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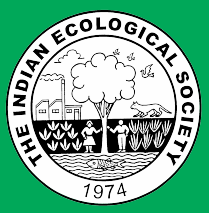

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Determining Factors of People's Participation in the Community Forest Program in the Central Dry Zone during the Rural Livelihood Transition in Myanmar

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***Abstract*:** The Forest Department initially facilitated the Community Forest (CF) program. People participation is more critical, and local people ideally implement and manage the CF. In Myanmar, a stagnant CF user group is one of the issues in CF program success after three decades of CF implementation. In the recent decade, rural livelihood strategies have been changing alongside political and economic reform since 2011. This research aims to explore the determining factors of local people's participation in the CF program. First, this research used binary logistic regression to understand people's decision to be part of the CF program through the socioeconomic characteristics of households. Then, multiple linear regression model was used to examine CF members' participation in the CF user group's collective activities. The following 7 factors among 10 independent factors determine CF membership: gender, education, the nonfarm income (remittance, wage labor income, salary) of the household, customary forest area, agricultural land-holding size, family labor, and livestock-holding unit. Then, among CF members, the increasing nonfarm income of households and CF members working outside the township area negatively affects CF members' participation in the collective activities of the user group, whereas family labor availability promotes participation.

***Keywords*:** Community forest, Rural livelihood, Migration, People participation, Collective activities

The Community Forest (CF) program has been used globally to protect forests and enhance local people's livelihoods (Gilmour 2016). The first step of the CF program in Myanmar was the development of the Community Forestry Instruction (CFI) in 1995 (MoF 1995). CF is the first breakthrough participatory forest management program in history. A part of the state-owned forest was officially transferred to Community Forest User Groups (CFUGs) for its development, conservation, utilization, and management. CFUGs are recognized as independent, autonomous, and self-governing institutions.

As of January 2021, more than (6,000) CFUGs with a total forest area of 352,163 ha (870,215 acres) were established, which is significantly less than the national target (919,000 ha (1.36% of land area) by 2030), according to an internal report of the Forest Department of Myanmar. A CFUG is a self-established group of community members responsible for forest management and benefits from forests. Interested villagers can apply the CF user certificates for 30 years by developing a Community Forest Management Plan.

Sustainable forest management (SFM) can be achieved through the active involvement of forest users, particularly in the CF program (Ostrom 1990, Glasmeier and Farrigan 2005, Maryudi et al 2012). Most CFUGs in Myanmar ended with stagnant user groups due to inactive people's participation in CFUG's activities after decades of CF program establishment (Springate-Baginski et al 2011).

Although local people's participation in CF programs is one of the policy challenges of the government of Myanmar, very limited research regarding people's participation in CF programs in Myanmar has been conducted. Hlaing and Inoue (2013) explored the relationship between people's social/institutional and physical factors and CF users' participation in the CF program. They found that social and institutional factors are the most important factors that determine people's participation in the collective activities of CF in dry forest. After that, Soe and Sato (2010) analyzed the socioeconomic condition of CF user group members and non-CF members and the reason that non-CF members were not involved in the CF program.

Rural livelihood transformation is underway in the CDZ in Myanmar, including increasing migration, mechanization of agriculture, livelihood diversification, development of the banking system, and increased accessibility to schools and transportation (Belton and Filipski 2019, CSO 2019). Concurrently, the population has also increased from 124 people per square kilometer in 1983 to 200 people per square kilometer in 2014, according to the national census in 2014, which resulted in the tightening of agricultural land availability in CDZ. In fact, the availability of agricultural land has

declined by 10% compared with their parent's land (Hein et al 2017).

In Myanmar, Filipski et al (2021) found that rural livelihood had transitioned from farm related livelihoods to nonfarm livelihoods, such as migration, casual labor in nonfarm works, and salary occupations. Remittances are a major source of income for rural households in the dry zone, received by 32% of households and accounting for 15% of total household income in the CDZ (Filipski and Belton 2019).

Previous research on people's participation in the CF program was conducted in the old livelihood setting and legal framework. As changes in Myanmar's rural livelihood are underway, the local people are facing opportunities and challenges during this transition period. Although Myanmar is an agrarian country, agriculture-based families rely on the forest for their livelihood. Together with changes in rural livelihood settings in Myanmar, the CF policy was reformed with the revised CFI in 2019, which aims to improve local people's livelihood through community forestry enterprise (World Bank 2019).

The relationship between the changes in rural livelihood and local people's participation is varied based on the socioeconomic and biophysical conditions of the study area in Nepal (Tamang et al 2014, Shahi et al 2022, Fox 2018). In Nepal, changes in rural livelihood with increasing migration and a decline in farm income at the household level have resulted in a decrease in forest dependency and changes in local people's forest management (Tamang et al 2014). Out-migration, which increases nonfarm income and livelihood diversification, did not negatively affect local people's participation in CF, although a significant decrease in forest dependency was found (Shahi et al 2022). By contrast, Fox (2018) found that nonfarm livelihood diversification has a negative impact on forest conditions and local people's participation in CF.

Socioeconomic and biophysical factors affect users' participation in CF activities (Agrawal and Gupta 2005, Coulibaly-Lingan et al 2011, Hlaing and Inoue 2013). Numerous studies emphasize that local people's socioeconomic factors determine people's participation in CF programs (i.e., to become a CF member) (Coulibaly-Lingan et al 2011). Hence, this study used the socioeconomic characteristics of the individual household as determining factors in people's participation in the CF program. Beyond that, the study considered new variables that highlight local people's livelihood diversification into a non-agrarian livelihood.

This paper explores the determinant of the socioeconomic characteristics of households to become CF members and further participation in the collective activities of the CFUGs. First, the socioeconomic model was used to analyze factors to become CF members using a binary logistic regression model to reach the primary research objective. Then, a multiple linear regression model was applied to explore the relationship between CF member participation in collective activities and members' socioeconomic characteristics.

MATERIAL AND METHODS

Study site: The Central Dry Zone (CDZ), the research area, is situated in the central part of Myanmar between 19°27′ and 23°16′ in the north latitude and 94°18′ and 96°24′ in the east longitude. As of January 2021, approximately 43% of the total CF area (152,201.761 ha of forest) is handed over to local people in the CDZ of Myanmar, according to Forest Department internal data. The CDZ receives an average of 672 mm of rainfall per year. The temperature ranges from 12°C to 42°C. April is the hottest month of the year. The study area is shown in Figure 1.

In 2013, the Forest Department implemented a

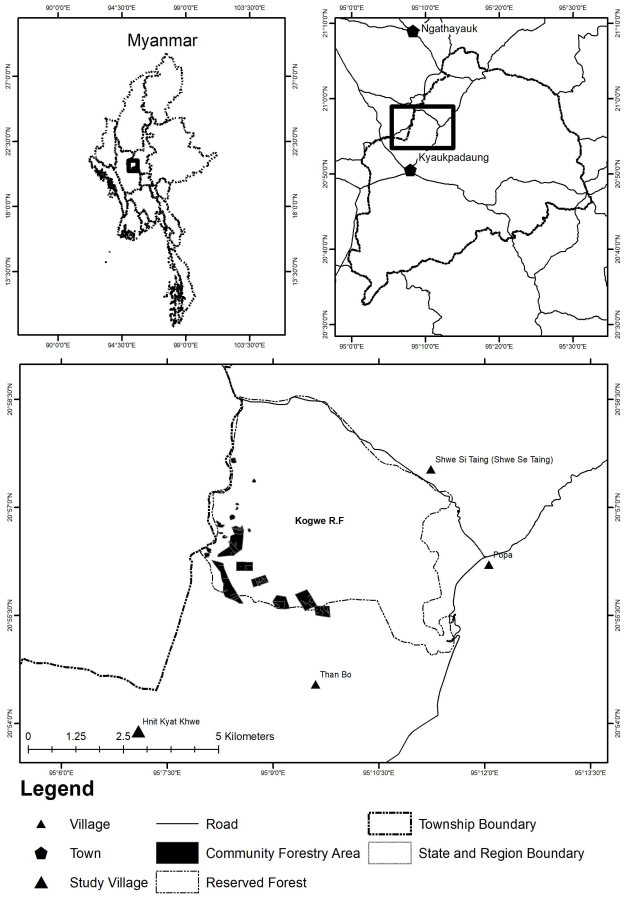

Fig. 1. Map showing the study village, which is located in Kyaukpadaung Township, Mandalay region, Myanmar

departmental instruction to provide CF certificates to local informal landowners if the land is in the Permanent Forest Estate (PFE). Consequently, interested customary forest owners in the village applied for the CF certificate in 2015.

The total CF members are 78 out of 954 households in the study village. The CF certificates were issued in 2017 to eight user groups, with a total area of 280.64 ac (113.57 ha). The land allocation of CF members is based on traditional land ownership in the Kogwe Reserved Forest area. The type of CF in this study village is agroforestry, where CF members use nearby farmland for cash crops such as sesame, green gram, maize/sorghum, chickpeas, groundnuts, and pigeon pea. The CF is the source of firewood, fodder, and cash crop income from agroforestry. However, there has yet to be a cash return from the forest because the forest has been degraded because of the overexploitation of firewood in the past.

Research methodology and analysis: A semi-structured questionnaire, a key informant interview, and a focus group discussion were used for primary data collection. Before the full household survey was conducted, a preliminary survey was conducted in October 2021 with 10 households and 1 focus group discussion to get the baseline information of the study village to structure the questionnaires. After that, a structural interview with 189 households was conducted in December 2021 and March 2022 through stratified random sampling to get quantitative data on socioeconomic data, natural resources ownership, awareness of CF, and participation in the collective activities of CF.

For statistical analysis, we used SPSS 28.0.0.0. In model 1, binary logistic regression was used to examine the influencing factors of local people's decision to become a member of CFUGs. Then, in model 2, multiple linear regression model was used to explore factors affecting CF members' participation in collective activities. Focusing on the findings from the previous literature, we selected socioeconomic characteristics of households related to the livelihood of local people affecting people's participation in the CF program, shown in Table 1. Then, the research aimed to explore the impact of the socioeconomic characteristics of rural households under rural transition on people's participation in the CF program. Household incomes were divided into farm and nonfarm incomes in the analysis to know the impact of increasing nonfarm livelihood activities under rural transition.

For model 1, the model's goodness of fit was assessed using Nagelkerke R^2 and chi-square values. To check the multicollinearity assumption of model 2, the variance inflation factor (VIF) test was conducted, and the VIF values of the independent variables in model 2 are from 1.05 to 5.5.

RESULTS AND DISCUSSION

Socioeconomic profile of the study area: Table 2 shows the socioeconomic profile of the respondents. Most of the households in our sample were dry land farmers with diverse livelihood systems that combined incomes from dry land agriculture, rearing livestock, migration, casual labor (nonfarm), farm labor, nonfarm employment (school teacher and administration staff of government/private businesses), and small businesses (e.g., incomes from small grocery shops, food services in town, and agricultural machine rental services). The income of CF members from farms is 74%, whereas nonfarm income contributed 26% of CF members' total gross household mean income. In the case of a non-CF member family, farm and nonfarm incomes contributed equally to a household's gross mean income.

Since the early 2000s, some toddy palm climbers have started working in foreign countries, Malaysia, Thailand, and cities in the country such as Yangon, Mandalay, and Naypyidaw due to the low price of toddy palm sugar. Then, with the connection of returnees, the number of households working outside of townships has increased, thereby increasing the nonfarm income of families in the study village. Before that, the village only depends on dry agriculture, toddy palm sugar production, and small-scale livestock. Owing to increasing investment cash for agriculture, many families pursue nonfarm income activities, mainly domestic and international migration, in this study area. However, farm income is still a significant contribution to most households.

Household food security levels were calculated based on sampled households' annual per capita income compared with the regional food poverty line (277,768 Myanmar Kyats, MMK/year = US$518.60/year) for the study area in the Mandalay region (Schmitt-Degenhardt 2013). Our analysis showed that CF and non-CF members' households in the study village had food security levels but were just above the food poverty line.

CF members (2.6 ± 1.3) are less occupied with family labor than non-CF members (3.2 ± 1.1). However, 30% of CF members work outside the township area, whereas 46% of non-CF members' families work outside the township. Non-CF members have more average school years in their families than CF members. Non-CF members owned bigger agricultural land than CF members did, whereas CF members kept more livestock and large customary forest areas.

Regarding forest dependency, fuel wood and fodder for livestock are the main products of forests that contribute to local people's livelihood in the study village. Although the non-CF user group used 3.6 m^3/year of firewood, CF members consumed 4.8 m^3/year of firewood. As per the field survey, the commercial income of the villagers from selling

firewood is not found at the time of the survey. Villagers traditionally used wood to cook toddy palm sugar (jaggery). In addition, villagers largely depend on small-scale livestock for livelihood. At least a couple of draught cattle are owned by each household for agricultural labor and transportation purposes. Villagers rear goats, pigs, dairy cattle, and chicken, which they can sell when in need of cash and which they use for subsistence consumption. CF members owned more livestock than non-CF members because CF provided more grazing land and fodder during summer.

Table 1. Description of coding and previous literature of dependent and independent variables used in the analysis

Variables	Description and coding in the analysis of models 1 and 2	Literature
AGRILAND	Agricultural land-holding size (ha)	Positivity toward forest conservation programs is connected with agricultural land ownership (Cynthia et al 2012).
LSU	Total livestock-holding unit (LSU[1]) (number)	Livestock fodder and firewood are the only forest products that local people get from the forest in CDZ. The more livestock a family reared, the more grazing land they needed, which encouraged them to participate in CF (Khaing 2018).
CUF	Area of customary forest (ha)	Recognizing customary rights and arrangements motivates customary forest owners to be involved in the institutionalized forest management system (Poudel,2019). When customary land is part of the Permanent Forest Estate (PFE), CF is the only option for the informal land owners to formally establish land ownership (Lin 2018).
EDU	Education The average school year of household member (year)	People with more education are more aware of the value of protecting forests and are more likely to participate in participatory forestry conservation programs (Jumbe and Angelsen 2007, Lise 2000, Oli and Treue 2015). The presence of highly educated family members in families has a positive effect on environmental conservation (Agarwalla & Saha 2021). Higher education levels lead to better employment and less reliance on forest resources, which decreases motivation to participate in actions to forestry activities (Agrawal and Gupta 2005). People who are more educated and aware of the environment's current condition are more likely to participate in conservation projects and implementation plans (Alkan et al 2009, Htun et al 2012).
FIREWOOD	Total firewood consumption of household (m^3/year)	Participation in forest management is motivated by high dependence on forests (Coulibaly-Lingani et al 2011, Dolisca et al 2006, Jumbe and Angelsen 2007, Oli and Treue 2015).
GEN	Gender of household head, Female =1, Male =0	Compared to female-headed households, male-headed households are more willing to assist in forest conservation (Coulibaly-Lingani et al 2011, Kugonza et al 2009, Oli and Treue 2015). Women's engagement in CF is nevertheless restricted by traditional gender stereotypes, and women with migratory husbands significantly burden their participation in activities in local community forest institution activities (Lama and Ghale 2017).
WOT	Family working outside of township area. Households have at least one household member working outside of township area at least 6 months in a year (Yes=1, No=0)	CF is affected by labor migration in terms of participation and forest condition (Fox 2018). When migration out of the community is low and resource dependence among users is strong, community-based natural resource management can be sustained (Ostrom 1990, Agrawal 2001).
FL	Family labor. Working age household member (16–62 years old)	The more working age household members a family has, the more likely to engage in labor-intensive forest conservation work (Coulibaly-Lingani et al 2011, Jumbe and Angelsen 2007, Maskey et al 2006, Soe and Yeo-Chang 2019).
Collective activities*	No. participation in collective activities CFUGs because CF certificate was granted in 2017.	Participation is defined as the households' involvement in CF activities by Lise (2000), where participation comprises resource utilization, forest protection, and decision-making.
FI	Farm income, including agriculture, agroforestry, toddy palm sugar, livestock, casual labor (agriculture+ toddy palm+ livestock)	Decreasing farm related income such as agriculture and forestry, families' attendance at CF meetings, and the average amount of time spent at each meeting have remained constant, and there has been a decline in their reliance on the forest (Tamang et al 2014). Agricultural cash income from agroforestry-type CF in the dry zone is one of the factor to participate in CF activities (Hlaing and Inoue 2013).
NFI	Nonfarm income, including remittance, casual labor (non-farm), small business, nonfarm employment (salary jobs)	Increasing opportunities in nonagricultural livelihood like the service sector, businesses, and migration (including the remittances from it) is triggering labor availability in the forest sector (Tamang et al 2014).

[1]Calculation of livestock using the Eurostat coefficient to get the common value of livestock based on the type of livestock
*Collective activities include nursery work, pruning, fire-break line, and gap filling

Factors affecting local people's decision to register as a CFUG member: This section will explore the socioeconomic characteristics of households affecting people's decision to become CF members. Model 1 is significant at a 0.1% significance level, and Nagelkerke R^2 is 54%. According to model 1, in Table 3, the first factor is the customary forest area of local people. The larger the customary forest area have more inclination to become a CF member ($p<0.001$). As explained in the characteristics of the study village, exclusively customary forest owners are invited to apply for CF certificates in this study area. According to the key informant interview with the township staff office of the Forest Department, the Forest Department aims to reduce land use change from forest cover to agricultural land in this study village. Therefore, informal landowners are invited to apply for a CF certificate to prevent further invasion into PFE.

Second, the livestock-holding unit (LSU) ($p < 0.01$) has a significant positive impact on joining CF members. Because the availability of fodder is one of the problems during summer, livestock-dependent livelihood households in the study area have more probability of becoming CF members.

The third and fourth factors, namely, agricultural land-holding size of household ($p < 0.05$) and nonfarm income ($p < 0.05$), show a significant negative correlation to being a member of CFUGs. The fifth and sixth factors, which are the female household head ($p < 0.05$) and the average school years of the household ($p < 0.01$), are the social characteristics of the household. Both negatively affect local people's decision to participate in CFUGs. The seventh factor is the availability of family labor ($p < 0.01$), which negatively influences local people's decision to become CF members.

Factors influencing CF member participation in the collective activities of the CFUGs: In this section, model 2 explains the factors influencing CF members' participation in the collective activities of the CFUGs. The model is significant at a 0.1% significance level, with an R^2 value of 68%. As shown in Table 4, in the case where the CF member family's income from nonfarm increases, the level of participation in collective activities ($p < 0.001$) tends to decrease. Furthermore, if the CF member family has

Table 2. Socio-economic profile of respondent households

Variables	CF member status	N	Mean	SD	SE
FL (No.)	No	111	3.230	1.068	0.101
	Yes	78	2.630	1.300	0.147
AGRILAND (ha)	No	111	1.310	1.318	0.125
	Yes	78	1.275	0.726	0.082
LSU (No.)	No	111	2.788	2.861	0.272
	Yes	78	5.812	5.439	0.616
INCOME (USD per capita/year)	No	111	544.130	324.802	30.829
	Yes	78	544.093	320.785	36.322
CUF (ha)	No	111	0.263	1.201	0.114
	Yes	78	2.179	3.161	0.358
EDU (year/HH member)	No	111	6.939	2.169	0.206
	Yes	78	5.046	2.067	0.234
FIREWOOD (m3/year)	No	111	3.632	3.188	0.303
	Yes	78	4.888	3.540	0.401
FI (USD/year)	No	111	1086.455	770.298	73.113
	Yes	78	1469.417	905.161	102.489
NFI (USD/year)	No	111	1097.224	1276.896	121.198
	Yes	78	512.581	817.390	92.551

Variables	Description	CF member households	Non-CF households
GEN (No.)	Female	14	38
	Male	64	73
WOT (No.)	Yes	24	51
	No	54	60

*1USD= 1630 Myanmar Kyat in 2021; SD= standard deviation; SE= standard error

someone working outside the Kyaukpadaung township area, the CF member family has fewer records of participating in collective activities ($p < 0.05$). However, family labor availability promotes CF member participation in collective activities at a 0.1% significant level.

Model 1 found that customary forest owners have more possibility to become CF members. The result is similar to that of Lin (2018) in Myanmar and Dolisca et al (2006) in Haiti. The landless or small land-holding size family is more interested in joining the CF program to get cultivation land. The increasing population in the CDZ tightened the availability of agricultural land because more than 60% of agricultural land is inheritance (Hein et al. 2017). The result suggests that small agricultural landowners or landless families are more interested in joining the CF program. In this context, a bigger agricultural landowner has less possibility to become a CF member in model 1. The result is opposite to that of the previous study by Cynthia et al (2012) in Madagascar.

With limited agricultural land availability in the CDZ, a customary landowner is more likely to register as a CF member, according to model 1. According to a discussion with Forest Department staff and CF chairman, the Forest Department exclusively aims for an informal landowner in the PFE to join the CF program in this study village, which means that it excludes local people from entering the CF program if they do not have customary land (informal land) in PFE. During discussions with non-CF members, some were interested in joining the CF program if the Forest Department allowed them to join, even though they do not have a customary forest in PFE, especially agricultural landless households. Therefore, the Forest Department should treat all villagers equally to join the CF program. If the Forest Department could provide a common pool forest area to interested villagers (local people) who are noncustomary forest owners, local participation in the CF program would be increased. Concurrently, local people can also earn cash income from agriculture while the forest is under rehabilitation.

Suppose that one of the family members works outside the township area and cannot commute daily, threatening family labor for the forestry sector, particularly for labor-demanding collective activities. Thus, CF member households with members working outside of the Kyaukpadaung township area show less participation in collective activities in model 2.

The increasing opportunities under the rural transformation with the improved banking system and transportation have been catalyzing local people to pursue nonfarm livelihood activities in the village and nearby town in Myanmar since 2011. Moreover, when the country opened to international communities in 2011, the increasing

Table 3. Result of the binary logistic model of factors affecting local people to become a CFUG member[1]

Variables (Model 1)	B	SE	p value
GEN	−1.106	0.506	0.029*
WOT	0.523	0.573	0.362
FL	−0.526	0.196	0.007**
AGRILAND	−0.66	0.269	0.014*
LSU	0.201	0.066	0.002**
CUF	0.374	0.108	<0.001***
EDU	−0.341	0.127	0.007**
√FI	1.279	0.742	0.085
√NFI	−0.398	0.194	0.04*
FIREWOOD	0.035	0.065	0.595
Constant	−0.236	2.201	0.915
Prob > chi-square X^2 (10,189)	79.886***		
Nagelkerke R^2	0.54		
No. of correct prediction	79.4		
N	189		

Significance level: *5%, **1%, ***0.1%, [1] Respondent is a CFUG member; B= coefficient of independent variables; SE= standard error

Table 4. Result of the multiple linear regression model of factors affecting CF member's participation in the collective activities of the CFUGs

Independent variables	Model 2 (Collective activities)		
	B	SE	p value
(Constant)	7.316	1.292	<0.001***
√FI	0.255	0.394	0.52
√NFI	−1.977	0.181	<0.001***
FIREWOOD	0.042	0.029	0.162
AGRILAND	0.114	0.138	0.411
WOT	−0.738	0.297	0.015*
LSU	0.009	0.022	0.674
GEN	−0.194	0.235	0.413
FL	0.638	0.126	<0.001***
EDU	0.011	0.062	0.858
CUF	0.009	0.033	0.781
	R^2	0.683	
	F(10,77)=108.515		
	p<0.001		
	N	78	

Significance level: *5%, **1%, ***0.1%; B= coefficient of independent variables, SE= standard error

international investment in the factory and services sector in big cities persuaded young educated people in the village to work in the cities. Thus, the result showed that bigger households choose more stable jobs and better careers instead of forestry, particularly joining the CF user group in model 1.

By contrast, in model 2, the availability of family labor among CF member families has a positive relationship with CF member participation in the collective activities of the CFUGs. Similar results were found by other scholars (Jumbe and Angelsen 2007, Coulibaly-Lingani et al 2011, Soe and Yeo-Chang 2019). Generally, a CF member does not consider forestry activities as a livelihood priority to share labor due to limited earnings from the forest in the study village. Thus, sharing family labor to participate in CFUG's activities is considered a voluntary contribution to the user group's development. In this context, if CF members have limited family labor, sharing labor to collective activities of CF is considered after agriculture and other livelihood work. Meaning that the lesser the family, the lesser the participation in CF collective activities. Thus, this study suggests that creating alternative income opportunities through participation in CF activities is urgently needed to give incentives to CF members to boost people's participation in CF activities.

In model 2, the CF member's farm income and agricultural land-holding size are found to have a positive relationship with the CF member's participation in the collective activities of the user group. However, these factors are not statistically significant. The finding is similar to that of the previous study by Hlaing and Inoue (2013) in the dry zone: agricultural income from the CF area is one of the main incentives to participate in CF collective activities, which means that if CF members' main livelihood is farm livelihood, they need more agricultural land from the CF area to increase household income. In addition, rural household wealth in Myanmar is generally measured by agricultural land-holding size. Therefore, creating socioeconomic opportunities, such as a revolving fund for poor CF members and a common livestock farm, would encourage participation by low-income families among CF members.

Model 1 shows that increasing nonfarm income significantly affects local people's decision to be CF members. All classes of wealth engage in nonfarm livelihood activities under the rural transformation: poor households are in the form of distressed livelihood diversification, and the well-off are in progressive livelihood diversification (Martin and Lorenzen 2016).

For the well-off and educated people, migration is for better job opportunities and stable income, which encourages them to work in urban areas instead of agriculture, forestry, and toddy palm. The trend is similar in Nepal; education catalyzed local people to grab regular salary jobs, which has a negative impact on local people's interest in forest management (Agrawal and Gupta 2005). Thus, the result suggests that educated households, largely dependent on nonfarm income are less interested in participating in CFUGs. The trend is opposite to that of previous studies by Soe and Yeo-Chang (2019). They mentioned that educated families are more interested in forest conservation.

Taking advantage of rural transformation, model 1 suggests that families with more educated members are less likely to join CFUGs, which means that educated households opt for nonfarm income opportunities such as salary jobs (teacher and regular clerk in a government department) and working outside the township area for better careers and a stable income.

The low interest of educated persons in the CF program in this study area will become an institutional challenge for the sustainability of CFUGs because the involvement of educated members is critical in communicating with external stakeholders and expanding user group networks to grab future economic opportunities for CF enterprises.

For poor people, irregular rainfall and the unstable price of agricultural commodities push them to pursue out-migration and nonfarm livelihood activities (low-income generating activities). However, most of the respondents keep agriculture as their main livelihood. They use the remittance and other nonfarm income as a cash investment for dryland agriculture, livestock, and the toddy palm sugar industry. Hence, this trend of livelihood transition to nonfarm livelihood makes them tighten labor availability to share with the forestry sector, which negatively impacts people's decision to be part of CFUGs.

Similarly, the increasing nonfarm income of CF members' households contributes to less participation in collective activities by its members in model 2. The result is the opposite to that of Shahi et al (2022) in Nepal: changing the livelihood of CF members to non-forest-dependent livelihood did not seem to reduce the participation records by CF members due to a clear CFUG fine rule for the absence of participation in meetings. However, owing to the absence of a clear institutional structure of CFUGs in the study village, CF member participation in collective activities is found to decrease when the nonfarm income of CF members increases, which means that if CFUGs have clear sanctions and fine systems in CFUGs, the participation of CF members in collective activities can be strengthened.

In addition, model 1 displayed a negative sign on the

female household head. The result is consistent with that of previous studies in Nepal and West Africa (Coulibaly-Lingani et al 2011, Oli and Treue 2015). In Myanmar, the result can be explained by the custom way of choosing livelihood: women think that forestry work is more relevant to males. However, model 2 shows that gender is not a significant factor determining CF member participation in collective activities, although women CF members show less participation in CFUGs.

Livestock rearing is one of the most critical livelihoods in this study area. Owing to severe weather and the uncertain yield of dry agriculture, most respondent families traditionally rear livestock, which can quickly be monetized as urgent needs arise. In summer, people need to depend on the forest for fodder and bedding due to limited fodder availability from agricultural products. Hence, livestock holding unit significantly influences people's decision to be CF members in model 1. However, the livestock-holding unit is not a significant factor in CF member participation in CFUGs' collective activities in model 2. The reason behind this is the CF management model of the study area, which is individual ownership and collective management (Feurer et al 2018). In the management plans of CFUGs, the land is allocated to each user individually as per traditional land ownership before CF was established. Eventually, land management is under each user's decision for grazing and cultivation of agricultural crops. The CF management committee cannot intervene in grazing land management once the CF management plan is approved. Only forest management activities especially silvicultural works are collective in the study area.

