – |
| 15–30 cm | 5.71 | 31.7 | 1.00 | 261.1 | 21.4 | 196.0 | – | – |
| CD (p=0.05) | NS | NS | 0.06 | 48.03 | 5.93 | 3.70 | – | – |

lack of sufficient natural regeneration of *Q. semecarpifolia* was due to anthropogenic effect and it didn't have clear relationships with soil factors.

The establishment stocking per cent and regeneration per cent were lowest at Chakla forest site. The performance of *Quercus glauca* regeneration was also the lowest of all the study sites. This site is more or less similar to Deothal from level of protection point of view. Human interference was very high and a few trees species accounted for their regeneration; most trees had no established regenerations. The site was also characterized by highest light radiation available on the forest floor but lowest soil moisture content,

organic carbon, and organic matter layer. These factors coupled with the presence of several old age *Quercus glauca* trees might have affected regenerations of all the species growing at the sites.

*Ficus sarmentosa, Machilus duthiei* and *Zanthoxylum alatum* had the highest recruits at Tikri, while the highest unestablished and established regenerations were recorded for *Zanthoxylum alatum* and *Toona ciliata,* respectively. Establishment stocking per cent and regeneration per cent was also high for *Toona ciliata. Quercus glauca,* had a moderate establishment stocking per cent and regeneration per cent. Its establishment stocking and regeneration was

**Table 4.** Correlation analysis of regeneration of *Quercus glauca* and associated species with site characteristics

| | | Regeneration | | Soil pH | | Moisture content | | Organic carbon | | Available nitrogen | | Available phosphorus | | Available potassium | | Organic matter layer |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Quercus glauca* | Associated species | 0–15 cm | 15–30 cm | 0–15 cm | 15–30 cm | 0–15 cm | 15–30 cm | 0–15 cm | 15–30 cm | 0–15 cm | 15–30 cm | 0–15 cm | 15–30 cm | |
| Regeneration | *Quercus glauca* | 1.00 | | | | | | | | | | | | | | |
| | Associated species | -0.07 | 1.00 | | | | | | | | | | | | | |
| Soil pH | 0–15 cm | -0.60** | 0.19 | 1.00 | | | | | | | | | | | | |
| | 15–30 cm | -0.58* | 0.16 | 0.99** | 1.00 | | | | | | | | | | | |
| Moisture content | 0–15 cm | 0.87** | 0.04 | -0.50* | -0.48* | 1.00 | | | | | | | | | | |
| | 15–30 cm | 0.85** | 0.15 | -0.46 | -0.45 | 0.98** | 1.00 | | | | | | | | | |
| Organic carbon | 0–15 cm | 0.54* | 0.21 | -0.57* | -0.59** | 0.54* | 0.60** | 1.00 | | | | | | | | |
| | 15–30 cm | 0.15 | -0.40 | -0.11 | -0.09 | -0.03 | -0.08 | -0.42 | 1.00 | | | | | | | |
| Available nitrogen | 0–15 cm | 0.20 | -0.04 | -0.52* | -0.53* | 0.04 | 0.05 | 0.41 | -0.26 | 1.00 | | | | | | |
| | 15–30 cm | 0.16 | -0.22 | -0.53* | -0.56* | 0.05 | 0.08 | 0.48* | -0.11 | 0.81** | 1.00 | | | | | |
| Available phosphorus | 0–15 cm | 0.05 | 0.30 | -0.01 | -0.04 | 0.02 | 0.05 | 0.17 | 0.07 | 0.35 | 0.26 | 1.00 | | | | |
| | 15–30 cm | -0.15 | 0.10 | 0.05 | 0.05 | -0.10 | -0.07 | -0.04 | -0.05 | 0.26 | 0.06 | 0.54* | 1.00 | | | |
| Available potassium | 0–15 cm | 0.01 | -0.37 | 0.03 | 0.05 | -0.11 | -0.14 | -0.17 | -0.13 | -0.12 | -0.14 | -0.43 | -0.04 | 1.00 | | |
| | 15–30 cm | 0.26 | -0.35 | -0.42 | -0.42 | -0.05 | -0.08 | 0.13 | 0.12 | 0.35 | 0.40 | -0.01 | -0.30 | 0.44 | 1.00 | |
| Organic matter layer | | 0.45 | 0.33 | -0.50* | -0.50* | 0.47* | 0.48* | 0.46 | -0.28 | 0.19 | 0.17 | -0.02 | -0.19 | 0.23 | 0.38 | 1.00 |
| Solar influx | | -837** | -0.03 | 0.43 | 0.41 | -0.95** | -0.92** | -0.42 | -0.02 | -0.09 | -0.09 | -0.12 | 0.10 | 0.22 | 0.04 | -0.40 |

* and **correlation is significant at the 0.05 and 0.01 level, respectively (2-tailed)

**Table 5.** Regression analysis of germination of *Quercus glauca* and site characteristics

| Equation* | $R^2$ | SE$\pm$ | $P_{0.05}$ |
|---|---|---|---|
| $Y = -17.21X_1 + 119.5$ | 0.363 | 11.9 | 0.008 |
| $Y = -16.5X_2 + 115.5$ | 0.335 | 12.1 | 0.012 |
| $Y = 1.71X_3 + -36.9$ | 0.753 | 7.4 | 0.000 |
| $Y = 1.6X_4 - 30.1$ | 0.718 | 7.9 | 0.000 |
| $Y = 42.8X_5 - 53.4$ | 0.289 | 12.5 | 0.021 |
| $Y = -1.8X_6 + 54.8$ | 0.700 | 8.1 | 0.000 |

*Y represents germination of *Quercus glauca,* whereas $X_1$= soil pH (0–15 cm), $X_2$= soil pH (15–30 cm), $X_3$= moisture content (0–15 cm), $X_4$= moisture content (15–30 cm), $X_5$= organic carbon (0–15 cm) and $X_6$ = solar influx

also the second highest among all the study sites. Like regenerations at Deothal and Chakla, much of the species failed to establish at Tikri. The higher number of tree species in the first phase of regeneration (recruits) and absence of established regeneration might indicate the progressive disturbance level of the site. Higher soil moisture content, the moderate soil nutrient contents and low light intensity on the forest floor indicated that disturbance was moderate as compared to Deothal and Chakla forest site. The positive influence of moderate disturbance on oak regeneration is also reported by Pande *et al*. (2014).

In general, regeneration of *Quercus glauca* at all the study sites was low; it ranged from 11.7–40 %. Evidenced from low regeneration percent of highly disturbed sites, anthropogenic factors appear to be a reason for lack of regeneration at the study sites. In addition to anthropogenic factor, site conditions do play a significant role in regeneration. The highest recruits, unestablished regeneration as well as regeneration percent were recorded at Narag. Better regeneration potential of this site was attributed to its moderate disturbance, better soil fertility, high crown width and higher shade (Amare and Bhardwaj, 2016). Tikri had better established seedlings and establishment stocking per cent. This site was presumed to be in transition from less disturbed site to highly disturbed site. The total absence of recruits and less number of unestablished seedlings of *Quercus glauca* might support this proposition. The low regeneration per cent was recorded at Deothal and Chakla, which is evidently an impact of sever disturbance on regeneration potential of *Quercus glauca.*

**Site characteristics of the study sites:** Site characteristics of *Quercus glauca* bearing forests of north-western Himalaya revealed the presence of significant differences among the study sites with respect to soil pH, organic carbon, available potassium and organic matter layer. However, differences in soil moisture content, available nitrogen, available phosphorus and solar influx were insignificant (Table 3).

Regeneration of *Quercus glauca* increased with increase in soil moisture content, organic carbon, available nitrogen and organic matter layer (Table 3). Some deviations from this general trend were observed at Shilli and Chakla in reference to available nitrogen and at Shilli and Oachghat in reference to organic matter layer; which might be due to other biotic and abiotic factors. On the other hand, *Quercus glauca* regeneration decreased with increase in soil pH, available phosphorus and available light on the forest floor/solar influx. Some deviations to this general trend were observed at Deothal in reference to soil pH, at Oachghat and Chakla in reference to available phosphorus, and at Oachghat and Tikri in reference to solar influx. These variations can be attributed to disturbance in the forest ecosystems and other site conditions. Available potassium has no clear relationship with *Quercus glauca* regeneration.

**Correlation and regression analysis between regeneration and site factors:** The soil pH in 0–15 cm and 15–30cm soil depths as well as solar influx showed a significant and negative correlation with regeneration of *Quercus glauca* (Table 4). However, soil moisture content in a soil depth of 0–15 cm and 15–30cm and soil organic carbon at the upper soil depth (0–15 cm) had a significant and positive correlation with regenerations of *Quercus glauca.* The simple linear regression analysis of these variables as independent variable and regeneration of *Quercus glauca* as dependent variable demonstrate that the ability of these site factors to explain regeneration dynamics of the species was very low, except for soil moisture content and solar influx (Table 5). The linear function presented for moisture content of 0–15cm, as independent variable, can explain 75% of the variation in natural regeneration of *Quercus glauca*; while the linear regression equation presented for solar influx, as independent variable, explained 70 % of variation in regeneration.

In conclusion, the overall natural regeneration at the study sites was very high although the natural regeneration and stocking per cent of *Quercus glauca* was quite low. In addition to stand characteristics and biotic disturbance, site conditions were found to be associated to natural regeneration. Soil pH, soil moisture, organic carbon and solar influx had a significant correlation with natural regeneration of *Quercus glauca*. However, soil moisture and solar influx are better explanatory variables to natural regeneration of the species. Therefore, proper regulation of soil moisture, available nutrients and light conditions can help to improve natural regeneration and successful establishment of *Quercus glauca*.

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# Effect of Moisture Conservation Methods and Nutrients Application on Biomass in *Eucalyptus pellita*

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**Abstract:** The study was carried out in one year old *Eucalyptus pellita* plantation. Moisture conservation treatments were trapezoidal staggered trench, conservation pit, ring trench, control and nutrient application were 200: 100:200, 250:125:250, 125:75:75, (kg) $N:P_2O_5: K_2O$ and control. Data were recorded after one year of treatments. Maximum biomass and carbon stock was recorded in trapezoidal staggered trench 4.624 t ha$^{-1}$ and 2.312 t ha$^{-1}$, respectively. Nutrient application 200:100:200 kg ha$^{-1}$ $N:P_2O_5: K_2O$ resulted maximum 4.284 t ha$^{-1}$ and 2.142 t ha$^{-1}$ biomass and carbon stock, respectively. These two treatments in combination also recorded maximum biomass and carbon stock among all other interaction treatments.

**Keywords:** Biomass, Carbon stock, *Eucalyptus pellita,* Moisture, Nutrients

Eucalyptus a fast growing, native of Australia comprises of genus *Eucalyptus* comprises of 700 species. The species was introduced in India by Tippu Sultan the Ruler of Mysore who planted few species on Nandi hills of Mysore in the late 18th century (Rajashekar *et al.,* 2006; Chauhan *et al.*, 2008). It is valuable tree that has number of benefits such as making wood pulp to manufacture paper, rayon and other synthetic fibre, timber, fly wood, essential oil from its leaves, bark is used for tanning. The *Eucalyptus* is of great importance in developing countries and regarded a remarkably versatile species, which adopt to varied climatic and soil condition.

Rain water is the main source of moisture during plantation activities, but it is often scanty and erratic in nature with massive runoff losses. Runoff water can be harvested in small catchment nearby plants, which help the plants to utilize moisture during scare season (summer). By adopting this technique, growth and establishment of plants were found to improve readily (Prinz, 2001; Bhardwaj *et al.,* 1995). There are many water conservation techniques but the technique that may be adopted will be based on climatic condition of the region and socio-economic condition of the farmers. Structures such as circular catchment of 1.5 m radius and 2 per cent slope runoff generating areas were found effective in improving the moisture content of the plant root zone.

*Eucalyptus* being a fast growing tree has huge potential to generate biomass. Hence, an investigation was conducted on effect of moisture conservation methods and nutrients application on biomass and carbon sequestration in *Eucalyptus pellita* plantation.

The present investigation was carried out on one year old plantation raised at 2x2m spacing by West Coast Paper Mill. Plantation is situated periphery of Uttarakannada district towards Haveri at 14°38' N latitude and 750.00 E longitudes and an altitude of 490 m above mean sea level. The study area falls under tropical climate. The climate is primarily monsoon during June–September. The average annual rainfall in the experimental area was 2000 mm. The soil of the experimental site was sandy clay loam (Kan Haplustalf). The soil was medium deep with fairly good drainage.

The experiment was laid out spit plot design with three replications. Gross plots (8 m x 8 m) of 16 plants were considered for each treatment [moisture conservation – Trapezoidal Staggered Trench Size: 60 cm top width. 30 cm bottom width. 30 cm depth. 1.5 m Length (M1), Conservation Pit: 45 X 30 X 30 cm (M2); Ring Trench: 1.5 diameter (M3); control (m4) and fertilizer application – 200:100:200 of $N: P_2O_5 : K_2O$ in kg ha$^{-1}$ (F1); 250:125:250 of $N: P_2O_5 : K_2O$ in kg ha$^{-1}$ (F2); 125:75:75 of $N: P_2O_5 : K_2O$ in kg ha$^{-1}$ + FYM (5 t ha$^{-1}$); control (F4)]. Five plants per treatment were randomly taken to record the data on height and diameter. Nitrogen (N), Phosphorus ($P_2O_5$) and Potash ($K_2O$) fertilizers were applied in the form of urea, rock phosphate, and Murat of potash respectively. Nitrogen was applied in two equal split dose, while phosphorus and potassium were applied as basal dose, 50 per cent of nitrogen and full dose phosphorus and potash were applied before on set of monsoon (May) and remaining 50 per cent of nitrogen was applied after four months of first application.

The plant height (m) was measured by using graduated pole and diameter at breast height (cm) was measured with digital caliper. Volume was calculated in order to calculate the

biomass as per Pressler's formula (1865) i.e., V = f×h×g Where, V = volume; f = form factor; h = total height and g = basal area.

Aboveground biomass = VOB × WD where, VOB = Volume over bark ($m^3ha^{-1}$); WD = Wood density

The study reveals that moisture conservation methods and fertilizers levels influenced the height and collar diameter significantly. The maximum plant height (4.65 m) and DBH (3.94 cm) was recorded in trapezoidal staggered trench (Table 1). Higher height in Trapezoidal staggered trench method than other methods might be due to more soil moisture available for the plants. Higher moisture available in this treatment for longer duration supported the plants in dry season. The



$y = 0.8706x - 10.476$
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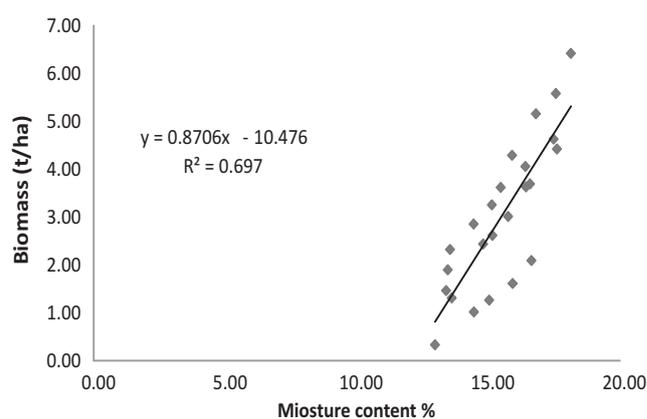
**Fig. 1**. Relation of biomass increment with moisture content

**Table 1**. Effect of moisture conservation measures and fertilizers on growth and biomass

| Treatments | DBH (cm) | Height (m) | Biomass (t ha$^{-1}$) |
|---|---|---|---|
| Main plots (M) | | | |
| Trapezoidal staggered trench (M1) | 3.94 | 4.65 | 4.624 |
| Conservation pit (M2) | 3.13 | 3.85 | 2.432 |
| Ring trench (M3) | 3.63 | 4.29 | 3.625 |
| Control (M4) | 2.57 | 3.24 | 1.462 |
| SEm ± | 0.02 | 0.08 | |
| CD (p=0.05) | 0.16 | 0.28 | |
| Subplots (F) | | | |
| 200:100:200 N P$_2$O$_5$ K$_2$O kg ha$^{-1}$ (F1) | 3.84 | 4.55 | 4.284 |
| 250:125:250 N P$_2$O$_5$ K$_2$O kg ha$^{-1}$ (F2) | 3.62 | 4.28 | 3.617 |
| 125:75:75 N P$_2$O$_5$ K$_2$O kg ha$^{-1}$+ FYM (5 t ha$^{-1}$) (F3) | 3.39 | 4.05 | 3.007 |
| Control (F4). | 2.43 | 3.16 | 1.261 |
| SEm ± | 0.03 | 0.08 | |
| CD (p=0.05) | 0.08 | 0.24 | |
| Interaction (M × F) | | | |
| M1F1 | 4.48 | 5.25 | 6.413 |
| M1F2 | 4.28 | 5.00 | 5.576 |
| M1F3 | 3.96 | 4.66 | 4.418 |
| M1F4 | 3.06 | 3.70 | 2.090 |
| M2F1 | 3.53 | 4.25 | 3.251 |
| M2F2 | 3.40 | 4.10 | 2.850 |
| M2F3 | 3.28 | 4.01 | 2.615 |
| M2F4 | 2.33 | 3.03 | 1.015 |
| M3F1 | 4.18 | 4.85 | 5.152 |
| M3F2 | 3.83 | 4.51 | 4.053 |
| M3F3 | 3.70 | 4.40 | 3.688 |
| M3F4 | 2.82 | 3.41 | 1.610 |
| M4F1 | 3.19 | 3.86 | 2.321 |
| M4F2 | 2.99 | 3.50 | 1.894 |
| M4F3 | 2.63 | 3.13 | 1.308 |
| M4F4 | 1.49 | 2.48 | 0.328 |
| SEm ± | 0.06 | 0.17 | |
| CD (p=0.05) | 0.17 | 0.50 | |

technique of soil moisture conservation helps in conserving the runoff water and in turn increases the productivity of lands. These results are in line with studies conducted by Bhardwaj *et al.* (1995) and Sharanabasappa *et al.* (2009) in *Celtis australia* and *Tectona grandis* species, respectively.