Another forest dependency of local people's livelihood in the study area is firewood collection for toddy palm sugar and cooking. In models 1 and 2, the firewood consumption rate of households is not statistically significant in becoming a CF member and in CF member participation in CFUG's collective activities. The result suggests that firewood consumption has no relationship with people's participation in CF in the study area. To explain this, toddy palm climbers who are agricultural landless consume more firewood in this study area, and only a few have access to customary forests. According to an interview with one of the toddy climbers, one firewood cart (0.509 m^3 of wood) can feed fuel for only two days for a toddy palm climber who can climb approximately 80 palms, which means that a toddy palm climber needs at least 25 to 27 m^3 of solid wood for one toddy palm season. The firewood consumption for household cooking between CF and non-CF members is similar. In this regard, people's participation in the CF program is not determined by firewood consumption in this study area. Thus, the result differs from that of previous studies in West Africa and Nepal (Oli and True 2010, Coulibaly-Lingani et al 2011): people's willingness to participate in the CF program is determined by forest dependency.

Conclusion and recommendation: The primary purpose of this paper is to explore the determinant of the socioeconomic characteristics of households to become CF member and further participation in the collective activities of the CFUGs. Among 10 independent factors, 7 factors determine local people's decision to become CF members: gender of household head, customary forest area, livestock-holding unit, education, agricultural land-holding size, family labor, and nonfarm income.

Among 10 independent characteristics of CF member households, the nonfarm income of the household, working outside of the township, and availability of family labor determine CF member participation in collective activities.

In contradiction to previous studies, the firewood dependency of local people does not influence local people's willingness to join the CF program due to changes in livelihood under the rural transformation in the CDZ. However, changes in rural livelihood from forest-dependent to non-forest-dependent livelihood determine people's willingness to join the CF program and to participate in the collective activities of the CFUGs.

The result is similar to that of Filipski (2019): the livelihood diversification of rural households to nonfarm livelihood has a negative impact on farm labor availability in the agricultural sector in the CDZ. This study found that the rural transition setting will have a negative effect on CF implementation due to reduced interest by local people in the forestry sector with limited labor availability. Local people cope with farm labor scarcity using mechanization in the agricultural sector because agricultural income is essential for their livelihood. However, in community forestry in the dry zone, where local people did not receive immediate income from CF, the less participation of CF members in CF management activities is the result of rural livelihood transformation.

Three recommendations are suggested to boost the participation of local people in the CF program in the CDZ. First, the Forest Department should consider a fair ground for noncustomary forest owners, particularly landless vulnerable households with a limited option to diversify their livelihood to join the CF program as an equal opportunity, and should then provide a shared pool CF area for them to manage.

Second, rural livelihood development programs should be considered in cooperation with the CF program, such as the introduction of community-owned livestock farms and skill development programs for women CF members to produce handicrafts from nontimber forest products or toddy palms.

Lastly, strengthening the institutional rule of CFUGs is

urgently needed to prevent from becoming stagnant user groups. User group participation will increase if there is a clear rule for the fine system.

Although this study only covered a sample population from the CDZ of Myanmar, the result can be applied to similar socioeconomic conditions in Myanmar, particularly the CDZ area, where the majority of the CF area is located. However, the CF model's land tenure, governance, and institutional factors are excluded from the study because the study wants to focus on the socioeconomic characteristics of local people regarding participation in the CF program.

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Stand Structure and Species Composition of Community Forests under Livelihood Transition in Two Villages in the Inle Lake Region, Myanmar

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Abstract: This study observed the stand structure and species composition of community-managed forests in two villages in the Inle lake region, Shan State of Myanmar. A total of 44 circular plots were established to sample trees ≥ 5cm diameter (DBH) (1.38 ha). Fifty-one species in thirty families are recorded. *Dipterocarpus tuberculatus, Shorea siamensis, Quercus brandisana, Melanorrhoea usitata, and Xylia xylocarpa* were the most important species in these community forests and accounted for about 73.17% of all recorded stems ≥ 5cm DBH. The site had a long history of fuelwood collection for local and regional needs until 2010. The presences of coppice and pollard trees are indicative of past disturbance activities. However, those forests are recovering after the demand for forest resources decreased, the breakdown of the fuelwood market, and applying specific village rules for harvesting. Dependency on the community forest resource was reduced after the electrification of the area, a marked shift to tourism-related livelihoods, and stabilized transportation access to the agricultural market. It can be concluded that livelihood transitions facilitated less dependency on forest resources and supported community forest regeneration.

Keywords: Forest transition, Community-managed forest, Disturbance, Harvesting methods, NTFPs

Nearly one third of tropical forests are now in the hands of local people. This share is likely to succeed in forest conservation, especially in places where deforestation and degradation are accelerated. Moreover, the communal ownership of forest land appears to be more common in developing countries than in developed countries (White and Martin 2002). Decentralized forest management has been introduced in developing countries where rural communities extensively use forest products and play crucial roles in their livelihoods (McShane 1990, De Boer and Baquete 1998 and Thapa and Chapman 2010).

Varying local livelihoods in a different landscape may differ the species composition, diversity, and stand structure of forest conditions. People living within and around forests use forest products for food, fuel, medicine, timber, fodder for livestock, and fallback when agricultural and other economic activities are inadequate to sustain the household economy (Charnley and Poe 2007). Many studies have found that forests near a human settlement with overexploitation of resources induced changes in the forest ecosystem (Thapa and Chapman 2010, Htun et al 2011 and Mon et al 2012).

However, only a few studies have assessed forest disturbances from the social-ecological perspective (Karanth et al 2006, Thapa and Chapman 2010). In our research, we will discuss forest conditions change from the perspective of socio-economic dynamics, and previous forest utilization, by representing the remaining forest stand structure and species composition. Furthermore, this paper examines utilization pattern changes in community forests in the Inle Lake region, where forest restoration and transition have occurred. The objective of this study is (i) to assess the impacts of livelihood transition on community forests (ii) to study localized community management systems, and (iii) to investigate CF's stand structure and species composition after disturbance.

MATERIAL AND METHODS

The selected area is in the Inle Lake region, famous for its broad diversity of cultural and natural assets; it has become one of the country's leading tourism destinations over the last decade. Deciduous forest types dominated the area, with the elevation ranging from 900 to 1100 m. The average temperature of the study area is 30 °C, and the annual precipitation is 1982mm.

Two community forests (CFs) in Nyaung Shwe township were selected because they both have similar histories of human disturbance activities and biophysical conditions (Fig. 1). These forests were heavily disturbed and exploited for commercial fuelwood and non-timber forest products (NTFPs) collection. The selected study villages established community forestry in the early 2000s. The area of the village community forest is 248.81 ha in L village and 511.93 ha in Y village.

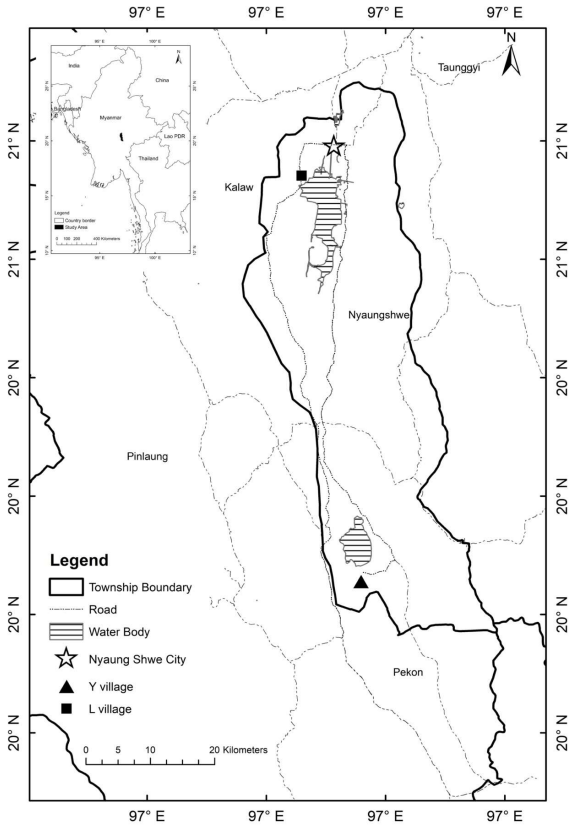

Fig. 1. Two community forest villages in Nyaung Shwe township, Myanmar

Study design: Focus group discussion and key informants with elderly villagers, village leaders, and villagers from different socio-economic backgrounds are conducted to know the history of forest utilization in the past and livelihood transition.

Vegetation was surveyed in 2017 (August- September) and 2018 (July-August). After making a local forest resource map with villagers, 44 sample plots (1.38ha), 0.72 ha in L village, and 0.66 ha in Y village were randomly selected. The layout was a circular plot of 10m, 5m, and 3m radius. The diameter at breast height (DBH) and tree height were measured for trees with a DBH≥5cm in a 10 m plot and 1-5 cm DBH in a 5m plot. Within a 1m plot, the number of seedlings (height ≤ 1.3m) was counted (Kabir and Webb 2006). Tree species, local names, and common usage of the trees were determined. Coppice and pollard trees were documented in a list to know past disturbances. The leaves and flowers of unknown species were sampled and later identified at the Forest Research Institute (FRI) in Naypyidaw, Myanmar.

Data analysis: The importance value index (IVI) is calculated based on relative abundance, relative density, and relative dominance. Abundance (ha^{-1}), basal area (m^2/ha) of each species are calculated and compared to know the past human disturbance activities and species preference. Regression analysis of normal and coppice trees were conducted to know forest recovery condition. The composition and structure of study forests were compared with other published studies with similar forest type.

RESULTS AND DISCUSSION

Stand structure: The percentage of preferred species in particular size and height compared to the total number of trees in two community forests is shown in Table 1. The average tree height of L and Y CF are 4.96± 0.71 m and 10.82 ± 0.24 cm respectively. In these forests, highly exploited species (*Dipterocarpus tuberculatus* in L CF and *Quercus brandisiana* in Y CF) are remained in smaller DBH (5-10 cm). 70% of In (*D. tuberculatus)* trees are less than 3 m in height due to the frequent leave collection in the past. *Q. brandisiana* species are commonly collected as household fuelwood in Y CF and 21% of them are found in smaller DBH class of 5-10cm. However, the recovery condition of *D.tuberculatus* and *Q.brandisiana* trees can be seen in the DBH classes of 11-16 cm with 21% and 15% respectively. *D.tuberculatus* trees were kept at 2-3 m for leave collection before, and now we can see that 17% of them reached between 4-6 m height.

Shorea siamensis in both forests were exploited as subsistence, commercial fuelwood and remained as coppice or pollard tree with smaller DBH class (24% in L CF and 37% in Y CF). The recovery condition of *S.siamensis* trees can be seen in the DBH 11-16 cm class. (44% in L CF and 35% in Y CF). Majority of them are found with bigger DBH ≥28 cm and height ≥10m. This is because the forest itself is Diptrocarpus dominated and some of the trees on pagoda compound were not cut for religious reason. The higher percentage in middle and higher DBH and height classes reflected that the forest conditions is improved. It is generally assumed that the more mature the succession of a forest can be seen, the greater the average height of the forest compared to others (Kijtewachakul et al 2004).

Species composition and diversity: Species composition and diversity is more or less similar in two CFs as both were highly disturbed in the past and have a similar forest type dominated by the virtual presence of Dipterocarpus and associated species. The individual species, abundance, BA(m^2/ha), and IVI are shown in Table 2 and 3. Data were shown into three categories: trees, saplings, and seedlings.

L CF has a higher tree density (1309 trees/ha) than Y CF (1077 trees/ha). A total of fifty-one species in thirty families are recorded. *D tuberculatus*, *S. siamensis, Q. brandisana*, *Melanohorrea usitata*, and *Xylia xylocarpa* trees were the

Table 1. Percentage of preferred species in particular size and height compared to total trees in two CFs

Species	Village	Usage	DBH class				Height class			
			5-10 cm	11-16 cm	17-27cm	≥28 cm	1-3 m	4-6 m	7-9m	≥10m
Dipterocarpus tuberculatus	L	Leaves	28%	21%	2%	10%	70%	17%	12%	2%
Shorea siamensis	L	Fuelwood (pole, post)	24%	44%	47%	52%	11%	33%	32%	63%
Shorea siamensis	Y	Fuelwood (pole)	37%	35%	34%	59%	25%	35%	37%	43%
Quercus brandisiana	Y	Fuelwood (household)	21%	15%	3%	18%	6%	14%	19%	20%

Table 2. List of trees, saplings and seedlings found in L CF in Inle Lake Region, A, abundance(ha^{-1}), BA, Basal Area(m^2/ha), IVI, Importance Value Index

Species	Family	Trees			Saplings			Seedlings
		A	BA	IVI	A	BA	IVI	A
Shorea siamensis	Dipterocarpaceae	546	5.63	102	25	0.47	48	26
Dipterocarpus tuberculatus	Dipterocarpaceae	460	3.19	74	21	2.26	81	24
Melanorrhoea usitata	Anacardiaceae	299	1.92	47	7	0.18	14	6
Quercus brandisiana	Fagaceae	121	0.49	17	28	0.55	54	7
Bombax ceiba	Bombacaceae	33	0.12	8	3	0.04	5	1
Grewia tiliifolia	Malvaceae	40	0.63	5	4	0.05	7	
Sterculia versicolor	Sterculiacea	31	0.20	5				6
Dalbergia cultrata	Leguminosae	25	0.06	4	7	0.14	14	20
Terminalia tomentosa	Combretaceae	27	0.10	4	1	0.01	2	
Morus alba	Lauraceae	25	0.15	3	3	0.07	6	
Croton oblongifolius	Euphorbiaceae	27	0.09	3				6
Buchanania lanzan	Anacardiaceae	23	0.10	3	3	0.02	5	
Strychnos nux-blanda	Loganiaceae	21	0.05	3	1	0.03	3	2
Emblica officinalis	Phyllanthaceae	19	0.04	2	10	0.19	19	1
Dillenia pentagyna	Dilleniaceae	17	0.06	2	1	0.05	3	
Macaranga denticulata	Lauraceae	10	0.04	2	4	0.10	8	
Xylia xylocarpa	Combretaceae	13	0.05	2	4	0.11	9	
Bombax insigne	Bombacaceae	4	0.11	2				
Lannea coromandelica	Malvaceae	10	0.03	1				
Ficus spp	Phyllanthaceae	4	0.03	1				
Sterculia angustifolia	Sterculiacea	8	0.03	1	4	0.08	8	
Litsea glutinosa	Lauraceae	8	0.03	1				
Albizia odoratissima	Mimosaceae	6	0.01	1				
Citrus medica	Rutaceae	6	0.01	1				
Streblus asper	Moraceae	6	0.16	1				
Polyathia simiarum	Annonaceae	4	0.02	1				
Garuga pinnata	Burseraceae	4	0.13	1				
Brassica rapa	Bombacaceae	4	0.02	1				
Antidesma bunius	Euphorbiaceae	4	0.02	0				
Anno spp	Anno spp	2	0.02	0	6	0.14	11	
Schleichera oleosa	Sapindaceae	2	0.02	0	1	0.03	3	
Premna tomentosa	Verbenaceae	2	0.02	0				
Stereospermum neuranthum	Bignoniaceae	2	0.00	0				
Terminalia chebula	Combretaceae	2	0.00	0				

most important species in these community forests and accounted for about 73.17% of all recorded stems ≥ 5cm DBH.

D. tuberculatus (25.4%), *S. siamensis* (30.2%), and *M. usitata* (16.5%) trees have highest IVI in L CF. The three most dominant species in Y CF are *S. siamensis, Q.brandisiana*, and *X.xylocarpa*. Species preference in the past shaped the current species composition (Bunyavejchewin 1983, Miller 1998 and Kabir and Webb 2006). *D. tuberculatus* and *S. siamensis* could easily get cash by selling leaves, fuelwood, pole, and post. *Q. brandisiana* is the most commonly used household fuelwood in both villages. *M. usitata* was used as

Table 3. List of trees saplings and seedlings found in Y CF in Inle Lake Region, A, abundance(ha^{-1}), BA, Basal Area(m^2/ha), IVI, Importance Value Index

Species	Family	Trees			Saplings			Seedlings
		A	BA	IVI	A	BA	IVI	A
Shorea siamensis	Dipterocarpaceae	391	5.69	102	36	0.03	147	40
Quercus brandisiana	Fagaceae	179	1.88	74	11	0.01	44	3
Xylia xylocarpa	Mimosaceae	76	1.00	47	8	0.002	23	4
Shorea obtusa	Dipterocarpaceae	74	0.91	17				10
Melanorrhoea usitata	Anacardiaceae	52	0.88	8				12
Emblica officinalis	Phyllanthaceae	39	0.19	5	3	0.00	13	9
Grewia tiliifolia	Malvaceae	39	0.22	5	15	0.01	50	6
Bauhinia acuminata	Caesalpiniaceae	24	0.23	4				
Morinda tinctoria	Rubiaceae	20	0.28	4				
Dalbergia cultrata	Leguminosae	18	0.31	3	2	0.001	6	1
Garuga pinnata	Burseraceae	18	0.18	3				
Macaranga denticulata	Euphorbiaceae	15	0.11	3				4
Bombax insigne	Bombacaceae	14	0.26	3	3	0.002	11	6
Sideroxylon burmanicum	Sapotaceae	14	0.09	2				
Lannea coromandelica	Anacardiaceae	12	0.09	2				
Strychnos nux-blanda	Loganiaceae	11	0.04	2	2	0.001	6	
Terminalia chebula	Combretaceae	11	0.14	2				
Eugenia spp	Myrtaceae	9	0.06	2				
Schleichera oleosa	Sapindaceae	8	0.08	1				1
Buchanania lanzan	Anacardiaceae	6	0.12	1				
Diospyros burmanica	Ebenaceae	6	0.07	1				
Heterophragma sulfureum	Bignoniaceae	6	0.08	1				
Vitex pubescens	Verbenaceae	6	0.04	1				1
Cassia fistula	Caesalpiniaceae	5	0.02	1				
Anno spp	Anno spp	3	0.01	1				
Dillenia pentagyna	Dilleniaceae	3	0.02	1				
Ficus spp	Moraceae	3	0.08	1				
Lagerstroemia tomentosa	Lythraceae	3	0.01	1				
Sterculia angustifolia	Sterculiaceae	3	0.01	0				
Antidesma bunius	Euphorbiaceae)	2	0.01	0				
Bombax ceiba	Bombacaceae	2	0.01	0				
Bridelia ovata	Euphorbiaceae	2	0.01	0				
Castanopsis armata	Fagaceae	2	0.14	0				
Dioscorea wallichii	Dioscoreaceae	2	0.05	0				
Homalium tomentosum	Flacourtiaceae	2	0.01	0				

lacquer on a boat to protect against water and required very little maintenance. However, due to easy transportation access, the lacquer business disappeared in the early 1990s. In addition, it is no use for fuelwood as it can cause severe irritation to the skin and eyes. *X. xylocarpa* species are commercially important in other parts of the region and are known as ironwood. However, it's not common in Y village as they believed that its wood is difficult to saw when it dries and considered to have a severe blunting effect on the cutting elements. Human disturbance may alter forest succession by giving an advantage to some species or selectively removing others. Dominance by a few tree species rose and stand density and tree diversity declined when a disturbance, primarily due to resource use by local people, increased (Htun et al 2011). Such domination by a few species in highly disturbed sites was found in other studies (Parthasarathy and Karthikeyan 1997, Mishra et al 2004, Brown et al 2006 and Htun et al 2011). The Dipterocarpaceae family composes a high percentage of saplings and seedlings in both forests, and future regeneration is secured in these forests. Fagaceae and Leguimosae families are the second dominant species in these CFs. Seedlings and saplings of *Emblica officinalis* and *Grewia tiliifolia* are found in deciduous forests as associated species.

Livelihood transition in Inle lake region: The site had a long history of fuelwood collection for local and regional needs until 2010. The previous forest utilization in the two villages included fuelwood extraction and NTFPs collection. For L village, previous forest utilization included fuelwood extraction for salt production, traditional snack making (rice crackers), household construction, and collecting *D. tuberculatus* (In) leaves and collection of poles and posts for construction. *D.tuberculatus* leaves were mostly used for fish packaging as Inle Lake was the major fish production area before.

However, Inle Lake experienced a massive tourism boom during the last decade and infrastructure and market conditions are developed. In L village, most villagers abandoned agriculture and forestry-related livelihoods and participated in tourism-related activities (e.g., boat piloting, selling souvenirs and local products to tourists).

Commercial fuelwood exploitation of *S.siamensis* was the major forest utilization in Y village's history. More than 40% of households relied on commercial fuelwood extraction as their primary livelihood in the past. However, in 2010, most villages inside Inle Lake were electrified, and fuel wood demand drastically decreased. Stabilized transportation access for the market attracted villagers to focus more on upland agriculture.

Forest resource use and community managed rules: Both villages have community-managed rules regarding resource utilization. As a result, only fuelwood and NTFPs (leaves, medicinal plants, mushrooms, bamboo shoots) can collect for subsistence use. Harvesting of the pole and fuelwood were also banned for the villagers. However, with the approval of the community forest user group, they are allowed to collect for household use. Penalties and reward system were introduced for the better participation of local people. For example: harvesting tools materials were taken and kept the first time, banned from entering community forests for the second time.

Coppice or pollard trees after livelihood transition: Both villages exploited *S. siamensis* commercially, and most of the remaining stands can be seen as coppice or pollard trees. DBH and height scatter diagrams of normal and coppice trees in both villages indicated that the trees were heavily disturbed in the past Figure 2 and 3. Previously disturbed

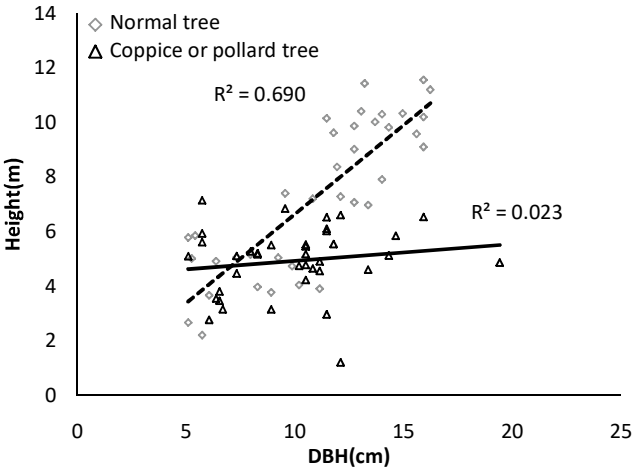

Fig. 2. *Shorea siamensis* normal and coppice or pollard tree in LCF

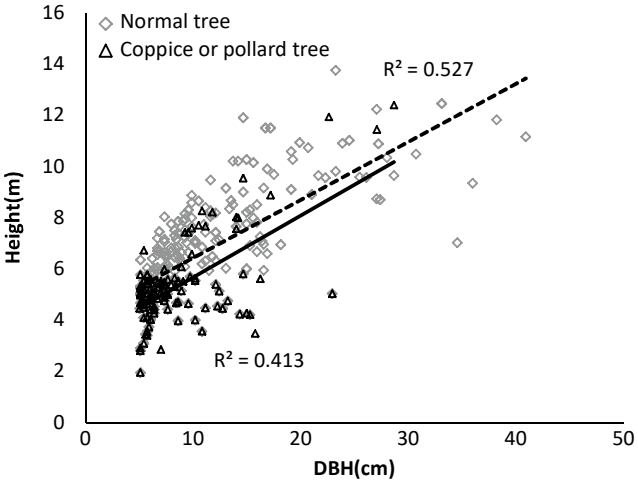

Fig. 3. *Shorea siamensis* normal and coppice or pollard tree in YCF

trees with higher DBH were accumulated under 5m as coppice trees. The R^2 value of the normal trees in L village is 0.69, and those of coppice or pollard tree is 0.02 (Fig. 2). *S. siamensis* trees were heavily exploited as fuelwood for cooking salt, making traditional snacks, and pole and posts for construction. Villagers cut the pole and post size trees for cooking salt as bigger size fuelwood can sustain the heat longer.

In Y village, some coppice trees have grown into DBH more than 10 cm and reached over 10m (Fig. 3). The R^2 value of a coppice or pollard tree is not much different from that of a normal tree. Y CF recovered faster as they only exploited pole-size trees and left the bigger tree as mother trees to reproduce seeds and regenerate the forest. They also shared the forest with nearby village, and resource extraction needs agreement from both parties. Accessing alternative income from agriculture and the breakdown of the fuel wood market speed up the forest regeneration process in Y CF. Different extraction forms may also have different levels of impact (Shaanker et al 2004, Shahabuddin and Kumar 2007 and Thapa and Chapman 2010). Economically valuable and efficient fuel species (*S. siamensis, D. tuberculatus, Q. brandisiana*) mostly remain as coppice and pollard trees in the study area. Repeated exploitation of forest resources has reduced diversity and density and altered structure with lower tree heights and smaller diameters.

The vigorous *S. siamensis* coppice trees in both villages showed a recovery condition after forest product demand decreased, community managed rules were developed and transited into non-forest-related livelihoods. The results showed that the species within this forest show an ability to recover by vegetative regrowth after being cut. Similar observations were made in tropical dry forests by Imbert and Portecop 2008, Lévesque et al 2011 and Van Bloem et al 2005. A similar case study of community forest regrowth conditions was investigated in Northern Thailand (Kabir and Webb 2006 and Chowdhury et al 2018). The forest appears to contain substantial levels of regeneration indicating a potential for structural recovery.

Comparison of different forest management system and disturbances: Table 4 describes tree density and structure according to different management system, and harvested forest products. The comparative studies were chosen based on similar forest types. Abundance and basal area were categorized into ≥2cm, ≥5cm, and ≥10cm to know the forest structure. In two CFs, Y CF regenerated faster than L CF because they only extracted pole-size fuelwood and were difficult to access due to poor transportation access and security reasons. On the other hand, L CF is easily accessible to the market to sell NTFPs and fuelwood. L CF, previously harvested for fuelwood, NTFPs, and household construction, remained with stems smaller than ≥10cm DBH in comparison with Y CF, which was collected for fuelwood alone. The forest condition of L CF is similar to the case study in Thailand (Kabir et al 2006), where forests were heavily harvested for charcoal making, railway sleepers, and construction wood. The abundance (stem/ha) is higher in the early stage of development (≥2cm and ≥5cm DBH) and then later declines in the bigger DBH class (≥10cm). Even with the same management type, the remaining forest structure is differed

Table 4. Comparative vegetation condition with previous studies in terms of forest type, management practice, abundance, and basal area different

Study	Forest type	Management	Harvested forest products	Area sampled(ha)	Abundance (stem/ha)			Basal area (m^2/ha)		
					≥2cm	≥5cm	≥10cm	≥2cm	≥5cm	≥10cm
This study	Mixed deciduous	Community (L)	NTFPs (Leaves) Fuelwood (pole/post)	0.72	1743	1309	339	13.77	13.57	10.49
	Mixed deciduous	Community (Y)	Fuelwood (pole)	0.66	1631	1077	477	13.40	13.34	11.05
Kabir et al (2006)	Deciduous	Community	Charcoal making Railway sleepers Construction wood	0.97	1936	1290	380	10.34	9.60	6.05
Htun et al (2011)	Mixed deciduous	Protected Area	NTFPs	1.68			790			24.47
Webb et al (Unpublished data)	Mixed deciduous and grass land	Protected area annual fires	-	4.0		601	343		21.6	20.6
	Mixed evergreen plus deciduous	Protected area Occasional fires	-	1.0		440	220		18.5	17.9

depending on the disturbance intensity and collection methods. However, we can say both forests' condition is better than the community forest condition in Thailand (Kabir et al 2006) by comparing the basal area of tree ≥10cm. Villagers in the study area did not cut certain species, such as *M. usitata* and *X. xylocarpa*, and they remained as vigorous stands. The basal area(m^2/ha) of bigger DBH class (≥10cm) in disturbed community forests (Kabir et al 2006 and our study) are smaller than that of protected forests.