Fertilizer application influenced the plant height and diameter at breast height (DBH) of *Eucalyptus pellita*. Among fertilizer treatments, application of N, $P_2O_5$ $K_2O$ 200:100:200 kg $ha^{-1}$ ($F_1$) recorded significantly higher height (4.55 m) and DBH (3.84 cm) whereas minimum respective values were recorded in unfertilized plants (3.16m and 2.43cm, respectively). The application of nitrogen showed improved the photosynthetic activity resulting in vigorous vegetative growth.

The interaction effect of trapezoidal staggered trench in combination with of N, $P_2O_5$ $K_2O$ 200:100:200 in kg $ha^{-1}$ ($M_1F_1$) recorded significantly higher plant height and DBH (5.25 m and 4.48 cm, respectively). Higher available soil moisture during dry season favored the nutrient absorption by plants, which in turn resulted in higher plant growth. Soil moisture conservation measures and nutrient management influence plant height and DBH growth (Rajendradu and Naidu, 1998). Biomass productivity is directly dependent upon plant growth, therefore, the trend of treatments was similar as observed in growth parameters, moisture conservation

favoured the biomass accumulation (Fig.1).

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# Lichens as Key Indicators of Forest Health in Sauni-Binsar Grove, Kumaun Himalaya, India

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**Abstract**: Sauni-Binsar grove is famous for a holy shrine Binsar-Mahadev and its natural beauty. People from around 200 villages come here for their various needs such as fuelwood, fodder, water, resin, stone queries, etc. The encroachment by so many people has affected the forest health of the grove. Lichens have been considered as significant indicators of forest health in any forest ecosystem. Lichen communities are used for biodiversity conservation and forest health monitoring. Lichen specimens were collected along the altitudinal zones (1500–2400 m) from all available substrates such as rock, soil, and trees of Sauni-Binsar grove, Kumaun Himalaya. Thirty nine lichen species of 22 genera belonging to 14 families were collected from the grove. Lichen species richness ranged from 10–28. Poor occurrence of both terricolous (soil inhabiting) and saxicolous (rock inhabiting) lichens in the grove is an alarming situation to the environmental condition of the grove and for its sustainability. The forest health could be improved only after restriction on grazing, controlling of forest fire, resin extraction, and prohibition of stone quarries.

**Keywords**: Anthropogenic activities, Forest health, Kumaun Himalaya, Lichens, Sacred grove

Lichens are not single entities, but a mutualistic symbiotic composite of a fungus, the mycobiont, and an organism capable of producing food via photosynthesis, the photobiont (Brodo *et al*., 2001). Depending upon the growth forms lichens may be of different types as foliose (leaf like), fruticose (thread like attached to the substratum at one or few points) and crustose (crust forming). On the basis of occurrence, lichens are corticolous (bark inhabiting), saxicolous (rock inhabiting) and terricolous (soil inhabiting). Lichens create microclimate and play a significant role for the colonization of other vegetation in any forest and help to regulate ecosystem functioning. In general terms, healthy forests maintain and sustain desirable ecosystem functions and processes (Thormann, 2006). A healthy forest is manifested by the occurrence of its health indicators including those are related to biodiversity, habitat, and resource sustainability. Lichens have been identified as one of the significant indicators of forest health. They are more sensitive towards habitat alteration and biodiversity change than other cryptogams and vascular plants (Bokhorst *et al*., 2007; Saipunkaew *et al*., 2007).

Sacred groves are the forest patches rich in biodiversity and provide good ecosystem services. Sauni-Binsar grove was mentioned as sacred grove by Gokhale and Pala (2011). The inhabitants of more than 200 villages are regularly benefitted by this sacred grove as it provides various ecosystem services such as fuelwood, timber, fodder, litter, mushrooms, agricultural implements and stones. Besides some natural water resources situated at Riddu (Upper altitudinal zone) in the grove are also fused by inhabitants. As a consequence of heavy anthropogenic activities, the forest cover in the grove has been deteriorated up to a greater extent in the recent past. To monitor the present condition of the grove, focus has been made on the study of its heath indicators i.e. lichens. Among the various forms of lichen habitats, terricolous lichens have been considered as crucial in ecosystem functioning in temperate to alpine regions of India (Rai *et al*., 2012). Several studies on lichens diversity, distribution, bio-monitoring and revisionary work have been carried out by various workers in the last decade from Central Himalayan region of India (Awasthi, 2007; Joshi *et al*., 2011; Mishra and Upreti, 2014). But Sauni-Binsar sacred grove has been investigated more systematically for the first time for lichen study as well as other botanical resources. The study was conducted with the aim to assess lichen diversity of the grove and their key role in forest ecosystem functioning.

## MATERIAL AND METHODS

The present study was carried out in three altitudinal zones viz. lower at Bhainskuri (1500-1800 m), middle at Titalikhet (1800-2100 m) and upper at Riddu (2100-2400 m) of Sauni-Binsar grove, Kumaun Himalaya (Uttarakhand). The grove is located along a local river named Kunjgaar-Bhainskuri. The grove is famous for a holy shrine Binsar-

Mahadev which is a place of faith and serenity for thousands of devotees and tourists who visit here throughout the year. Geographically, the study area lies between 29˚ 35' 59.92" N latitude and 79˚ 17' 28.25" E longitude (Fig. 1). The upper altitudinal zone is dominated with *Quercus leucotrichophora,* while middle and lower regions are dominated by *Pinus roxburghii* and associated with *Cedrus deodara*, *Myrica esculenta* and *Lyonia ovalifolia.*

Lichen specimens were collected during June 2015 to June 2016 from each altitudinal zone on all available substrates such as rock, soil, and trees along with their ecological notes. The type of forest vegetation, substratum, altitude and other ecological notes were also recorded.

The collected specimens were identified morphologically, anatomically and chemically at Lichenology Laboratory of the CSIR-National Botanical Research Institute, Lucknow. The collected specimens were packed on hard card sheets inside a lichen herbarium packet (17cm x 10 mm) with details of the locality and are preserved at Biodiversity Conservation Laboratory, Department of Botany, S.S.J. Campus Almora (Uttarakhand). The broad outline of the study has been provided in Fig. 2.
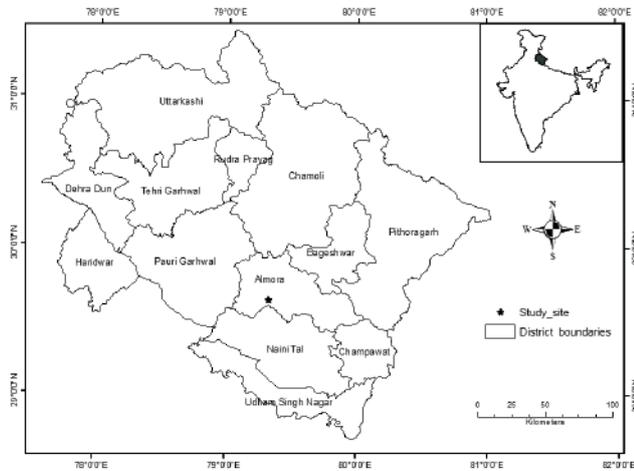


**Fig. 1.** Location map of study area (Sauni–Binsar sacred grove)

### RESULTS AND DISCUSSION

In the present investigation a total of 39 lichen species of 22 genera belonging to 14 families were enumerated from Sauni–Binsar grove (Table 1). Lichen family Parmiliaceae represented by 18 species was dominant followed by Physciaceae, Collemataceae and Ramalinaceae each having three species. However, each lichen family−Chrysothriaceae, Dermatocarpaceae, Lobariaceae, Umbilicariaceae, Usneaceae, Graphidiaceae, Pertusariaceae, Porpidiaceae and Trapeliaceae represented by a single species (Fig. 3).

Species richness was maximum (28) at upper altitudinal
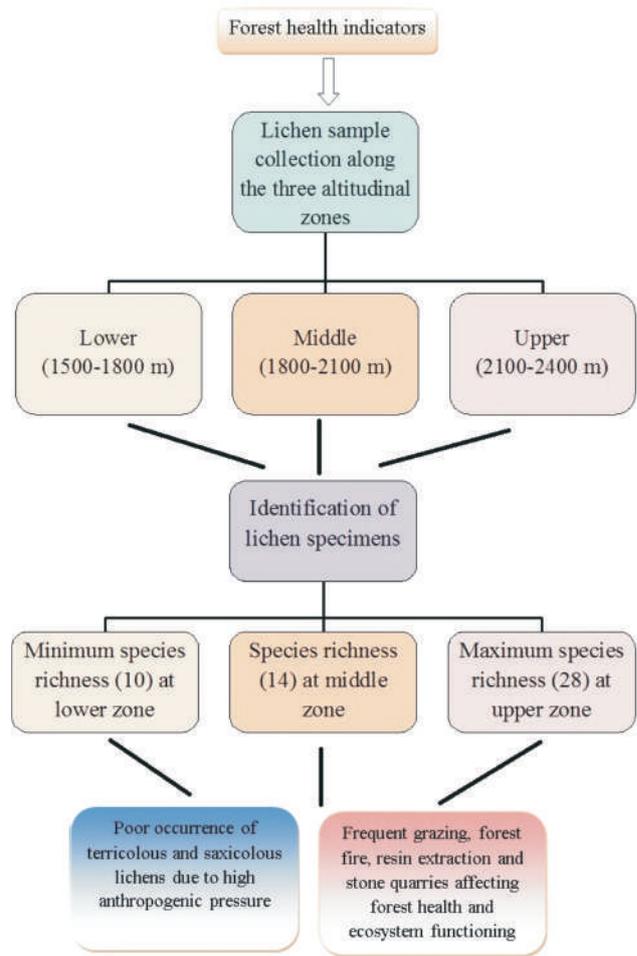


**Fig. 2.** Broad outline of the study

zone followed by 14 at middle zone and 10) at lower altitudinal zone (Fig. 4). Only two species of lichens viz. *Everniastrum cirrhatum* and *Flavoparmelia caperata* were common in all the three altitudinal zones. 18 species of lichens−*Bulbothrix meizospora, Cetrelia cerarioides, Flavoparmelia tinctorum, Leptogium askotense, L. papillosum, L. pedicelatum, Lobaria retigera, Parmotrema austrosinense, P. melanothrix*, *P. nilgherrense*, *Pertusaria leucosona, Phaeophyscia hispidula, Porpidia macrocarpa, Punctelia rudecta, Ramalina hossei*, *R. sinensis, Trapelia coarctata* and *Usnea orientalis* were not found below 2100 m altitude. Most of the lichen species known from lower altitudes (up to 1500 m) of the other Himalayan region were recorded above 2100 m in the study area. The migration of species from lower altitudes (1500 m) to 2100 m and above may be due to the fact that the habitat have destroyed due to heavy anthropogenic activity.

Out of 39 lichen species, maximum (28) species were found as foliose form followed by seven crustose and only

**Table 1.** Distribution of different lichen species in the study area

| Lichen taxa | Family | Growth form | Substratum/Altitude (m) | | |
|---|---|---|---|---|---|
| | | | 1500–1800 | 1800–2100 | 2100–2400 |
| *Bulbothrix meizospora* (Nyl.) Hale. | Parmeliaceae | Foliose | – | – | Bark |
| *Bulbothrix setschwanensis* (Zahlbr.) *Hale* | Parmeliaceae | Foliose | – | Bark | Bark |
| *Canoparmelia aptata* (Krempedh) Elix & Hale | Parmeliaceae | Foliose | – | Bark | Bark |
| *Canoparmelia pustulescaris* | Parmeliaceae | Foliose | – | Bark | – |
| *Canoparmelia texana* | Parmeliaceae | Foliose | Bark | – | Bark |
| *Cetrariopsis wallichiana* (Taylor) Kurokawa | Parmeliaceae | Foliose | – | Bark | Bark |
| *Cetrelia cerarioides* (Delise ex Duby) Culb & C. Culb | Parmeliaceae | Foliose | – | – | Bark |
| *Chrysathrix candelaris* (L.) Laundon | Chrysothriaceae | Crustose | Bark | – | – |
| *Dermatocarpon vellereum* Zschacke | Dermatocarpaceae | Foliose | – | Rock | Rock |
| *Diorygma sp.* | Graphidaceae | Crustose | Bark | – | – |
| *Everniastrum cirrhatum* (E Fries) Hale ex Sipman | Parmeliaceae | Foliose | Bark | Bark | Bark |
| *Everniastrum nepalense* (Taylor) Hale ex Sipman | Parmeliaceae | Foliose | – | Bark | Bark |
| *Flavoparmelia caperata* (L.) Hale | Parmeliaceae | Foliose | Bark | Bark | Bark |
| *Flavoparmelia tinctorum* (Nyl.) Hale | Parmeliaceae | Foliose | – | – | Bark |
| *Heterodermia diademata (Taylor)* D.D. Awasthi. | Physciaceae | Foliose | Bark | – | – |
| *Heterodermia formula* | Physciaceae | Foliose | – | Bark | – |
| *Heterodermia speciosa* (Wulf.) Trevis. | Physciaceae | Foliose | Bark | – | – |
| *Lecanora achroa* (Nyl) | Lecanoraceae | Crustose | – | Rock | – |
| *Lecanora polytropa* | Lecanoraceae | Crustose | – | Bark | – |
| *Leptogium askotense* D. Awasthi | Collemataceae | Foliose | – | – | Bark |
| *Leptogium papillosum* B. de Lesd Dodge | Collemataceae | Foliose | – | – | Bark |
| *Leptogium pedicelatum* M. P. Jorg | Collemataceae | Foliose | – | – | Bark |
| *Lobaria retigera* (Bory) Trevisan | Lobariaceae | Foliose | – | – | Bark |
| *Parmotrema austrosinense* (Zahlbr.) Hale | Parmeliaceae | Foliose | Bark | – | Bark |
| *Parmotrema melanothrix* (Mont.) Hale | Parmeliaceae | Foliose | – | – | Bark |
| *Parmotrema mesotropum* | Parmeliaceae | Foliose | – | Bark | – |
| *Parmotrema nilgherrense* (Nyl.) Hale | Parmeliaceae | Foliose | – | – | Bark |
| *Parmotrema reticulatum* (Taylor) Chiosey | Parmeliaceae | Foliose | Bark | – | Bark |
| *Parmotrema tinctorum* | Parmeliaceae | Foliose | – | Bark | – |
| *Pertusaria leucosona* | Pertusariaceae | Crustose | – | – | Bark |
| *Phaeophyscia hispidula* (Ach.) Moberg | Physciaceae | Foliose | – | – | Bark |
| *Porpidia macrocarpa* (DC) Hertel & Knoph in Hertel | Porpidiaceae | Crustose | – | – | Rock |
| *Punctelia rudecta* (Ach.) | Parmeliaceae | Foliose | – | – | Bark |
| *Ramalina conduplicans* (Vainio) | Ramalinaceae | Fruticose | Bark | – | – |
| *Ramalina hossei* | Ramalinaceae | Fruticose | – | – | Bark |
| *Ramalina sinensis* Jatta | Ramalinaceae | Fruticose | – | – | Bark |
| *Trapelia coarctata* | Trapeliaceae | Crustose | – | – | Rock |
| *Umbilicaria virginis* Schaerer | Umbilicariaceae | Foliose | – | Rock | Rock |
| *Usnea orientalis* Mot. | Usneaceae | Fruticose | – | – | Bark |

four species as fruticose form (Fig. 5). On the basis of the occurrence of lichen species on different substratum, maximum 24 species were preferred to grow as corticolous at upper altitude followed by 4 species grows as saxicolous. However, terricolous lichens were not found in the study area (Fig. 6). The absence of terricolous lichens in all the three zones indicates that study area is influenced by anthropogenic activities. It is observed that most of the localities of the zones have no trace of forest regeneration due to heavy grazing. The saxicolous lichens also exhibit
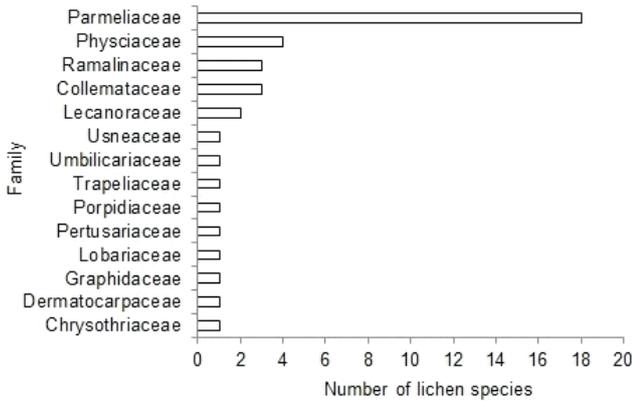
**Fig. 3.** Occurrence of lichen families in the study area
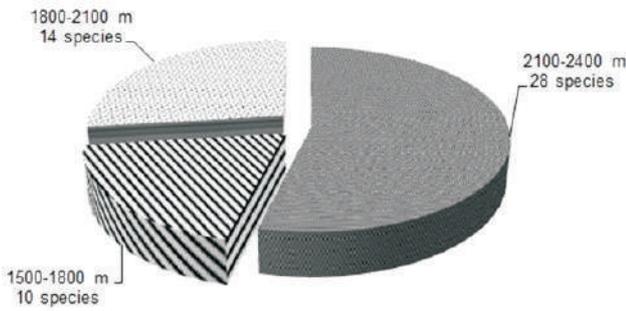


**Fig. 4.** Lichen species richness in the study area
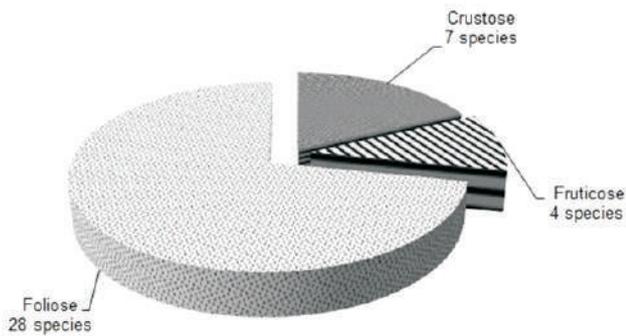


**Fig. 5.** Distribution of lichen growth forms in the study area
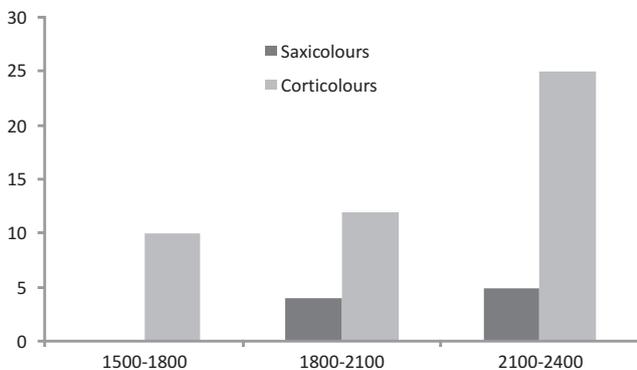


**Fig. 6.** Lichen species growing on different substratum

their complete absence in the lower altitudes. The probable reason for absence of rock inhabiting lichens may be due to the fact that being an area dominated with pine forest which is more prone to frequent forest fire due to resin containing needles of pine, easily catch fire and destroy the lichen growing on rocks. Stone quarries, resin extraction from pine trees, cutting and pruning of tree branches for firewood and fodder and litter collection from the forest floor are other activities leading to poor diversity of both epiphytic and lichens on other substrates. Riddu locality and surrounding areas at higher altitudes are rich in moisture as have water bodies exhibit rich diversity of lichens needs conservation.

**CONCLUSIONS**

Among the various bioindicator communities/species, terricolous lichens clearly indicate extend of grazing, trekking by tourist and other human activities in a particular area. Poor occurrence of both terricolous and saxicolous lichens in the grove is an alarming situation to the environmental condition of the grove and for its sustainability. The pleasant and natural beauty of the grove attracts thousands of devotees and tourists throughout the year. The grove is influenced by heavy anthropogenic activities in terms of trekking, grazing, fuelwood, fodder, and resin extraction, sand and stone quarries. The health of the grove could be improved only after restriction on grazing, controlling of forest fire, resin extraction, and prohibition of stone quarries.

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# Performance of Pre-treatment on Germination and Initial Growth Attributes of *Hydnocarpus pentandra* (Buch. Ham.) Oken

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**Abstract**: The present study was aimed to evaluate the various pretreatments on seed germination, its subsequent seedling growth behaviour and nutrient accumulation of *Hydnocarpus pentandra*. Seeds were treated with cold water, sulphuric acid, gibberellic acid (GA) and its combination and untreated seed as control. The highest germination percentage was recorded with seeds treated with 300 ppm GA without seed coat (86.3 %) followed by 200 ppm GA without seed coat (76.6 %). It was also observed that GA 300 ppm without seed coat resulted in fast germination compared to other treatments. With regards to growth attributes of seedlings, 300 ppm GA without seed coat was produced maximum height, collar girth, number of leaves, shoot and root biomass except shoot: root ratio, which was observed maximum on seeds with complete removal of seed coat.

**Keywords**: *Hydnocarpus pentandra,* Pretreatment, Germination, Growth parameter, Gibberellic acid

*Hydnocarpus* is an Indo‑Malasian genus belonging to the family Flacourtiaceae. This family consists of about 85‑89 genera and 800‑1250 species distributed throughout tropical and subtropical (Sambamurty, 2005). Five species of Hydnocarpus *viz., H. alpina, H. kurzii, H. macrocarpa, H. pentandra* and *H. pendulus* are reported from India (Nayar *et al.,* 2006). Out of the five species, *H. pentandra, H. macrocarpa* and *H. pendulus* are endemic in Western Ghats region of India (Balakrishnan, 2005). *Hydnocarpus pentandra* is a medicinal tree and most widely distributed species in moist deciduous and semi‑evergreen forests of Western Ghats, India upto 850 m (Joshi and Harijan, 2014) while *H. pendulus* is a newly reported species restricted to Silent Valley National Park, Palakkad, Kerala, India (Manilal *et al.,* 1983). *H. pentandra* is a potential biodiesel tree as it meets the specifications of biodiesel (Karthikeyan *et al.,* 2013). Over exploitation of fruits from wild has resulted major threat for the regeneration of *H. Pentandra* and perusal of literature indicated that species has low germination in natural forest. Seed germination may be reduced by the presence of impermeable seed coats that restricts the availability of water, which can penetrate into seeds at their optimum concentration (Kumar *et al.,* 2015a,b). However, no such studies have been carried out specifically on seed germination, growth and vigour of this species. Therefore, the present study has been designed to explore the enhanced seed germination as well as seedling growth performance of *H. pentandra* under different pre‑sowing treatments avoiding the laborious process of depulping the dry fruits of the species.

## MATERIAL AND METHODS

The present investigation was carried out at the Tree Nursery, College of Forestry, Vellanikkara Kerala Agricultural University, Thrissur, Kerala. The nursery area is located at 40 meters above mean sea level at $10^0 32'$ N latitude and $76^0 26'$ E longitude. The area experiences a warm and humid climate with maximum temperature is 28.6 $^0$C to 36.5 $^0$C and the minimum temperature is 21.6 $^0$C (July) to 25.6 $^0$C (April). Fully matured fruits of *H. Pentandra* were collected from the College of Forestry campus and the seeds were extracted. The average number of seeds per fruit and the number of seeds per kg were determined in 10 replications. The extracted seeds were cleaned and thoroughly hand mixed to improve the homogeneity during the sampling. The individual seed length, width, weight, volume, density and specific gravity were recorded from 100 randomly selected samples from the seed lot. Specific gravity was determined by specific gravity module (Schimadzu AUY220). The pulp content of the seeds was also determined. Seeds were subjected to pre‑sowing treatments (Table 1). The pre‑treated seeds (100 x 3) were sown in polythene bags (11.43 x 15.24 cm, gauge 75 micron) containing the rooting medium soil, sand and FYM in the ratio 1:1:1 and daily germination counts were recorded. At the end of the trial, the percentage and peak value of germination and germination value (GV) of the species were calculated as suggested by Czabator (1962). Twelve seedlings per treatment were destructively sampled at

monthly intervals till 112th day of sowing to record height, collar girth, tap root length, fresh and dry weight of root and shoot and shoot root ratio. Seedling quality indices were deduced from these observations. Quality index, which is a measure to assess the quality of seedling based on the height, stem diameter and dry biomass was also calculated using the method given by Hatchell (1985). The data generated were analysed using the IBM SPSS Statistics 20. The data on germination, seedling growth, biomass, and quality indices were subjected to one way analysis of variance and the treatment means were compared.

## RESULTS AND DISCUSSION

**Effect of pre-treatments on seed germination:** Germination parameters of the *Hydnocarpus pentandra* seeds were significantly influenced by pre-sowing treatments. The maximum germination percentage (86.3 %), mean daily germination (2.43), peak value of germination (4.32) and germination value (10.49) recorded for treatment with 300 ppm GA without seed coat followed by 200 ppm GA without seed coat (Table 1). The result indicated that a decreasing trend in germination when the seeds soaked for more time in water i.e. soaking for long duration after treating

with hot water. It was also observed that untreated seeds and the treatments with sulphuric acid gave no germination. Similarly, the results were obtained for cold water and hot water treatment combinations, because in both circumstances the increased soaking time reduces the germination percentage to zero (Table 1). Such wide variation indicated the effectiveness of soaking in cold water in controlling the germination of seeds. Indeed, GA treatments were found to be having good impact on germination when compared to other treatments. Among different GA treatments, those without seed coat gave higher germination. Treatment with similar concentration (300 ppm), maximum germination (86.6%) effected for seeds without seed coat when compared to seeds with seed coat (50 %) (Table 1). It indicated that the hormone treatment had an enhanced effect in seed germination as they showed higher germination percentage than normal cold water treatment. In seeds with coat dormancy, light and gibberellins (GA) both can release (coat) dormancy and promote germination (Kumar *et al.,* 2015a,b; Chauhan *et al.*, 2017).

The days required for fifty percentage germination is an indication of the fastness in germination. Treatment in 300 ppm GA without seed coat was the fastest taking 26.7 days

**Table 1.** Effect of treatments on germination of *Hydnocarpus pentandra*

| Pre-treatment | Germination per cent | Days for 50 per cent germination | Mean daily germination | Peak value of germination | Germination value |
|---|---|---|---|---|---|
| T₁−Cold water for 12 hours | 30[e] | 63[ab] | 0.58[e] | 1.10[e] | 0.64[e] |
| T₂−Cold water for 24 hours | 43.3[d] | 66.3[b] | 1.08[d] | 0.89[ef] | 0.96[d] |
| T₃−Boiling water for 2 minutes | 23.3[e] | 61.6[b] | 0.37[e] | 0.39[f] | 0.14[e] |
| T₄−Boiling water for 2 minutes followed by cold water for 12 hours | 0[f] | 0[f] | 0[f] | 0[g] | 0[f] |
| T₅−Boiling water for 2 minutes followed by cold water for 24 hours | 0[f] | 0[f] | 0[f] | 0[g] | 0[f] |
| T₆−Sulphuric acid for 2 minutes | 0[f] | 0[f] | 0[f] | 0[g] | 0[f] |
| T₇−Treatment with sulphuric acid for 2 minutes followed by cold water treatment for 12 hours | 0[f] | 0[f] | 0[f] | 0[g] | 0[f] |
| T₈−Treatment with sulphuric acid for 2 minutes followed by cold water treatment for 24 hours | 0[f] | 0[f] | 0[f] | 0[g] | 0[f] |
| T₉−Complete removal of seed coat | 53.3[cd] | 44.3[c] | 1.75[cd] | 1.83[cd] | 3.20[cd] |
| T₁₀−Control | 0[f] | 0[f] | 0[g] | 0[f] | 0[f] |
| T₁₁−treatment in 300 ppm GA without seed coat | 86.3[a] | 26.7[e] | 2.43[a] | 4.32[a] | 10.49[a] |
| T₁₂−100 ppm GA without seed coat | 60[c] | 39.6[d] | 1.89[c] | 2.03[c] | 3.84[c] |
| T₁₃−200 ppm GA without seed coat | 76.6[b] | 31[e] | 2.17[b] | 2.21[b] | 4.79[b] |
| T₁₄−300 ppm GA for 2 minutes | 30[e] | 36.3[d] | 0.58[e] | 1.10[e] | 0.64[e] |
| T₁₅−300 ppm GA for overnight with seed coat | 50[cd] | 39[d] | 1.68[cd] | 1.78[d] | 2.99[d] |

Figures with same super scripts do not differ significantly

for 50 per cent germination, whereas, cold water treatment for 24 hours was the slowest treatment as it took 66.3 days for completing 50 per cent germination (Table 1).

**Growth attributes of seedlings:** The observation on height, collar girth and number of leaves produced by seedlings selected a positive impact of pre-treatments on the growth and biomass production. Treatment with cold water for 24 and 12 hours produced almost uniform results with other treatments. At first fortnight, treatment in 300 ppm GA without seed coat (T11) showed the maximum height (6.23 cm), collar diameter (2.65 mm), number of leaves (2.70) and followed by 200 ppm GA without seed coat. Cold water treatment for 24 hours, showed the minimum values in all the above parameters followed by boiling water for 2 minutes (Table 2). In the last fortnight, both treatment in 300 ppm GA without seed coat showed maximum height (29.4 cm), collar diameter (3.45 mm), number of leaves (11.37) and followed by 200 ppm GA without seed coat (Table 2). The growth regulators like gibberellic acid ($GA_3$), benzyl adenine (BA) and nitrogenous like $KNO_3$, and thiourea are commonly used chemicals to enhance seed germination (Kumar *et al.,* 2015 a,b).

Many mechanisms have been proposed by which the seed coat can impose dormancy on a seed, which include the mechanical restriction of germination of the embryo (Ajeesh *et al.,* 2014; Vidyasagarn *et al.,* 2016; Chauhan *et al.*, 2017), presence of chemical inhibitors of germination ( Pons, 2000), restriction of water uptake and restriction of oxygen uptake. In the present study, the initial nursery performance of the seedlings as measured by height, collar girth, root length and total biomass varied significantly due to pretreatment over different periods. Seed traits such as seed size, seed coat thickness, shape and moisture content are already known to affect seed germination, dormancy and early seedling establishment in various other species (Chauhan *et al.*, 2017).

Perusal of data on seedling biomass indicated that the above ground biomass as well as below ground biomass both fresh weight and dry weight was recorded maximum for T11 followed by T13 and T12. The similar trend was observed for root length also. (Table 3). Shoot: root ratio of seedlings studied under different treatments revealed that the treatment with complete removal of seed coat recorded highest value followed by T13 and T11 (Table 3). Treatments

**Table 2.** Effect of treatment on vegetative growth parameters of *Hydnocarpus pentandra*

| Treatments | Height (cm) | | | | Collar girth (mm) | | | | Number of leaves | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 14 days | 42 days | 84 days | 112 days | 14 days | 42 days | 84 days | 112 days | 14 days | 42 days | 84 days | 112 days |
| $T_1$ | 1.31[c] | 3.68[c] | 6.81[c] | 8.97[c] | 0.51[c] | 0.85[cd] | 0.99[dc] | 1.08[e] | 0.50[c] | 1.37[c] | 2.63[c] | 3.40[c] |
| $T_2$ | 1.76[c] | 4.43[c] | 8.38[c] | 11.11[c] | 0.78[c] | 1.23[c] | 1.47[cd] | 1.59[cd] | 0.80[c] | 1.77[c] | 3.03[c] | 4.07[c] |
| $T_3$ | 1.56[c] | 3.01[c] | 5.33[c] | 6.79[c] | 0.62[c] | 0.68[d] | 0.77[e] | 0.83[f] | 0.75[c] | 1.37[c] | 2.17[c] | 2.54[c] |
| $T_9$ | 3.78[b] | 7.75[b] | 13.92[b] | 17.2[b] | 1.61[b] | 1.76[b] | 1.96[bc] | 2.08[c] | 2.00[b] | 3.33[b] | 5.6[b] | 7.23[b] |
| $T_{11}$ | 6.23[a] | 12.78[a] | 23.08[a] | 29.4[a] | 2.65[a] | 2.89[a] | 3.22[a] | 3.45[a] | 2.70[a] | 5.17[a] | 8.70[a] | 11.37[a] |
| $T_{12}$ | 4.21[b] | 7.96[b] | 13.95[b] | 17.81[b] | 1.75[b] | 1.93[b] | 2.18[b] | 2.34[c] | 2.23[ab] | 3.67[b] | 6.07[b] | 7.90[b] |
| $T_{13}$ | 5.72[a] | 11.65[a] | 21.06[a] | 26.75[a] | 2.43[a] | 2.66[a] | 2.99[a] | 3.22[b] | 2.77[a] | 5.00[a] | 8.34[a] | 10.8[a] |
| $T_{14}$ | 2.06[c] | 3.7[c] | 6.48[c] | 8.37[c] | 0.84[c] | 0.93[cd] | 1.06[de] | 1.16[d] | 1.10[c] | 1.90[c] | 3.20[c] | 4.07[c] |
| $T_{15}$ | 3.83[b] | 7.80[b] | 13.94[b] | 17.64[b] | 1.56[b] | 1.71[b] | 1.9b[c] | 2.08[c] | 1.83[b] | 3.33[b] | 5.77[b] | 7.33[b] |

Figures with same super scripts don't differ significantly

**Table 3.** Effect of treatment on biomass production of *Hydnocarpus pentandra* at 112 days

| Treatments | Fresh weight of above ground (g) | Dry weight of above ground (g) | Fresh weight of below ground (g) | Dry weight of below ground (g) | Root length (cm) | Shoot: Root ratio |
|---|---|---|---|---|---|---|
| $T_1$ | 14.08[e] | 4.39[e] | 13.84e | 2.99[e] | 3.56[d] | 1.47[d] |
| $T_2$ | 29.65[c] | 7.19[c] | 15.53[d] | 3.24[d] | 6.08[c] | 2.21[b] |
| $T_3$ | 13.81[f] | 4.12[f] | 14.31[de] | 3.15[de] | 2.09[d] | 1.97[c] |
| $T_9$ | 33.58[bc] | 6.37[bc] | 17.94[c] | 3.88[c] | 8.15[c] | 2.49[a] |
| $T_{11}$ | 36.81[a] | 9.96[a] | 21.30[a] | 4.24[a] | 11.50[a] | 2.33[ab] |
| $T_{12}$ | 35.19[b] | 8.80[b] | 18.86[b] | 4.05[b] | 8.26[b] | 2.17[b] |
| $T_{13}$ | 36.58[a] | 9.89[a] | 20.28[a] | 4.18[a] | 11.72[a] | 2.38[ab] |
| $T_{14}$ | 18.71[de] | 4.94[de] | 18.92[ab] | 4.06[ab] | 3.25[d] | 1.21[e] |
| $T_{15}$ | 24.92[d] | 5.88[d] | 17.74[bc] | 3.83[bc] | 7.01[bc] | 1.53[cd] |

Figures with same super scripts don't differ significantly

with higher biomass did not show significant variation among all the parameters studied. Researching the way to strike a balance for the shoot: root ratio is beneficial for the normal growth and development of wheat plants even from the early stages of vegetation. The root and shoot systems of plant have been studied in different stages of vegetation, in order to assess the nutrition and vegetation state of plants, plant state in relation with soil works, fertilizer concentration (Kang, 2004), plants-nutrient relation (Erenoglu *et al*., 2010), plant tolerance to temperature and water stress (Essemine *et al*., 2010; Wasson *et al.*, 2012) and plant tolerance to salinity (Tammam *et al.*, 2008).