The long history of human-induced disturbance has resulted in a degraded forest with reduced species diversity and virtually no large trees. Forests harvested for fuelwood, charcoal and NTFPs remained with a smaller basal area. The ownership, management, and utilization shaped the abundance of the remaining stand and basal area. Forest nearby community villages tend to experience more human disturbance than protected and reserved forests because it is challenging to manage shared resources without stakeholder consent. Different extraction forms may have different levels of impact, too (Shaanker et al 2004, Shahabuddin and Kumar 2007 and Thapa and Chapman 2010).

CONCLUSION

The site had a long history of fuelwood collection for local and regional needs until 2010. The presences of coppice and pollard trees are indicative of past disturbance activities. However, those forests are recovering after the demand for forest resources decreased, the breakdown of the fuelwood market, and applying specific village rules for harvesting. Dependency on the community forest resource was reduced after the electrification of the area, a marked shift to tourism-related livelihoods, and stabilized transportation access to the agricultural market. It can be concluded that livelihood transitions facilitated less dependency on forest resources and supported community forest regeneration. However, only this case study cannot represent all community forests in the Inle lake area. Therefore, the accumulation of longitudinal case studies will be required. Furthermore, community forests may vary significantly in forest structure and composition, intensity of use, and trends. Significantly, more research is required on the impacts of livelihoods on forest conditions in different geographic and socio-economic conditions.

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Threatened and Near Threatened Underutilized Edible Fruit Species of Southern India for Food Security and Diversifying Agroecology

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Abstract: Southern India (SI) is bestowed with several threatened and near threatened (TNT) underutilized edible fruit species (UEFS) that contributes to food and nutritional security, particularly to the indigenous people. Unfortunately, information related to these natural products is fragmentary and least researched. The PRISMA Protocol was used to conduct a systematic review of the TNT-UEFS of the SI. The study confirmed that, of the total of 69 species of TNT-UEFS recorded, most of the species were reported to have medicinal, economical, and many other values, which need instant sustainable initiatives for conservation, consumption and cultivation. Among these species, 10 (14.5 %) were near threatened (NT), and 59 (85.5 %) were threatened. According to the IUCN Red List, the threatened species were further divided into three categories: Vulnerable (31 species), Endangered (20 species), and Critically Endangered (8 species). The provision of various ecosystem services is aided by integrating native and naturalised TNT-UEFS in various ecosystem restoration efforts through afforestation and reforestation. Consecutively, it helps India meet its commitment to the Sustainable Development Goals (SDGs) and neutralise land degradation by 2030. Hence, the study will provide baseline information for future research and be useful for policymakers to develop region-specific, scientific, and sustainable policies for SI.

Keywords: IUCN Threatened list, Wild fruits, Food security, Agroecosystem, Restoration ecology

Southern India (SI), also known as Peninsular India or South India, includes mainly five Indian states such as Andhra Pradesh, Karnataka, Kerala, Tamil Nadu and Telangana. SI covers around one fifth of India's land area and population (Census of India 2011). This region is diverse with two mountain ranges, the Western and Eastern Ghats and drained by several perennial rivers like Bharathappuzha, Godavari, Kaveri, Krishna, Tungabhadra and many more (Pullaiah and Rao 2002). Generally, climate of the SI is tropical and receiving monsoon rainfall of 400 to 1700 mm annually. The temperature normally ranges from 10° to 15°C during winter and 25° to 32°C during summer (Renuka 2020). SI still fails to address its complete food and nutritional security despite markedly sustained growth in per-capita income and economy. The average operational land holdings in all the states of SI were markedly reducing (Agriculture Census Division 2019). Most of the farmers are in the marginal and small categories, with an average land holding of ≤ 2 hectare, posing challenges to use of mechanization and expensive inputs that are economically non-viable. Most of the SI practices rain-fed agriculture with supplementary irrigation. Crops like jowar, cotton, sugarcane, bajra, pulses and oilseeds are predominantly grown in the semi-arid region with black cotton soils (Agriculture Census Division 2019). Arecanut, banana, paddy and sugarcane are cultivated in the sub-humid and humid region. Though, SI is blessed with an environment and resources favourable for agricultural practices but fragmentation of land, land degradation, urbanization and other anthropogenic activities result in farmers' reluctance to achieve food and nutritional security.

According to the state of food security and nutrition in the world, 2020 report India is still suffering from hunger and malnutrition though it holds the distinction of being the world's second-largest producer of fruits. In the food security index-2020, India ranked 71 out of 113 major countries. Though there is a remarkable advance in science and technology, we still fail to meet the food demand of a growing population for adequate, nutritious, safe and sustainable food. Nowadays,

soil degradation is becoming a global challenge as it poses a high risk on land productivity, food and livelihood security. The worst impact of soil degradation includes poverty, malnutrition, disease, forced migration, increased conflicts and cultural damage. Some parts of Eastern and Western Ghats are still pristine and rich in floral biodiversity with a high occurrence of UEFS. Among these UEFS, species like *Eugenia argentea*, *Garcinia imberti*, *Madhuca insignis*, *Myristica fragrans*, *Syzygium travancoricum*, etc., were reported to be threatened in the IUCN Red list. These are native and naturalized, unique and underutilized rare minor fruits that are collected from the wild and consumed mostly by the native population during different seasons (Suresh et al 2014). UEFS are the most important component in agriculture, forestry and other land use (AFOLU) systems, contributing to dietary needs, food security, livelihood and diverse ecosystem services. These are rich sources of antioxidants, fibers, minerals, polyphenols, and vitamins; consumption of these fruits lowers the risk of various diseases and illnesses while also enhancing human health and nutrition (Suresh et al 2014, Shankar et al 2020). It has the potential to play a significant role in eliminating hunger and malnutrition as well as eradicating poverty. These fruit species integrated LUS also helpful in the conservation and utilization of indigenous or underutilized fruit resources, reducing pressures on remaining forests and effectively tackling forests resource overexploitation. Most of these UEFS are native, easy to grow with less cultural and technical inputs and hardy in nature. Some of these UEFS are integrated with the farming systems for large-scale plantation and other ecosystem restoration programs (Suresh et al 2014). Unfortunately, due to a lack of awareness, discontinuity in traditional culture and knowledge, intensive agriculture, limited research, insufficient policy support, western influence, and urbanisation, diminished the utilisation of local UEFS (Shankar et al 2020). Even though, they have many advantages but received less attention on research or extension and they have been largely neglected. Hence, it is an alarming sign to conserve the country's biodiversity and maintain ecological balance. This systematic review aims to survey, summarize and annotate the published information related to TNT-UEFS of SI to assess potentiality, conservation, cultivation and sustainable utilization, particularly to diversify agroecology and improve the socio-economical status of SI community.

MATERIAL AND METHODS

Study location and protocol: This study implements the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) protocol (Moher et al 2009, Liu et al 2021) to review the published information pertaining to the angiospermic native and naturalized TNT-UEFS of SI (Fig. 1). Study provides implications for the cultivation assisted conservation, institutional and policy support, future research etc., for improving farmers' income and diversifying agroecology. A systematic review was executed using the flow diagram (Fig. 2, adapted from Moher et al 2009). A precise explanation and categorization of fruits may be challenging due to their complexity and it dependents on several aspects like availability, awareness, consumption, distribution, exploitation, knowledge, popularity, production, and use (Suresh et al 2014). The definition of UEFS excludes species that fall under widespread to moderate commercial importance category and species that have been widely naturalized, cultivated and well known in SI. However, this review includes only a lesser known, lesser distributed, underutilized, undomesticated and wild edible fruit species from forests, agroforestry systems, gardens, vacant and other marginal lands.

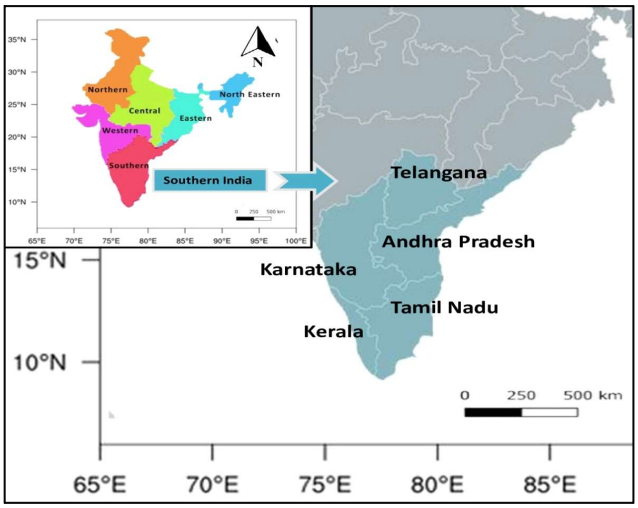

Fig. 1. Outline map of the study area

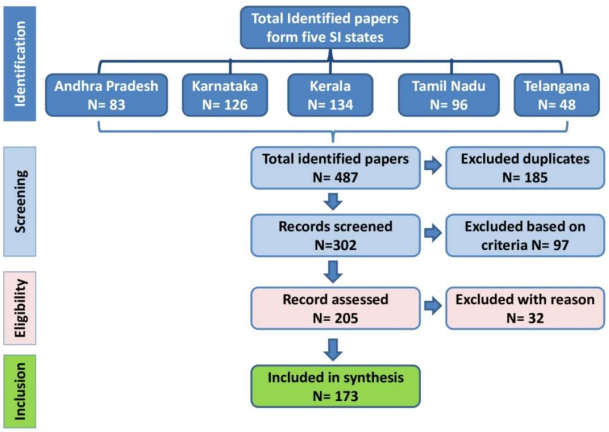

Fig. 2. Flow diagram of TNT-UEFS of SI

Survey and analysis: Analysis was focused on angiospermic native and naturalized TNT-UEFS of SI and reviewed the published articles, book chapters, and scientific reports to analyze the ethno medicinal/ traditional/ indigenous use, growth pattern and seasonal availability of TUEFS in SI. The search keywords considered are forest fruits, wild edible fruits, underutilized fruits and lesser-known fruits in the search engines like Google, Google Scholar, J-gate, Scopus and Web of Science, for all time. A total of 302 articles were screened which includes the UEFS conservation, documentation, ecology, economics and ethnobotany in topics of agriculture, botany, environment, forestry and climate change studies, food science, horticulture, medicinal and aromatic plants, social science, and urban studies. On the other hand, articles related to nutrient composition, evolution, genetics, chemistry, pharmacology, toxicology, immunology, medicine, etc., of UEFS were screened further and considered as an accessory literature. Finally, 173 articles were included in the synthesis (Fig. 2). Similarly, for TNT-UEFS, completely threatened and Near Threatened (NT) species were considered, whereas Non-threatened category species were excluded. The recorded threatened species were divided into three categories as per the IUCN conservation status namely Vulnerable (VU), Endangered (EN) and Critically Endangered (CR) and verified from the IUCN website https://www.iucnredlist.org/search. The published scientific names of the TNT-UEFS were verified from online sources like http://www.the plantlist.org (Plantlist) and http://www.ipni.org (International Plant Names Index). The information from the literature found in the mentioned sources was used to prepare a comprehensive list of species with scientific names, common names, family, life form, origin, flowering and fruiting time, state-wise distribution, nutritional status, uses, products, other significance, IUCN status and sources (Table 2). An Excel spread sheet and the IBM SPSS version 2020 were used to analyze the recorded data using descriptive statistics.

RESULTS AND DISCUSSION

Diversity of the UEFS of SI: Analysis showed a repository of 394 fruit species belonging to 216 genera in 83 families. Further, the habit-wise analysis has revealed that around 224 species (57 %) were trees, 80 species (20 %) were shrubs, 31 species (8 %) were herbs and 58 species (15 %) were climbers. Among these fruit species, 359 species (91 %) were native and 34 species (9 %) were naturalized to SI. Study confirmed that, of the total 394 species of UEFS recorded, most of the species reported to have medicinal, economic and many other values, which need immediate sustainable initiatives for conservation, consumption and cultivation. Around, 7000 species of UEFS were documented worldwide (Grivetti and Ogle 2000). Out of which, 1200 species were identified in Africa, 1000 species in America and 800 species in Asia (Verheij et al 1991). Out of the 800 documented species in Asia, around 394 species (present study) were prevalent in the SI.

Diversity of the TNT-UEFS of SI: From the recorded 394 species of UEFS, 69 species were categorized into TNT-UEFS. Among these species, 59 species (85.5 %) were threatened and 10 species (14.5 %) were NT (Fig. 3). The threatened species were further divided into three categories

Table 1. Constraints and solutions of TNT-UEFS of SI

Criteria	Constraints	Solutions
Lesser-known	Poor availability and utilization.	Awareness, research and extension activities are vital.
Lesser-knowledge	Lack of cultivation practices, economical, nutritional and medicinal knowledge.	Linking practitioner, researcher and policymaker. Training and capacity building.
Less researched	Lacking of mass cultivation, nutrition, pest and disease management.	Research on nutrition, preference and genetic improvement are vital
Less technology	Un availability of quality planting materials, varieties, production, post harvest technologies.	Need of certification, grading, processing, value addition, packaging and adequate storage.
Less popular	Long gestation period, poor yield, less economic, no value addition and awareness.	These issues can be dealt with the application of scientific interventions and policy support.
Less utilized/exploited	Less market, lower commercial exploitation and perishable. Low consumer preference, improper economics, policy and institutional arrangements.	Low preference can be optimized by natural ripening and genetic improvement. Processed into value added products by using traditional and advanced techniques.
Less production	Poor yield and quality	Application of scientific and technological interventions, and genetic improvement.
Less distribution	Confined, storage and processing constraints	Creating awareness, research and extension activities through training and capacity building.
Less conservation	Genetic erosion, over exploitation and habitat loss.	Linking society, economics and ecology, and cultivation assisted conservation are needed.

Table 2. General account on reported TNT-UEFS, significance, IUCN status and references

Scientific name and family	Common name	L F and origin	Flowering - fruiting	State	Phytochemicals and utility	IUCN status	References
Aegle marmelos (L.) Correa.; Rutaceae	Bael	Tree; Native	March - May	KL, KA, TN, AP, TS	Aeglemarmelosine, beta sitosterol; Digestive, anti-diarrheal	NT	Vizhi and Lohidas 2020
Aglaia perviridis Hiern.; Meliaceae	Cheruchokla	Tree; Native	April - Sep	KL	Sesquiterpenoid, diterpenoid; Nutrient dense pulp is used	VU	Med 2017
Ampelocissus latifolia (Roxb.) Planch.; Vitaceae	Kaadu drakshi	Climber; Native	Sep - June	KA	alkaloids, flavonoids, reducing sugars and gums; folk medicine	VU	Kalaivani and Sumathi 2018
Antidesma menasu (Tul.) Miq. ex Muell.-Arg.; Euphorbiaceae	Pali eechi	Tree; Native	May - Sep	KL	Fleshy and sour taste pericarps are separated from seeds and consumed	VU	Med 2017
Aphananthe cuspidata (Bl.) Planch.; Ulmaceae	Kodithani	Tree; Native	Feb - May	KA	Phenols, lactones, flavonoid, and ascorbic acid; folk medicine	VU	Gunaga et al 2015
Aporosa acuminata Thw.; Euphorbiaceae	Vetti	Tree; Native	Jan -June	KL	Rich in minerals; folk medicine	VU	Med 2017
Aporosa lindleyana (Wight) Baill; Euphorbiaceae	Lindley's Aporosa	Tree; Native	Jan - June	KL, KA	Antioxidants and flavonoids; antimicrobial and antifouling	VU	Sathish et al 2017
Artocarpus hirsutus Lam.; Moraceae	Hebbalasu	Tree; Native	Dec - Mar	KA	Thiamine, riboflavin and niacin; reduces constipation	VU	Gangaprasad et al 2019
Baccaurea courtallensis (Wight) Mull. Arg.; Phyllanthaceae	Mootikaya	Tree; Native	Jan - June	KL, KA	Tannin, terpenoid and phenols; diarrhoea, dysentery and skin infection	NT	Narayanan et al 2011
Borassus flabellifer L.; Arecaceae	Palmyra palm	Tree; Native	March -Sep	KL, TS, AP	Anti-oxidant Anti-inflammatory and antibacterial	EN	Narayanan et al 2011
Buchanania lanceolata Wight; Anacardiaceae	Kulamavu	Tree; Native	Nov -March	KL, TS, AP	Flavanoids, tannins and sterols; Seeds are eaten by roasting	VU	Raj et al 2020
Canthium dicoccum (Gaertn.) Merr.; Rubiaceae	Ceylon boxwood	Tree; Native	Nov -March	KL, KA, TN	Antidiabetic, nephroprotective, antifungal and antibacterial property	VU	Sathish et al 2017
Carissa carandas; Apocynaceae	Kalakai	Shrub; Native	March-July	KL,KA, TN, TS, AP	Vitamins and minerals; heart disease and digestive trouble	VU	Vizhi and Lohidas 2020
Cassia senna ; Fabaceae	Nilaavaarai	Tree; Native	March - April	TN	Rhein,aloe-emodin, kaempferol; folk medicine	EN	Sivasankari et al 2014
Citrus latipes (Swingle) T. Tanaka; Rutaceae		Shrub; Naturalized	June -Jan	KL	Flavonoids, phenolics, limonoids; folk medice and cosmetics	NT	Singh 2017
Citrus medica; Rutaceae	Lungamu	Shrub; Native	Mar-Feb	TS, AP	Fruits are stomachic and have potent anti-scorbutic activity	NT	Pendem saidulu et al 2015
Corallocarpus epigaeus (Rottl.) C.B.Clark; Cucurbitaceae	Haavina kodda	Climber; Native	Dec -Mar	KA	Amino acids, vitamins; laxative, hypoglycemic, anti-inflammatory and analgesic	EN	Narayan 2016
Cycas circinalis L.; Cycadaceae	Sago-palm	Palm; Native	Dec -Feb	KL	Neurotoxin, cycasin; high doses are Carcinogenic	EN	Haridas and Kunhikannan 2020
Dimocarpus longan Lour.; Sapindaceae	Dragon's eye	Tree; Naturalized	March -Aug	KL, KA	Glucopyranose, gallic acid and ellagic acid; antioxidant	NT	Singh 2017
Diospyros candolleana Wight; Ebenaceae	Karemara	Tree; Native	April -Mar	KA	Minerals, carotene and vitamins; anti-inflammatory and antipyretic	VU	Gowthami et al 2021
Diospyros discolor Willd.; Ebenaceae	Velvet apple	Tree; Native	April -July	KL	Alkaloids, flavonoids, tannin, terpenoid and essential oils	VU	Maridass et al 2008

Cont...

Table 2. General account on reported TNT-UEFS, significance, IUCN status and references

Scientific name and family	Common name	L F and origin	Flowering - fruiting	State	Phytochemicals and utility	IUCN status	References
Diospyros ferrea (Willd.) Bakh; Ebenaceae	Philippine Ebony	Tree; Native	Feb -May	KA	alkaloids, flavonoids, terpenoids and essential oils; Folk medicine	EN	Ved et al 2016
Diospyros malabarica (Desr.) Kostel.; Ebenaceae	Toopura	Tree; Native	Feb -Nov	KA, AP	diarrhea, hemorrhoids, diabetes, skin diseases, fever	EN	Gowthami et al 2021
Diospyros melanoxylon Roxb.; Ebenaceae	Tendu	Tree; Native	Sep -Dec	KL, KA	Tannin, alkaloids, glycosides, proteins, phenolics; stomach, skin	NT	Kumawat et al 2019
Diospyros vera (Lour.) A.chev; Ebenaceae	Gaura koli	Tree; Native	June -july	TS, AP	Rich in sugars, fat, minerals and vitamin; Folk medicine	EN	Pendem saidulu et al 2015
Dysoxylum binectariferum Hook. f. ex Bedd.; Meliaceae	Kempu Devadaru	Tree; Native	Mar -Jan	KA	Minerals and vitamins; Treatment of Osteomylitis, cancer	EN	Arya et al 2017
Dysoxylum malabaricum Bedd. ex C. DC.; Meliaceae	Bili Agilu	Tree; Native	Feb -June	KA, TS	Flavopiridol; Medicinal properties and immuno- modulatory properties	EN	Bodare et al 2013
Ehretia microphylla; Boraginaceae	Vetrilai	Shrub; Native	March- May	TN, TS	Vitamins and minerals rich; Cough, diarrhea and dysentery	NT	Vizhi and Lohidas 2020
Elaeocarpus munronii (Wl.) Masters; Elaeocarpaceae	Nari bikki	Tree; Native	Sep -April	KL, KA	Flavonoids, Phenols, Quinones, Triterpinoids; antidiabetic property	NT	Devi et al 2018
Embelia tsjeriam-cottam (Roem. & Schult.)A.DC.; Myrsinaceae	Malabar Embelia	Shrub; Native	Feb -Mar	KA	Embelin; antibacterial, antitubercular, antidiabetic and anti-inflammatory properties	VU	Bohara and Nagalakshmi 2021
Eugenia argentea Bedd.; Myrtaceae	-	Shrub; Native	Jan -March	KL	Essesntial oils, caryophyllanes	CE	Raj et al 2020
Eugenia indica (Wight) Chithra; Myrtaceae	Nara	Shrub; Native	March -May	KL	Folk medicine	EN	Remesh et al 2016
Euphorbia hirta L.; Euphorbiaceae	Spurge	Herb; Native	Throughout the year	TN, TS, AP	Beta carotene, vitamin; Asthma, hypertension, dengue and malaria	VU	Sasi et al 2011
Garcinia gummi-gutta (L.) Robs.; Clusiaceae	Malabar tamarind.	Tree; Native	Feb -Aug	KL, KA, TN	Tannin, pectin and fat; Rheumatism, obesity and ulcers	VU	Sathish et al 2017
Garcinia imberti Bourd.; Clusiaceae	Manjakanji	Tree; Native	Feb -Sep	KL	Alkaloids, flavanoids, glycosides, phenols, terpenoids; antioxidant	CE	Kandhasamy et al 2021
Garcinia indica (Thouars) Choisy.; Clusiaceae	Kokum butter	Tree; Native	Sep -May	KL, KA, TS	Benzophenones and garcinol; medicinal and nutraceutical	VU	Ananthakrishnan and Kumar 2016
Garcinia morella (Gaertn.) Desr.; Clusiaceae	Indian gamboge	Tree; Native	Nov -July	KL, KA	Benzophenones, triterpenoids; antimicrobial and anticancer	VU	Murthy et al 2020
Garcinia travancorica Bedd.; Clusiaceae	Mangosteen	Tree; Native	May -Sep	KL	Biflavonoid fukugiside, superoxide; Antitumor	VU	Aravind and Kumar 2016
Gymnema sylvestre (Retz.) Schult; Apocynaceae	Gymnema	Climber; Native	July -Jan	KA	Saponins; diuretic and antidiabetic	EN	Tejaswini and Jayashankar 2021
Hydnocarpus alpina Wight; Achariaceae	Attuchankala	Tree; Native	Feb -July	KL	Coumarins, quinones, alkaloids, and steroids; leprosy, cancer	VU	Ganesh et al, 2019
Hydnocarpus pentandra (Buch.-Ham.) Oken; Flacourtiaceae	Chaulmugra	Tree; Native	Dec -May	KL, KA	Hydnocarpin, flavonolignan, Fatty acids; antirheumatic, antidiabetic, anticancer	VU	Arun and Kiran 2014

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Scientific name and family	Common name	L F and origin	Flowering - fruiting	State	Phytochemicals and utility	IUCN status	References
Kingiodendron pinnatum (DC.)Harms; Fabaceae	Chou Paini	Tree; Native	Feb -Dec	KA	Saponins, tannins,terpenoids, oleo-gum resin; Gonorrhoea	EN	Mepani and Cruz 2022
Litchi chinensis var. euspontanea; Sapindaceae	Litchi	Tree; Naturalized	April -June	KL	Flavonoids, phenols, anthocyanins; diabetes, obesity, hernia	VU	Singh 2017
Maba buxifolia (Rottb.) A.L. Juss.; Ebenaceae	Chinna-ullingi	Tree; Native	Jan -July	TS, AP	Protein, sugar; folk medicine	EN	Pullaiah 2015
Madhuca insignis (Radlk.) H. J. Lam.; Sapotaceae	Ibbe gida	Tree; Native	Dec -April	KL, KA	Terpenoids, hetrocyclics, phenolics; antidiabetic, antitumor	CE	Ravikumar et al 2004
Madhuca longifolia (Roxb.) A.Chev.; Sapotaceae	Mahua	Tree; Native	March -april	KL, TS	Alkaloid, glycoside, phenol; diarrhea, and antidote in snakebite	VU	Suryawanshi and Mokat 2019
Madhuca neriifolia (Moon) H.J.Lam; Sapotaceae	Ulinannil	Tree; Native	Nov -Mar	KA	Flavonoids and tannins; Kidney complaints, rheumatism, asthma	VU	Minu et al 2022
Mesua ferrea L.; Guttiferae	Iron wood	Tree; Native	Jan -Oct	KL	Xanthones, terpenoids; immunomodulatory	EN	Asif et al 2017
Momordica charantia ; Cucurbitaceae	Pakarkkai	Climber; Naturalized	June-Sep	TN	Vitamins and minerals; Diabetes	CE	Vizhi and Lohidas 2020
Morinda reticulata Benth.; Rubiaceae	Mapoon Bush	Climber; Native	March -Sep	KL	Flavonoids, anthraquinone glycosides; cancers, diabetes	EN	Singh et al 2022
Myristica fragrans Houtt.; Myristicaceae	Nutmeg	Tree; Native	Dec -June	KL	Terpenoids, anthraquinones; narcotic, antidiabetic	CE	Asgarpanah and Kazemivash 2012
Myristica malabarica Lam.; Myristicaceae	Malabar nutmeg	Tree; Native	Jan -Sep	KL, KA	Isoflavones, diarylnonanoids; anti-cancer, anti-diabetic	VU	Chelladurai and Ramalingam 2017
Olax psittacorum Roxb.; Olacaceae	Mekabanda	Tree; Native	Sep-oct	AP	Folk medicine	CE	Sathyavathi and janardhan 2014
Passiflora edulis ; Passifloraceae	Odey pannu	Climber; Naturalized	July-Oct	TN	Vitamins and minerals; Headache	EN	Sasi et al 2011; Sathyavathi and janardhan 2014
Phoenix acaulis Roxb. Ex Buch.-Ham.; Arecaceae	Stemless palm	Tree; Native	Feb -Sep	KA, TS, AP	Glycosides, phenols; diuretic, vertigo and unconsciousness.	NT	Charu et al 2021
Phyllanthus indofischeri ; Phyllantaceae	Nelli	Tree; Native	July-Feb	TN	Vitamins; Diabetes	VU	Rasingam 2012
Physalis peruviana ; Solanaceae	Pitlannu	Herb; Naturalized	July-Aug	TN, TS, AP	Thiamine, niacin, linoleic acid, oleic acid; Vomiting	CE	Sathyavathi and janardhan 2014; Sasi et al 2011
Psydrax dicoccos Gaertn.; Rubiaceae	Oppai	Shrub; Native	Oct-Nov	TN	flavanols, tannins; Antioxidant, antimicrobial, diarrhea	VU	Rasingam 2012
Santalum album L.; Santalaceae	Srigandha	Tree; Native	Mar -Oct	KA	omega-9 oils, fibre; folk medicine	VU	Swaminathan and Kennedy 2019
Syzygium arnottianum ; Myrtaceae	Nerli annu	Tree; Native	April-June	TN	Thiamine, riboflavin, vitamins; Toothache	VU	Sathyavathi and janardhan 2014
Syzygium calophyllifolium ; Myrtaceae	Kadu nerli	Tree; Native	Feb-May	TN	Rutin and ellagic acid; Toothache	EN	Sathyavathi and janardhan 2014

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Table 2. General account on reported TNT-UEFS, significance, IUCN status and references

Scientific name and family	Common name	L F and origin	Flowering - fruiting	State	Phytochemicals and utility	IUCN status	References
Syzygium caryophyllatum (L.) Alston; Myrtaceae	Kunta Nerale	Tree; Native	Feb -dec	KL, KA, TS, AP	Flavanoids, tannings, saponins; anti-inflammatory, anti-bacterial	EN	Heendeniya et al 2018
Syzygium densiflorum Wall. ex Wt. & Arn.; Myrtaceae	Ayuri (Mal)	Tree; Native	April -June	KL, KA	Flavonoids, sterols, terpenoids; antiulcerogenic, cardioprotective	VU	Shareef and Kumar 2020
Syzygium occidentale (Bourd.) Ghandhi; Myrtaceae	Karinjara	Tree; Native	Dec -June	KL	Total phenols, saponins; anti-oxidant, Antifungal	VU	Varghese and Sreekala 2017
Syzygium travancoricum Gamble; Myrtaceae	Poriyal	Tree; Native	April -July	KL	Essential oil, fibre, amino acids; arthritis, diabetes and hypoglycemic	CE	Rajalakshmi et al 2016
Syzygium zeylanicum (L.) DC.; Myrtaceae	Chaliyakkani	Tree; Native	Jan -April	KL	Phenolics, flavonoids, terpenoids; folk medicine,	EN	Anoop and Bindu 2015
Toddalia asiatica ; Rutaceae	Massikai	Shrub; Native	Sep-Jan	TN	Coumarins, alkaloids, triterpenes; skin allergy	VU	Sathyavathi and janardhan 2014
Trichosanthes cucumerina ; Cucurbitaceae	Kattu-padavalam	Climber; Native	July - Sep	TN	Cucurbitacin, sterols and stigmasterol; Headache, Diarrhea	NT	Kumar et al 2014
Vaccinium leschenaultii ; Ericaceae	Cranberry	Tree; Native	Feb - April	TN	Anthocyanins and Flavonoids; Antiulcer, antioxidant	EN	Sasi et al 2011

namely VU (31 species), EN (20 species) and CR (8 species). Further, our habit-wise analysis has revealed that around 53 species (76.8 %) were trees, 7 species (10.1 %) were shrubs, 2 species (2.9 %) were herbs and 7 species (10.1 %) were climbers (Fig. 4). Maximum number of species was recorded in Myrtaceae family (9 species), followed by Ebenaceae (7 species) and Clusiaceae (5 species) (Fig. 5, Table 2). Among these fruit species 63 species (91.3 %) were native and 6 species (8.7 %) were naturalized to SI. Maximum number of species was recorded in Kerala (37 species), followed by Karnataka (31 species) and minimum (13 species) in Andhra Pradesh (Table 2). Among 173 recorded documents, maximum were published in 2020-2021 followed by 2014-2015 (Fig. 6). Prior to 2000, there were only five publications regarding TNT-UEFS while these numbers were increased in the recent years. Many studies have provided a comprehensive review of UEFS in the context of World, Continent, India and portion of SI (Verheij et al 1991, Suresh et al 2014). Native and naturalized UEFS are primarily organic, nutritious and have substantial cultural, medicinal, regulatory and supporting values. Also constitute

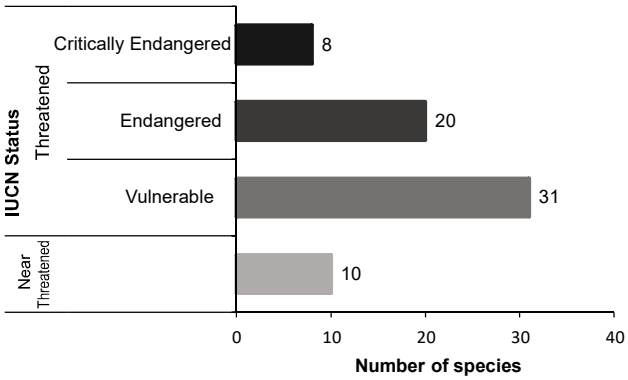

Fig. 3. Conservation status of UEFS of SI as per IUCN categories

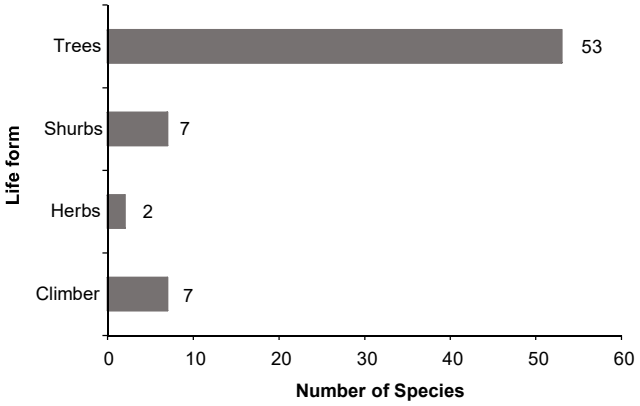

Fig. 4. Habit-wise species distribution

the major source of subsidiary nutrients to the tribal, forest dwellers and marginalized local communities. Studies suggested that integrating fruit trees are a key solution to address food and nutrition insufficiency while providing various ecosystem services (Tag et al 2012, Suresh et al 2014).

Collection calendar and documentation of TNT-UEFS: Collection calendar includes the flowering and fruiting period in the months for each species were recorded (Table 2). The most concentrated period of TNT-UEFS collection was April-August; some species were collected year-round. Round the year availability of TNT-UEFS of different species supplement diverse and nutrient-dense food and enhance the livelihood of the dependent communities. *Aporosa acuminata* was collected from January to June; *Aegle marmelos* from March to May, *Physalis peruviana* in the month of July to August, *Todalia asiatica* from September to January, *Artocarpus hirsutus* from December to March. The flowering and fruiting periods of these species are also important for developing appropriate conservation measures to save these species from extinction in the near future (Tag et al 2012). These UEFS were majorly collected from forests and other unmanaged landscapes. The extent of collection of UEFS depends on various factors such as agriculture failure, adverse climatic conditions and other lean times (Tag et al 2012, Suresh et al 2014). The cultivar fruits are less familiar and not accessible to tribal and local communities. The urban communities are unfamiliar with the UEFS that tribal and local communities utilize. Most of the forest dwellers and tribal populations are largely dependent on forest resources for their livelihood and sustenance. They collect a portion of the excessively available fruits from the forest for sustenance. Documentation of indigenous knowledge through ethnobotanical studies is significant for the sustainable bioprospecting, conservation and utilization of these natural fruit products (Tag et al 2012). UEFS like amla, bael, jamun, wood apple and many others find a place in various ancient literatures including Charaka Samhita and Vrikshayurveda since 4th century BC to date. Documentation helps in appraisal, cultivation, domestication, improvement, conservation and sustainable utilization which can diversify food and livelihood of rural poor and tribals. Hence, there is a need for scientific collating and validation of available traditional knowledge in Vedas, Grantas, Vrikshayurveda and many more. UEFS covers major portions of wild food plants and are eaten worldwide mostly in raw or unripe form as compared to cooked form (FAO 2011). The exploitation of UEFS in a particular area depends on availability in terms of duration and distance, demand, preferences, tastes and traditional knowledge (Tag et al 2012). UEFS have been playing dual role such as source of hidden harvest i.e. supplementing the community with food and income, and buffer food i.e. rescuing lives during food shortages and famines (Grivetti and Ogle 2000).

UEFS connection with culture, folklore and socio-economy: UEFS has been integrally associated with the culture, socio-economy and folklore of aboriginals (Grivetti and Ogle 2000, Tag et al 2012). UEFS are often the only fruits consumed by rural poor and tribals as they cannot afford cultivated commercial fruits in the many developing nations (Verheij et al 1991). Tribals of various ethnic groups like Savara, Khonds, Nayaka, Pulayans, Maratis, Irular, Uraly, Gonds, Koyas and others are predominant in the various parts of SI and follow traditional farming practices (Pullaiah and Rao 2002). Most of the UEFS are used in their diverse and seasonal diet and are often shared or sold in the local

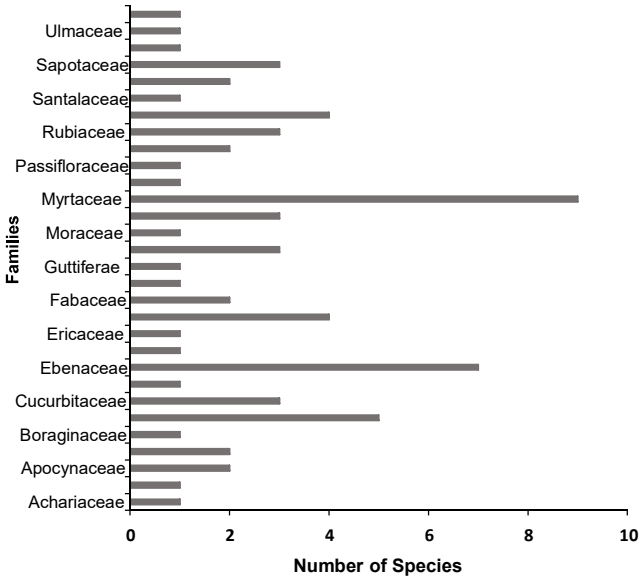

Fig. 5. Familywise dirstributionof TNT-UEFS recorded in SI

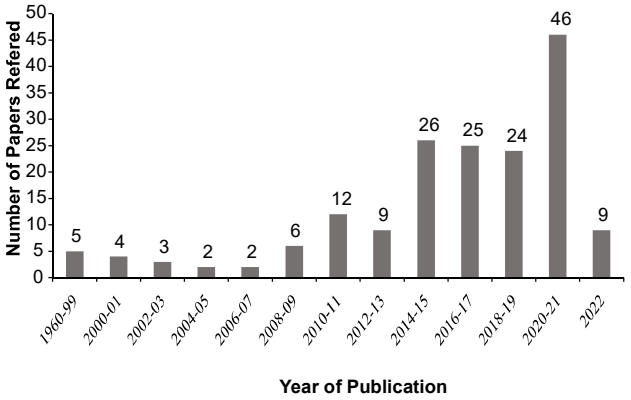

Fig. 6. Number of TNT-UEFS Paper refered

markets. They are consumed either raw or in the form of beverages, pickled or cooked with other dishes (Shankar et al 2020). The knowledge and utilization of UEFS depends on availability, habit, habitat, frequency of food shortages and people's way of life interms of their cultural, religious and socio economical domains (Grivetti and Ogle 2000, Tag et al 2012). Studies showed that different tribes had used a number of similar wild fruits with different uses which proving the diversification of knowledge in the region to region and nation to nation (Grivetti and Ogle 2000). In most parts of India, species such as *Aegle marmelos, Limonea acidissima,* Ficus species *etc.* have a strong connection with the culture, folklore and religious belief. Presently, this long-established culture and knowledge is loosing importance due to the western influence and intensive agriculture which result in the degradation of a harmonious relationship among biodiversity, farming and folklore (Pretty 2003). Hence, it is imperative to renew, document and utilize the traditional knowledge systems for sustainable development of the community.

Implications for food security: Contribution of UEFS to food security and livelihood of the dependent community is well documented even during erratic times such as agriculture failure, adverse climatic conditions and other lean times (Mahapatra et al 2012). Most of the UEFS are available round the year and they constantly supplement diverse and nutrient dense food to the dependent rural and tribal communities in many regions of the world. Ensuring food security of the dependent community further strengthens the education, employment, health and many other sectors. These UEFS can be processed into various products such as dry fruits, jam, jelly, juice, pickle, wine etc., by amalgamation of traditional knowledge and advanced techniques. The quality and shelf life of the products could be improved and reduce post-harvest losses with the application of modern techniques and scientific interventions (Meghwal and Singh 2016) Promoting UEFS-based small food and agro-based industries will contribute to sustainable rural development by providing many fold employment opportunities round the year. It can fulfil the growing need of alternative bio-nutritional sources and also used as a dietary supplement since it has valuable ingredients such as Fe, Na, K, Ca and many more (Tag et al 2012). Many studies showed its significance in meeting the nutrient demand of rural poor and tribal population (Grivetti and Ogle 2000, Tag et al 2012). The nutritional values of many TUEFS were found to be at par with the popular domesticated fruits such as banana, mango, papaya and many more (Mahapatra et al 2012). It improves household food and nutritional security under normal circumstances and food scarcity. Many studies have shown that good quality dry fruits, jams, juices and other drinks can be produced using simple procedures suitable for small-scale commercial production (Mahapatra et al 2012). Apart from the edibility, most of these UEFS are multiuse biological resources that are potential for cosmetic, medicinal, nutraceutical, ornamental, religious, therapeutic, wood, non-wood and other ancillary purposes. Most of these species are used for the preparation of medicinal Ayurvedic formulations like *Triphala*, *Chavanprash*, etc. UEFS are used in the treatment of a wide range of ailments as they are rich sources of antioxidants, minerals, and vitamins. Some UEFS have great antioxidant properties thus, they can be used to cure insomnia, constipation, rejuvenate skin cells and hair growth. Phytochemicals of some UEFS such as *Atalantia monophylla, Diploknema butyracea, Protium serratum, Sterculia foetida* and *Terminalia bellirica* are used in numerous therapeutic advantages (Mahapatra et al 2012).

Implications for diversifying agroecology: In the ancient Indian times, natural resource-based traditional subsistence farming was practiced with the indigenous knowledge and experience. The people worshipped some indigenous fruit species which became an integral part of their life. In recent times, rapid increase in population demanded intensive cultivation. Non-judicious use of chemical inputs in agriculture led India to sacrifice its ecological balance. Many indigenous traditional landraces have been forced to extinct because of the introduction of hybrids. Nowadays, people are realizing the potential of UEFS and hence demand for quality planting material is increasing. To meet this demand, vegetative propagation techniques such as cuttings, grafting, layering and stooling are adopted for commercial multiplication. Diverse, region specific, fruit crop-based models are adopted to diversify farm income, reduce the risk and enhance the productivity (Singh et al 2020). Most of the semi-arid region of SI is water deficient and suitable for the cultivation of horticulture crops, hence attention to be paid to develope region-specific fruit-based agroforestry (AF) models having high resource use efficiency. Plantation of UEFS should be done at proper spacing with locally available perennial organic mulches and proper canopy management (Singh et al 2020).

The Convention on Biological Diversity recognizes the sovereign right of each country over their biodiversity and assists in maintaining countries biological diversity (Prakash 2011). Many of the native species are severely TNT due to various natural and manmade factors. It is estimated that around 26,106 plant species are globally threatened whereas in India, 1700 angiospermic plants are categorized under the threatened list (Singh and Dash 2014). Prioritization of endemic and threatened plant species and their conservation

for sustainable utilization is a vital concern (Singh et al 2020). Genetic resources of some UEFS of SI are remained neglected or still available in outfields or forests. Apart from this, few are in the hands of local or tribal communities and they use mainly for their subsistence while protecting as part of their folkloric responsibilities. There is a great necessity for species-specific surveys and explorations in diverse areas of SI for systematic evaluation, characterization and conservation of indigenous UEFS germplasm.

The degradation and denudation of various land use systems (LUS) through various anthropogenic activities is a major driver to biodiversity loss, carbon (C) emission and uncertainty of food and livelihood for various dependent communities. Greenhouse gases are increasing the earth's temperature; among them, CO_2 is the key gas in global warming, leading to climate change. Additionally, human-induced intensive agriculture degrades productive soils (Rakesh et al 2022). Thus, sequestration of atmospheric C would be the possible solution for soil quality, yield sustainability, and environmental security (Rakesh et al 2022, Dinesha et al 2023). Planting fruit trees through various afforestation and reforestation programmes would help restore degraded lands. Plantation forestry helps improve the soil C sequestration and nutrient dynamics through continuous litter fall (Dinesha et al 2020). Earlier study showed successful integration of fruit species in various degraded and denuded ecosystems such as degraded forests, mined areas, marginal and wastelands (Ghosh et al 2021). Most of the farmers' surveys reported increased soil microbial status, earthworms and beneficial insects like pest antagonists', pollinators, etc. These LUS are explicitly focused on agro ecological balance and on-farm biodiversity. The most prominent effects on the success of restoration of the planted native and naturalized species depend on climate, existing natural vegetation, hydrological features, and soil quality. However, region-specific models of UEFS like bael, jamun, tamarind, chironji, khirni, custard apple, etc. are immensely constructive and climate-smart by surviving in harsh agroclimatic conditions and can be established on degraded lands (Singh et al 2020). These resilient fruit species also augment biodiversity, ecosystem and landscape. Most of these UEFS contribute to the region's diversity of flora and fauna and offer potential niches to various biotas. UEFS are being exploited continuously and unscientifically from the wild without any conservation efforts to propagate them. These UEFS have also been acknowledged as critical resources for long-term ecological security as they are providing multifarious ecosystem services, growing in varied climatic conditions and resistant to biotic and abiotic stresses (Suresh et al 2014).

Challenges and solutions of UEFS cultivation: Concerning UEFS cultivation, challenges and solutions were noted (Table 1). Farmers' awareness and attitude towards UEFS integration in farming are poor due to long gestation period of native and natural fruit species which require long-term investment. They are not aware about the various tangible and intangible benefits, and ecosystem services of UEFS. Many indigenous fruits such as *Adansonia digitata* and *Citrullus lanatus* in Africa and *Parkia timoriana* and *Pithocelobium dulce* in India have high nutraceuticals but very poorly organized production and marketing systems as well as research and policy support. Size of land, poor irrigation facility and lack of awareness induce negative perception and attitude among farmers towards fruit species adoption. Generally, the productivity of native UEFS are very low compared with other major fruit crops grown in India. Hence, there is a critical need of developing improved varieties, quality planting materials, the package of practices, transfer of technologies, post-harvest management, storage, marketing and transport to overcome these challenges and popularize the UEFS among growers. Many research organizations related to fruit crops are tremendously working under the GOI assistance to standardize cultivation practices and further improve UEFS. Developed varieties and standardized several production technologies like propagation methods, plant spacing, canopy management, crop compatibility, nutrient and water management, crop regulation, plant protection, post-harvest management and value addition (Singh et al 2020). In order to cope up with various abiotic stresses, UEFS like *Emblica officinalis*, *Aegle marmelos* and *Feronia limonia* are modified or improved to assure critical morpho-physiological functions i.e., strong deep root system, high root-to-shoot ratio, selective absorption, uptake and storage of more water and nutrients, reduced transpiration and heat shocks (Meena et al 2022). Additionally, synchronized flowering and fast fruiting, biosynthesis of antioxidants, proteins and drought-responsive genes, assist in growth and development under adverse conditions (Meena et al 2022). Generally, breeding and biotechnological interventions are hindered by major obstacles like genetic heterogeneity, long juvenile phase, self and cross incompatibility, sterility and many more (Gill et al 2022). Successful approaches to overcome these challenges are genetic diversity and phylogenetic studies, hybridization, omics-based interventions, linkage mapping, marker-assisted selection and mutation breeding (Gill et al 2022).

Outcome of the UEFS cultivation and farmers' messages: Recent studies reported the shifting of farmers from conventional farming to organic and natural farming

(NF), especially in the SI states namely Andhra Pradesh, Karnataka, Tamil Nadu and Telangana (Nayana and Veni 2020). Farmer's survey in these states reported better plant health, vigour and climate resilience in fruit crops integrated organic and NF system even under dry spells, flooding and cyclone situations (Bharucha et al 2020). Apart from increased incomes, farmers also experienced encouraging outcomes across a range of farm health indicators, agro biodiversity and sustaino-resilence of the agroecosystem (Bharucha et al 2020). Another study reported successful integration of UEFS like aonla (*Emblica officinalis*), bael (*Aegle marmelos*), ber (*Zizyphus* spp.) and jamun (*Syzigium* spp.) based cropping models to minimize the risk and enhance the yield and productivity in Arid and Semi-arid regions of India (Singh et al 2020). Furthermore, integration of leguminous crops under these UEFS models increased the income two to three-fold (Singh et al 2020).

Policy and institutional support: Government of India (GOI) is promoting fruit species incorporation through Mission for Integrated Development of Horticulture, Rashtriya Krishi Vikas Yojana, Sub-Mission on Agroforestry, National Mission for Sustainable Agriculture and National Food Security Mission. In addition, Ministry of Tribal Affairs in association with Ministry of Food Processing Industry and Tribal Cooperative Marketing Development Federation of India Limited (TRIFED) started a scheme called “Trifood” for value addition of forest produce including UEFS by establishing food processing centres in tribal areas. Therefore, the synergy among these missions encourages to improve farmers' income through improved cultivation practices, integrated farming practices, enhancing resource use efficiency, pest, disease and nutrient management, insurance, credit and market support. Some organizations like Food and Agriculture Organization (FAO), World Agroforestry Centre (ICRAF) etc., are fulfilling policy space, conducting scientific studies, providing best practices and publishing guidelines. Earlier studies reported that the growing support by the GOI for agriculture and horticulture over the past decade, including initiatives like the Mission for Integrated Development of Horticulture and the Crop Insurance Scheme (Khandelwal et al 2019). However, policies to support high-value fruit crops both economically and nutritionally are limited and few are embedded in broader agricultural policy initiatives. Furthermore, these policies mainly focus on exports, employment, livelihood and economic growth while lacking importance on its local consumption or inclusion in daily diets (Khandelwal et al 2019).

CONCLUSION

Studies suggested that integrating fruit species in various agroecosystems is a key solution to address food and nutrition insufficiency while providing various ecosystem services. Many of these enlisted TNT-UEFS were economically potential for edible, medicinal, ornamental, timber and many other uses. Hence, there is a need for coordinated research efforts for the survey, documentation, evaluation, cultivation and conservation (*ex-situ* and *in-situ*). Developing standard cultivation protocols, supply of quality planting materials, transfer of technology, processing and marketing through small-scale industries, self-help groups and farmer producer organizations are considered necessary. The government should also actively participate in this regard by providing necessary inputs and technical support. States of SI should come up with the clear-cut policy to encourage and promote UEFS cultivation, marketing and utilization. Furthermore, linking policy makers, researchers and practitioners may build a strong association between national policies and agroecosystem restoration initiatives. As these fruits are highly adaptable and show resistance for various insect-pest and diseases, hence pesticide and other chemical requirements are almost negligible. Thus, they can fit well into organic and natural farming, as they require less attention by the cultivars. On the other supporting hand, the government is emphasizing on promoting indigenous species through the slogan 'vocal for local' and 'local for global'.

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Optimisation of Genomic DNA Extraction and PCR Procedure for Sal (*Shorea robusta*)

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***Abstract*:** In the present study, leaves of Sal were collected from natural populations to isolate the genomic DNA. Unfortunately, genomic DNA extracted using protocols developed by Agbagwa et al (2012) and Doyle and Doyle (1987) was inadequate and failed to obtain genomic DNA of high quality and quantity. Woody tree species are rich in higher concentrations of polyphenols, polysaccharides, and secondary metabolites and interfere in the genomic DNA isolation process. To obtain the genomic DNA in good quality and quantity, the cetyl-trimethyl ammonium bromide (CTAB) method was modified. Modifications were carried out in the concentration of PVP and 2-mercaptoethanol and the process of utilising these reagents. The quantity of extracted DNA was evaluated through a NanoBio spectrophotometer (Analytical Technologies Ltd.), and the quality was checked by 0.8% agarose gel electrophoresis. The standardised protocol yielded high molecular weight DNA in the range of 830.12 ng μl^{-1} to 1597. 23 ng μl^{-1} with an average of 1278.28 ng μl^{-1}. The absorbance ratio at 260 to 280 nm ranged from 1.81 to 1.90, with an average of 1.84, which confirmed the purity of isolated DNA and indicated the presence of very low levels of protein, RNA, and polysaccharide contaminants. The extracted DNA was used to optimise the PCR conditions for microsatellite markers and obtain sharp polymorphic bands in an agarose gel.

***Keywords*:** Sal, Genomic DNA, CTAB, PCR, Microsatellite markers

Sal is one of the most important timber species and belongs to the family Dipterocarpaceae. As a perennial and long-lived tree species, shows higher genetic diversity within the population than among populations (Surabhi et al 2017). Pandey and Geburek (2011) reported a good amount of genetic variability present in natural populations of Sal, which may be due to continuous gene flow among the populations as evidenced by population genetic structure studies. Molecular biology techniques are helpful in understanding the genetic diversity and genetic makeup of a species. For the achievability and reproducibility of most molecular biology experiments, the isolation and availability of pure genomic DNA are pre-requisites. Quality is a key issue in genomic studies and most amplification-based assays because DNA amplification can be affected by co-purification inhibitors that reduce the efficiency of subsequent PCR (Anuradha et al 2013, Youssef et al 2015). To maximise the isolation of pure DNA in large quantities, researchers are continuously working on, modifying, and developing species-specific DNA extraction protocols. The principal goal of numerous DNA isolation techniques is the improvement of a pretty quick, cheaper, and steady protocol to extract excessively fine DNA with a higher yield (Singh and Singh 2015). Obtaining a high amount of DNA from small amounts of tissue is usually a difficult task (Pereira et al 2011). In the extraction process, objective is to minimise the quantity of polyphenols and polysaccharide content (Karthikeyan et al 2010, Sandip 2013). Commercially available DNA isolation kits provide higher throughput but are mostly specific to certain species. Additionally, their availability and excessive fees may be limiting factors. The most commonly used DNA extraction procedures are based on the cetyltrimethylammonium bromide (CTAB) method. Different procedures work best for different groups considering the great diversity of plant secondary metabolites that, in many cases, may interfere with a particular method of DNA isolation (Doyle and Doyle 1987). There are multiple procedures that have been standardised for the isolation of plant DNA, e.g., Doyle and Doyle (1987), Agbagwa et al (2012), Llongueras et al (2012). The purity and quantity of genomic DNA play an important role in downstream molecular studies. Therefore, it was needed to develop a DNA isolation protocol that could yield a good concentration of DNA with high purity. The primary objective of the present study was to develop a reproducible and efficient technique for the isolation of pure, high-quantity DNA from Sal leaf tissues and standardise their PCR conditions for SSR markers. To achieve the objective, several elements affecting DNA isolation from the leaf tissue have been investigated, together with buffer composition, replacement of reagents, and the addition of reagents that

improve the DNA quality. The reagent addition steps, as well as the reagent concentration, were modified to create a robust DNA isolation protocol that yielded high-quality and large amounts of genomic DNA from Sal. By utilising an optimised protocol, the extracted DNA yielded polymorphic and sharp bands when amplified using SSR markers.

MATERIAL AND METHODS

Plant leaf tissue sample collection and preparation: Fresh leaves were collected during the period May 2021 to August 2021 from natural populations of Sal distributed in different agro-climatic sub-zones of Jharkhand, India. The collected leaves were kept in an airtight polybag with silica beads to avoid excess moisture retention. The polybags were stored in an icebox, and, on return from the field, leaf samples were kept in a -40°C deep fridge to retain the freshness of the leaves. The DNA isolation experiment was carried out during the period October 2021 to March 2022 at the Forest Biotechnology Laboratory at latitude 23°2128 NL and longitude 85°1442. The collected samples were washed with distilled water to remove particulate contaminants and unwanted materials from the leaf surfaces. The extraction buffer was freshly prepared, containing 100 mM Tris-HCl (pH 8), 20 mM EDTA (pH 8), 1.4 M NaCl, 2% CTAB, 3% PVP, and 4% 2-mercaptoethanol.

Grinding of plant materials: The leaf samples were taken from a -40^{0}C deep fridge and washed vigorously through distilled water to remove particles on the leaf surfaces. For 200 mg of leaf sample, midribs were removed for better grinding and chopped into pieces. The chopped samples were taken into a mortar and pestle with 1 mL of CTAB extraction buffer (preheated at 65^0 C for at least 30 minutes). During grinding, a little pinch of PVP powder may be added. After grinding, an equal volume of approximately 1 mL of the resultant paste was distributed into two separate 2-ml microcentrifuge tubes.

Extraction and purification protocol: Samples were incubated in a hot water bath at 65^{0}C for 1 hour and then kept at room temperature for 10–15 minutes. An equal volume of Chloroform: Isoamyl alcohol (24:1) was added to each microcentrifuge tube for extraction. This was mixed gently but thoroughly by continuous rocking and inverting of the tubes for up to 10 minutes. Then the samples were centrifuged at room temperature (22^{0}C) at 14,000 rpm for 12 minutes to separate the phases. The top aqueous phase was transferred to new 2-ml microcentrifuge tubes by micropipette. The last step was repeated another time for better extraction. Then the top aqueous phase was transferred to new 1.5 ml microcentrifuge tubes along with 66% of the supernatant volume; chilled isopropanol was added to precipitate the DNA sample. This was gently mixed by continuous inversion for 2 minutes and kept at 4°C overnight. The next day, samples were centrifuged at 10,000 rpm for 10 minutes at 4^{0}C. The supernatant was discarded carefully to save the DNA pellets. The pellets were washed in 70% ethanol twice at 4000 rpm for 5 minutes and air-dried till the ethanol evaporated completely from the samples. This was facilitated by inverting tubes on tissue paper for a few minutes. After proper drying, the DNA pellets were rehydrated and dissolved in 100 µl $T_{10}E_1$.