## CONCLUSION

The highest germination percentage, vegetative growth and growth attributes in *Hydnocarpus pentandra* were recorded in seeds treated with 300 ppm GA without seed coat and followed by 200 ppm GA without seed coat. It also that a decreasing trend in germination when soaked for more time in water soaking seeds for long duration after treating with hot water.

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# Comparative Assessment of Radioactive Strontium and Cesium Contents in the Feedstuffs and Dairy Products of Western Siberia

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**Abstract**: In this study, we investigated the accumulation of radioactive strontium and cesium in different types of plant feedstuffs and dairy products from some Siberian regions including the Novosibirsk, Kemerovo and Tomsk regions. It was shown that the content of radionuclides in the five types of plant feedstuffs did not exceed maximum permissible concentration (MPC). The content of $^{90}$Sr in the hay from the Kemerovo region was higher as compared with the hay from the Novosibirsk or Tomsk regions, but the content of $^{90}$Sr in concentrated feedstuffs in the Tomsk region was higher than that in Novosibirsk or Kemerovo regions. The concentration of $^{137}$Cs in silage and concentrated feedstuffs from the Tomsk region was higher as compared with other regions, while the content of this radionuclide in hay and silage was lower in Novosibirsk region as compared with Kemerovo or Tomsk regions. The content of both radionuclides in dairy products (whole and dry milk) from Novosibirsk and Tomsk regions was by several times (up to 100) below than the MPC. In conclusion, the territory of Western Siberia is excellent for the production of ecologically safe forage and animal husbandry, and it can be used for the production of dietary.

**Keywords**: Radioactive strontium and cesium, Environmental safety, Fodder crops, Feedstuffs, Dairy products

Creature of the multidisciplinary approach to the production of ecologically safe food products should take into account a natural cycle, which includes the soil, vegetation, animals, produced herbal and animal products, and human beings at the end of the food chain (Baranovskaya, 2010; Petukhov *et al.,* 2007, 2013). In this context, one of the most important objects of ecological monitoring should be not only arable lands, grazing and lands used for fodder production, but also various types of vegetable feedstuffs used for livestock and food products (Chysyma *et al.,* 2003 a,b). The contamination of plant feedstuffs significantly influences the safety indicators of food products for human consumption (Afonina *et al.,* 2003; Petukhov *et al.,* 2014). The contamination of soil with radionuclides is of a particular danger to flora and fauna. The main radionuclides, which could determine the nature of contamination in the West Siberian region of Russia, are cesium‒137 ($^{137}$Cs) and strontium‒90 ($^{90}$Sr), which are differently sorted by soil (Syso, 2007). At the time of emission of cesium‒137 into the environment, the radionuclide is initially in a soluble state (vapor phase, fine particles, etc.). In this case, plants are easily absorbed cesium‒137 from the soil. Further, the radionuclide in the soil can enter into different reactions. Its mobility is reduced, while the fixing strength increases, the radionuclide "ages" entering into the complexes of soil crystallochemical reactions and bonded in the structures of secondary clay minerals (Rikhvanov and Baranovskaya, 2013; Syso, 2007).

Sorption of radionuclides depends on many factors among which the soil mechanical and mineralogical composition is one of the main factors. In heavy soils, in terms of their granulometric composition, the absorbed radionuclides, especially cesium‒137, are bonded stronger than in the light soils, and with decrease in the size of mechanical fractions of the soil the bonding strength of strontium‒90 and cesium‒137 increases, for example, radionuclides are bonded most firmly in the clay fraction of the soil (Syso, 2002, 2004). It is revealed that strontium‒90, located on the soil surface, is washed away by rain into the lowermost layers. It should be noted that the migration of

radionuclides in soil is slow and their main part is in the upper layer from 0 to 5 cm. Accumulation (removal) of radionuclides by agricultural plants depends largely on soil properties and biological peculiarities of plants. In acidic soils, the radionuclides are delivered to plants in large amounts as compared to slightly acidic soils. Reduction of soil acidity usually contributes to reducing the transfer of radionuclides into plants. Thus, depending on the soil properties, the content of strontium-90 and cesium-137 in plants may vary on average by 10–15 times (Korotkevich *et al.,* 2014).

Contamination of the environment by radionuclides is usually estimated using soil and vegetation sampling, however radionuclides affects productivity, dairy product quality and resistance of cattle to diseases At the same time, the contamination of territory with radionuclides had no effect on the incidence of cattle tuberculosis and brucellosis (Petukhov *et al.,* 2001, 2003). Thus, a transfer of radionuclides from contaminated soil and vegetation through water or forage pathway to farm animals is dangerous not only to humans, as radionuclides enter in human through livestock products, but they also worsen the health status of farm animals (Kizilstein, 2002; Krotkevich *et al.,* 2009; Narozhnykh *et al.,* 2013).

Thus, a necessary and promising recent trend of monitoring environmental parameters of farmlands is the assessment of the level of radionuclides ($^{137}$Cs and $^{90}$Sr) in different types of vegetable feedstuffs, and dairy products that is which are the most important indicators for environmental survey of ecological safety of agricultural regions in our country. This study presents the results on the content of radioactive elements in various types of the plant feedstuffs, stored up in different regions of Western Siberia, as well as in some dairy agricultural products (whole and dry milk) produced in the concerned regions.

## MATERIAL AND METHODS

Studies on accumulation of strontium-90 and cesium-137 by plants were carried out at Veterinary laboratories located in the territories of Novosibirsk, Kemerovo and Tomsk regions. Samples of feedstuffs were collected in different agricultural farms. Preparation of samples in terms of content of radionuclides was carried out in accordance with the requirements of SanPiN 1.3.2.560-96 (Russian sanitary regulations and norms) and MUK 2.6.1.1194-03 (Russian procedulal guidelines). In total, we investigated 179 samples of hay from natural acreages, 85 haylage samples, and 147 samples of silage, 117 samples of grass from natural acreages, and 186 samples of concentrated feedstuffs.

Samples of herbage were taken before animals grazing or cutting for forage. Composite samples were formed from the three portions, taken on the areas located within the triangle and spaced from each other at a distance of 50–100 m. The height of cut was 3–5 cm from the ground. Distance from highways was more than 200 m. The samples' mass varied within the range from 4 to 5 kg. Hay samples were collected in 4–8 points at the height of 1–1.5 m from the ground surface from all available sides of haystacks at a depth of at least 0.5 m. Composite sample with the weight of 2–3 kg was prepared combining individual samples. Analytical feed sample weighing 1–2 kg was prepared by mixing 3–5 portions taken from different bags. Simultaneously with the sampling, natural background radiation rate was measured using radiometers IMD-5 and RKS-107. The activity of $^{137}$Cs and $^{90}$Sr was measured employing a universal spectrometric complex (USC) "Gamma+". The device includes two detection units. To assess the content of $^{137}$Cs we used the method of native samples. The measurements were performed using a semiconductor gamma-spectrometer with the detector unit located in a lead shield (γ -path). The same device was employed to determine the activity of $^{90}$Sr using a beta spectrometer (β-tract). Hygienic assessment of feedstuff in terms of radiation safety criteria was performed using the current regulatory documents.

Sampling of milk and milk products as well as their preparation for analysis was conducted in accordance with the requirements of GOST 26809-86 "Milk and dairy products. Acceptance rules, sampling and preparation methods for analysis" (All-Union State Standard, Russia). In total, 290 samples of whole milk and 98 samples of dry milk taken in Novosibirsk and Tomsk region were studied. Measurement of $^{137}$Cs and $^{90}$Sr activity was performed employing a universal spectrometric complex (USC) "Gamma+" using the "Progress" software. Preparation of samples in terms of radionuclides content was carried out in accordance with the requirements of SanPiN 1.3.2.560-96 and MU-13-7-2/1056. It included samples grinding and dry ashing in a furnace at controlled temperature regime. For measuring $^{137}$Cs, we used a semiconductor gamma-spectrometer with the detector unit located in a lead shield (γ-path). When measuring $^{137}$Cs, we employed a beta spectrometer (β-tract).

The data are presented as mean standard error. The data were analyzed by 2-way ANOVA using the package «STATISTICA 8.0». All data were tested for normality before statistical analysis by the Shapiro-Wilk criterion. In the absence of the normal distribution, we used methods of nonparametric statistics: Kendall correlation coefficient (τ) instead of the Pearson correlation coefficient (r), and the Wald-Wolfowitz run test, which is an analog of Student's t-test.

## RESULTS AND DISCUSSION

The South of Western Siberia is the territory most developed in terms of industry and agriculture as well as most complicated by its biogeochemical conditions. Here, natural and anthropogenic factors cause a significant negative change in soil quality and forage crops in agricultural lands, both arable and natural. Radionuclide contents in different plant feedstuffs from three regions of West Siberia. It was revealed that the maximum content of $^{90}$Sr and $^{137}$Cs was detected in the natural grass hay, regardless of region. At that, the average concentration of $^{137}$Cs in Kemerovo region is much higher than that in other regions. This is probably due to anthropogenic pollution of environmental objects and orographic specifics of the Kemerovo region, which is located mostly in the Kuznetsk basin. The content of $^{137}$Cs in the silage and compound feedstuffs from Tomsk region was significantly higher (p<0.001) than that in samples from the Novosibirsk and Kemerovo regions. The highest phenotypic variation in radionuclide accumulation was revealed in silage from the Tomsk region, as well as compound feedstuffs and natural grass hay of from the Kemerovo region (Table 1). There was a notable variability of the $^{137}$Cs content in animal feedstuffs. Thus, in some samples, the content of $^{137}$Cs was 35 times higher than that in other samples. Nevertheless, none of the studied samples contained radionuclides at amounts exceeding the permissible levels (VP 13.5.13/06−01).

The degree of radionuclides accumulation depended not only on type of feedstuffs, but also on region. The concentration of $^{137}$Cs in the natural grass hay in the Kemerovo region was significantly higher than that in Tomsk and Novosibirsk regions (3.1 and 2.4-fold, Table 1). In the samples of natural grass hay in Kemerovo region, the concentration of $^{90}$Sr was 2.0 and 1.3 times higher than in the samples from Novosibirsk and Tomsk regions (p<0.001). The highest variability in the accumulation of this radionuclide was noted in natural grass hay from Tomsk and Kemerovo regions, as well as silage from Tomsk region. In some feedstuff samples the concentration of $^{90}$Sr was at 56.8% higher than in the other samples, though, neither of the

samples contained strontium in concentrations exceeding permissible and reference levels (SanPiN 2.3.2.1078-01. 13.5.13/06-01).

Table 1 shows the impact of ecological and climate factors on the accumulation of radioactive $^{137}$Cs and the influence of technogenic pollution on its accumulation in by various types of feedstuffs. The content of this radionuclide in concentrated feedstuffs and haylage depends to the greatest extent on ecological zone (p<0.001). It may be supposed that this is, partly, due to peaks in its concentration in the feedstuffs of the Tomsk region. The differences in the accumulation degree of $^{90}$Sr and $^{137}$Cs in forage, depending on the region that was 1.6 times lower for the first radionuclide than for the second one. Influence of ecological−climatic zone on the content of both radionuclides in the silage was consistently high (for $^{90}$Sr $r_w = 0.58$, for $^{137}$Cs $r_w = 0.52$. A sharp decline in the influence of environmental factors on the concentration of $^{90}$Sr was observed in the grass of natural pastures and silage, which respectively was 9 and 3 times lower than that for $^{137}$Cs. The influence of foodstuff type on the level of $^{90}$Sr in the Novosibirsk region was the highest ($r_w = 0.70$), 1.7 and 1.3 times higher than that in Tomsk and Kemerovo regions, respectively. Thus, monitoring of the radionuclides concentration ($^{90}$Sr and $^{137}$Cs) in feedstuffs of plant origin allowed estimating the role of ecological and climatic factors, as well as the type of plant feedstuff in the radionuclides accumulation. The minimum content of radionuclides in most types of feedstuffs was observed in the Novosibirsk region in comparison to the Tomsk and Kemerovo regions; however, their levels did not exceed the MPC.

The content of $^{137}$Cs and $^{90}$Sr in whole and dry milk produced at the enterprises of Novosibirsk and Tomsk regions indicated that their concentration was many times lower than MPC (Marmuleva *et al.,* 2003). Thus, concentrations of $^{90}$Sr and $^{137}$Cs in the whole milk from the Novosibirsk region were respectively 18 and 62 times below the MPC. Similar data were obtained with regard to concentrations of radionuclides in dry milk produced in Novosibirsk and Tomsk regions (Table 2). Dry milk was characterized by greater accumulation of

**Table 1.** The content of $^{90}$Sr and $^{137}$Cs (nKu kg$^{-1}$) in various types of feedstuffs from different regions of Western Siberia

| Type of feedstuffs | Kemerovo region | | Novosibirsk region | | Tomsk region | |
|---|---|---|---|---|---|---|
| | $^{90}$Sr | $^{137}$Cs | $^{90}$Sr | $^{137}$Cs | $^{90}$Sr | $^{137}$Cs |
| Grass of natural pastures | 38.0±2.0 | 31.0±1.0 | 32.0±2.0 | 16.0±2.0 | 39.0±2.0 | 25.0±1.0 |
| Natural grass hay | 92.0±8.0 | 85.0±6.0 | 46.0±2.0 | 46.0±2.0 | 54.0±3.0 | 72.0±2.0 |
| Haylage | 30.0±2.0 | 25.0±1.0 | 23.0±1.0 | 13.0±0.9 | 11.8±1.0 | 59.4±6.3 |
| Silage | 25.0±1.0 | 24.0±1.0 | 21.0±2.0 | 13.0±1.0 | 20.0±1.0 | 29.0±1.0 |
| Concentrated feedstuffs | 21.0±1.0 | 22.0±2.0 | 16.0±1.0 | 9.0±1.0 | 36.0±2.0 | 58.0±2.0 |

**Table 2.** The content of $^{90}$Sr and $^{137}$Cs in whole and dry milk (Bq kg$^{-1}$) from Novosibirsk and Tomsk regions

| Product | | Novosibirsk region | | Tomsk region | |
|---|---|---|---|---|---|
| | | $^{90}$Sr | $^{137}$Cs | $^{90}$Sr | $^{137}$Cs |
| Whole milk | | 0.80±0.15 | 2.70±0.2 | 0.80±0.02 | 0.24±0.03* |
| | lim | 0–18.0 | 0–22.0 | 0–2.8 | 0–2.8 |
| | MPC | 25 | 50 | 25 | 50 |
| Dry milk | | 0.80±0.40 | 7.2±0.50 | 0.48±0.03 | 0.20±0.03* |
| | lim | 0–24.0 | 0–32.5 | 0–0.60 | 0.17–0.40 |
| | MPC | 200 | 360 | 200 | 360 |

\* −significant differences between the regions (p<0.001)

$^{137}$Cs as compared to whole milk, whereas such dependence was not noted for $^{90}$Sr.

There are significant interspecies differences in agricultural crops in terms of the ability to accumulate radionuclides. L legumes absorb strontium-90 and cesium-137 by 2–6 times more intensively than cereals. Intake of strontium-90 and cesium-137 by grass stand in the meadows and pastures is determined by the nature of their distribution in the soil crossover. The accumulation of radionuclides in the herbaceous plants depends on the characteristics of the sod layer structure. In the meadow grass with strong dense sod layer, the content of cesium-137 in the phytomass is 3–4 times higher than that in the forb meadow with a loose thin sod layer (Syso, 2007).

Intake of cesium-137 in plants depends on soil type. In terms of cesium accumulation, the soils can be ranged in the following order: soddy–podzolic loamy sandy soils, soddy–podzolic loamy soils, gray forest soil, chernozems, etc. The accumulation of radionuclides in plants depends not only on soil type, but also on the biological characteristics of plants. It is noted that the calcium-demanding plants usually absorb more strontium-90 than plants poor in calcium. Strontium-90 is accumulated most of all by legume crops, in a less degree by root crops and tubers, and even less–by cereals. Cultures with low content of potassium accumulate less amount of cesium. Grasses accumulate less amount of cesium in comparison with legumes. Though plants are relatively resistant to radioactive exposure, they can accumulate such amount of radionuclides that they become not suitable for use as animal feedstuff or food production.

The accumulation of radionuclides in plants depends on the content of nutrients in the soil. Thus, it is recognized that mineral fertilizer applied in doses of N 90, P 90, increase the concentration of cesium-137 (Nezavitin et al., 2000; Syso, 2002). All the activities carried out currently to improve the soil fertility will help to reduce the intensity of the radionuclides transfer in plants at pollution of agricultural land with radioactive fallout. The simplest and cheapest method of reducing the content of radionuclides in agricultural products is the selection of crops and varieties, characterized by their ability to accumulate the minimum amount of strontium-90 and cesium-137. As a rule, these are varieties with a low content of potassium and calcium. Effective techniques include also plowing the contaminated topsoil, liming of acid soils and applying mineral and organic fertilizers. The correct choice of soil tillage depth and methods can significantly reduce the intake of radionuclides into the plants several fold (Syso, 2002).

## CONCLUSION

The findings of the current study allow concluding that the agricultural lands in Novosibirsk, Tomsk and Kemerovo regions are suitable for the production of ecologically safe forage and, consequently, crop production and animal husbandry, especially for the production of dietary and functional products as well as baby foods. However, it becomes clear that the radionuclide monitoring strategy should include food chain continuous monitoring of radionuclide content not only in the soil and in feedstuffs as the most popular monitor system, but also all kinds of agricultural foodstuff produced in Western Siberia.