Quantification and purity of genomic DNAL DNA yield and purity were determined through NanoBio UV spectrophotometer analysis. The yield was measured by checking the optical density (OD) with a UV spectrophotometer. The purity of DNA was determined with an absorbance ratio of A260 to A280 nm. The molar absorption coefficient is a property associated with a sample and is a measure of how strongly a sample absorbs light at a specific wavelength. The concentration is simply the molar mass L^{-1} (M) of the sample dissolved in solution, and the length is the length of the cuvette used for absorbance measurement, typically 1 cm. Absorbance is dimensionless and therefore should have no units. In the context of absorption spectroscopy, optical density is an older term synonymous with absorbance (Trumbo et al 2013).

Nano Bio UV spectrophotometer: The NanoBio UV spectrophotometer is a spectrophotometer capable of highly accurate analysis of 1 µl samples for DNA, RNA, protein, pigment, the entire UV-VIS (220–700 nm) spectrum and cell density testing, and has remarkable reproducibility. It is used to measure the concentration and purity of DNA, RNA, or protein samples for various downstream applications.

Agarose gel electrophoresis: The quality was checked by 0.8% agarose gel electrophoresis. Genomic DNA products were electrophoresed on a 0.8% agarose gel with 10x TBE buffer (100 mM TrisHCl and 20 mM EDTA, pH 8.0) and stained with ethidium bromide. The bands were digitally photographed and stored in the gel documentation system.

Efficacy comparison of extraction protocols: A comparison between the standard CTAB-based protocol and protocols suggested by Doyle and Doyle (1987) and Agbagwa et al (2012) was used. Using a spectrophotometer and 0.8% agarose gel electrophoresis, the yielded DNA from all protocols was compared for quality and quantity.

PCR amplification of extracted DNA through SSR markers: The PCR reaction conditions were optimised for the extracted genomic DNA for getting polymorphic bands. A 10µl of reaction mixture was prepared containing1X Taq DNA buffer, 0.6 U Taq DNA Polymerase, 0.2 mM dNTP Mix, 50ng template DNA, 0.25 µM of each forward and reverse primer

and nuclease free molecular grade water to maintain the volume. The details of chemicals used and their concentration are given in Table 1. The reaction mixture was mixed and a short spin was given. The thermal cycler [Eppendorf Mastercycler X50] was utilised for the amplification process (Table 2).

Results and Discussion

Agarose gel electrophoresis analysis of genomic DNA : Isolated genomic DNA through all the utilized protocols were assessed through 0.8 % agarose gel electrophoresis using 10x TBE buffer (100 mMTrisHCl and 20 mM EDTA pH. 8.0) and visualized by ethidium bromide staining. The DNA obtained through the Agbagwa et al (2012) protocol was not of good quality, as a smear of DNA was found with higher RNA contamination (Fig. 1). Doyle and Doyle (1987) method also yielded a very small amount of DNA with less purity (Fig. 2). The modified CTAB method yielded good quality and quantity of genomic DNA (Fig. 3).

UV Spectrophotometric analysis of DNA: The qualitative and quantitative analysis of genomic DNA samples was performed using UV spectrophotometry. DNA absorbs UV light very efficiently, making it possible to detect and quantify it's concentration. Nitrogenous bases in nucleotides have an absorption maximum of around 260 nm. The ratio of absorbance at 260 nm/280 nm is a measure of the purity of the DNA sample, should be between 1.80 and 1.90. The first-used protocol (Agbagwa et al 2012), yielded low quality and quantity of genomic DNA. The 260 nm/280 nm data was found to be greater than 2 in all samples, which indicated the presence of RNA as an impurity (Table 4). The second utilised protocol (Doyle and Doyle 1987) also yielded a low quantity and quality of DNA with RNA as an impurity (Table 5). The modified and standardised protocol yielded substantial quantity of genomic DNA ranged from 830.12 ngµl^{-1} to 1597.23 ngµl^{-1} with an average of 1278.28 ng µl^{-1} (Table 6). The obtained DNA was also found to be pure, as the absorbance ratio at 260 to 280 nm varied from 1.81 to 1.90, with a mean of 1.84, which indicated the absence of polysaccharides, polyphenols, and RNA.

Table 1. Chemicals and their concentration used in the PCR reaction

Chemicals	Concentration utilised
10X Taq DNA buffer	1X
Taq DNA Polymerase	0.6U/reaction
dNTP Mix	0.2 mM
Forward Primer	0.25 µM
Reverse Primer	0.25 µM
Template DNA	50ng

Table 2. Thermal profile utilised for PCR reaction

PCR steps	Temperature	Time	Cycle
Initial denaturation	95°C	5 min	33X
Denaturation	95°C	45 s	
Annealing	55°C to 60°C	30 s	
Extension	72°C	45 s	
Final extension	72°C	7 min	
Storage	4°C	∞	

Annealing temperature varied for different SSR primers

Table 4. Quantitative estimates of DNA concentration revealed by UV spectrophotometry isolated using Agbagwa et al (2012) method

Quantitative estimates of DNA concentration of Sal revealed by Nano-Bio Spectrophotometer			
Genotypes	Sample weight (mg)	DNA Concentration	
		ng/µl	A260/A280
JP-15-1	200 mg	80.21	1.70
JP-15-2	200 mg	145.56	2.19
JP-15-3	200 mg	200.23	2.10
JP-15-4	200 mg	204.45	2.25
JP-15-5	200 mg	217.62	2.28
JP-15-6	200 mg	187.65	2.20
JP-15-7	200 mg	233.90	2.22
JP-15-8	200 mg	170.80	2.14
JP-15-9	200 mg	10.43	1.63
JP-15-10	200 mg	90.78	2.15

Table 3. Details of SSR primers used in the PCR amplification

Locus	Forward/Reverse	Sequence (5' to 3')	Repeat Motif	No. of bases	T_a (°C)
SSR-53	Forward	TTGCATCTCCCTGGTAGAGA	(GAA)17	20	59.9
	Reverse	TCCACAGACTTCCTACCATCT		21	
SSR-54	Forward	GCTTCTTCTGCATGGCGATG	(TTG)21	20	59.9
	Reverse	CCTCTTTTGCATGGCATCAGT		21	
SSR-74	Forward	TCAGTTTTCCTTTGACAATGAGCA	(AG)18	24	55.6
	Reverse	TGAAGCTAGATGATACTGGCAGT		23	
SSR-80	Forward	CGTCCGGGCCAAAACATTTT	(AG)26	20	56.1
	Reverse	TGTTTGATGCGTATGTGTTGCA		22	

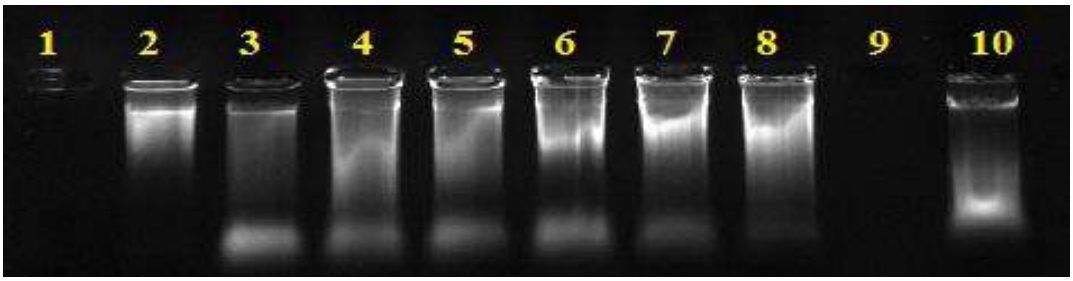
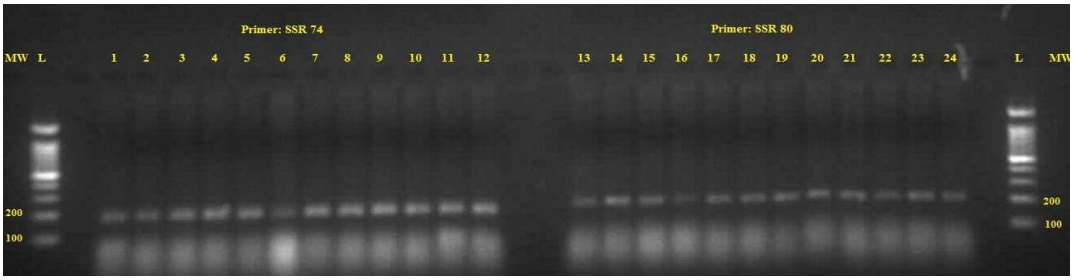


Fig. 1. Agarose (0.8%) gel electrophoresis of genomic DNA extracted using Agbagwa et al (2012) method

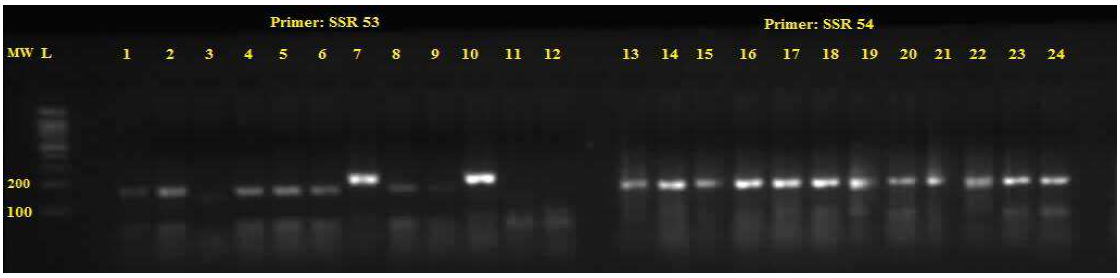
Fig. 2. Agarose (0.8%) gel electrophoresis of genomic DNA extracted using Doyle and Doyle (1990) method

Fig. 3. Agarose (0.8%) gel electrophoresis of gDNA extracted using our standardised modified CTAB method



Lane L: 50 bp DNA step ladder, Lane 1-12: Amplicons of Sal genomic DNA using primer SSR 74, Lane 13-24: Amplicons of Sal genomic DNA using primer SSR 80

Fig. 4. 2% agarose gel image of amplicons of Sal genomic DNA through PCR reaction using SSR primers (SSR 74 &SSR 80)



Lane L: 50 bp DNA step ladder, Lane 1-12: Amplicons of Sal genomic DNA using primer SSR 53, Lane 13-24: Amplicons of Sal genomic DNA using primer SSR 54

Fig. 5. 2% agarose gel image of amplicons of Sal genomic DNA through PCR reaction using SSR primers (SSR 53 & SSR 54)

Amplification of extracted genomic DNA using modified CTAB procedure by SSR markers: The genomic DNA extracted through an optimised protocol was subjected to amplification using SSR primers, viz., SSR-53, SSR-54, SSR-74, and SSR-80 (Table 3). The utilised primers amplified the targeted region of the extracted DNA and produced sharp and polymorphic bands in a 2% agarose gel.

Table 5. Quantitative estimates of DNA concentration revealed by UV spectrophotometry isolated using Doyle and Doyle (1990) method

Quantitative estimates of DNA concentration of Sal revealed by Nano-Bio Spectrophotometer			
Genotypes	Sample weight (mg)	DNA Concentration	
		ng/µl	A260/A280
JP-15-1	200 mg	120.23	1.99
JP-15-2	200 mg	94.45	2.00
JP-15-3	200 mg	50.62	1.68
JP-15-4	200 mg	187.65	2.10
JP-15-5	200 mg	263.90	2.02
JP-15-6	200 mg	71.80	2.08
JP-15-7	200 mg	150.43	2.04
JP-15-8	200 mg	172.67	2.12

Table 6. Quantitative estimates of DNA concentration revealed by UV spectrophotometry isolated using standardised modified CTAB method

Quantitative estimates of DNA concentration of Sal revealed by Nano-Bio Spectrophotometer			
Genotypes	Sample weight (mg)	DNA Concentration	
		ng/µl	A260/A280
JP-15-1	200 mg	1396.20	1.81
JP-15-2	200 mg	1528.34	1.86
JP-15-3	200 mg	1492.60	1.89
JP-15-4	200 mg	1597.23	1.82
JP-15-5	200 mg	1526.45	1.85
JP-15-6	200 mg	1228.68	1.81
JP-15-7	200 mg	1525.04	1.85
JP-15-8	200 mg	1267.91	1.86
JP-15-9	200 mg	1026.10	1.87
JP-15-10	200 mg	1324.13	1.82
JP-15-11	200 mg	1288.88	1.88
JP-15-12	200 mg	1450.24	1.81
JP-15-13	200 mg	1394.20	1.90
JP-15-14	200 mg	1487.81	1.85
JP-15-15	200 mg	1104.78	1.83
JP-15-16	200 mg	1304.24	1.86
JP-15-17	200 mg	982.76	1.84
JP-15-18	200 mg	974.15	1.84
JP-15-19	200 mg	835.77	1.85
JP-15-20	200 mg	830.12	1.88

CONCLUSION

The modified DNA isolation protocol successfully yielded high quality and quantity of genomic DNA with very low levels of protein, RNA, polyphenol, and polysaccharide contaminants compared to other utilised protocols. The genomic DNA isolated using the standardised and modified CTAB procedure underwent PCR amplification using primers such as SSR-53, SSR-54, SSR-74, and SSR-80 and resulted in polymorphic bands. This optimisation of PCR and genomic DNA isolation protocols for Sal can be utilised for its genetic improvement and molecular breeding programmes across the laboratory.

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Epicormic Shoot Induction and Rooting of *Tectona grandis* from Branch Cuttings: Influence of Growing Condition and Hormone Application

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***Abstract*:** The purpose of this study was to understand the effect of controlled circumstances and exogenous hormones on epicormic shoot development and rooting ability of teak epicormic shoots obtained from teak branch cuttings. The growing conditions had an effect on physiological and shoot quality parameters with an average of 8 to 10 harvestable shoots per branch cutting. Naphthalene acetic acid (NAA) inhibited the production of epicormic shoots on branch cutting. Rooting percentage increased with increasing IBA concentration, whereas the combinations of indole butyric acid (IBA) and NAA encouraged the callusing. NAA was inhibited either rooting or forming callus in teak cuttings. Shorter and thinner cuttings failed to produce roots as all rooting parameters were lower. The highest rooting percentage (67.40%) was exhibited in the cuttings of diameter sizes 5.50 to 7.00 mm, and the least (45.03%) was in 2.50 to 4.00 mm cuttings.

***Keywords*:** Epicormic shoot, Vegetative propagation, Auxin, Teak, Branch stick cutting

Tectona grandis is perhaps the most well-known tropical timber species grown widely to meet the demand of wood-based industries in the tropics. Teak is a deciduous species, naturally distributed in South Asian Countries with substantial genetic diversity owing to its wide occurrence. The teak plantations managed traditionally for rotation of 60-80 years are no longer well-adapted to the present scenario of demand crisis. Even though teak cultivation in India was geared up in recent decades, productivity is very low (Shahapurmath et al 2016, Chelliah et al 2021, Sasidharan 2021). According to the Food and Agricultural Organization, about 70% of teak logs consumed in India are imported from Africa and Latin America. The low productivity of teak plantations is attributed to low-quality planting material and poor management. Propagation of teak through seeds has limitations because of low germination percentage, failure of teak plantations in flower induction, asynchrony in flowering among the clones, pollination, and fruit set (Vasudeva 2004, Palupi et al 2010, Florence and Mohanadas 2011). The problems with seed production and demand for planting material for rapid expansion of plantations outside the natural forest have encouraged different vegetative propagation methods. Selecting superior parents and rejuvenation by vegetative means is vital for any improvement program. The vegetative propagation of tree species aims to produce exploitable yield earlier than seed-origin plants. Vegetative propagation helps retain physiological age and improve the genetic base through maintaining genetic consistency. Despite several attempts at vegetative propagation, there is little information on the teak's macro-propagation technique through epicormic shoot production from branch stick cuttings. Most vegetative propagation investigations were done on apical shoot cutting, hard-stem cutting, softwood cutting, and grafts, coupled with exogenous growth hormone treatments (Husen 2013, Guleria and Vashisht 2014, Packialakshmi and Sudhagar 2019).

The use of juvenile shoots for plant production has an advantage over the stem cuttings through an improved root system with taproot production, rooting potential, and reduced lignification. The epicormic shoots or mini juvenile cuttings show orthotropic growth by reduced topophysis effect as the presence of shoot apex (Packialakshmi and Sudhagar 2019). In general, juvenile propagules in tree species are produced by either coppicing or girdling the matured tree, which is lethal to the tree. Production of juvenile shoots on stick branch cutting by forced epicormic bud bursting without losing the mother tree is an option. These then can be used for developing a mother-clonal-hedge garden or planting directly after rooting (Pinon et al 2021). This study was designed to investigate the effect of controlled environmental settings and exogenous hormones on the development of epicormic shoots from branch cuttings of teak and its rooting.

MATERIAL AND METHODS

Study site: The study was conducted at the College of Forestry, Kerala Agricultural University, Kerala, India (10°32'N, 76°26'E) from October 2021 to April 2022. The location experiences a warm, humid climate with an average annual rainfall of about 2100 to 2500 mm. The average temperature ranges from 24.4°C to 42.8°C with a relative humidity of 80 to 100%.

Experimental material: Branch cuttings were obtained from the middle to lower portion of the crown from trees located in the College of Forestry, Vellanikkara, Trichur tree garden. Branch cuttings were prepared by removing the side branches and cutting them into pieces of 1.25 to 1.5 m in length and 3 to 5 cm in diameter. Damaged and split ends were avoided to reduce the impairment of dormant buds. The juvenile epicormic shoots produced from branch cuttings in the experiment were used for the rooting experiments. The branch cuttings and epicormic shoot cuttings were treated with carbendazim 50 %WP (2%) for ten minutes to avoid fungal infection.

Experimental Design and Data Collection

Effect of growing condition on growth, production, and physiology of epicormic shoots: Branch cuttings were planted in the mist chamber and in the open to investigate the effect of growing conditions on epicormic shoot yield and quality. A 30-second intermittent mist was employed in the mist chamber at 20-minute intervals (7.00 am to 6.00 pm) to maintain the appropriate humidity. In the open, branch cuttings were manually watered every 2 to 3 hours (8.00 am to 5.00 pm). The weather conditions in both environments were recorded using a portable weather recorder (Table 1). The experiment was laid out with three replications, each replication having five branch cuttings each. The biometric parameters of epicormic shoots such as shoot length (cm), shoot diameter (mm), leaf length (cm), leaf width (cm), and leaf area (cm^2) were measured and recorded. The physiological traits such as Photosynthetic Rate (µmol CO_2 m^{-2} s^{-1}), Canopy Air Temperature Difference (CATD) (°C), Stomatal Conductance (µmol H_2O m^{-2} s^{-1}), and Transpiration Rate (µmol H_2O m^{-2} s^{-1}) were measured for the third leaf from the tip using an IRGA (LI6400 portable photosynthetic system). The total chlorophyll content of leaves was recorded with the help of SPAD. The relative water content (%) of leaves was estimated (Barrs 1968).

Exogenous growth hormone and epicormic shoot production: The prepared branch cuttings (1.25 to 1.5 m in length and 3 to 5 cm in diameter) were planted after treatment with various concentrations (liquid form) of growth hormones (Table 2) and maintained in the mist chamber. The lower cut end of branch cuttings was dipped in the required concentration of plant growth regulator for eight hours. The treated branch cuttings were planted in polybags containing a mixture of soil and coco-coir pith (1:1). Each treatment was laid out as a CRD with three replications with five branch cuttings forming one replication. The total number of live buds (buds with a length of less than 1 cm), sprouts (buds with a length greater than 1 cm), and harvestable shoots (shoots with a length greater than 5 cm) produced during the study period were recorded until the majority of the branch cuttings dried. The biometric parameters of epicormic shoots such as shoot length (cm), shoot diameter (mm), and the number of leaves were recorded.

Effect of growth hormones on rooting of epicormic shoots: The epicormic shoots produced from the branch cuttings were excised at the three to four pair leaf-stage. Immediately after detaching from the main branch, cuttings were immersed in water to avoid desiccation. The 2/3 parts of the leaves were carefully trimmed to prevent transpiration. The cut ends of the excised shoots were treated with carbendazim 50 % WP (2%). The cut end of the shoots was then dipped in different concentrations of growth hormones (Table 2), prepared in talc (powder form), and planted in the root trainers filled with sterile vermiculite. The root trainers were maintained in the mist chamber. During day time, an intermittent mist of 30 seconds every 30 minutes was used to maintain the relative humidity between 80 and 95 percent. After 55 days, rooted cuttings were observed for root parameters such as root length (cm), the number of roots, and rooting percentage (%).

Table 1. Weather parameters under different growing conditions during the experimental period

Weather parameter	Inside mist-chamber	Open condition
Temperature (°C)	30±1°C to 33±1°C	27±1°C to 30±1°C
Relative humidity (%)	85% to 95%	65% to 80%
Soil moisture (%)	24.3	12.4

Table 2. Different growth hormone treatment combinations used in the study

Treatments	IBA (mg/L)	NAA (mg/L)
T1	----	----
T2	3000	----
T3	4000	----
T4	----	2000
T5	----	4000
T6	1000	1000
T7	2000	2000
T8	3000	3000

IBA: Indole butyric acid; NAA: Naphthalene acetic acid

Influence of shoot size on rooting of juvenile epicormics shoots: The juvenile epicormic shoots produced from the branch cuttings were collected at three to four pair leaf-stage and categorized into three diameter classes *viz.,* 2.50-4.00 mm, 4.00-5.50 mm, and 5.50-7.00 mm. Cuttings after fungicide treatment were treated with IBA (6000 mg/L), prepared in talc (powder form) and planted in root trainers filled with the sterile vermiculite, and maintained under mist conditions for rooting. The experiment was laid out as CRD with three replications with five shoot cuttings forming one replication. After 45 days of planting, rooted cuttings were prickled out and observed for rooting.

Data analysis: Data collected from the above experiments were analysed using R software version 4.0.4. The effect of growing conditions on epicormic shoot production and physiology under two growing conditions was compared using a t-test. The effects of growth hormone on epicormic shoot production and rooting were studied using DMRT postdoc analysis. Spearman correlation was performed to know the association between the size of the shoot and rooting.

RESULTS AND DISCUSSION

Production of epicormic shoots: The epicormic shoots are the juvenile shoots produced due to dormant budburst. The shoot production was varied under growing conditions and hormonal application.

Effect of growing condition on growth, production, and physiology of epicormic shoots: The prevailing environmental conditions such as light availability, temperature, and humidity are always considered as the primary factor for the epicormic bud dormancy release, physiological activity, and elongation of shoots (Gordon et al 2006). The temperature and relative humidity significantly influence shoot production, shoot biometric and shoot physiological traits. Budburst and other shoot growth parameters except shoot diameter were higher in the mist chamber condition than in the open shed (Table 3). The shoot initiation was observed on the same date between the conditions as an immediate response of branch cuttings to the stress level. An average of 18 shoots with a mean length of 12.17 cm was recorded under mist conditions, whereas only ten shoots of nearly half-length (6.63 cm) were observed under open shed conditions (Plate 01b). Growing conditions were found to have a significant influence on the leaf area too. The leaf area was 36.87 cm^2 under the moist condition, whereas in the open condition was only 16.28 cm^2. The variation in leaf length, width, and expansion under favourable conditions have resulted in this difference. The growing condition significantly influenced relative water content and the photosynthetic rate. The photosynthetic rate was also higher in the mist chamber (4.11 µmol CO_2 m^{-2} s^{-1}) than the open condition (3.63 µmol CO_2 m^{-2} s^{-1}). However, the growing condition did not significantly affect other physiological parameters like stomatal conductance, transpiration rate, and total chlorophyll.

The temperature, natural light has positively impacted epicormic shoot production (Akram and Aftab 2009). Greenhouse condition has been shown to improve the shoot production in teak (Palanisamy and Subramanian 2001, Badilla et al 2017); *Casuarina junghuhniana* (Palanisamy et al 2020); *Pterocarpus indicus* (Pinon et al 2021) and other temperate and tropical tree species (Brondani et al 2009, Wendling et al 2013). Light, humidity, and hormone dynamics are significant influencers for shoot production in plants. The enforced production of shoots on a live detached branch under a mist chamber with higher humidity and temperature is an excellent alternative for coppicing, which can also be used to develop a mini-clonal-hedge garden.

Growth hormone-induced epicormic shoots production: The production of epicormic shoots is mainly dependent on the number of dormant buds present in the trunk or the branch. Hormonal activity plays a significant role in juvenile epicormic shoot production from these dormant buds. The cytokinins and auxin interaction have a significant role in the budburst. When higher cytokinin is present than the auxin, it helps sprout dormant buds and promotes the growth of buds (Tworkoski et al 2006, Pallardy 2008). The exogenous application of auxin has a significant influence on shoot induction (Table 4). The combination of auxins, *viz.,* IBA and NAA, slowed down the shoot emergence and production of epicormic shoots. The first shoot initiation was observed in untreated branch cuttings after 7 to 8 days of planting, followed by IBA treated branch cuttings and then IBA+NAA. The production of live buds after dormancy release was influenced by auxin treatment. Auxin treatment was found to delay and reduce the number of live buds. The untreated branch cuttings have produced a maximum number of buds (average 20.27/ branch cutting) followed by IBA treatment (Plate 01a). In contrast, NAA had a negative impact either alone or in combination with IBA. The least live buds were observed in NAA 2000 mg/L (average 11.87/cutting) and NAA 4000 mg/L (12/ branch cutting). The NAA was found to be inhibiting auxin, which reduces the production of shoots (Okao et al 2016).

The harvestable shoot development after bud bursting was on par among the treatments except for NAA treatments. The differences between the live buds produced and harvestable shoots within the same treatment. The maximum harvestable shoots were produced in Control (11.73/branch

cutting), followed by IBA treatments, a Combination of IBA and NAA, and finally, NAA alone. This difference in the production of live bud to the final harvestable shoot within the same treatment is also a result of competition among the individual shoots in the epicormic complexes (a group of buds formed at a single position) from the period of bud production to final harvest period. Each complex will produce more than one bud, during the process of development and elongation, limited potential shoots will retain causing the death of weak shoots within the complex (Colin et al 2010). The nutrient uptake by the sprouts in an epicormic complex impacts the growth and development of potential shoots. The tiny buds are likely weaker than the prominent ones; hence, they are suppressed in growth and may die (Cochard et al 2005). The average shoot diameter among the treatments ranged from 5.14 mm (IBA + NAA 2000 mg/L) to 5.97 mm (control). The highest shoot length was recorded in the control (11.29 cm), and the least was found in IBA+NAA 1000 mg/L (5.70 cm). The variation in shoot length is attributed to early bud production, emergence, and elongation of epicormic shoots. The average number of leaves produced was 6 to 9 per shoot. A similar reduction in shoot production after treating the cutting with sucrose and auxin was observed in *I. paraguariensis.* The potential of epicormic shoot production on the trees is influenced by the factors such as light availability, stress, physiology, hormone dynamics, and genetics of species (Bowersox and Ward 1968, Burrows et al 2008). The stress or damage in the tree trunk associated with hormonal changes is the most critical factor influencing epicormic shoot production. Stress-initiated and hormonal signalling plays a crucial role in producing epicormic shoots. The bud dynamics controlled eco-dormancy (Meier et al 2012) and endo-dormancy (Burrows et al 2008) are well explained in many tree species.