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# Biogas Production from Paddy Straw using Thermophilic Fungi

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**Abstract**: The potential of thermophilic fungal pretreatment of paddy straw was investigated by determining the lignolytic enzyme production, changes in chemical composition of paddy straw like cellulose, hemicellulose, lignin and silica contents and biogas production. The fungal isolates namely *Aspergillus* sp ($T_{14}$), *Paecilomyces* sp ($T_{17}$) and *Penicillium* sp ($T_{22}$) displayed production of lignolytic enzymes viz. laccase and lignin peroxidase. Maximum production of lignolytic enzymes was achieved with isolate $T_{17}$ and minimum with isolate $T_{22}$. Cellulose, hemicellulose, lignin and silica contents decreased by 9.6, 13.3, 32.6 and 25.0 percent, respectively in paddy straw pretreated with isolate $T_{14}$. In case of isolate $T_{17}$ pretreated paddy straw, cellulose, hemicellulose and lignin contents decreased by 17.5, 14.1 and 47.9 percent, respectively but change in silica content was non significant. A reduction of 41.3, 13.7 and 43.5 percent was observed in cellulose, hemicellulose and silica contents in paddy straw pretreated with $T_{22}$. Paddy straw pretreated with all the isolates ($T_{14}$, $T_{17}$ and $T_{22}$) showed enhanced biogas production by 37.0, 41.6 and 25.2 percent, respectively in comparison to untreated paddy straw containing cattle dung and digested cattle dung slurry.

**Keywords:** *Aspergillus* sp, *Paecilomyces* sp, *Penicillium* sp, Biogas

The lignocellulosic biomass is found to be abundant on earth, which is not gainfully utilized. This can be converted to biogas via anaerobic digestion (Kashyap *et al.,* 2003). It is a biological process in which biodegradable organic material is decomposed in the absence of oxygen to produce biogas. The organic matter of biomass can be degraded by the sequential action of hydrolytic, acetogenic and methanogenic bacteria to produce biogas. Paddy straw is one of the most abundant organic celulosic waste. In India, approximately 100 million tons of paddy straw is disposed of by burning which releases 3 kg particulate matter, 60 kg CO, 1460 kg $CO_2$, 199 kg ash and 2 kg $SO_2$ per ton of paddy straw (Jenkins and Bhatnagar, 2003). Despite the presence of high cellulose content i.e. 35-40 per cent, biogas production from paddy straw is not sufficient because the lignin complex and silica incrustation shields the microbial action for degradation. Therefore, the paddy straw needs to be pre treated in order to enable cellulose to be more accessible to the microbial/enzymatic attack. Many physical (mechanical and non-mechanical), chemical (alkaline hydrolysis, acid hydrolysis, oxidative delignification and solvent extraction), physico-chemical (ammonia fibre explosion, $CO_2$ and steam explosion) and biological pretreatments (lignocellulolytic micro-organisms and the enzymes) have been explored in the recent years (Hendriks and Zeeman, 2009). However, the physical and chemical pre treatments require high energy,

corrosion resistant and high-pressure reactors, which increase the need of sophisticated equipments and cost of pre treatment (Keller *et al.,* 2003). Thus, an alternative approach is microbial pre treatment which employs the use of micro-organisms especially fungi to increase digestibility of paddy straw. Advantages of biological pre treatment include cost effectiveness, low energy requirement and mild environmental conditions. Most of the white-rot fungi degrade lignin and cellulose simultaneously. A selective white-rot fungus, *Ceriporiopsis subvermispora* is known to selectively degrade lignin in softwood and hardwood (Okano *et al.,* 2005). Thermophiles are a good source of novel catalysts that are of great industrial interest. The main advantage of performing process at higher temperatures are reduced risk of microbial contamination, lower viscosity, improved transfer rates and improved solubility of substrates (Li *et al.,* 2005). The *Aspergillus* s*p., Paecilomyces sp.* and *Sporotrichum sp.* were most efficient lignocellulose degraders. Reports are available for biological pre treatment of paddy straw; however, reports are limited on the effect of pre treatment on lignolytic enzyme production and its correlation with biogas production from paddy straw. Therefore, the present study was undertaken to optimize the conditions for biological pre treatment of paddy straw by three thermophilic lignolytic isolates and their effect on lignolytic enzyme production, paddy straw digestibility and biogas production.

## MATERIAL AND METHODS

**Procurement of the materials:** Fresh paddy straw used as feedstock was obtained from the research field of Punjab Agricultural University, Ludhiana. The paddy straw was chopped (3–5 cm) and stored in polythene bags at room temperature. Digested cattle dung slurry was procured from a working biogas plant of School of Energy Studies for Agriculture, Punjab Agricultural University, Ludhiana and cattle dung was procured from dairy farm complex, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana.

**Cultures:** Thermophilic fungal isolates *Aspergillus* sp. ($T_{14}$), *Paecilomyces* sp.($T_{17}$) and *Penicillium* sp. ($T_{22}$) used for pre treatment study were selected from the previously isolated cultures by Sahni and Phutela (2013). The cultures were stored in refrigerator after sub–culturing at monthly intervals. The isolates were identified by slide culturing technique using microscope at 100 X using immersion oil on Magnus Binocular photographic microscope.

**Pre treatment of paddy straw:** For the pre treatment, chopped paddy straw was mixed with salt solution containing 0.1% yeast extract and 0.3% $CuSO_4$ in 1:4 ratio (w/v) in Erlenmeyer flasks. The flasks were autoclaved at a pressure of 121˚C for 20 minutes and then cooled at room temperature and inoculated with spawn (10% w/w) of thermophilic fungal isolates i.e. T14, T17 and T22 separately (Phutela *et al.,* 2011). The inoculated flasks were incubated at 50±2ºC and were removed at a regular interval of 2 days for a period of eight days. Crude enzyme was extracted from the substrate using 0.1 M sodium acetate buffer (1:10 w/v ratio) and shaking the contents for 30 min in shaking incubator (250 rpm) at 4ºC. The extract was filtered using muslin cloth to obtain a clear filtrate. The filtrate was centrifuged in a cooling centrifuge at 10,000 rpm for 20 minutes at 4ºC and supernatant was used for estimation of lignolytic enzyme activities i.e. laccase, lignin peroxidase and manganese peroxidase by spectro–photometric method. The experiment was performed in triplicate.

Laccase activity was determined by measuring oxidation of 2, 2'– Azinobis–3–ethyl benzthiazoline–6–sulphonic acid (ABTS) at 420 nm and expressed as IU/ml (Mishra *et al.,* 2008). Lignin peroxidase activity was measured by the method of Tien and Kirk (1988) at 310 nm and expressed as IU/ml. The pre treated paddy straw (left after enzyme extraction) was then dried and grinded for the determination of proximate (total solids, volatile solids, total organic carbon and ash content) and chemical composition (cellulose, hemi–cellulose, lignin and silica content) of paddy straw by standard methods of AOAC (2000).

**Biogas production from pre treated paddy straw:** Biogas production experiments were carried out, in triplicate, in glass biogas digesters of 2 litre capacity following single phase digestion and biogas produced was measured by water displacement method for a period of 45 days. The pre treated and untreated (as control) paddy straw was mixed with digested cattle dung slurry and cattle dung in ratio of 1:1:0.5 i.e. 250 g paddy straw + 250 g digested cattle dung slurry + 125 g cattle dung. The mixture was fed to the biogas digesters which were incubated at 37±2˚C for 45 days. Paddy straw mixed with minimal salt solution containing 0.1% yeast extract and 0.3% $CuSO_4$ was pre treated separately with isolates *Aspergillus* sp. ($T_{14}$), *Paecilomyces* sp.($T_{17}$) and *Penicillium* sp. ($T_{22}$) and their effect on lignolytic enzyme production, change in chemical composition of paddy straw and biogas production were determined.

## RESULTS AND DISCUSSION

**Identification of isolates:** The morphological and sporulation pattern of the isolates $T_{14}$, $T_{17}$ and $T_{22}$ were identified as *Aspergillus* sp, *Paecilomyces* sp and *Penicillium* sp by slide culturing with their classification as shown in Fig. 1.

**Lignolytic enzyme production by isolates $T_{14}$, $T_{17}$ and $T_{22}$:** The lignolytic enzyme profile of $T_{14}$, $T_{17}$ and $T_{22}$ using paddy straw as a substrate showed significant increase in laccase
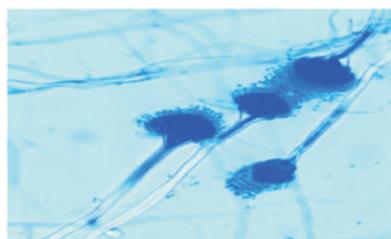


Phylum–Ascomycota
Class–Eurotiomycetes
Order–Eurotiales
Family–Trichomaceae
Genera–*Aspergillus*
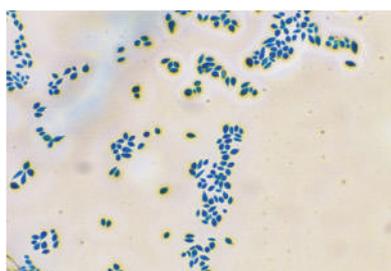
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Class–Eurotiomycetes
Order–Eurotiales
Family–Trichomaceae
Genera–*Paecilomyces*
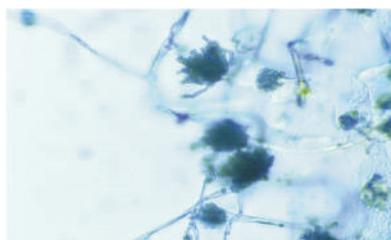
Phylum–Ascomycota
Class–Eurotiomycetes
Order–Eurotiales
Family–Trichomaceae
Genera–*Penicillium*

**Fig. 1.** Identification of isolates T14, T17 & T22 by slide culturing technique at 100X using immersion oil

and lignin peroxidase activity until 6th day and it started decreasing due to depletion in carbon sources (Table 1). In $T_{14}$ pre treated paddy straw, a maximum of 600.6 U g$^{-1}$ of laccase and 500.8 U g$^{-1}$ of lignin peroxidase activity were found on 6th day of incubation, while a maximum of 640.8 U g$^{-1}$ of laccase and 448.5 U g$^{-1}$ of lignin peroxidase activities were found, in case of $T_{17}$ pretreated paddy straw. In $T_{22}$ pre treated paddy straw, a maximum of 100.6 U g$^{-1}$ of laccase and 102.8 U g$^{-1}$ of lignin peroxidase were found on 6th day of pretreatment. Isolate $T_{17}$ was best culture for lignolytic enzyme production followed by $T_{14}$. Vikineswary *et al.* (2006) reported that the productivity of laccase in solid state fermentation of selected agro-residues by *Pycnoporus sanguineus* reached a maximum of 487.0 Ug$^{-1}$ while *Fomessclerodermeus* grown on wheat straw produced 270.5 Ug$^{-1}$ of laccase activity (Papinutti *et al.,* 2003).

**Paddy straw degradation:** The change in proximate and chemical composition of paddy straw with enhanced lignolytic enzyme activities was taken as criteria for paddy straw digestibility. There was a smooth and significant decrease in cellulose, hemicellulose, lignin and silica

contents in $T_{14}$ pretreated paddy straw (Table 2). The cellulose, hemicellulose, lignin and silica contents kept on decreasing with the increasing incubation period, with the maximum reduction of 9.6, 13.3, 32.6 and 25.0 percent, respectively after 8 days pretreatment. This indicates that $T_{14}$ fungus did not have much active cellulase and hemicellulase as compared to lignolytic and/or silicolytic enzymes. In case of $T_{17}$ pre treated paddy straw cellulose, hemicellulose and lignin contents kept on decreasing with the increasing incubation period, with the maximum reduction of 17.5, 14.1 and 47.9 percent, respectively after 8 days pre treatment. The decrease in silica was non-significant. Hence, isolates $T_{14}$ and $T_{17}$ are ligno-cellulolytic in nature. There was a smooth and significant decrease in cellulose, hemicellulose and silica contents in $T_{22}$ pre treated paddy straw. The cellulose, hemicellulose and silica contents kept on decreasing with the increasing incubation period, with the maximum reduction of 41.3, 13.7 & 43.5 per cent, respectively after 8 days pre treatment. This indicates that the $T_{22}$ fungus have much active cellulase and silicolytic enzymes as compared to hemicellulase and ligninase. However, drastic decrease in

**Table 1.** Lignolytic enzyme profile of different isolates from pretreated paddy straw

| Isolate no. | Enzyme activity (U/g) | Pretreatment period (days) | | | | | CD (p= 0.05) |
|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | |
| $T_{14}$ | Laccase | 0.9 | 140.0 | 380.7 | 600.6 | 375.3 | 2.1 |
| | Lignin Peroxidase | 1.1 | 180.5 | 330.9 | 500.8 | 375.0 | 2.9 |
| $T_{17}$ | Laccase | 0.9 | 150.0 | 350.7 | 640.8 | 425.3 | 2.3 |
| | Lignin Peroxidase | 1.1 | 167.5 | 305.9 | 485.5 | 375.0 | 4.0 |
| $T_{22}$ | Laccase | 0.9 | 31.3 | 65.3 | 100.6 | 75.3 | 1.2 |
| | Lignin Peroxidase | 1.1 | 40.2 | 85.1 | 102.8 | 55.3 | 1.4 |

0 day (control): uninoculated paddy straw; Cultural conditions: paddy straw mixed with minimal salt media (1:4); Temperature 50±2˚C; pH 6

**Table 2.** Change in chemical composition of paddy straw pretreated with different isolates

| Isolate no. | Composition (%) | Pre-treatment period (days) | | | | | CD (p= 0.05) |
|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | |
| $T_{14}$ | Cellulose | 37.3 | 36.2 | 35.4 | 34.9 | 33.7 | 0.9 |
| | Hemicellulose | 25.6 | 25.0 | 24.7 | 23.2 | 22.2 | 0.6 |
| | Lignin | 9.8 | 8.9 | 8.0 | 7.6 | 6.6 | 1.7 |
| | Silica | 9.2 | 8.7 | 8.0 | 7.4 | 6.9 | 1.1 |
| $T_{17}$ | Cellulose | 37.3 | 36.0 | 33.8 | 32.1 | 30.8 | 1.1 |
| | Hemicellulose | 25.6 | 24.8 | 23.9 | 22.7 | 22.0 | 1.0 |
| | Lignin | 9.8 | 8.4 | 7.8 | 6.4 | 5.1 | 1.1 |
| | Silica | 9.2 | 9.0 | 8.5 | 7.8 | 7.1 | NS |
| $T_{22}$ | Cellulose | 37.3 | 34.9 | 31.8 | 25.5 | 21.9 | 1.9 |
| | Hemicellulose | 25.6 | 25.0 | 24.1 | 23.0 | 22.1 | 0.5 |
| | Lignin | 9.8 | 9.0 | 8.6 | 8.1 | 8.4 | NS |
| | Silica | 9.2 | | 7.2 | 6.5 | 5.2 | 0.8 |

: %decrease; : %increase; See footnotes of Table 1

**Table 3.** Biogas production (l kg⁻¹ paddy straw) from pre-treated paddy straw with different isolates

| Isolate no. | Biogas (l kg⁻¹ paddy straw) | | | | | CD (p=0.05) |
|---|---|---|---|---|---|---|
| | 0 d | 2 d | 4 d | 6 d | 8 d | |
| T$_{14}$ | 221.0 | 230.8 (4.4 ) | 268.9 (21.7 ) | 302.8 (37.0 ) | 192.9 (12.7 ) | 10.5 |
| T$_{17}$ | 221.0 | 242.1 (9.5 ) | 281.5 (27.4 ) | 312.0 (41.6 ) | 201.0 (9.0 ) | 9.8 |
| T$_{22}$ | 221.0 | 232.1 (5.1 ) | 251.5 (13.8 ) | 276.6 (25.2 ) | 184.9 (16.3 ) | 11.1 |

See footnotes of Table 1 and 2

silica content indicates that T$_{14}$ and T$_{22}$ could be a potential organism for silica removal. Reports regarding silica solubilizing microbes for weathering of rocks in nature are also available (Ehrlich, 2006).Shi *et al.* (2009) pre treated the cotton stalks with *Phanerochaete chrysosporium* and found significant decrease in lignin i.e. 19.38 and 35.33 percent for submerged and solid state cultivation respectively.

**Effect of pre treatment on biogas production:** The biogas production increased significantly in 4 and 6 days pre treated paddy straw and maximum of 37.0, 41.6 and 25.2 percent increase in biogas production was observed in T$_{14}$, T$_{17}$ and T$_{22}$ pre treated paddy straw respectively after 6 days of pre treatment (Table 3). However, further increase in pre treatment time led to decrease of biogas production as 8 days pre treated straw reflected decrease in biogas production. The increase in biogas production might be due to the increase in digestibility of paddy straw by decrease in silica content and breakage of bonds between cellulose, hemicellulose and lignin content (Fox and Noike, 2004). Further reduction in biogas production can be correlated with the decrease in substrate, preferably cellulose, which is preferred source for methanogens and hindrance caused by lignin barrier. Lignin degradation is primarily an aerobic process and in an anaerobic environment lignin can persist for very long periods (Van Soest, 2006). Because lignin is the most recalcitrant component of the plant cell wall, the higher the proportion of lignin the lower the bioavailability of the substrate. The effect of lignin on the bioavailability of other cell wall components is thought to be largely a physical restriction, with lignin molecules reducing the surface area available to enzymatic penetration and activity.

The thermophilic fungi, isolates T$_{14}$ (*Aspergillus* sp), T$_{17}$ (*Paecilomyces* sp) are efficient lignolytic fungi where as T$_{22}$ (*Penicllium* sp) is efficient cellulolytic fungus. Isolates T$_{14}$ and

T$_{22}$ are also silica solubilizer.

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# Impact of Dietary Intervention of High Bioactive Compound Beverages on the Lipid Profile of Hyperlipidemic Subjects

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**Abstract**: High bioactive compounds of the fruits like amla (*Embliciaofficinalis*), orange (*Citrus aurantium*) and kinnow (*Citrus sinensis*) were used to make antioxidants rich fruit beverages for hyperlipidemic patients. The subjects were equally divided into two groups i.e. C‒Control (given only nutrional counseling) and E‒Experimental (supplementation of beverages with nutritional counseling) and dietary intake, anthropometric measurements and blood lipid profile of the subjects were recorded before and after experimentation. There was a slight reduction in body weight i.e from 72.8 ± 7.74 kg to 71.00 ± 7.69 in the experimental group as compared to the controlled group because of the beverage supplementation. Thus, study recommends intake of antioxidant rich beverage with diet counselling can improve the lipid profile of hyperlipidemic subjects.

**Keywords**: Cardiovascular diseases, Cholesterol, Hyperlipidemic, Reactive oxygen species (ROS), Lipid profile, Antioxidant

The free radicals alter lipids, protein and DNA. They are the root cause of a number of diseases. The phenolic compounds are used as a natural drug in curing various sickness of breathing, blood, skin and also in prevention of disease like atherosclerosis (Cesar, 2010). Citrus fruits provide 60‒106 mg of flavonoids per day. Orange juice contain flavonoids mainly hesperid in that has anti‒inflammatory, anticarcinogenic, antiallergic and hypolipidemic properties (Constans, 2015). Flavonoids help to lower the serum levels of LDL‒cholesterol (Eliat‒Adar, 2010), apolipoprote in B and triglycerides and increases HDL cholesterol reducing the risk of coronary artery diseases (Fattahi, 2011). The vitamin C antioxidant effect is vasoprotective (Fletcher, 2005). Dyslipidemia is the major cause for the development of atherosclerosis, obesity which is related to higher rates of morbidity and mortality from coronary artery disease (Fossati, 1982). Citrus juices like orange juice and grapefruit juice are rich source of flavonoids,folate and vitamin C. Flavonoids like hesperetin from oranges and naringenin from grapefruit are similar to genistein, a flavonoid from soya bean postulated to be hypocholestrolemic. The main objective of the research was to lower down the total cholesterol levels in the hyperlipidemic patients using the fruit beverages, some patients were counselled for their health issues whereas some patients were supplemented with the beverages for the research purpose.

## MATERIAL AND METHODS

**Determination of ascorbic acid**: (Knekt et al., 2002): Weighed 2‒5 ml of the fresh sample and it was grounded in a pestle mortar to form a slurry by adding 20 ml of 6 % metaphosphoric acid. After filtering through Whatman no.1 filter paper, 30 ml metaphosphoric acid was added to the residue and it was filtered again. Added 5 ml of filterate into a separating funnel which were labelled as A and B containing dye and 0.1 ml of standard ascorbic acid solution into funnel C. Added 5 ml acetate buffer to all three funnels followed by 2 ml of dye solution and then added 10 ml of xylene solution quickly and shaked the contents for 5‒10 seconds. Allowed the layers of the solution to get separated into two layers. Discarded the bottom layer and transferred xylene layer into a test tube and read the optical density in a spectrophotometer at 500nm.

**Determination of Antioxidant (DPPH) activity**: (Lopes‒Virella, 1977): Two milligram of sample was extracted with 20 ml methanol (99.5 %). The extraction process was done twice (20 ml+20 ml) each for 2 hrs in a shaking machine. Supernatant was filtered using Whatman No .1 filter paper after centrifuging the suspension at 10,000 rpm for 15 min. An aliquot of 0.1 ml of the sample filtrate was taken in a test tube and then 2.9 ml of 0.05 mM DPPH reagent was added and vortexed and let to stand at room temperature in the dark for 30 min. Discolouration of DPPH was measured against blank at 517 nm. Antioxidant activity was expressed as percent inhibition of the DPPH radical and was determined by the following equation.

% inhibition = $[(A_B - A_A \times 100)]/A_B$

Where, $A_B$ = Absorption of blank sample; $A_A$ = Absorption of tested extraction solution

**Determination of total phenolic content (Madhuri *et al.*, 2011):** Total phenolics were determined using the Folin−Ciocalteu reagent. Samples (2 ml) were homogenized in 80% aqueous ethanol at room temperature and centrifuged in cold at 10,000 rpm for 15 min and the supernatant was saved. The residue was re-extracted twice with 80% ethanol and supernatants were pooled, put into evaporating dishes and evaporated to dryness at room temperature. Residue was dissolved in 5 ml of distilled water and 100 micro litres of this extract was diluted to 3 ml with water and 0.5 ml of Folin−Ciocalteu reagent was added. After 3 min, 2 ml of 20% of sodium carbonate was added and the contents were mixed thoroughly. The colour was developed and absorbance measured at 650 nm after 60 min using catechol as a standard. The results were expressed as mg catechol/100 g of fresh weight material.

**Preparation of the beverages**: Fruits namely− amla (*Embliciaofficinalis*), orange (*Citrus aurantium*) were procured from the local market of the Ludhiana city where as kinnow (*Citrus sinensis*) was procured from the Department of Horticulture, PAU, Ludhiana. Based on the high bioactive compound content, these fruits were used for the preparation of beverages.

**Experimental subjects:** Sixty males in the age group of 45−55 yrs were selected from Punjab Agricultural University, Ludhiana. The subjects were selected on the basis of being hyperlipidemic (total Cholesterol 200 mg dl$^{-1}$, triglycerides 150 mg dl$^{-1}$, HDL (High density lipoproteins) 70, LDL (Low density lipoproteins) 160, VLDL (Very low density lpoproteins) 40), free from serious complications and were equally divided into two groups of 30 each. The groups were named as C and E group. C was given only nutritional counselling and E group was supplemented with 200 ml of the beverage. The experimental period was for 100 days. Anthropometric parameters−height, weight, BMI,(Body mass index)waist circumference, waist to hip ratio was measured before and after the experiment (Mansfield *et al.*, 1999).

**Plasma lipid profile estimation:** The serum lipid profile of sixty selected subjects was checked before and after

supplementation and nutrition counselling. The serum was analysed for cholesterol (Mathur *et al.*, 1996), triglycerides (Miller *et al.*, 2008), high density lipoprotein cholesterol (Okcuz, 2009), low density lipoprotein cholesterol (Patil and Alva, 2002), very low density lipoprotein, using the formula VLDL = Triglycerides/5.

## RESULTS AND DISCUSSION

**Antioxidant activity of beverages:** Amla beverage having the highest content of ascorbic acidamong all the three beverages (Table 1) whereas kinnow having the least antioxidant capacity in comparison to other two beverages. The DPPH (2, 2−diphenyl−1−picrylhydrazyl) scavenging activity was highest in amla beverage (84.8 %) followed by citrus fruit and kinnow beverage. The phenolic content was highest in amla beverage followed by orange beverage and kinnow beverage respectively.

**Anthropometric profile of the respondents:** The anthropometric data revealed that in group C there was no change in body weight where as in group E there was reduction in body weight. There was no change in the height of the respondents, where as the BMI values of the subjects decreased from 24.84 to 24.44 kg/m$^2$ and 26.34 to 26.24 kg/m$^2$ after the study period in E and C group, respectively. The mean value of WHR (Waist Hip Ratio) before the study was 0.91 in group C and 0.92 group E and after the study it was 0.870.17 and 0.91, respectively.

**Lipid profile of the respondents:** A decrease ($P < 0.01$, $P < 0.05$) in the level of total cholesterol, LDL−C, VLDL−C and triglycerides levels were seen in both the groups where as the HDL−C levels were increased in the subjects (Table 3). Because of the administration of high antioxidant rich

**Table 1.** Antioxidant activity of beverages

| Fruits | Ascorbic acid (mg) | DPPH Radical scavenging activity (% Inhibition) | Total phenolic content (mg) |
|---|---|---|---|
| Amla | 780.2 ± 1.3 | 84.8 ± 0.9 | 96.0 ± 1.3 |
| Kinnow | 30.3 ± 1.5 | 66.3 ± 1.5 | 74.1 ± 1.0 |
| Orange | 40.4 ± 1.4 | 78.1 ± 0.1 | 77.6 ± 0.2 |

## RECIPES

| Beverage | Ingredients | Method |
|---|---|---|
| Amla | Amla (after destonning)−150g, mint leaves−3gms, sugar−30g ,Water−100 ml | Amla was destined and mint leaves were chopped. Both the ingredients were then blended together by adding water in the grinder. Then the juice was filtered and sugar was added. |
| Kinnow | Kinnow (after peel)−350 g, sugar−30g, black salt−pinch | Kinnow was peeled, the outer covering and the seeds were removed. The pulp was used for extracting the juice. Sugar was added and mixed well till it was dissolved. |
| Orange | Orange (after peel)−350 g, ginger – 3g, sugar−30g | Orange was peeled, the outer covering and the seeds were removed. A slice of ginger was added with the pulp for extracting the juice. Sugar was added and mixed well till it was diluted. |

**Table 2.** Anthropometric profile of the subjects

| Parameters | Control (n=30) | | t−value | Experimental (n=30) | | t-value |
|---|---|---|---|---|---|---|
| | Pre test | Post test | | Pre test | Post test | |
| Total cholesterol (mg dl$^{-1}$) | 245±50 | 227± 48 | 3.44* | 286± 30.2 | 190± 25.5 | 18.89* |
| HDL (mg dl$^{-1}$) | 37±9 | 38±7 | 4.54* | 35 ± 9 | 44 ± 7 | 9.65* |
| LDL (mg dl$^{-1}$) | 154± 27 | 141±28 | 16.57* | 171± 24 | 144±15 | 10.61* |
| VLDL (mg dl$^{-1}$) | 48 ± 8 | 46±9 | 3.37* | 48 ± 8 | 42±7 | 21.59* |
| Triglycerides (mg dl$^{-1}$) | 181± 16 | 176±15 | 36.27* | 199±29 | 147 ± 20 | 16.60* |

* Significant at 1%:  ** Significant at 5%

**Table 3.** Lipid profile of the respondents

| Parameters | Control (n=30) | | t−value | Experimental (n=30) | | t-value |
|---|---|---|---|---|---|---|
| | Pre test | Post test | | Pre test | Post test | |
| Total cholesterol (mg dl$^{-1}$) | 245±50 | 227± 48 | 3.44* | 286± 30.2 | 190± 25.5 | 18.89* |
| HDL (mg dl$^{-1}$) | 37±9 | 38±7 | 4.54* | 35 ± 9 | 44 ± 7 | 9.65* |
| LDL (mg dl$^{-1}$) | 154± 27 | 141±28 | 16.57* | 171± 24 | 144±15 | 10.61* |
| VLDL (mg dl$^{-1}$) | 48 ± 8 | 46±9 | 3.37* | 48 ± 8 | 42±7 | 21.59* |
| Triglycerides (mg dl$^{-1}$) | 181± 16 | 176±15 | 36.27* | 199±29 | 147 ± 20 | 16.60* |

* Significant at 1%

beverage for 100 days in the experimental group, a significant decrease in the cholesterol levels was observerd in group E as compared to group C.

## CONCLUSION

This study suggested that long-term consumption of antioxidant rich beverages made from amla, kinnow and orange by hyperlipidemic subjects was associated with reduced total cholesterol, LDL-C, VLDL-C, TG and increase in HDL-C. Hence, it proves the significant effects of the beverages on the various risk factors for cardiovascular and other diseases caused by free radical reactions in the body.

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# Mineral and Antinutritional Composition of Doughnuts from Pearl Millet Blended Composite Flour

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**Abstract**: The present investigation was undertaken to expand the utility of pearl millet through value addition and to ascertain the chemical and sensory characteristics of developed doughnuts. The pearl millet flour was blended with refined wheat flour and whey protein isolates for the development of doughnuts. The developed doughnuts, were stored for 90 days to ascertain the changes in chemical and sensory characteristics. The maximum magnesium and phosphorus content of 139.91 mg per 100 g, and 311.69 mg per 100 g, respectively in 00:100:00:: Pearl millet flour: Refined wheat flour : Whey protein isolate. Antinutrients (phytic acid and polyphenols) decreased with the storage period. The free fatty acid content in flat bread and doughnuts increased with the increase in the storage period in all the treatments. Highest phytic acid, polyphenols and free fatty acids were recorded in (40:50:10:: pearl millet flour: refined wheat flour: whey protein isolates which differed significantly with rest of the treatments. On the basis of sensory evaluation, 00:100:00:: Pearl millet flour: Refined wheat flour : Whey protein isolate recorded highest score for colour (8.96), texture (8.74) and taste (8.87) whereas, 40:50:10:: Pearl millet flour: Refined wheat flour : Whey protein isolate recorded lowest score for sensory parameters. Overall,the incorporation of pearl millet flour upto 20 per cent level was considered acceptable for preparation of doughnuts beyond which the products reduced their acceptability.

**Keywords**: Pearl millet, Whey protein, Minerals, Antinutritional factors, Sensory parameters

Doughnut is a fried snack which is one of the most popular fried products throughout the world (Rehman *et al*., 2007). Doughnuts are made by deep-frying dough that is a mixture of flour, water, egg, oil, sugar and milk (Hatae *et al*., 2003). Doughnut made from non-enriched wheat flour lacks other essential nutritional composition such as dietary fibre, vitamins and minerals that are lost during the process of wheat flour refinement. Pearl millet (*Pennisetum glaucum*) is the most widely grown millet. Its production is concentrated in the developing countries. India is the single largest producer of pearl millet in the world (Basavaraj *et al*., 2010). Pearl millet forms a staple food for large population living below poverty line. The fat, proteins and minerals of millet is comparable to other cereals but rough texture, lack of gluten and typical flavour of grain limit their uses in various food preparation. Iron deficiency anaemia is widely prevalent amongst women and children in India. Dietary fiber is one of the important nutraceutical components with wide range of health benefits. Pearl millet is one of the richest sources of iron and dietary fiber (Singh & Sehgal, 2008). Estimates of the worldwide production of whey indicate that about 700,000 tonnes of true whey proteins are available as valuable food ingredient (Wit, 1998). Supplementation of pearl millet flour with whey protein isolate makes it nutritionally superior and produces acceptable food products. Functionality of food proteins is defined as those physical and chemical properties, which affect the behaviour of proteins in food systems during processing, storage, preparation and consumption. A functional property is any non-nutritional property of a food additive that affects its utilization (Fennema, 1996). Whey proteins have been used widely in foods for their distinctive physiochemical and functional properties as well as nutritional values. These proteins are available to the food industry in the form of concentrates and isolates with respective protein contents of 40% −50%, 70% and 90% or more. These proteins, when added to a variety of foods, supply desirable functional properties, such as emulsification, fat absorption, moisture holding, thickening and foaming (Rhee, 1985). Thus; the aim of this research was to study the substitution of wheat flour by pearl millet in the development of doughnuts with added whey protein isolates.

## MATERIAL AND METHODS

The pearl millet grains were obtained in a single lot from the Dryland Research Sub Station, Dhiansar, SKUAST−Jammu. The grains were sorted, milled and were ground to flour and used for further product development. The refined wheat flour was procured from market and was blended with pearl millet flour along with soy protein and whey protein

isolates to make protein enriched products. This composite flour mixture was used for development of doughnuts which were prepared by incorporation of 10, 20, 30 and 40 per cent of pearl millet flour with refined wheat flour and 10 per cent of whey protein isolates. There was following six treatments.

For preparation of doughnuts all the ingredients (refined wheat flour, pearl millet flour, whey protein isolates, egg, sugar, baking powder, sodium bicarbonate, salt and water) were mixed manually so as to get uniform dough (Fig. 1). The dough was rolled out on a floured table, cut with a ring shaped doughnut cutter and fried.

| Treatments | Per cent pearl millet flour | Per cent refined wheat flour | Per cent whey protein isolates |
|---|---|---|---|
| $T_1$ | 0 | 100 | 0 |
| $T_2$ | 0 | 90 | 10 |
| $T_3$ | 10 | 80 | 10 |
| $T_4$ | 20 | 70 | 10 |
| $T_5$ | 30 | 60 | 10 |
| $T_6$ | 40 | 50 | 10 |

Mix all the ingredients

↓

Dough formation

↓

Rolling on board and cutting with doughnut cutter

↓

Frying
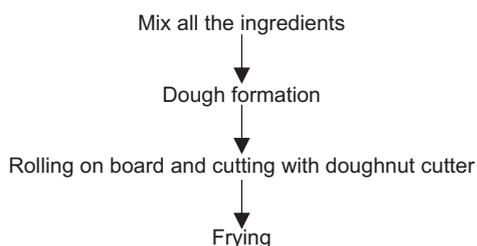
**Fig. 1.** Flow chart for preparation of doughnuts

**Minerals (AOAC, 2005):** The organic matter present in the sample (1g) was wet digested with 25 ml of diacid mixture ($HNO_3$: $HClO_4$ in 5:1) and kept overnight. Digestion was done next day by heating till clear white precipitates settled down at the bottom. The crystals were dissolved by diluting in double distilled water. The contents were filtered through Whatman filter paper no. 42. The filtrate was made upto the volume of 25 ml with double distilled water. The digested samples were analysed for the determination of magnesium, phosphorus and calcium using Atomic Absorption Spectrophotometer.

**Antinutritional factors:** Phytic acid content was estimated by method of Sadasivam and Manickam (2008). The colour intensity was read to 480 nm. Polyphenols were estimated by the method of Sadasivam and Manickam (2008). Absorbance was measured at 650 nm against a blank. A standard curve was prepared using different concentrations of catechol.