Table 3. Effect of growing condition on production, growth, and physiology of epicormic shoots

Parameters	Mist chamber	Open condition	T-value
Number of shoots produced	18.83	10.67	9.50 *
Shoot length (cm)	12.17	6.63	3.84 *
Shoot diameter (mm)	5.67	4.85	1.78
Leaf area (cm^2)	36.87	16.28	4.09 *
Length (cm)	21.65	11.98	4.83 *
Width (cm)	9.22	6.06	3.84 *
Relative water content (%)	69.63	53.22	3.75 *
Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})	4.11	3.63	5.53*
CATD (°C)	-2.33	-0.55	1.90
Stomatal conductance (µmol H_2O m^{-2} s^{-1})	0.048	0.062	0.95
Transpiration rate (µmol H_2O m^{-2} s^{-1})	0.95	0.98	0.12
Total Chlorophyll (SPAD units)	37.47	39.73	1.15

*Significant at 5 %

Table 4. Exogenous growth hormone influence on epicormic shoot production

Treatment	First shoot (DAP)	Live bud (<1cm)	Sprout (>1cm)	Harvestable shoot (>5cm)	Shoot Diameter (mm)	Shoot Length (cm)	Number of leaves
T1	7.07 ± 0.90 ^{c}	20.27 ± 2.42 ^{a}	16.07 ± 2.20 ^{a}	11.73 ± 0.90 ^{a}	5.97 ± 0.30 ^{a}	11.29 ± 1.02^{a}	9.27 ± 0.70 ^{a}
T2	9.80 ± 1.93 ^{bc}	17.07 ± 0.61^{abc}	13.07 ± 0.58 ^{abc}	9.40 ± 0.87 ^{ab}	5.57 ± 0.22^{abc}	8.65 ± 1.40 ^{b}	8.27 ± 0.31^{abc}
T3	7.33 ± 0.23 ^{c}	19.33 ± 4.03 ^{ab}	14.67 ± 1.80 ^{ab}	11.40 ± 0.20 ^{a}	5.63 ± 0.20 ^{ab}	8.66 ± 1.24 ^{b}	6.80 ± 0.40 ^{bc}
T4	11.93 ± 1.22 ^{ab}	11.87 ± 1.86 ^{c}	8.53 ± 1.60 ^{d}	5.20 ± 0.53 ^{c}	5.17 ± 0.33 ^{bc}	6.84 ± 0.33 ^{bc}	6.73 ± 0.83 ^{c}
T5	12.53 ± 1.90 ^{ab}	12.33 ± 2.23^{c}	9.47 ± 1.86 ^{cd}	5.93 ± 0.83 ^{bc}	5.33 ± 0.12 ^{bc}	8.71 ± 0.23 ^{b}	9.00 ± 0.53 ^{a}
T6	12.53 ± 0.42 ^{ab}	15.07 ± 1.42^{abc}	11.67 ± 0.61 ^{bcd}	8.33 ± 0.58 ^{abc}	5.27 ± 0.09^{bc}	5.70 ± 0.94 ^{c}	8.07 ± 0.64^{abc}
T7	11.93 ± 0.58 ^{ab}	14.33 ± 1.72^{bc}	11.00 ± 0.87 ^{bcd}	8.33 ± 0.81 ^{abc}	5.14 ± 0.28 c	7.03 ± 0.24 ^{bc}	7.87 ± 2.08^{abc}
T8	13.67 ± 1.10 ^{a}	16.73 ± 2.20^{abc}	12.40 ± 1.80 ^{abcd}	10.20 ± 0.72 ^{a}	5.46 ± 0.10^{bc}	7.07 ± 0.98 ^{bc}	8.80 ± 0.53 ^{ab}
MSE	2.668	10.481	6.191	5.242	0.074	1.793	1.472
P	0.000*	0.048 *	0.027 *	0.166	0.003 *	0.000 *	0.931

Significant at 5%, DAP: Days after planting

Large and potential buds are expected to sprout early in favorable environmental conditions, whereas smaller buds will remain dormant and act in the later phase. Owing to this nature of bud dynamics, branch cuttings in the present study continuously produced buds even after two harvests of the epicormic shoot (first harvest on the 40th day after planting, second harvest, and third harvests in 30 days intervals). However, the quality of shoots (diameter and intermodal length) and rooting ability deteriorated, and it was not economically feasible to maintain the branch stick cuttings after the third harvest. Progressively, the stored food material in cutting is reduced, epicormic shoot production is reduced, and eventually, the cutting dies. Such observations were made in species like *I. paraguariensis* (Wendling et al 2013) and *A. angustifolia* (Wendling et al 2009). The potential of epicormic shoot production on the trees is influenced by the factors such as light availability, stress, physiology, hormone dynamics, and genetics of species (Bowersox and Ward 1968, Burrows et al 2008). The stress or damage in the tree trunk associated with hormonal changes is the most critical factor influencing epicormic shoot production. Stress-initiated and hormonal signalling plays a crucial role in producing epicormic shoots. The bud dynamics controlled eco-dormancy (Meier et al 2012) and endo-dormancy (Burrows et al 2008) are well explained in many tree species.

Rooting of epicormic shoots: The clonal technology for mass production has significantly impacted the quality of the cuttings and the quantity of hormones used for rooting. The growth hormone application during the rooting will help enhance the rooting potential as an act of metabolic changes. Auxins also help mobilise the carbohydrates to the rooting zone (Husen and Pal 2007). The size of cuttings affects the rooting potential because the food material stored is directly related to the size of cuttings. The rooting potential or ability is directly influenced by the size of cuttings used in the propagation methods.

Effect of growth hormones in rooting of epicormics shoots: Auxins are widely recognised for fostering adventitious roots from vegetative propagules. Both exogenous and endogenous auxins affect the rooting potential and the number of roots produced in cuttings. The effect of exogenous growth hormone application on rooting has shown significant variation between the auxins IBA and NAA. NAA to inhibited the rooting of teak cuttings (Table 5). The highest rooting percentage was recorded in IBA 4000 mg/L (49.19%), followed by IBA 3000 mg/L (41.71%) and control (35.98%) (Plate 01d). Packialakshmi and Sudhagar (2020) observed similar results in teak shoots; IBA was found to increase the rooting potential compared to IAA and NAA. IBA at its highest concentration (6000 mg/L) was better compared to the lower concentrations. Rooting parameters such as root length, diameter, and rooting percentage

Table 5. Effect of exogenous growth hormones on rooting of juvenile epicormic shoots

Treatments	Rooting percentage (%)*	Callusing percentage (%)*	Root length (cm)	Number of roots
T1	35.98 (36.85) ^{c}	17.27 (24.48) ^{f}	9.00±2.05 ^{a}	2.17±0.76 ^{a}
T2	41.71 (40.23) ^{b}	27.98 (31.93) ^{e}	8.78±0.38 ^{a}	3.17±1.15 ^{a}
T3	49.19 (44.53) ^{a}	36.44 (37.11) ^{d}	8.08± 1.47 ^{ab}	3.06± 0.59 ^{a}
T4	5.61 (13.64) ^{e}	5.08 (12.86) ^{g}	1.33± 2.31 ^{c}	1.00± 0.58 ^{b}
T5	14.01 (21.97) ^{d}	5.78 (13.86) ^{g}	6.50± 2.00 ^{ab}	1.00± 0.58 ^{b}
T6	--	50.92 (45.52) ^{b}	--	--
T7	14.15 (22.08) ^{d}	42.87 (40.89) ^{c}	5.50±0.50 ^{b}	1.20± 0.00 ^{b}
T8	13.73 (21.03) ^{d}	55.26 (47.75) ^{a}	7.00±0.50 ^{ab}	1.80± 0.00 ^{b}
MSE	2.06	5.89	2.16	0.36
P (0.001)	0.00	0.00	0.00	0.00

* Values in parenthesis is square rooted transformed values

Table 6. Influence of shoot size on rooting of juvenile epicormics shoots

Shoot diameter class	Mean shoot diameter (mm)	Shoot length (cm)	Number of leaves	Rooting percentage (%)*	Root length (cm)	Number of roots
2.50-4.00 mm	3.43±0.41 ^{c}	8.03±0.60 ^{b}	5.39±0.98 ^{a}	50 (45.03) ^{b}	7.48±1.61 ^{b}	1.33±0.29 ^{b}
4.01-5.50 mm	4.79±0.34 ^{b}	12.27±2.66 ^{ab}	5.33±2.01 ^{a}	65 (53.87) ^{b}	10.94±0.74 ^{a}	2.06±1.11 ^{b}
5.51-7.00 mm	6.72±0.57 ^{a}	12.61±2.50 ^{a}	6.03±1.78 ^{a}	85 (67.40) ^{a}	11.83±1.09 ^{a}	3.83±0.45 ^{a}
MSE	0.205	4.564	2.819	37.401	1.445	0.503
P (0.05)	0.0003	0.0699	0.8569	0.0118	0.0098	0.0126

* Values in parenthesis is square rooted transformed values

increased with increasing IBA concentration compared to other auxins. These effect of exogenous application of IBA has been reported to increase the rooting in teak (Husen and Pal et al 2007, Husen 2013, Guleria and Vashisht 2014, Packialakshmi and Sudhagar 2019) and other tree species (Kala et al 2018, Pinon et al 2021, Olaniyi et al 2021). However, a very high concentration of IBA may result in mortality of shoots followed by necrosis. However, the concentrations tried in the experiment were not to these toxic levels.

NAA inhibited rooting and promoted callus formation at the cut ends in teak shoots. The cutting treated with NAA (T4-2000 mg/L, T5- 4000 mg/L) showed only 5.61% and 14.01% rooting. A similar inhibitory effect of NAA has been reported earlier (Gordon et al 2006, Kesari et al 2009, Packialakshmi and Sudhagar 2019). The highest callusing was observed in the combination of IBA and NAA 3000 mg/L (55.26 %), followed by IBA + NAA 1000 mg/L (50.92 %), IBA + NAA 2000 mg/L (42.87%), and IBA 4000 mg/L (36.44 %). The combination of IBA and NAA encouraged maximum callusing at a given period. The cumulative response percentage for rooting callus formation was highest in IBA 4000 mg/L (85.63%) followed by IBA 3000 mg/L, IBA + NAA 3000 mg/L, IBA + NAA 2000 mg/L , control (53.25%) (Fig. 1). The IBA+NAA @3000 mg/L recorded high percentile rooting as synergistic action. Similar inhibitory effects of NAA were reported in *Pongamia pinnata* (Kesari et al 2009) and *Ficus schlechteri* (Henselova 2002). The highest root length was observed in control and IBA treatments. The least root length was in NAA 2000 mg/L. The maximum number of roots were in IBA treatments, and the least was in NAA treatments.

Influence of Shoot size on rooting of epicormics shoots: The size of epicormic cuttings greatly influenced the rooting percentage, root length, and the number of roots. The epicormic shoots under the different size classes had significant variation in mean shoot length as the selection was made according to size class (Table 6). All root parameters were lower in smaller cuttings. The rooting percentage was highest (67.40%) in size class - 5.50 to 7.00 mm and least (45.03%) in size class 2.50 to 4.00 mm. Root biometric parameters such as root length and the number of roots were highest in 5.50-7.00 mm size cuttings (11.84 cm and 3.83 respectively) with an average shoot length of 12.61 cm. 4.01 to 5.50 mm size shoots with 10.94 cm root length, 2.06 cm number of roots and 12.27 cm shoot length (Plate 01c). The lesser shoot diameter was observed with the least root length and other root and shoot parameters.

The results of the present study are also supported by other research findings on different tree species (Kathiravan 2009, OuYang et al 2015, Okao et al 2016, Kala et al 2018, Olaniyi et al 2021). Cutting diameters has shown a significant effect on the root regeneration and shoot formation in teak (Guleria and Vashisht 2014). Packialakshmi and Sudhagar (2019) reported maximum rooting in 3 to 5 mm diameter, 5 cm length teak cuttings. In *Picralima nitida,* cuttings of length >8 cm had higher rooting properties than 6 cm and lower (Olaniyi et al 2021). Kala et al (2018) recommended using 25mm diameter semi-hardwood stem cutting with 15 cm length for propagation in *Pongamia pinnata* compared to the smaller sizes. The size class effects are attributed to the carbohydrate stored in cuttings and the number of leaves retained in cuttings. Leaves present in the cuttings help in carbon assimilation and hence affect the development of root and shoot structures.

Correlation analysis was used to study the relationship between the size of the epicormic shoot and the rooting parameter (Fig. 2). The rooting percentage showed a significantly positive correlation with shoot diameter (r=0.89) and was not affected by the number of leaves. The number of

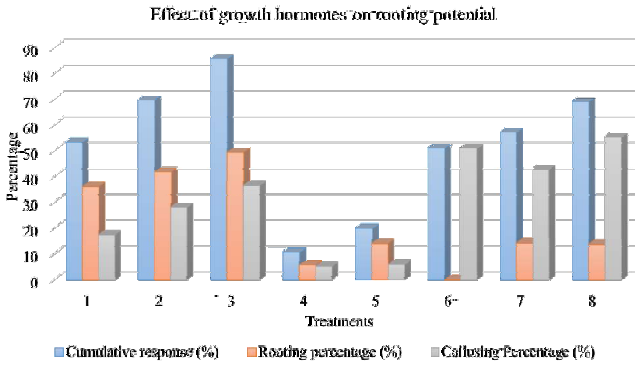

Fig. 1. Effect of exogenous growth hormone on rooting potential

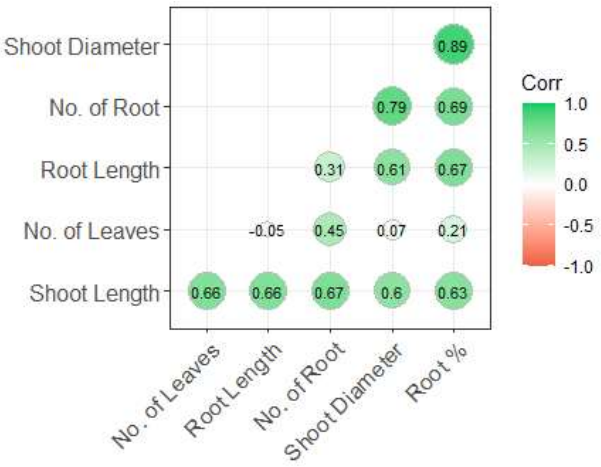

Fig. 2. Relation between the shoot parameters and rooting character

roots positively correlated with shoot diameter and shoot length . The number of leaves had no relation to rooting. A similar relation between the shoot character and rooting potential was reported in earlier studies (Kathiravan 2009, OuYang et al 2015).

The study indicates that the induction of juvenile shoots on branch cutting is more efficient for the vegetative propagation of matured trees. Under mist chamber, the average number of epicormic shoots production was 12 per branch. The untreated and lower concentration of IBA was on par and found to have no effect on epicormic shoot production on branch cuttings, whereas NAA alone and in combination with IBA had negatively influenced the shoot production. The study also supports the use of larger cuttings

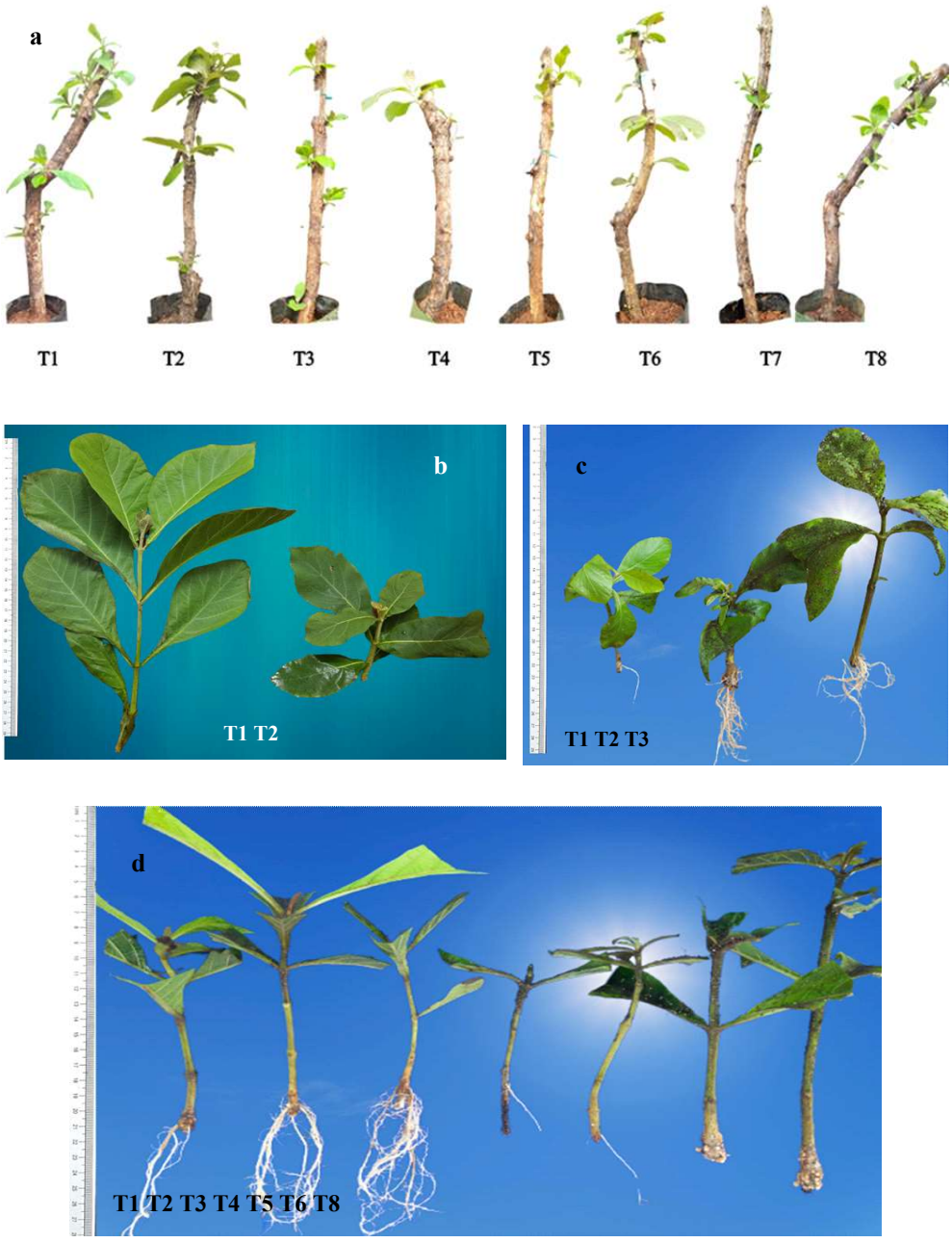

Plate 1. Production and Rooting of epicormic shoots; a. Influence of exogenous growth hormone on shoot production (25th day after planting), b. Effect of growing condition on juvenile shoots- (T1) inside mist chamber and (T2) open shed condition (cuttings taken on 55th days after planting), c. Influence of shoot size on rooting of juvenile Epicormics shoots- T1 (2.50 - 4.00 mm), T2 (4.00 -5.50 mm), and T3 (5.50 - 7.00 mm) (45th day after treatment), d. Effect of exogenous growth hormones on rooting of juvenile epicormic shoots (55th day after treatment)

(>4 mm) together with exogenous application of IBA (@ 3000mg/L and more) for successful rooting of the excised shoots. There is scope for developing specific studies on the shoot production from branch cuttings to determine the best season with compatible hormonal studies, nutrient management for maximising the production, physiology of bud dormancy release, and anatomical and ontogenetic studies.

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Morphometric and Reproductive Phenophases in *Bauhinia* Species

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***Abstract*:** The present paper deals with the morphometric and reproductive phenophases of two important tropical tree species, namely, *Bauhinia variegata* and *Bauhinia purpurea*. The study was carried out on nine trees selected from Punjab Agricultural University, Ludhiana and Dr. YS Parmar UHF Nauni, Solan. Phenophases like leaf bud swell, leafing, opening of bud, and flowering characteristics were recorded in year 2018-19. Phenophases were species-specific and dependent on the research area's environmental and meteorological circumstances. The present investigations revealed that mean days of leafing, bud opening, and leaf bud swell remains were similar in both species, but leaf area (cm^2) was higher in *B. purpurea* than in *B. variegata*. The mean days of flowering were different in both species. Petal length and breath, filament length, were all higher in *B. purpurea* species, and the maximum anther dehiscence was observed between 7.30 and 8.30 am in selected genotypes. These discoveries are important for botany, especially in the fields of forestry and ecology, where they will help with work to improve genetics.

Keywords: *Bauhinia variegata*, *Bauhinia purpurea,* Phenophases, Reproductive biology

Bauhinia L. is an extremely variable genus of shrubs, medium-sized and large trees of the family Caesalpinioideae, with a pan tropical distribution of about 600 species. It is native to South and Southeast Asia (China, India, Pakistan, Burma, Sri Lanka, and Nepal). In India, around 30 species are found and generally prevalent in the sub and outer Himalayas from the Indus River eastwards, ascending to an altitude of 1,830 m.s.l. in Assam, Burma and other parts of the Indian Peninsula. *Bauhinia variegata* is a moderately sized tree and the bark is ashy to dark brown in color, almost smooth. Dropsy, rheumatism, convulsions, insanity, and septicemia have all been treated using the complete *Bauhinia purpurea* L. plant. The plant *Bauhinia purpurea* L. may have anti-proliferative and antioxidant properties (Zakaria et al 2011). Various extracts of *Bauhinia racemosa* L. leaves have been investigated in developing a novel pharmaceutical medication to prevent enteric infection (Dahikar et al 2011). *B. purpurea* leaves are rich in nutrient content and are fed to lactating buffaloes, sheep, goats, and cattle, with crude protein content estimated at 12.6 per cent (Orva et al 2009). Taking into consideration their importance, it is important to study their morpho-metric and reproductive characteristics. Studies of phenology, in general, and blooming phenology, in particular, are important for establishing conservation strategies and creating methods for large-scale cultivation of such species (Bernardello et al 2001). The purpose of this study is to investigate the variation in leaves and flowers characters of *Bauhinia variegata* and *Bauhinia purpurea*.

MATERIAL AND METHODS

Five parent trees V_1, V_2, V_3, V_4, V_5 of *Bauhinia variegata* and two trees P_1, $P_2$of *Bauhinia purpurea* were selected at Dr. Yashwant Singh Parmar, University of Horticulture and Forestry, Nauni, Solan (Latitude: 30°51'N, Longitude: 76°11'E). The area experiences sub-tropical weather with relatively hot summers and cold winters (Fig. 1). The hottest months are May and June, while the coldest months are December and January. The site's mean annual rainfall is 1000–1300 mm/yr, with heavy rainfall during the monsoon. Two trees of *Bauhinia purpurea* $P_3$and P_4were selected by the Punjab Agricultural University, Ludhiana. The experimental site is located at an altitude of 247 m above mean sea level in the central zone of Punjab and lies between 30°-50'N latitude and 75°-52'E longitude. The climate of this area is sub-tropical to tropical and average annual rainfall of 700 mm per year.

The selection of superior trees was made on the basis of various morphological characteristics in the natural distribution region of Himachal Pradesh and Punjab. For their floral characters (floral bud swell, floral bud formation, flowering span), petal length and breath, stigma length, pollen size, leafing, *etc.* were observed. Three branches on each tree will be marked, and on each branch, five buds and

flowers were observed. Randomized block design was used to analyze data on phenotypic characteristics of *Bauhinia* genotypes. The significance of differences among the treatment means was tested by using SPSS software.

RESULTS AND DISCUSSION

Vegetative (leafing) characters: The mean number of days of vegetative bud opening, leaf bud swells and leafing was statistically on par with all the selected trees of *Bauhinia* species with a maximum in V_3(98.0 days) (Table 1). Blooming periods are significantly determined by the timing of vegetative phenology, according to a study of the proximal controls of flowering in tropical deciduous forest species, and therefore flowering is at least indirectly dependent on environmental periodicity (Rivera et al 2002). Leaf initiation in *T. bellirica, S. colorata,* and *C. arborea* started in April and in *B. variegata, S. villosa,* and *D. pentagyna* during May. Bud swell and bud burst are essential adaptation characteristics because they govern the tree's vegetative period's co-adoption to periodic variations in climatic components in the environment where it flourishes. Leafing is initiated during the dry-summer period and those species which can produce new leaves during the dry season depend on water stored in the tree stem or water remaining in the subsoil (Sayer & Newbery 2003). Moreover, deep rooting canopy trees do not experience a water deficit condition during dry season and can continue leaf-flushing activity. Seasonal duration of leafing, flowering and fruiting mainly determine phenological behavior in tropical trees. These phenological events are not mutually independent in woody species, and flowering may be partly or wholly dependent on leafing activity. The leaf area of certain genotypes showed that *Bauhinia purpurea* (96.9-99.3 cm^2) has a lot more leaf area than *B. variegata* (85.9-89.4 cm^2) (Table 1).

Floral Characters

Mean day flower character: Floral bud swell of different species was statistically significant with maximum days of reproductive bud swell in P_3 (67.8) and P_4 (68.6), which is on par with each other. Floral bud formation was significant with mean days for floral bud formation in P_1 (16.7), which are on par with species of *Bauhinia purpurea* but attain mean maximum days in comparison to *Bauhinia variegata* (Table 2). The flower bud development starts with the onset of physiological activity within the plant, and buds take a maximum of 16.7 days. There was significant difference between the flowering span of both the species. P_3(88.7) and P_4 (87.4) had the highest day flowering than remaining, which was statistically higher than the other parents. All species of

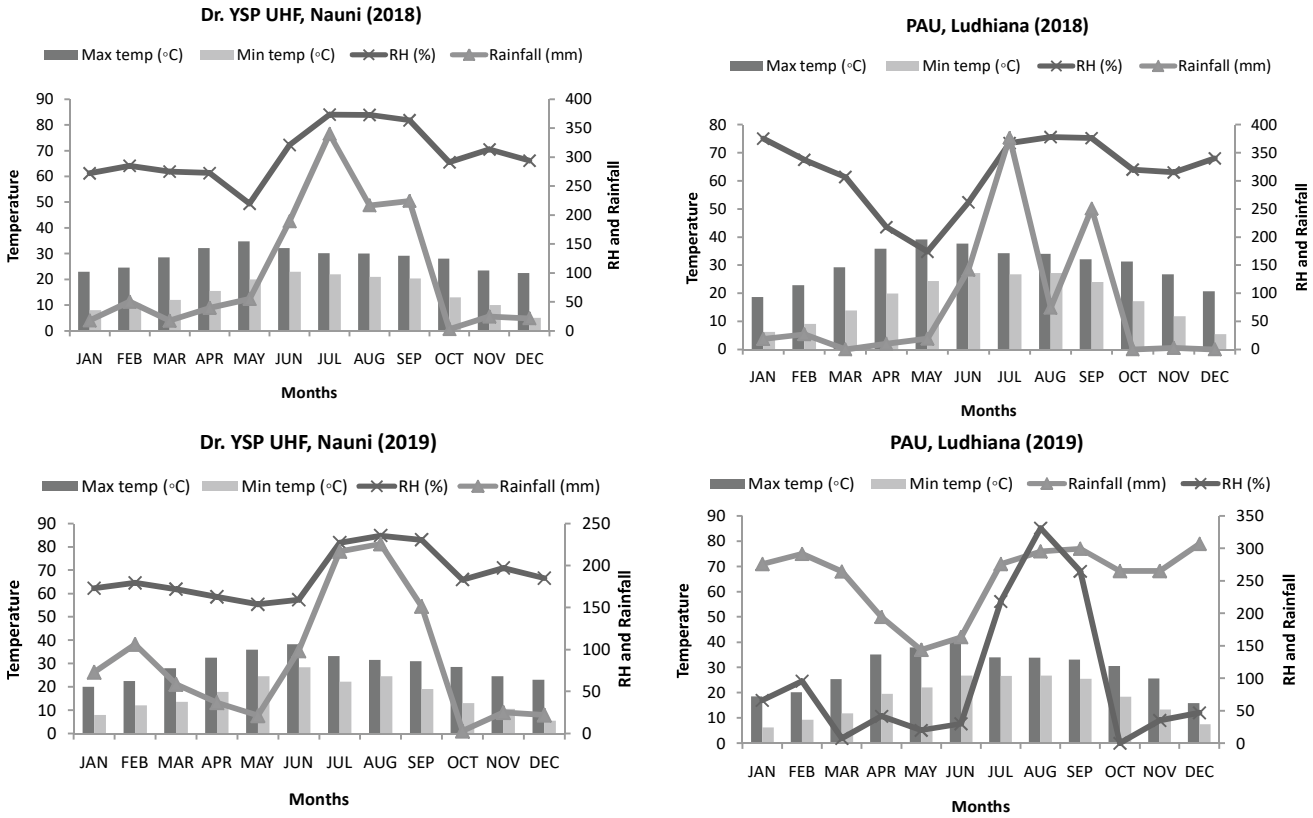

Fig. 1. Mean monthly meteorological data of Dr. YS Parmar UHF Nauni, Solan (HP) and PAU Ludhiana (Pb) for the year 2018-19

B. variegata remain at par; *B. purpurea* genotypes P_1 and P_2 also remain at par (Table 2). Flower initiation occurs during the dry season. This is in conformity with the report of Yadav and Yadav (2008) for dry deciduous forest trees. Flowering in the dry season indicates the availability of water from many sources, such as intermittent winter rainfall, soil absorption, and water retained in the stem (Singh and Kushwaha 2006). Other researchers have noted a peak blooming time prior to the rainy season (Kikim and Yadava 2001), and been suggested that moisture, temperature, and photoperiod are all factors influencing flowering (Pandey et al 2002). Pollen size varies statistically among different selected species. Maximum pollen size was obtained in V_5 (80.99 m), which is on par with V_1 (70.3 m) and V_2 (70.3 m), while minimum pollen size was in P_4 (54.6 m) (Table 2).