**Free Fatty Acid (AACC, 2000):** Ground sample (5 g) was taken to determine the free fatty acids. To the sample in stoppered flask benzene (50 ml) was added and kept for 30 minutes with frequent shakings. After filtration, measured aliquot (10 ml) of supernatant liquid was added with equal amount of alcohol (95 per cent) and few drops of indicator and titrated against 0.02 N KOH till permanent pale colour persisted. Results were expressed as per cent oleic acid.

**Sensory evaluation;** Sensory evaluation depends upon the responses given by different sense organs. The samples were evaluated on the basis of colour, texture and taste by semi‑trained panel of 7‑8 judges by using 9 point hedonic scale assigning scores 9‑like extremely to 1‑dislike extremely. A score of 5.5 and above was considered acceptable (Amerine *et al.*, 1965).

### RESULTS AND DISCUSSION

**Storability of composite flour blended doughnuts**

**Minerals:** There was a significant decrease in the magnesium, phosphorus and calcium content during 90 days of storage. Significant differences were also observed between treatments with respect to calcium, phosphorus and magnesium contents of doughnuts prepared from composite flours. The values for magnesium, phosphorus and calcium ranged from 136.46 to 139.89 mg $100g^{-1}$, 303.21 to 311.69 mg $100g^{-1}$ and 41.57 to 53.49 mg $100g^{-1}$, respectively. The mineral content of doughnuts also showed significant differences with storage for 90 days but non‑significant differences were observed in case of calcium content. The interaction between treatment and storage was found to be non‑significant in all the three mineral elements. Similar results have been reported in the findings of Lakra and Sehgal (2011) in potato flour blended doughnuts.

**Antinutritional factors:** Phytic acid content significantly increased in all the treatments with the increase in the percentage of pearl millet flour. The highest mean phytic acid content after 90 days of storage was recorded in $T_6$ (40:50:10:: pearl millet flour: refined wheat flour: whey protein isolates) and lowest in $T_2$ (00:90:10:: pearl millet flour: refined wheat flour: whey protein isolates). However, phytic acid content decreased with the advancement of the storage period. This might be due to the hydrolysis of phytic acid, enzymatically to phytases or chemically to lower inositol phosphates such as inositol pentaphosphate (IP5), inositol tetraphosphate (IP4), inositol triphosphate (IP3) and possibly the inositol di and mono phosphate (Burbano *et al.*, 1995). Similar increase in phytic acid with have been reported by Rehman *et al.* (2007) in vetch flour blended doughnuts and Singh and Srivastava (2012) in millet blended fried products. Polyphenol content increased significantly from the mean value of 243.60 mg per 100g in $T_2$ (00:90:10:: pearl

millet flour: refined wheat flour: whey protein isolates) to 335.03 mg per 100g in $T_6$ (40:50:10:: pearl millet flour: refined wheat flour: whey protein isolates). However, there is a decrease in the polyphenol content (298.58 to 267.21 mg $100g^{-1}$) with the storage period from 0 to 90 days. Similar results have been reported by Lakra and Sehgal (2011) in potato flour blended doughnuts. There was a significant increase in free fatty acid content during 90 days of storage from 1.79 to 2.19 per cent as oleic acid. Treatment $T_6$ (40:50:10:: pearl millet flour: refined wheat flour: whey protein isolates) observed maximum increase in free fatty acid content, however, minimum value was observed in $T_1$ (00:100:00:: pearl millet flour: refined wheat flour: whey protein isolates). Free fatty acid content increased with storage due to the higher activity of lipase and lipoxydase which accelerates the release of free fatty acid (Kent and Evers, 1994). The results are in accordance with the finding of Kumari *et al.* (2009) and Anitha and Rajyalakshmi (2013) in sorghum based seviya and rice products, respectively.

**Sensory evaluation:** The mean scores for colour, texture and taste significantly decreased with the addition of pearl millet and whey protein isolates. Maximum of 20 per cent substitution was accepted by the judges without any adverse effect. However, low appearance was observed at 30 and 40 per cent pearl millet flour incorporation. The mean scores for colour, texture and taste decreased from 8.96 to 7.33, 8.74 to 6.11 and 8.87 to 6.53 with increasing level of pearl millet flour, respectively. Similar results have been reported by Echendu *et al*. (2004) in maize–pigeon pea flour doughnuts and Chong and Noor (2008) in banana flour blended doughnuts. The

**Table 1.** Effect of treatments and storage period on mineral content (mg $100g^{-1}$) of composite flour blended doughnuts

| Treatment | Magnesium | | | | | Phosphorus | | | | | Calcium | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Storage period (days) | | | | | Storage period (days) | | | | | Storage period (days) | | | | |
| | 0 | 30 | 60 | 90 | Mean | 0 | 30 | 60 | 90 | Mean | 0 | 30 | 60 | 90 | Mean |
| $T_1$ | 140.16 | 139.95 | 139.85 | 139.61 | 139.89 | 303.38 | 303.27 | 303.14 | 303.06 | 303.21 | 41.78 | 41.69 | 41.43 | 41.36 | 41.57 |
| $T_2$ | 139.69 | 139.51 | 139.44 | 139.33 | 139.49 | 302.89 | 302.77 | 302.32 | 302.03 | 302.50 | 50.38 | 50.27 | 50.01 | 49.89 | 50.14 |
| $T_3$ | 139.31 | 139.23 | 139.19 | 138.96 | 139.17 | 304.12 | 304.01 | 303.85 | 303.52 | 303.87 | 50.95 | 50.76 | 50.58 | 50.47 | 50.69 |
| $T_4$ | 138.94 | 138.88 | 138.79 | 138.67 | 138.82 | 304.76 | 304.58 | 304.39 | 304.27 | 304.50 | 51.78 | 51.57 | 51.32 | 51.19 | 51.54 |
| $T_5$ | 138.65 | 138.57 | 137.49 | 137.38 | 138.02 | 306.02 | 305.17 | 305.09 | 305.01 | 305.32 | 52.96 | 52.72 | 52.38 | 52.09 | 52.53 |
| $T_6$ | 137.35 | 137.27 | 136.19 | 135.04 | 136.46 | 312.02 | 311.84 | 311.59 | 311.32 | 311.69 | 53.81 | 53.59 | 53.32 | 53.24 | 53.49 |
| Mean | 139.02 | 138.90 | 138.49 | 138.16 | | 305.53 | 305.37 | 305.12 | 304.88 | | 50.27 | 50.15 | 49.83 | 49.71 | |

| CD (P= 0.05) | | | | |
|---|---|---|---|---|
| Treatment | 0.82 | | 1.70 | | 0.90 |
| Storage | 0.67 | | N.S. | | N.S. |
| Treatment × Storage | N.S. | | N.S. | | N.S. |

**Table 2.** Effect of treatments and storage period on antinutritional factors (mg/100g) and free fatty acid (% as oleic acid)content of composite flour blended doughnuts

| Treatment | Phytic acid | | | | | Polyphenols | | | | | Free fatty acid | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Storage period (days) | | | | | Storage period (days) | | | | | Storage period (days) | | | | |
| | 0 | 30 | 60 | 90 | Mean | 0 | 30 | 60 | 90 | Mean | 0 | 30 | 60 | 90 | Mean |
| $T_1$ | 242.97 | 241.75 | 239.49 | 235.84 | 240.01 | 266.38 | 257.47 | 248.61 | 240.28 | 253.19 | 1.33 | 1.56 | 1.73 | 1.87 | 1.62 |
| $T_2$ | 238.24 | 237.83 | 236.78 | 235.53 | 237.09 | 257.66 | 245.44 | 239.77 | 231.55 | 243.60 | 1.47 | 1.59 | 1.78 | 1.92 | 1.69 |
| $T_3$ | 252.09 | 248.58 | 245.89 | 242.73 | 247.32 | 272.71 | 265.37 | 256.46 | 247.92 | 260.62 | 1.63 | 1.81 | 1.99 | 2.03 | 1.87 |
| $T_4$ | 255.69 | 251.99 | 247.63 | 244.41 | 249.93 | 306.49 | 287.19 | 281.85 | 273.53 | 287.26 | 1.92 | 2.01 | 2.13 | 2.19 | 2.06 |
| $T_5$ | 258.87 | 256.84 | 249.98 | 246.00 | 252.92 | 329.55 | 310.59 | 298.10 | 290.14 | 307.09 | 2.10 | 2.17 | 2.23 | 2.29 | 2.20 |
| $T_6$ | 262.84 | 258.76 | 252.90 | 248.03 | 255.63 | 358.67 | 334.11 | 327.54 | 319.82 | 335.03 | 2.31 | 2.39 | 2.65 | 2.83 | 2.54 |
| Mean | 251.78 | 249.29 | 245.44 | 242.09 | | 298.58 | 283.36 | 275.39 | 267.21 | | 1.79 | 1.92 | 2.08 | 2.19 | |

| CD (P= 0.05) | | | |
|---|---|---|---|
| Treatment | 1.59 | 1.70 | 0.02 |
| Storage | 1.30 | 1.39 | 0.01 |
| Treatment × Storage | 3.19 | 3.40 | 0.03 |

**Table 3.** Sensory scores of composite flour blended doughnuts during storage

| Treatment | Colour | | | | | Texture | | | | | Taste | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Storage period (days) | | | | | Storage period (days) | | | | | Storage period (days) | | | | |
| | 0 | 30 | 60 | 90 | Mean | 0 | 30 | 60 | 90 | Mean | 0 | 30 | 60 | 90 | Mean |
| T₁ | 8.99 | 8.97 | 8.95 | 8.94 | 8.96 | 8.78 | 8.75 | 8.74 | 8.70 | 8.74 | 8.99 | 8.86 | 8.84 | 8.80 | 8.87 |
| T₂ | 8.92 | 8.91 | 8.88 | 8.86 | 8.89 | 8.60 | 8.50 | 8.48 | 8.46 | 8.51 | 8.75 | 8.72 | 8.69 | 8.65 | 8.70 |
| T₃ | 8.72 | 8.67 | 8.53 | 8.49 | 8.60 | 8.22 | 8.10 | 7.82 | 7.68 | 7.96 | 8.59 | 8.57 | 8.51 | 8.42 | 8.52 |
| T₄ | 8.41 | 8.38 | 8.35 | 8.31 | 8.36 | 8.00 | 7.81 | 7.73 | 7.59 | 7.78 | 8.31 | 8.23 | 8.09 | 7.86 | 8.12 |
| T₅ | 8.28 | 8.21 | 8.16 | 8.10 | 8.19 | 6.88 | 6.76 | 6.64 | 6.58 | 6.71 | 6.97 | 6.91 | 6.87 | 6.80 | 6.89 |
| T₆ | 7.78 | 7.71 | 6.97 | 6.84 | 7.33 | 6.29 | 6.18 | 6.00 | 5.98 | 6.11 | 6.81 | 6.62 | 6.40 | 6.28 | 6.53 |
| Mean | 8.52 | 8.47 | 8.31 | 8.25 | | 7.79 | 7.68 | 7.57 | 7.50 | | 8.07 | 7.98 | 7.90 | 7.80 | |

CD (P= 0.05)
Treatment            0.03                        0.02                        0.01
Storage              0.03                        0.01                        0.01
Treatment × Storage  0.07                        0.03                        0.03

maximum mean texture score of 8.74 was in T₁ (00:100:00:: pearl millet flour: refined wheat flour: whey protein isolates). However, with progression of storage period and incorporation of pearl millet flour, the texture score declined. Similar results have been reported by Echendu *et al*. (2004) in maize‑pigeonpea flour doughnuts and Chong and Noor (2008) in banana flour blended doughnuts. The taste score decreased with the increase in the storage period as well as with the incorporation of pearl millet flour, which might be due to the typical flavour of pearl millet flour imparted to the product. Sharma (2002) also reported the decrease in taste scores when soy flour was supplemented to maize flour products. Echendu *et al*. (2004) in maize‑pigeonpea flour doughnuts and Melito (2013) in gluten‑free doughnuts also reported similar results.

## CONCLUSION

The acceptable doughnuts with high protein content could be prepared from composite flour blends of pearl millet flour: refined wheat flour: whey protein isolates. Based on sensory properties, level of 20 per cent pearl millet flour and 10 per cent whey protein isolates against wheat flour (70 per cent) yielded in good quality doughnuts. Thus the use of these crops will go a long way in reducing dependence on wheat flour.

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# Dust Interception Capacity of Common Plant Species Growing Alongside National Highway Sirmaur, Himachal Pradesh, India

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**Abstract**: Dust fall on leaf surfaces was estimated on four plant species namely *Ficus roxburghii*, *Mallotus philippensis, Shorea robusta* and *Woodfordia fruticosa* to ascertain the highest dust capturing potential of common roadside plants. The observed trend of dust accumulation was in the order order *Ficus roxburghii > Shorea robusta > Mallotus philippensis > Woodfordia fruticosa*. Dust accumulation was highest during pre-monsoon season as compared to post monsoon months. The use of *Ficus roxburghii* to monitor dust pollution is recommended.

**Keywords**: Vehicular emissions, Dust, *Ficus roxburghii*

Air pollution is one of the most severe environmental concerns faced globally. A major contributor to the air pollution problem is the transport sector which contributes through the vehicular emission. The increasing number of industries and vehicles contribute to amplified pollutant levels in the air due to the increase in harmful gases like CO, $NO_2$, $SO_2$ and particulate matters (Joshi *et al*., 2014). Roadside plant leaves are in direct contact with air pollutants, and may act as stressors for pollutants, hence need to be examined for their biomonitoring potential (Sharma *et al*., 2007). Biomonitoring of plants is an important tool to evaluate the impact of air pollution (Rai, 2011a, b). Accumulation of dust particles depends on internodal distance, petiole length, leaf area, orientation, margin, folding and arrangement, hair density, hair type and length (Yan and Hui, 2008). Due to surface characteristics of twigs, bark and foliage of the plants particulate matters are captured by them and remain there for extended time period. Leaf orientation and the sessile or semi sessile nature of leaves play important role in dust deposition as they determine the surface available for dust deposition. Air movement easily disturbs leaves having thin lamina, smooth surfaces, and long petioles. Consequently such leaves can hold lesser amounts of dust while thick leaves having rough surfaces or hairs on the surface and short petioles can hold large amount of dust and hence, are better collectors of dust. The objective of the present study was to assess dust accumulation on leaves of selected plant species growing alongside National Highway.

## MATERIAL AND METHODS

The study area for the present investigations was Markanda to Paonta Sahib National Highway (NH-7) in Sirmaur District of Himachal Pradesh (Fig. 1). The 40 km stretch of the National highway from Markanda to Paonta Sahib was divided into four equal parts of ten kilometres each (four study locations). In order to study the dust interception capacity of plants, plants were selected from 0-10 m and 10-20 m horizontal distances from both sides of the road.

During the study period (2015-2016) in the selected stretch of National Highway (Markanda to Paonta Sahib), no rainfall was experienced during the months of October-November (post monsoon season). Whereas, a rainfall of 18.7 mm was recorded during April-May (pre monsoon season). Maximum temperature varied from 28.95 to 37.45$^\circ$C and minimum temperature varied from 8.8 to 18.5$^\circ$C. Relative Humidity varied from 53.4 to 67.34% (Fig. 2).

The commonly occurring plant species namely *Ficus roxburghii, Mallotus philippensis, Woodfordia fruticosa* and *Shorea robusta* were selected for the study. The sample collection and analysis of selected plant species was done during two seasons i.e. post monsoon season (October-November, 2015) and pre monsoon (April–May, 2016). Fully matured leaves of the selected tree species were taken for the present studies. The upper surface of the leaves was cleaned with fine brush and identification mark was put on them. These leaves were kept for 24 hours for dust accumulation and were collected in the pre weighed butter paper bags with the help of fine brush. After taking the data for dust accumulation, the leaves were cut from petiole, kept in ice box and brought to the laboratory for further analysis. The amount of dust accumulated on leaves was weighed on top pan electronic balance and calculated by using the equation:

**Fig. 1.** Map showing sampling locations of plant species alongside Markanda to Paonta National Highway (NH–7) in Himachal Pradesh
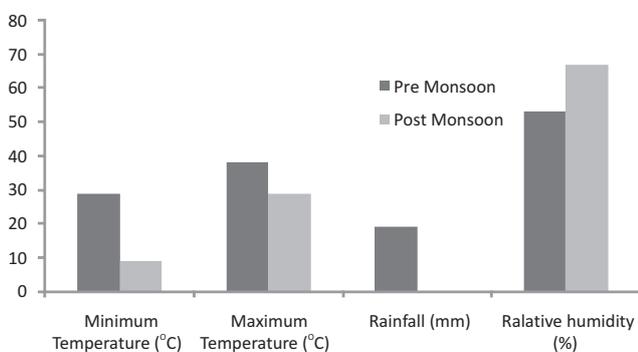


**Fig. 2.** Distribution of minimum, maximum temperature, rainfall and relative humidity during sampling seasons (2015–2016)

$$W = \frac{W_2 - W_1}{A}$$

Where: W = Dust content (mg m$^{-2}$) $W_1$= Initial weight of butter paper bag  $W_2$ = Final weight of butter paper bag with dust  A = Total area of the leaf (m$^2$)

## RESULTS AND DISCUSSION

The dust accumulation of the selected plant species varied significantly with species, horizontal distances and seasons of the year (Table 1). The order of selected plant species according to dust accumulation was *Ficus roxburghii* >*Shorea robusta* > *Mallotus philippensis* >*Woodfordia fruticosa*. The highest dust accumulation on the leaves of *Ficus roxburghii* may be attributed to its coriaceous leaf texture which might have accumulated more dust on leaves and prevented dust fall from leaf surface. These  results are in line with the findings of Madan and Chauhan (2015),  who have  reported that plants having leaves of broad and coriaceous   accumulate   more

dust from air. Lowest amount of dust observed on *Woodfordia fruticosa* may be attributed to its smaller leaf size and smooth surface due to which dust settled on the leaf surface may slip down due to gravitational force or even by wind. The present findings are also in congruence with observations that t dust deposition capacity of plants depends on their surface geometry and leaf external characteristics (Thakar and Mishra, 2010; Joshi and Bora, 2011). The leaf dust accumulation of the selected plant species also varied with horizontal distances from the Highway (Fig. 4). The highest dust load of 49.35 mg m$^{-2}$ was on leaves of plant species growing at the distance of 0–10 m from the National Highway and lowest dust load of 2.77 mg m$^{-2}$ in leaves of plant species growing at the distance of 10–20 m from the National Highway (Table 1). This may be ascribed to dense traffic movement nearby the roadside as compared to the distance away from the road. These results are in corroboration with the findings of Rahul and Jain (2014), who reported that high dust deposition on leaf surface at road side with heavy vehicular traffic may be due to spray of unburnt oil residue of diesel or petrol on the leaf surface. Dust accumulation is more in plants growing at roadsides due to high dust intensity which results by the vehicles activity and capturing dust with a gentle wind (Younis *et al.*, 2013). Accumulation of dust on the leaves of selected plant species was highest during pre–monsoon season (30.70 mg m$^{-2}$) as compared to post–monsoon months where it was only 21.42 mg m$^{-2}$ (Fig. 3). The higher leaf dust accumulation in the pre monsoon season may be attributed due to higher temperature conditions in the study area as compared to post monsoon months (Fig. 1). Further this may be attributed to the road making activities in the study area in pre monsoon season. High values of dust are seen in some months largely due to construction or road making activities at the sites (Joshi *et al.*, 2014).