Flower length and breadth: Maximum flower length was in P_3 (9.53 cm), which is on par with all the rest of the species except V_2 (8.86 cm) and P_4 (7.55 cm). Higher flower breath was obtained in *Bauhinia purpurea, i.e.,* P_3 (50.5 cm), which is on par with all the other *B. purpurea* species and superior to other species of *B. varigeta* (Table 3).

Petal length (mm): Posterior petal length was significantly superior in all the genotypes of *Bauhinia purpurea* and P_3 was having maximum posterior petal length (50.5 mm) which was statistically at par with P_1, P_2 and P_3 (50.5 mm to 50.1 mm), whereas value of *Bauhinia variegata* ranges between 49.8 mm to 46.5 mm and V_5 was having minimum length. Anterior petal length 1 and 2 showed significant difference with maximum length in B. *purpurea* (53.9 mm, 51.1 mm) and *B. variegata* (44.1 mm, 42.8 mm), respectively. Lateral petal length 1 and 2 of both species was also differing significantly. Lateral petal length 1 has maximum value in V_2 (45.3 mm) which was at par with P_1, P_2, P_3 but superior than remaining genotypes. Lateral petal length 2 was higher in P_2 (44.8 mm) which were at par with P_1, P_3, but statistically more than remaining genotypes (Table 3).

Petals width (mm): Posterior petal width of all the parents were also statistical differ with each other with maximum width in P_1 (36.3 mm) which was at par with P_2, P_3 and higher than remaining selected genotypes. Anterior petal width 1 and 2 was significantly more in P_1 (30.8 mm), V_4 (26.6 mm) and lesser in V_1 (21.7 mm) and V_5 (22.9 mm), respectively.

Table 1. Duration of different vegetative phase in Bauhinia species (days)

Parents	Bud opening	Leaf bud swell	Leafing	Leaf area (cm^2)
V_1	4.07	95.2	97.8	86.8
V_2	4.17	93.2	95.5	85.8
V_3	4.07	94.8	98.0	86.9
V_4	3.80	97.5	95.8	89.4
V_5	4.10	96.1	97.1	86.7
P_1	4.03	95.9	97.8	96.7
P_2	3.93	95.9	95.5	96.9
P_3	4.03	98.4	90.7	97.5
P_4	3.97	95.1	95.7	99.3
CD (p=0.05)	NS	NS	NS	7.1

Table 2. Mean days for different floral parameter of *Bauhinia* species

Parents	Floral bud swell	Floral bud formation	Flowering span	Pollen size
V_1	36.9	15.8	41.3	70.3
V_2	37.6	14.5	41.5	70.3
V_3	38.8	15.8	40.2	64.7
V_4	44.2	15.1	40.9	62.7
V_5	38.3	15.3	40.3	81.0
P_1	37.7	16.7	55.7	57.5
P_2	39.2	16.1	54.2	60.4
P_3	67.9	15.7	88.7	57.7
P_4	68.6	16.6	87.4	56.5
CD (p=0.05)	4.92	1.22	3.43	14.0

Lateral petal width 1 and lateral petal width 2 was higher in P_1 (25.7 mm) and P_1 (24.8 mm) and lesser in V_5 (19.7 mm) and V_5 (19.5 mm) (Table 3).

Filament length (mm): Anterior filament length was more in P_2 (45.4 mm) which are at par with all the other species except V_3 (43.0 mm) and V_4 (42.9 mm). Posterior filament length 1 was statistically more in P_2 (35.5 mm) which was at par with P_1 and P_3 and inferior in V_4 (25.5 mm) and posterior filament length 2 was higher in P_3 (32.7 mm) that was at par with P_2, P_1 and least was in V_5 (24.2 mm). Lateral filament length 1 were more in V_4 (43.5 mm) which was at par with V_1, V_2, V_5 lesser in all the rest parents and lateral filament length 2 was more in V_5 (43.3 mm) that was at par with V_1 and V_2 (Table 3).

Floral Bud Diameter (mm)

Stage 1 to 3: At stage one reproductive bud diameter shows statistical significant differences with maximum in P_1 (2.69 mm) which was at par with P_4 (2.41 mm) and minimum in P_3 (2.21 mm). Stage two shows maximum bud diameter in P_1 (3.63 mm) which was at par with V_4 and P_3 and minimum bud diameter was in V_5 (2.87 mm) and stage three shows maximum bud diameter was in P_1 (4.54 mm), which was at par with P_2, P_4 and V_1 and least diameter in V_5 (3.92 mm) (Table 4).

Stage 4 to 6: Maximum value of bud diameter in stage four was in P_1 (7.58 mm) and least in P_2 (6.71 mm). In stage five, genotype V_1 (9.07 mm) was maximum bud diameter which was at par with V_2 and minimum in P_2 (7.66 mm) and at stage seven, bud diameter of V_3 (10.9 mm) and V_1 (10.9 mm) significantly superior to other parents (Table 4).

Stage 7 to 9: Bud diameter of P_3 (13.2 mm) was found to be more than other selected genotype in stage seven; minimum was in V_4 (12.0 mm). At stage eight maximum diameter of bud was in P_3 (15.3 mm) that was at par with V_3, V_2, V_1, P_2 and P_4

Table 3. Flower parameters of Bauhinia species

Parents	Flower size(cm)		Petals										Stigma (mm)		Anthers length(mm)				
			Length					Breath					Length	Breadth	A	P1	P2	L1	L2
	L	B	P	A1	A2	L1	L2	P	A1	A2	L1	L2							
V_1	9.04	10.00	46.8	44.1	45.7	41.3	41.7	23.3	21.7	23.8	20.5	20.1	40.6	2.6	44.1	26.3	25.6	43.1	42.4
V_2	8.86	10.80	48.1	44.9	45.8	45.3	41.5	21.7	22.8	23.7	23.2	20.3	39.6	2.5	44.5	26.2	25.9	42.1	42.2
V_3	9.17	9.95	47.8	45.2	44.6	44.0	43.5	28.1	27.0	26.1	24.1	23.1	40.4	2.6	43.0	25.6	25.8	41.0	41.5
V_4	9.35	10.50	49.8	45.7	45.0	43.4	42.9	27.6	26.9	26.6	25.4	23.0	39.6	2.8	42.9	25.5	24.9	43.5	41.6
V_5	9.11	10.10	46.5	42.1	42.8	40.3	38.7	25.5	24.0	22.9	19.7	19.5	40.5	2.5	43.2	26.0	24.2	43.3	43.3
P_1	9.07	9.48	50.1	53.5	50.2	44.9	44.7	36.3	30.8	25.9	25.7	24.8	43.4	1.9	45.1	35.3	32.5	32.1	32.9
P_2	9.22	9.58	50.4	53.9	50.1	44.6	44.8	35.9	29.2	25.7	25.5	24.3	43.7	2.0	45.4	35.5	32.6	32.1	33.2
P_3	9.53	9.60	50.5	53.9	49.5	44.5	44.3	35.6	28.9	25.0	25.3	24.6	44.1	2.0	45.2	35.4	32.7	32.3	33.2
P_4	7.55	9.20	50.3	51.1	46.8	42.5	42.4	27.1	26.7	23.7	23.9	21.1	38.5	2.20	44.2	30.2	29.3	37.5	37.3
CD (p=0.05)	0.64	0.65	1.37	2.33	2.59	1.12	1.38	1.65	2.62	1.87	2.28	2.98	2.86	0.32	1.44	1.37	2.15	2.84	2.60

Table 4. Floral bud diameter at different developmental stages of bauhinia species

Parents	Diameter of buds at different stages								
	Stage-1	Stage-2	Stage-3	Stage-4	Stage-5	Stage-6	Stage-7	Stage-8	Stage-9
V_1	2.48	3.52	4.29	7.08	9.07	11.0	12.2	14.9	18.0
V_2	2.40	3.33	4.07	6.77	8.90	11.0	12.2	14.9	18.0
V_3	2.34	3.42	4.22	6.85	8.21	9.60	12.1	15.1	18.2
V_4	2.27	3.57	4.24	6.91	8.00	9.59	12.0	15.1	18.1
V_5	2.23	2.87	3.92	7.01	8.16	10.2	12.1	14.0	18.0
P_1	2.69	3.63	4.54	7.58	8.45	9.72	11.9	14.0	16.3
P_2	2.23	3.42	4.48	6.71	7.66	9.90	12.9	14.9	18.1
P_3	2.21	3.29	4.52	6.87	8.03	10.2	13.2	15.3	18.1
P_4	2.41	3.50	4.32	7.11	8.63	10.1	12.2	14.8	17.6
CD (p=0.05)	0.21	0.20	0.25	0.31	0.42	0.40	0.33	0.49	0.59

were superior to other genotypes. At stage nine maximum bud diameters was obtained in P_2 (18.1 mm), it was at par with V_1, V_3, V_4, P_3 (Table 4).

Changes in the timing, length, and synchronization of phenological episodes in tropical forests may be influenced by global climate change. Because tropical trees differ greatly in terms of adaptations to seasonal dryness and signals for bud break of vegetative and floral buds, are predicted to respond differently to variations in rainfall and temperature (Singh and Kushwaha 2005). As a result of climate change, several studies considerable variation (earlier or later) in blooming beginning dates (Fitter and Fitter 2002) and fruiting responses (Chapman et al 2005) in tree species. Climate change impacts are likely to be best examined at the functional type level, depending on deciduousness length and reproductive phase beginning time (first-visible-flower).

Floral bud length (mm)

Stage 1 to 3: Bud length in first stage was found to be higher in P_1 (6.57 mm) than all other parents and minimum in V_4 (4.33 mm) which were at par with V_1, V_3 and P_2. In stage two, maximum bud length was obtained in V_1 (8.70 mm) and minimum in V_5 (7.39 mm) which was at par with each other and inferior then V_1. Higher bud length was found in stage three for genotype V_4 (12.6 mm) that was at par with all other parents except P_1, P_2 and P_3 (Table 5).

Stage 4 to 6: Stage four shows statistically more bud length in P_3 (23.0 mm) which was at par with V_2 and minimum was found in P_1 (19.5 mm). Maximum bud length was reported in stage five for genotype V_1 (35.1 mm) which was at par with V_2 and P_3, minimum in P_2 (34.9 mm). Stage six shows that maximum value of bud length in V_2 (47.7 mm) and at par with V_1, statistically superior to all other parents, and minimum bud length was found in V_3 (43.5 mm).

Stage 7 to 9: Stage seven shows maximum value of bud length in P_3 (58.6 mm) that was at par with P_2, and minimum in V_3 (54.20mm). Stage eight shows maximum value of bud length in P_3 (69.2 mm) which was at par with P_2, minimum value was found in V_3 (63.2 mm). Stage nine has maximum value of bud length in V_1 (81.9 mm), and was at par with all the species except P_1(Table 5).

Several phenological studies have concluded that changes in water availability from shifts in precipitation regimes and soil moisture are the essential proximate causes affecting phenological patterns. Tree species with similar leaf phenology often differ in the timing of their flowering and fruiting. Many deciduous tree species show flowering and fruiting during the leafless period, exhibiting wide separation between leafing and flowering phenophases. In many evergreens and in some deciduous species leaf flush and flowering occur close in time on the same new shoot.

Phenophase: Time Period for floral and vegetative bud swell and burst, leafing, fruiting, senescence and seed maturity of studied species are presented (Figure 2). Phenological characters of *Bauhinia variegata* and *Bauhinia purpurea* presented in Table 6.

Bauhinia Variegata: Bark color of this species was grey and flower color was whitish with mostly purple tinge. Its pollen vector was honey bees, ants; moth etc. Seed color of *B. variegata* was grayed orange. Its flower opening time was 6-9 am. Anther dehiscence time was 6.30 -9.30 am. Pod color was dark brown.

Bauhinia purpurea: Bark color of this species is pale grey brown and flower color was purple to white. Its pollen vector was honey bees, ants, bumble bees etc. Seed color was grayed orange. Its flower opening time was also 6-9 am. Anther dehiscence time is 7.00 -10.30 am. Pod color was dark brown.

Table 5. Floral bud length at different developmental stages of bauhinia species

Parents	Length of reproductive buds at different stages								
	Stage-1	Stage-2	Stage-3	Stage-4	Stage-5	Stage-6	Stage-7	Stage-8	Stage-9
V_1	4.8	8.7	12.3	22.2	35.1	47.5	55.1	64.0	81.9
V_2	5.0	7.6	12.1	22.2	34.9	47.7	55.5	65.4	81.7
V_3	4.5	7.6	12.4	21.4	32.2	43.5	54.2	63.2	81.5
V_4	4.3	7.9	12.6	22.0	32.5	43.8	54.3	65.0	81.3
V_5	4.3	7.4	12.0	21.5	33.0	45.1	55.6	65.4	80.5
P_1	6.6	7.8	11.5	19.5	31.6	43.7	54.3	66.0	75.3
P_2	4.5	7.6	11.8	22.0	33.3	44.4	58.2	69.1	81.7
P_3	4.6	7.7	11.9	23.0	34.3	45.2	58.6	69.2	81.0
P_4	4.9	7.7	12.1	21.0	33.2	44.8	55.0	64.1	80.1
CD (p=0.05)	0.5	0.4	0.5	0.8	1.2	1.7	1.6	1.0	1.5

Flowering and fruiting occur during the leafless period in many deciduous tree species, with a large gap between the leafing and flowering phenophases. In many evergreen and deciduous plants, leaf flush and blooming occur at the same time on the same young stem. The main pollinators for cross-pollination in *Bauhinia variegata* are Bumbus spp. and *Metasyphris conferato*r. Controlled crosses between *B. variegata* and *B. purpurea* resulted in maximum fruit and seed set after 8 hours of anthesis and developed into viable seedlings. Broeck et al (2003) observed that *Populus nigra* x *P. canadensis* and *Maughania macrophylla* x *M. chappar*. Anthesis brings about exposure of anthers and stigma to the pollen vector. Flower ordinarily opens and closes at definite hours. The present investigations revealed that the dehiscence of anthers took place between 6:30 to 9:30 am in a longitudinal fashion, maximum anther dehiscence was observed between 7.30 to 8.30 am in selected genotypes. Variation in flowering time relative to vegetative phenology, induced by a variety of factors (significant rain in winter/summer, decreasing or increasing photoperiod, or drought-induced leaf fall), results in a number of flowering patterns in tropical trees (Borchert et al 2004). In *Dalbergia sissoo*, Chauhan et al (2004) observed that stigma become responsive a few hours prior to anthesis and stay receptive a few hours following anthesis. Wani (2008) discovered anther dehiscence in the morning in *Bauhinia variegata*. Aguiara et al (2016) observed that anther dehiscence in *Cenostigma macrophyllum* occurred about 9 a.m. In Senna cana Torres et al (2008), the timing of anthesis was found to be approximately 7am. Wani and Chauhan (2008) in *Bauhinia*

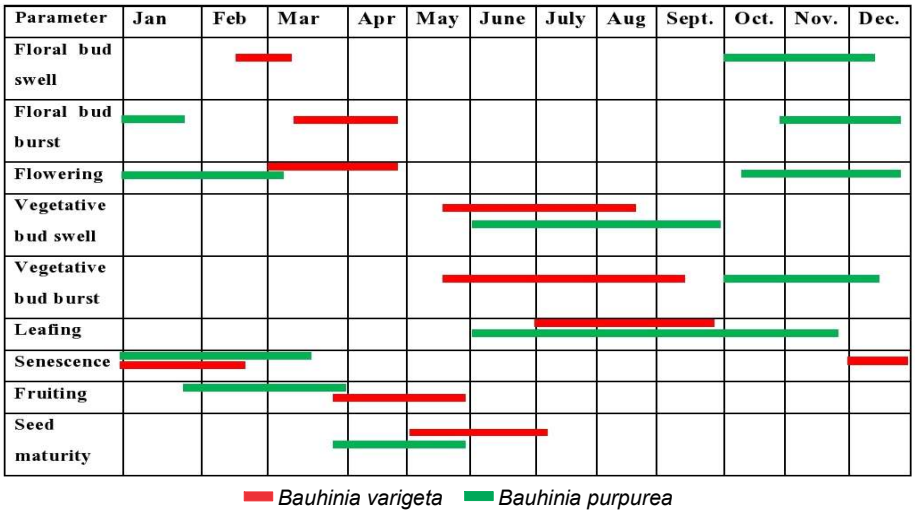

Fig. 2. Phonogram showing different phenophases in *B. variegata* and *B. purpurea*

Table 6. Various phonological characters of Bauhinia species

Parameters	*Bauhinia variegata*	*Bauhinia purpurea*
Bark color	Grey and smooth to slightly rough	Pale grey brown
Flower color	Whitish with purple tinge	Purple to nearly white or at least purple marked
Pollen size	50-60 um	55-65 um
Pollen vectors	Honey bee(Apics sps), ant, bumble bees, moth etc	Honey bee (Apices sps.), ant, bumble bees, moth etc
Seed color	Greyed orange gp. 162b to 163 c	Greyed orange gp.164b
Flower type	Entomophilous	Entomophilous
Odour	Present	Present
Flower opening time	6-9 am	5.30-9 am
Anther dehiscence time	6.30-9.30 am	6 -10.30 am
Stamens	5 (2+2+1)	5 (2+2+1)
Anther dehiscence mode	Longitudinal	Longitudinal
Pollen shape	Triangular with 3 apertures	Prolate-spheroidal, iso-pollar, tricolporate
Gynoecium	Monocarpellary, unilocular stalked, superior ovary	Monocarpellary, unilocular stalked, superior ovary
Seed dispersal	2-8 days after maturity	2-8 days after maturity

variegata and Smitha and Thondaiman (2016) observed that anthesis in *Saraca asoca* (Roxb.) occurred between 3.00 and 5.00 in the morning.

CONCLUSION

This study can uncover phenological patterns of examined species and give valuable insights into the biology of the plants involved. Maximum petal length and breath, filament length, and stigma length were all in *B. purpurea*. This study also showed the timing of vegetative and reproductive characters. It also provided a difference in seed color, flower color, bark color, pollen vectors, etc. The information from these studies is important for genetic improvement work in botany, especially in the fields of forestry and ecology.

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Nursery Performance of *Murraya koenigii* from Seed in Relation to Potting Media and Hormonal Treatment of Branch Cuttings

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***Abstract*:** Two experiments were laid out to study the effect of potting media and hormonal treatment on nursery performance using seed and branch cuttings respectively in *Murraya koenigii*. In the first experiment, four potting media- pure soil, soil: sand (1:1), soil: sand :FYM (1:1:1) and soil: sand :vermicompost (1:1:1) were used in five replications. In the second experiment, the stem cuttings were taken from healthy plants and treated with thirteen hormonal concentrations: IBA (500, 1000, 1500 and 2000 ppm), NAA (500, 1000, 1500 and 2000 ppm), IBA (500, 1000, 1500 and 2000 ppm) and control (no treatment). This experiment had three replications. The seeds sown in soil: sand: vermicompost (1:1:1) in mid July showed significantly higher germination percentage, germinative energy, survival percentage, collar diameter, number of leaves, length of primary root, fresh shoot weight, fresh root weight, dry weight, root:shoot ratio and seedling quality index. The cuttings treated with treatment IBA 2000 ppm had significantly higher above ground and belowground parameters. The study implies that *Murraya koenigii* can be either regenerated in nursery through seed sowing in mid July using potting mixture of soil: sand: vermicompost (1: 1: 1) and also be propagated by planting branch cutting in first week of August treated with IBA 2000 ppm.

***Keywords*:** Propagation, Germination, Rooting, Growth, Planting stock

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial Recent estimates suggest that over 9,000 plants have known medicinal applications in various cultures and countries (Gopal et al 2014). The medicinal and aromatic crops are now occupying a significant role in Indian agriculture. *Murraya koenigii* Spreng is one such plant that has gained immense attention over the years. *Murraya koenigii*, commonly known as curry leaf or karipatta in Indian dialects, belongs to family Rutaceae and is well known for its characteristic aroma and medicinal value (Jagadeeshkanth et al 2017). *Murraya koenigii* is well distributed in tropical and subtropical areas of Sri Lanka, Malaysia, Indonesia, Southern China and India (Dahlia et al 2017). It is also common in subtropical parts of Jammu and Kashmir. This plant adorns every house yard of Southern India. Curry leaf is a popular leaf-spice used in very small quantity for their distinct aroma due to the presence of volatile oil and their ability to improve digestion. Curry leaf is widely used in Indian cookery for flavouring foodstuffs. The leaves have a slightly pungent, bitter and feebly acidic taste, and they retain their flavour and other qualities even after drying. Curry leaf is also used in many of the Indian Ayurvedic and Unani prescriptions (Singh et al 2014). Leaves and roots are traditionally used as analgesic, curing piles, inflammation, itching and are useful in leucoderma and blood disorders.

In spite of having lots of medicinal values and good revenue, not much work has been done on its propagation in order to produce quality planting material Therefore, the present study was undertaken to standardize the propagation of *Murraya koenigii* through seeds in different potting media and branch cuttings treated with different hormones to obtain its quality planting material for its artificial regeneration.

MATERIAL AND METHODS

The study was carried out in homogeneous conditions in the open nursery conditions of Division of Agroforestry, Sher-e-Kashmir University of Agriculture Sciences and Technology of Jammu (SKUAST-J) located at Chatha, Jammu, India. The experimental site is situated at an altitude of 332m above msl at 32^0 40' N latitude and 74^0 58' E longitudes. The experimental site falls in subtropical zone.

Seed collection, extraction, preparation of potting media and seed sowing: The disease-free plants of *Murraya koenigii* were identified and marked after an initial survey. Ripened fruits (berries) were collected in July 2019. The berries were brought to the laboratory and the extraction of seeds was done manually by removing the mucilaginous substance by squeezing in water and then washing with water three to four times. Soil for media preparation was taken from the nursery. The soil was sandy loam. Soil, sand, well decomposed farm yard manure (FYM) and vermicompost (VC) were mixed in proper proportions to get the requisite media (treatments), pure soil (soil only),

soil:sand (1:1), soil:sand:FYM (1:1:1) and soil: sand: vermicompost (1:1:1). There were five replications in this experiment. The experiment was laid in CRD design. Two seeds per polybag (16cm x 24cm) were sown uniformly to a depth of one cm in each treatment. The polybags were watered and weeded regularly per requirement. Germination parameters (number of days taken for germination, germination percentage, survival percentage and germinative energy) were estimated since the beginning of experiment and remaining parameters [number of leaves, average shoot length (cm), collar diameter (mm), primary root length (cm), total seedling fresh weight (g), fresh shoot weight (g), dry shoot weight (g), fresh root weight (g), dry root weight (g), root: shoot ratio (dry weight basis), sturdiness quotient and seedling quality index] were recorded after 90 sowing. Sturdiness quotient was calculated by using the formula given by Chauhan and Chauhan (1997) and seedling quality index was estimated using the formula given by Dickson et al (1960).

Collection, preparation and treatment of branch cuttings: Branches from the plants already marked for seed collection were harvested in first week of August 2019. Cuttings of length 12-15 cm with at least 3-4 buds were immediately prepared from these branches. The prepared cuttings were bundled in the sets of 15 in order to dip in 13 different treatments (3 hormones each at 4 concentration levels viz. 500, 1000, 1500 and 2000 ppm and one control). Thereafter, basal portion of each bundle of cuttings was dipped in requisite concentrations of rooting hormones for 10-15 seconds (a quick dip method) and were planted 7-8 cm deep in the polythene bags of 16cm x 24 cm containing field soil. The soil was sandy loam with 7.81-pH. A light overhead watering was given immediately after planting so that cuttings get settled. Afterwards, the cuttings were irrigated as per requirement. Weeding was done at equal interval with proper care without disturbing the planted cuttings. The experiment was conducted in Completely Randomized design (CRD) with 3 replications. The growth and development characteristics of stem cuttings were recorded after 90 days from their planting by uprooting the whole plant except sprouting percentage, which was recorded after 45 days from planting. The observations included: sprouting percentage, number of shoots, average shoot length (cm), number of leaves per plant, number of lateral roots, root length (cm), fresh root biomass (g) and dry root biomass (g).

The dry weight of the roots was recorded using digital electronic balance by drying the roots separately in the oven at 60°C for 48 hours and weighing the dried samples.

Data analysis: The data of both the experiments were analysed using the technique of variance (ANOVA) in accordance with procedure outlined by Gomez and Gomez (1984). The effect of different treatments was tested at 0.05 level of significance. The percentage data were transformed using angular transformation.

RESULTS AND DISCUSSION

Germination parameters and survival per cent: All the germination parameters (germination percentage, number of days taken for germination and germination energy) were significantly influenced by the potting media (Table 1). The survival percentage was also significantly influenced by the potting media (Table 1).

The treatment soil:sand:vermicompost (1:1:1) exhibited highest seed germination (96.00%), minimum number of days taken for germination (13.40 days) and maximum germinative energy (52.00%) (Table 1). The value of germination percentage and germination energy in treatment soil: sand: vermicompost (1:1:1) was statistically as par with respective values in soil: sand: FYM (1:1:1) but superior to soil only and soil: sand (1:1) respectively. In case of survival per cent, the value in soil:sand:vermicompost (1:1:1) was statistically superior to all the remaining treatments (Table 1). The numbers of days taken for germination was minimum in soil:sand:vermicompost (1:1:1) which was statically as par with soil: sand: FYM (1:1:1) but inferior to soil only and soil:sand (1:1), respectively.

Table 1. Effect of different potting media on germination, days taken to germination*, germination energy and survival percentage*

Treatments	Germination (%)	Number of days taken for germination	Germination energy (%)	Survival (%)
Soil only	90.00 (73.54)*	16.2	34.00	86.68 (68.78)*
Soil:Sand(1:1)	78.00 (64.87)*	18.4	14.00	79.45 (63.24)*
Soil:Sand:FYM (1:1:1)	94.00 (78.92)*	14.0	46.00	95.78 (78.71)*
Soil:Sand: VC(1:1:1)	96.00 (82.61)*	13.4	52.00	98.00 (82.41)*
Mean	89.50 (72.48)*	15.5	36.50	89.97 (73.28)*
CD (p=0.05)	11.31 (9.21)*	1.2	10.90	7.39 (10.71)*

*Figures in parenthesis are transformed (angular) value

Above ground parameters: The treatments (potting media) exhibited significant effect on all the studied aboveground seedling parameters (shoot length, collar diameter, number of leaves per plant, number of leaves, fresh shoot weigh and dry shoot weight) (Table 2).