**Table 1.** Leaf dust accumulation (mg m$^{-2}$) on the leaves of the selected plant species growing at different horizontal distances alongside the Markanda to Paonta Sahib National Highway (NH‒7)

| Plant species | Horizontal distance | | | Season | |
|---|---|---|---|---|---|
| | 0‒10 m | 10‒20 m | Mean | Pre‒monsoon | Post‒monsoon |
| *Ficus roxburghii* | 71.06 | 5.54 | 38.30 | 43.03 | 33.57 |
| *Mallotus philippensis* | 42.90 | 1.71 | 22.31 | 27.26 | 17.35 |
| *Shorea robusta* | 51.33 | 2.56 | 26.94 | 32.09 | 21.80 |
| *Woodfordia fruticosa* | 32.11 | 1.28 | 16.70 | 20.44 | 12.95 |
| Mean (distance) | 49.35 | 2.77 | Mean (season) | 30.70 | 21.42 |
| Pre monsoon | 58.33 | 3.08 | 30.70 | | |
| Post monsoon | 40.37 | 2.46 | 21.42 | | |
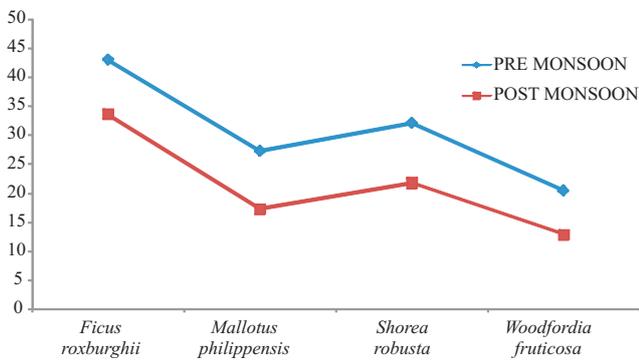


**Fig. 3.** Seasonal dust accumulation pattern of selected plant species growing alongside Markanda to Paonta Sahib National Highway (NH‒7)



**Fig. 4.** Variation in dust accumulation pattern of selected plant species at different horizontal distances from the Markanda to Paonta Sahib National Highway (NH‒7)

## CONCLUSIONS

The deposition of atmospheric dust on plant leaves varies with structure, geometry, epidermal and cuticular features and phyllotaxy of leaves. Smaller plants with short petioles and rough leaf surfaces accumulate more dust than larger plants with long petioles and smoother leaf surfaces. The study indicated that *Ficus roxburghii* can effectively be used as active monitors for dust fall. Thus, this plant species can be suggested for plantations alongside the Markanda to Paonta Sahib National Highway to withstand the impact of dust pollution in that area.

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# Amelioration of Perturbed Phosphorus Metabolism in Mungbean Seedlings Exposed to Sodium Arsenate by Phytohormones and Phosphate Salts

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**Abstract:** The effect of Sodium arsenate ($Na_2HAsO_4.7H_2O$) on phosphorus content and activities of phosphatase enzymes was examined in mungbean (*Vigna radiata* (L.) Wilczek seedlings. With the increase in concentration of Sodium arsenate (5, 10 and 20µM) there was significant decrease in length of mungbean seedlings. Under arsenate induced stress, phosphorus metabolism was perturbed. The effects were manifested by a decline in phosphorus content and inhibition of activity of key phosphatise like alkaline phosphatase (EC 3.1.3.1) whereas activity of acid phosphatase (EC3.1.3.2) was elevated. These alterations in P metabolism might contribute to metabolic perturbations and decreased growth of mungbean plants in an arsenate polluted environment. Reduction in growth inhibition was achieved by pretreating the seeds with phytohormones ($GA_3$, IAA at 50 µM concentration) and phosphate salts ($KH_2PO_4$, $NaH_2PO_4$, $2H_2O$ at 10mM concentration). Pretreatment of mungbean seeds with $GA_3$, $KH_2PO_4$ and $NaH_2PO_4.2H_2O$ could ameliorate As induced toxicity to different extent in terms of growth and phosphorus metabolism. Thus, the use of $GA_3$ and phosphate salts may help to resist the arsenic toxicity in seedling stage to some extent, in arsenic contaminated areas.

**Keywords:** Arsenate, Phosphorus metabolism, Macroelements, Phytohormones, Phosphatases, *Vigna radiata*

Soil and water contamination with arsenic occurs through natural and anthropogenic pathways (Meharg and Rahman, 2003). Arsenic is known to have many toxic effects in humans and is ranked first, in the priority list of hazardous substances compiled by the US Environmental Protection Agency (USEPA) (ATSDR, 2011). Besides groundwater, food is also an important pathway of arsenic in to human system (Norra *et al*., 2005). People are also working to manage arsenic toxicity in plants through various fertilization and nutrient strategies like macronutrients and plant hormones. Earlier studies conducted have shown that phytohormones have profound role in counteracting the effects of toxic metals and metalloids. Phosphorus has a vital functional role in energy transfer, and acts as modulator of enzyme activity and gene transcription. Hydrolytic breakdown of phosphate esters is brought about by phosphatases, which occurs in both acidic and alkaline medium. Acid phosphatases (EC 3.1.3.2) catalyze non-specific hydrolysis of Pi from phosphate monoesters and play a major role in the supply and metabolism of phosphate in plants (Tabaldi *et al.,* 2007). Similarly alkaline phosphatases (EC 3.1.3.1) have a potential role in utilization of phosphomonoesters as the source of Pi required for maintenance of cellular metabolism (Orhanovi´c and Pavela-Vrancic, 2000). However, little information is available on the effect of arsenic on phosphorus metabolism and activity of phosphatase enzymes in pulse plants. Therefore, the present investigation was undertaken to examine the effect of different concentrations of sodium arsenate on the growth and development of mungbean seedlings and to study the altered phosphorus metabolism of the growing mungbean seedlings due to stress induced by sodium arsenate. Phytohormones and phosphate salts were tried as pre-sowing chemical treatment to combat the serious problem of arsenic toxicity and to provide a possible method of arsenic tolerance.

## MATERIAL AND METHODS

**Plant material and arsenic treatments:** Mungbean seeds, collected from pulses and oilseed Research Station, Berhampore, West Bengal, India, were treated with 0.1% w/v HgCl2 solution for 2 min for surface sterilization and washed repeatedly with distilled water. Around 20 seeds were allowed to germinate on petridishes lined with filter paper and sodium arsenate, $Na_2HAsO_4.7H_2O$, (from Merck) used as test solution at concentrations-5, 10 and 20µM. The seeds soaked in distilled water served as control. The pH of the solution was 6.5 throughout. The seedlings were harvested after 5 days for further studies. The above mentioned arsenate concentrations are comparable to soil conditions and are environmentally relevant.

**Pretreatment with growth regulators:** For the amelioration of arsenate toxicity, seeds were pretreated with growth regulators like phytohormones, $GA_3$ and IAA–both at 50µM concentration and phosphate salts– $KH_2PO_4$, and

$NaH_2PO_4.2H_2O$ solutions each at 10mM concentration, for 24 h and then transferred to sodium arsenate solution-10µM (sub lethal dose) for next 4 days, total germination time being maintained for 5 days in all the cases. Another set of seeds were pretreated with distilled water for 24 h and transferred to sodium arsenate solution (10µM) for next 4 days. The set which received distilled water for 5 days served as control.

**Morphological studies**: After 5 days, arsenate induced damaging effects were observed on root and shoot length of mungbean seedlings. Seedling length of 10 seedlings were determined after excising the cotyledons and averaged. All the experiments were repeated thrice and analyzed statistically.

**Estimation of phosphorus content:** Total phosphorus released was determined spectrophotometrically by the method of Fiske and Subbarow (1925) with slight modifications. This method is based on the fact that orthophosphate and molybdate ions condense in acidic solution to give molybdophosphoric acid or phospho molybdic acid which upon selective reduction with a reducing agent produces a blue colour. The intensity of the blue colour was measured in HITACHI U‑2000 Spectrophotometer at 660 nm.

**Assay of acid phosphatase activity:** Acid phosphatase activity was assayed according to the method of Malik and Singh (1980). 1 g fresh tissue was extracted in 0.1 M acetate buffer, pH 4.8 and centrifuged in cold at 10,000 ×g for 20 minutes. The supernatant was used for enzyme assay. The absorbance of the reaction mixture was recorded at 430 nm and compared with a standard curve of p‑nitrophenol. Enzyme activity was expressed as mg p‑nitrophenol released per g fresh tissue.

**Assay of alkaline phosphatase activity:** Alkaline phosphatase enzyme activity was assayed according to Sadasivam and Manickam (1996) with slight modifications. 1 g fresh tissue was extracted in 50 mM glycine‑NaOH buffer, pH 10.4 and centrifuged in cold at 10,000×g for 20 minutes. The supernatant was used as enzyme source. The absorbance of the reaction mixture was measured at 430 nm and compared with a standard curve of known concentration of p‑nitrophenol. The enzyme activity was expressed as mg p‑nitrophenol released per g fresh tissue.

### RESULTS AND DISCUSSION

**Effect of arsenate and growth regulators on the growth of seedlings:** The sodium arsenate, $Na_2HAsO_4.7H_2O$ treatments resulted in significant (P≤0.01) inhibition of initial growth of mungbean seedlings. Inhibition of elongation of mungbean seedlings started at a concentration of 5µM As (V) and it was remarkably pronounced at 20µM (Table 1). The inhibition increased at higher doses, the effect being more

**Table 1.** Effect of sodium arsenate on shoot and root length of non‑pretreated and pretreated mungbean seedlings

| Treatment | Shoot length (cm) | Inhibition (%) | Root length (cm) | Inhibition (%) |
|---|---|---|---|---|
| Control | 11.5 | – | 4.5 | – |
| 5µM As (V) | 7.0 | 39.1 | 1.8 | 60.0 |
| 10µM As (V) | 2.5 | 78.3 | 0.7 | 84.4 |
| 20µM As (V) | 1.0 | 91.3 | 0.5 | 88.9 |
| *$H_2O$ | 3.2 | 72.2 | 0.8 | 82.2 |
| *$GA_3$ | 9.5 | 17.4 | 3.7 | 17.8 |
| *IAA | 7.5 | 34.8 | 3.0 | 33.3 |
| *$KH_2PO_4$ | 8.0 | 30.4 | 3.2 | 28.9 |
| *$NaH_2PO_4$ | 8.6 | 25.2 | 3.3 | 26.7 |

*pretreated

pronounced on root than shoot. Roots were characteristically stubby and brittle and root tips gradually turned brown. Similar reports were observed in rice seedlings where shoot height decreased (Abedin and Meharg, 2002) along with significant reduction in root growth (Rofkar and Dwyer, 2011).With the increase in concentration of As(V), primary leaf area of mungbean was reduced considerably also the water content of the seedlings declined (Swarnakar, 2017). Among all the growth regulators used for amelioration, phytohormones were best capable of reversing arsenate toxicity. IAA and $GA_3$ could ameliorate arsenate toxicity to different degrees when the seeds were pretreated with them followed by transfer to sodium arsenate (Table 1). Best result was brought about by $GA_3$ followed by IAA. Both the phosphate salts viz., $KH_2PO_4$ and $NaH_2PO_4.2H_2O$ brought about significant (P 0.01) enhancement in growth of shoot and root length, when used as pre‑sowing chemical treatment (Table 1). Similar results were obtained in Canola (*Brassica campestris*) where addition of higher dose of P (500 µM) in hydroponic solution could prevent the plant form As toxicity (Cox *et al*., 1996). Again, amelioration of arsenate toxicity was achieved only by pretreating the seeds with the above mentioned chemical regulators while post‑treatment or simultaneous treatment of these growth regulators with sodium arsenate bore no fruitful result (Swarnakar and Mukherji, 2005).

**Effect of sodium arsenate and growth regulators on phosphorus content:** Phosphorus content showed a decreasing trend with increasing arsenate concentrations (Fig. 1) being 28, 49 and 67 per cent with 5, 10 and 20µM sodium arsenate respectively in comparison to the control. Pre-treatment with growth regulators, especially phosphate salts, viz., $KH_2PO_4$ and $NaH_2PO_4$ brought about significant (P 0.01) increase in Phosphorus content of mungbean seedlings (Fig. 1).
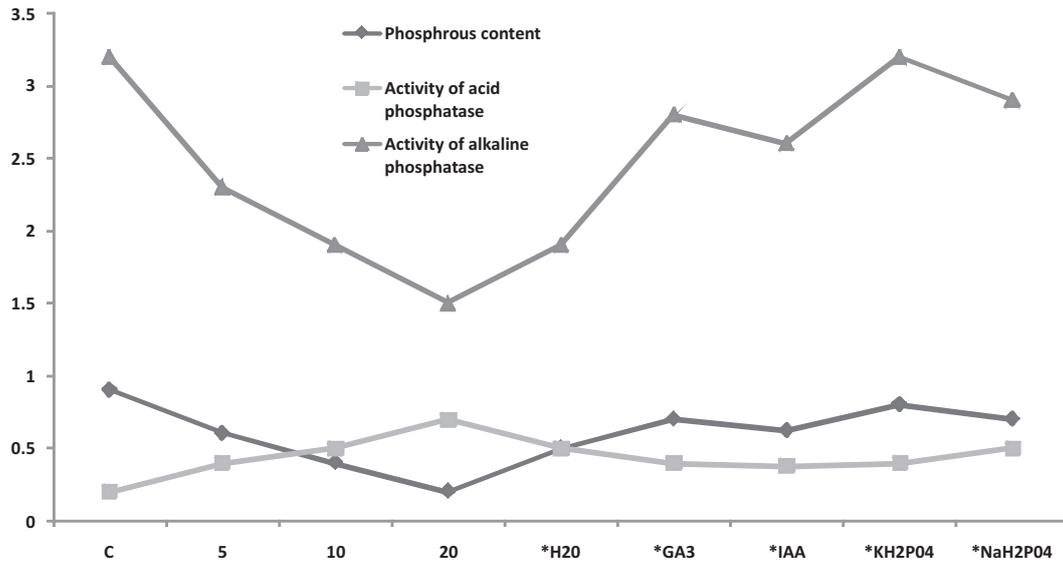
**Fig. 1.** Effect of Sodium arsenate (μM) on Phosphorus content (mg P released/g f wt) , the activity of acid phosphatase (mg p‒nitrophenol released/g f wt) and activity of alkaline phosphatase (mg p‒nitrophenol released/g f wt) of non‒pretreated and *pretreated (with growth regulators) mungbean seedlings. Vertical bars represent standard error of mean of 3 replicates

**Effect of sodium arsenate and chemical regulators on the activity of acid phosphatase and alkaline phosphatase enzyme:** A stepwise increase in acid phosphatase activity with increasing concentration of sodium arsenate was noted. Acid phosphatase activity increased with increasing concentrations of sodium arsenate to the extent of 37, 49 and 86 percent due to 5, 10 and 20μM sodium arsenate respectively in comparison to control (Fig. 1). The acid phosphatase showed increased activity, the alkaline phosphatase showed gradual decline in activity with the increase in concentration of arsenic. The fall in the activity of this enzyme was to the extent of 31, 44 and 53 percent in 5, 10 and 20μM sodium arsenate respectively in comparison to control. However, phosphorus metabolism could be restored to some extent due to pre-treatment of mungbean seeds with the growth regulators (also evident from the results of total P content). Activity of acid phosphatase decreased and activity of alkaline phosphatase (Fig. 1) increased significantly (P 0.01) with the help of these growth‒promoting chemicals to various extents, which are thought to be reversal of stress effects. GA$_3$ and KH$_2$PO$_4$ and NaH$_2$PO$_4$ could possibly mitigate the toxic effects of As (V) to some extent and perturbations in the activities of phosphatase enzymes of mungbean seedlings were restored to different levels. Exogenous application of phosphate might have compensated the decrease in inorganic phosphate level. Therefore, Arsenate tolerance could be enhanced by increasing Pi uptake (Pigna *et al*., 2009). P fertilization may reduce the impact of arsenic toxicity without increase in arsenic concentrations in above ground parts of plants. Thus, GA$_3$ and KH$_2$PO$_4$ and NaH$_2$PO$_4$ can be used as effective ameliorative chemicals as these could reduce the toxic effects of sodium arsenate in the mungbean seedlings and thus their promoting effects on seedling growth parameters were well visible. This may have some practical importance in agricultural systems, as it can reduce yield losses and also improve quality of pulse crops.

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# CONTENTS

# CONTENTS