The media soil: sand: vermicompost (1:1:1) had the maximum value for parameters- shoot length (14.05 cm), collar diameter (2.42 g), number of leaves (10.6), fresh shoot weight (2.01 g) and dry shoot weight (0.92 g). Shoot length and number of leaves in soil: sand: vermicompost (1:1:1) were statistically at par with the respective values in soil: sand FYM (1:1:1) and soil only but statically superior to soil:sand(1:1). In case of collar diameter, fresh shoot weight, the values in soil: sand: vermicompost (1:1:1) were statistically at par only with the respective values in soil: sand: FYM (1:1:1) but superior to both soil only and soil: sand (1:1). The dry shoot weight in soil: sand: vermicompost (1:1:1) was statistically superior to all the remaining treatments (Table 2).

Below ground parameters: Table 3 shows that the effect of treatments was significant on length of primary root, fresh root weight and dry root weight.

The maximum root length (19.1 cm), fresh root weight (2.70 g) and dry root weight (1.39 g) were recorded in soil: sand: vermicompost (1: 1: 1). The values of length of primary root, fresh root weight and dry root weight were statically superior to the respective values in the remaining treatments (Table 3).

Whole seedling parameters: The potting media significantly influenced all of the whole seedling quality parameters except the sturdiness quotient (Table 4).

The maximum values of total seedling fresh weight, total seedling dry weight, root: shoot ratio and seedling quality index were observed in soil: sand: vermicompost (1:1:1) which were statistically superior to respective values in all other treatments (Table 4).

Effect on aboveground parameters of branch cuttings: The effect on hormonal treatment was significant on sprouting per cent, shoot length and number of leaves (Table 5). However, the effect on number of shoots was non-significant. The treatment IBA 2000ppm had the highest sprouting per cent (56.48%) which was statistically higher than all the remaining treatments. The values of sprouting per cent, shoot length and number of leaves were higher in IBA (500, 1000, 1500 and 2000 ppm) than the respective values in respective concentrations of NAA and IBA (Table 5).

Table 3. Effect of different potting media on belowground seedling parameters

Treatments	Length of primary root (cm)	Fresh root weight (g)	Dry root weight (g)
Soil only	15.7	0.85	0.39
Soil: Sand(1:1)	10.6	0.50	0.26
Soil: Sand: FYM (1:1:1)	17.3	1.35	0.82
Soil:Sand: VC(1:1:1)	19.1	2.70	1.39
Mean	15.7	1.35	0.71
CD (p=0.05)	0.5	0.25	0.05

Table 2. Effect of different potting media on aboveground parameters of seedlings

Treatments	Shoot length (cm)	Collar diameter (mm)	Number of leaves plant^{-1}	Fresh shoot weight (g)	Dry shoot weight (g)
Soil only	12.46	2.04	8.6	1.50	0.55
Soil: Sand(1:1)	10.36	1.63	6.2	0.70	0.36
Soil: Sand: FYM (1:1:1)	13.45	2.29	9.2	1.91	0.75
Soil: Sand: VC(1:1:1)	14.05	2.42	10.6	2.01	0.92
Mean	12.58	2.04	8.65	1.40	0.65
CD (p=0.05)	1.67	0.24	2.3	0.31	0.04

Table 4. Effect of different potting mixture on whole seedling parameters

Treatments	Total seedling fresh weight	Total seedling dry weight	Root: shoot ratio (dry weight basis)	Sturdiness quotient	Seedling quality index
Soil only	2.35	0.92	0.71	13.88	0.006
Soil:Sand (1:1)	1.20	0.61	0.73	12.94	0.004
Soil:Sand:FYM (1:1:1)	3.26	1.55	1.08	13.58	0.011
Soil:Sand: VC(1:1:1)	4.71	2.34	1.51	13.72	0.016
Mean	2.79	1.33	1.00	13.53	0.009
CD (p=0.05)	0.43	0.05	0.07	NS	0.002

Effect on belowground parameters of branch cuttings: Rooting percentage, number of lateral roots and root length varied significantly with hormonal concentration (Table 6). The highest rooting percentage (40.19) and highest number of lateral roots (18.20) were recorded in IBA 2000ppm which were statistically superior to all the remaining hormonal treatments (Table 6). The average root length was also highest (4.47 cm) in IBA 2000ppm which was statistically at par with IBA1500ppm and IBA1000ppm but superior to remaining hormonal treatments. The values of rooting per cent, number of lateral roots and root length were higher in IBA (500, 1000, 1500 and 2000 ppm) than the respective values in respective concentrations of NAA and IBA (Table 6).

Effect of potting media on germination and growth of seedlings: The germination parameters (germination percentage, germination energy and survival percentage), above ground parameters (shoot length, collar diameter, number of leaves, and fresh and dry shoot weight) and below ground parameters (length of primary root, fresh root weight, dry root weight) and whole seedling parameters (total seedling fresh weight, total seedling dry weight, root: shoot ration and seedling quality index) and whole seedling parameters (total seedling fresh and dry weights, root: shoot ratio and seedling quality index) in soil:sand: VC (1:1:1) were

Table 5. Effect of hormonal applications on aboveground parameters of branch cuttings

Treatments	Sprouting percentage	Number of shoots	Shoot length(cm)	Number of leaves
IBA500ppm	22.09 (28.02)	1.600	5.21	6.43
IBA1000ppm	38.81 (38.51)	1.967	5.94	8.93
IBA1500ppm	46.30 (42.86)	3.200	6.17	9.30
IBA2000ppm	56.48 (48.70)	2.967	7.07	13.33
NAA500ppm	20.00 (26.54)	1.667	4.14	5.13
NAA1000ppm	25.56 (30.35)	2.273	4.36	5.43
NAA1500ppm	29.89 (33.12)	1.527	4.76	5.80
NAA2000ppm	33.34 (35.25)	2.010	5.01	6.10
IAA500ppm	19.76 (26.37)	1.700	4.10	5.00
IAA1000ppm	24.67 (29.77)	2.067	4.28	5.53
IAA1500ppm	28.67 (32.35)	2.097	4.75	5.67
IAA2000ppm	32.64 (34.83)	1.670	5.05	6.03
Control	8.78 (17.22)	0.00	0.00	0.0
CD (p=0.05)	2.11 (1.39)	NS	0.45	0.87

Table 6. Effect of hormonal applications on belowground parameters of branch cuttings

Treatments	Rooting Percentage	Number of lateral roots	Root length (cm)
IBA500ppm	18.78 (25.66)	9.83	2.95
IBA1000ppm	23.67 (29.10)	12.17	3.50
IBA1500ppm	31.11 (33.89)	15.43	3.93
IBA2000ppm	41.67 (40.19)	18.20	4.47
NAA500ppm	7.69 (16.06)	3.80	1.23
NAA1000ppm	12.39 (20.59)	5.90	1.60
NAA1500ppm	14.80 (22.61)	6.87	1.94
NAA2000ppm	16.67 (24.07)	7.07	2.17
IAA500ppm	6.97 (15.29)	3.73	1.16
IAA1000ppm	12.11 (20.35)	5.77	1.58
IAA1500ppm	14.39 (22.28)	6.47	1.88
IAA2000ppm	16.34 (23.83)	7.00	2.08
Control	0.00 (0.00)	-	-
CD (p=0.05)	1.69(1.38)	1.82	1.04

superior to remaining potting media. Similarly, the number days taken for germination were lowest in soil:sand:VC (1:1:1), which was lower than remaining potting media. This implies treatment soil:sand:VC (1:1:1) was overall superior with respect to germination parameter, above ground, below parameters. Mugloo et al (2015), Sood et al (2018), Ram and Sood (2019) also reported similar findings in respective seedlings of *Picea smithiana, Oroxylum indicum* and *Terminalia bellirica*. The highest germination parameters (germination per cent, germination energy and survival per cent in soil:sand:VC (1:1:1) in the current study could be due to fact that media containing soil:sand:VC (1:1:1) could have higher aeration porosity and water holding capacity which ultimately increases the speed of seed emergence (Bharadwaj 2013). Speady and early emergence of seedlings in Soil:Sand: VC (1:1:1) might have provided longer period of growth in this media leading to better performance of seedlings. Further, vermicompost possesses higher nutrient content than FYM (Sheikh and Dwivedi 2017) and also reported to have bioactive principles, which are considered to be beneficial for root growth, root initiation, germination and growth of plants (Zaller et al 2007). Ram (2017) found higher contents of available N, P and K in soil:sand:VC (1:1:1) than soil:sand:FYM (1:1:1) and soil only respectively. All of this might have also accelerated the growth of seedlings in soil:sand:VC (1:1:1) in the present study.

Effect of hormonal treatment of growth and development of branch cuttings: The above ground parameters (sprouting per cent, number of shoots, shoot length and number of leaves) were significantly affected by hormonal treatment. All the pre-said parameters in treatment IBA 2000ppm were significantly superior to remaining hormonal applications. The below ground parameters namely rooting percentage and number of lateral roots was significantly higher in treatment of cuttings with IBA 2000ppm than the remaining treatments. However, root length in IBA 2000ppm was highest but statically at par with IBA 1500ppm and IBA 1000ppm but statistically superior to remaining treatments. This also implies that overall IBA 2000 ppm is also superior in belowground parameters. Further, a majority of growth parameters had higher values in IBA (2000ppm, 1500 ppm, 1000ppm and 500pm) than in the respective concentrations of NAA and IAA. These results are consistent and corroborates with the findings of earlier workers, who demonstrated that IBA was most effective auxin in triggering rooting in stem cuttings than IAA and NAA (Babaie et al 2014). The role of IBA in improving both rooting percentage and development of roots have been reported by Hartman et al (2011). Amri et al (2010) reported similar findings in IBA treated African Blackwood (*Dalbergia melanoxylon*) cuttings that produced maximum number of roots. Rana and Sood (2012) also reported IBA to be more effective than NAA and IAA in inducing root and development of *Ficus roxburghii* branch cuttings. The overall better growth and development with IBA treatment in the current study could be because of its greater stability, transportability, ability to produce roots and consequently results in lower morality in plants in the current study. Further, this could also be attributed to the fact that growth hormones determine cell elongation and cell division thereby promoting roots length (Abidin and Baker 1984) and consequently resulting better overall growth of the cuttings.

CONCLUSION

The current study indicates potting media plays a significant role in germination and development of seedlings from seed. The hormonal concentration also influenced rooting and development of branch cuttings of *Murraya koenigii*. To obtain better growth germination and growth of plants from seed origin, potting media containing soil:sand:VC (1:1:1) should be used. The seedlings can also be obtained by treating branch cuttings IBA 2000 ppm.

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Species and Provenance Testing in India: Field Application

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***Abstract*:** Provenance in forestry refers to the original place of origin of seed/propagule or trees. Provenance research aims at defining the genetic and environmental components of phenotypic variability between trees from different geographical origins. However, it has great significance in introduction of the species for their testing at the new location. A wrong choice of species or provenance can lead to a big loss than benefit. This paper reflects some examples for successful choice for provenances.

Keywords: Forest area, Species, Provenances, Short rotation trees

The Forest Survey of India undertakes assessment of country's forest resources including trees outside forests after every two years. The salient features of ISFR, 2021 include a total forest cover of 7,13,789 sq km (21.71 % of the geographical area), and tree cover of 95728 sq. km (2.91 % of the geographical area).The Forest cover has increased by 1540 sq.km (0.22 %) and tree cover by 721(0.76 %) sq. km as compared to the previous assessment of 2019. The top five States in terms of forest area are Karnataka, Andhra Pradesh, Kerala, Jammu and Kashmir and Himachal Pradesh. However, the forest and tree cover are still well below the national target of 33%. The importance of this target was reinforced in a study commissioned by ICFRE, Dehra Dun. The study conducted a detailed review dating back to 1850 and concluded that based on ecosystem approach, the target of 33% forest area was justified. The study points out a forest area loss of 18.5 million ha during 1950-80 and presents alternative approaches on the subject. It recommends the ecosystem approach, which considers the dynamic interaction of forests with other system components, viz. abiotic, biotic, atmospheric and cultural factors. It then provides an assessment of land use and forest cover of the country from an ecosystem perspective in order to improve forest ecosystem services. Since 1980, the forests are managed under sustainability and conservation approach. India's forests have very low productivity. The increment of India's forests is less than 1 m^3ha^{-1} yr^{-1}as compared to the world average of 2.1 m^3 ha^{-1} yr^{-1} (Lal and Bhandari 2020). The total carbon stock in the forest is estimated as 7,124.6 million tonnes and soil organic carbon contribution is 56% of total carbon stock.

On the other hand, the growing stock of TOF is 1642 million m^3. This is due to very high increments (25-30 m^3 ha^{-1} yr^{-1}) of trees on farmers' field especially clonal plantations of eucalyptus, poplars, leucaena, dek, casuarinas and other species. The low productivity of India's forests underscores the importance of conserving forests and preventing degradation. To this end, trees outside forests particularly agroforestry points to the way forward. Agroforestry constitutes the major source (nearly 90 %) of timber required for industrial purposes- saw mills, ply and veneer mills, and paper mills (Pandey and Roy 2020). Under the Paris Agreement on Climate Change, India has committed to grow additional trees to capture 2.5-3.0 billion tonnes of carbon dioxide by 2030. In the short time available, TOF and particularly agroforestry has the potential to contribute significantly to achieve this nationally determined target. High productivity agroforestry plantations have the potential to sequester up to 60 t C/ha (IPCC, 2000) while emission avoidance via conservation through forest protection and management have a much higher carbon mitigation potential (4-252 and 41-102 t C/ha respectively).

Thus, greater emphasis on agroforestry may be the path to conserve forests and environment as also to meet India's wood requirements. Presently, as reported by Pandey and Roy (2020) imports of wood and wood products for the years 2016-19 amounted to Rs 48,099.9 crores (around Rs 10,000 crores per year). Bamboo import alone is around 400crores annually. This demand is growing rapidly with the rise in India's GDP and the general standard of living.

The tremendous increase in demand for wood in the form of pulp and panel products due to economic and industrial development has brought about a revolution in forestry. With limited availability of wood from natural forests, foresters

have adapted to new means of wood production since the 1980s. Frequently, natural species did not meet the growing industrial requirement and it became necessary to introduce exotics with high productivity per unit area. Eucalypts and Poplars are prime examples.

Introduction of Species/provenances and achievements: It may be emphasized that climatic and ecological matching of new sites and the original habitat of a species that is being considered for introduction is usually not enough since it cannot reveal adaptability of a species to grow satisfactorily on a range of sites in a new environment. It is expected that the best way forward may be through trials of the candidate species on representative locations. It is common to find that foresters and forest scientists are not fully aware of the need for species/provenance testing. Systematic research work is followed in developed nations since 1930s, unfortunately, because of the hurry to introduce new species this step is generally not followed in India. The authors have observed several examples of this involving species such as *Leucaena leucocephala, Prosopis flroa, Paulonia fortunei*, *Jatropha curcus*, etc. The notable exceptions are eucalypts and poplars where adequate testing has been done. Even here it is observed that for some inexplicable reasons species like *E. grandis* were not given a fair trial under the Australian assisted project that commenced in the late 1970s. The authors have observed some outstanding specimens of the species. The lead author also had the opportunity to see excellent clonal plantations of *E. grandis* in South Africa, where it is used in the paper industry. Some foresters continue to advocate species like Paulownia and Melia. In the latter case it is still not clear whether *Melia composita* and *M. dubia* are different or same species, or perhaps one is a subspecies of the other.

The advisability of species trials is accepted but the need for their careful planning and high standards of maintenance and assessment has often been less appreciated. For species with naturally wide geographical/ecological ranges, provenance testing is considered essential (Burley and Wood 1976). There is a danger that foresters who have a readily accessible seed or clone of a satisfactory species or provenance may feel it unnecessary to test others that could be potentially better. This has sometimes been experienced by the authors during his trips to a few places in India.

Two examples relevant to the Indian situation are those of *Eucalyptus tereticornis* Smith and *Eucalyptus camaldulensis* Dehnh. The former has been the most successful in summer rainfall areas with moderate to severe drought season while the latter is perhaps the world's most widely planted tree species in arid and semiarid areas (Eldridge et al1993). *E camaldulensis* is also found to be fairly salt tolerant and some populations were found to have greater salt tolerance than others (Karschon and Zoher 1975). Trials in India have shown superiority of some provenances over others (Chaturvedi et al 1989, Kapur and Dogra 1987a, b, c, Dogra and Sharma 2005, Dogra and Chauhan 2021).

Species/provenance testing: The size of the trial plots depends on the purpose, duration and expected growth rate of trees. Leaving aside the arboretum phase where 1-2 trees would suffice and when a large number of species and provenances are being screened in the elimination phase line plots of 5-7 tree plots may be suitable with a few replications. The next stage is the species elimination phase during which a large number of possible species may be tested in small plots for about 10% to 20% of the rotation length. Here 20-30 species may be involved (Callaham 1964, Burleyet al 1976).The next is the species testing phase which involves critical testing of a reduced number of species in larger plots (16-25 trees) with a 1-2 row surround for longer periods: about half the rotation length. Here 5-10 species may be involved. The third stage is the species proving phase which is designed to confirm under normal plantation conditions the superiority of a few species/provenances in 100 tree plots with a surround of 2 rows. Here growth and yield estimates are important. Some authors suggest that the width of the surround may be approximately equal to the expected height of trees. The trials may be done sequentially or, if there is a hurry to obtain usable results, in parallel. A similar approach was followed in species and provenance trials of eucalypts in Punjab (Kapur and Dogra 1987a,b,c, Dogra and Sharma 2005). Here the local Eucalyptus hybrid was used as a control.

Three similar stages apply to provenance testing for a species with a wide natural distribution: a range-wide provenance sampling phase, a restricted provenance sampling phase, and a provenance proving phase. In range-wide provenance phase 25 trees with no surrounds are recommended. Here we may test 10-30 provenances. In restricted provenance phase 25-49 tree plots are suggested. In provenance proving phase we may go in for 100 tree plots and one or two row surrounds. Plots are generally square or rectangular. In case of sloping land, replicates may be aligned along the contours. The spacing of trees generally varies from 2-3 m. Since these are generally applied to species that are promising or probable their plot size needs to be larger for longer duration. Plots should be large enough to provide data on growth and yield for full rotation and plot surrounds should be large enough to minimize the edge effects. At this stage, it is also appropriate to investigate wood quality and other management techniques including techniques of nursery growing, site preparation, planting methods, and spacing, pruning and thinning trials.

The objective of an experimental design for species or provenance trial is to ensure precise and accurate estimates of differences between populations and between environments. Enough replications are required to reduce the residual variance associated with any comparison of population means. As a rule of thumb, we may never have degrees of freedom of less than 10 and preferably 15. This may entail increase in the number of replicates, or the number of species or provenances being tested. Usually, a well-known local species is included in trials to have a reference point against which to judge the performance of the unknown species/populations.

Other species and provenance trials include those of *Paulownia* and tropical pines. Unfortunately, the trials of *Paulownia* and tropical pines were not diligently followed even though pine species like *Pinus caribaea, P. elliotii, P. patula* and several other species showed promise in tropical/sub-tropical areas.It is well known that tropical pines are fast growing compared to indigenous pines and yield general purpose timber and long fibre pulping material (Chaturvedi1982 and Guha 1982). In his report on *Paulownia*, Dhiman (2008) recommended that a wide-range provenance trial of selected species, and their germplasm was urgently needed. The fate of tropical pines introduced in 1960s was similar, although countries like Australia and New Zealand have adopted these pines in a big way. In both countries, *Pinus radiata* is the most widely planted softwood species with plantations over 773000 ha in Australia alone and around 89% of New Zealand plantations forests include *Pinus radiata*. The species is used as a general-purpose timber and for pulping. In Australia, plantations of *P. caribaea* and *P. elliottii* called southern pines also have around150,000 ha (Singh et al 2013). As a matter of fact, Australia is now also considered a leader in teak, chandan, neem, etc. plantations while the species did not exist there till the 1980s. The above facts should make us sit up and take a serious note of developments elsewhere. Tropical pines have the potential to meet our requirements for long fibre pulp as well as general purpose timber just like chir pine and kail. By planting these species in degraded forests and under agroforestry plantation, India can potentially emerge as a country surplus in wood production. The said pines are normally grown over a rotation of 30 years and under agroforestry contract farming is one possible route.

CONCLUSION

Decline in timber harvests from natural forests because of ecological and environmental considerations has shifted focus to planted forests or trees outside forests.The increase in tree planting activities is also due to the increase in demand for wood as a result of economic development and population growth. In India, agroforestry is the single biggest component of planted forests and meets more than 90% the demand for wood. Currently stress is on a limited number of fast-growing species. However, foresters and scientists are expected to look at a much wider spectrum of species. In other words, it is appropriate to grow comparatively fast-growing timber species and develop appropriate agroforestry models for them as well. It is therefore imperative that we choose appropriate species/clones to meet the demand for timber; of furniture grade, general purpose timber and for veneer and ply mills; and long-fibre tropical pines for paper mills.

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Variability of Fruits among Different Improved Landraces and Seedling Origin Tree of *Terminalia chebula* Retz.

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***Abstract*:** The survey was conducted in Hamirpur district of Himachal Pradesh. The 20 random fruits were taken from five improved landraces and seedling origin tree for the fruit variability analysis. Results revealed that Pahlu was found statistically superior among all the landraces with the highest average green weight of fruit (61.3 gm) and pulp weight (57.4 gm). The maximum fresh stone weight was in Kallar strain (4.6 gm). Tamber recorded the highest dry weight of whole fruit (20.3 gm). The maximum dry pulp weight was recorded in Pahlu (15.5 gm). The highest fruit length was recorded in Kallar (7.9 cm). Pahlu (4.7 cm) showed the highest fruit diameter among all the landraces whereas; Seedling origin tree was recessive among all the landraces and showed the lowest value of all the fruit parameters and fruit weight.

***Keywords*:** Fruits, Landraces, Superior, Weight, Recessive

The genus *Terminalia* includes about 250 species of trees and shrubs (Zhang et al 2019). In India, 20 species belonging to four sections namely; *Catappa, Myrobalans, Chuncea and Pentaptera* include *T. alata*, *T.citrina*, *T. coriacea*, *T. crenulata*, *T. arjuna*, *T. bellirica*, *T. berryi*, *T. bialata*, *T. pallida*, *T. paniculata*, *T. parviflora*, *T. catappa*, *T. chebula*, *T. gella*, *T. manii*, *T. moluccana*, *T. myriocarpa*, *T. procera*, *T. tomentosa* and *T. travancorensis* (Raju et al 2012). Harar or Haritaki (*Terminalia chebula* Retz.) fruit of Harar has healing powers. It has astringent, purgative, rejuvenating, antibacterial, antifungal and laxative activity. This activity is due to the presence of substances-tannic acid, chebulinic acid, gallic acid, anthraquinone and sennoside. It is used in India to treat many diseases such as urinary, digestive diseases, diabetes, skin diseases, parasitic infections, heart ailments, fever, flatulence, constipation, ulcers, vomiting, colic pain and hemorrhoids (Bag et al 2013). The edible fruit tissue of Harar also contains nutrients, vitamin C, protein, amino acids and minerals (Mahesh et al 2007, Chander and Chauhan 2014). The present study is an attempt on fruit variability of *T. chebula* with an aim to ascertain the nature and extent of diversity present among improved landraces and seedling variety tree from different geographical regions of Himachal Pradesh.

MATERIAL AND METHODS

The observation on three replicates of samples, each consisting of 20 fruits from five different improved landraces and from the seedling variety planted at Khaggal farm [College of Horticulture and Forestry, Hamirpur H.P.)]. Fruits of harar were randomly selected from each bulked fruit lot of each landrace and seedling variety after discarding the damaged fruits. The parameters viz. fruit length, fruit diameter, stone length, stone diameter was measured using vernier caliper whereas, fresh and dry fruit weight was recorded with the help of weighing balance.

RESULTS AND DISCUSSION

A large variation was observed with respect to size and weight of fruits of different harar landraces (Table 1). The shape of Harar fruits varied from obovate to ovoid, obovoid, elliptical and ovate while fruit colors noted were dark green, light green and pale green. Significant difference was observed with regard to fresh weights of whole fruit, pulp and stone.

The maximum fresh weight of whole fruit was observed in Pahlu strain (61.3 gm), followed by the strains of Kallar and Paluri while the minimum was in seedling of origin tree (22.0 gm). All the improved areas showed that the fresh whole fruit weight was significantly higher over seedling origin tree. Pahlu was statistically superior to all other landraces, exhibiting the highest weight of fruit. The fruit weights of Kallar, Tamber and Paluri strains were, however, statistically alike. The highest fresh pulp weight was registered in Pahlu (57.4 gm) followed by Paluri and Kallar while the lowest was in seedling origin tree (19.2 gm). All the improved landraces recorded significantly higher fresh pulp weight over seedling variety trees. However, as Pahlu showed the highest pulp weight and was statistically higher than all other landraces.

Fresh stone weight was recorded maximum in Kallar (4.6 gm) followed by Tamber and Paluri while the minimum was registered in seedling origin tree (2.7 gm). The fresh stone weight of all improved landraces over seedling trees was significantly higher. Landrace, Kallar showed the highest stone weight was statistically greater than all other landraces. The highest dry weight of whole fruit was in Tamber (20.3 gm) followed by Pahlu and Paluri while the lowest was registered in seedling origin tree (5.8 gm). All the improved landraces significantly exhibited higher dry fruit weight over seedling origin trees. Tamber recorded the highest dry fruit weight was statistically better over all other landraces. Pahlu (15.5 gm) recorded the maximum dry pulp weight followed by Paluri and Kallar whereas, the minimum dry pulp weight was registered in Seedling origin tree (4.7 gm). All the improved landraces significantly recorded higher dry pulp weight than seedling variety. The strain Pahlu, which was statistically superior to all other landraces, had the highest dry pulp weight. The dry stone weight exhibited no significant effect with respect to improved landraces and seedling origin tree. However, dry weight of stone was recorded highest in Tamber (3.3 gm) followed by Kothi and Kallar whereas, the lowest was in Seedling origin tree (1.1 gm).

The significant effect was observed on fresh weight of whole fruit, fresh pulp weight, fresh stone weight, dry weight of whole fruit and dry pulp weight whereas, had non-significant influence on dry stone weight w.r.t. improved landraces and seedling origin tree. Among all the landraces, the maximum values for fresh weight of whole fruit, fresh pulp weight and fresh stone weight were in Pahlu (61.3 gm), Pahlu (57.4 gm) and Kallar (4.6 gm), respectively. The highest values for dry weight of whole fruit and dry pulp weight were in Tamber (20.3 gm) and Pahlu (15.5 gm), respectively. However, the entire minimum values for all the fruit parameters *viz*., fresh whole fruit weight, fresh pulp weight, fresh stone weight, dry whole fruit weight and dry pulp weight were recorded only in seedling variety.

The significant difference was observed on different parameters of fruit and stone viz., fruit length, fruit diameter, stone length and stone diameter (Table 2). The maximum fruit length was in Kallar (7.9 cm) followed by Paluri and Tamber while the minimum was registered in Pahlu (5.1 cm). Pahlu (4.7 cm) showed the highest fruit diameter among all the landraces which was significantly followed by Kallar (4.2 cm) and Tamber (4.1 cm) whereas, the lowest fruit diameter was recorded in seedling origin tree (2.9 cm). Pahlu strain that registered the maximum fruit diameter was statistically better over all other landraces. The maximum stone length was in Kallar (3.5 cm) followed by Paluri and Pahlu while the minimum was shown in Tamber and seedling origin tree (2.6 cm). The highest stone diameter (1.7 cm) was in the landrace, Tamber followed by Kallar and Pahlu while the lowest stone diameter was registered in seedling origin tree (1.2 cm). The two landraces i.e. Kallar and Tamber which had maximum stone length and stone diameter respectively were statistically superior over all other landraces.

Among different improved landraces and seedling variety, the values for highest fruit length, fruit diameter, stone length and stone diameter were in Kallar (7.9 cm), Pahlu (4.7 cm), Kallar (3.5 cm) and Tamber (1.7 cm), respectively. However, the lowest minimum fruit length, fruit diameter, stone length and stone diameter were in Pahlu (5.1 cm), Seedling variety (2.9 cm), Seedling variety; Tamber (2.6 cm) and Seedling variety (1.2 cm), respectively. Thakur et al (2008) showed significant variation in fruit size, weight and seed/pulp ratio. Fruit diameter showed significant and positive correlation with green fruit weight, the most important character from market point of view.

Substantial variation was observed with regard to size and weight of Harar fruits of different landraces due to their inherent potential/genetic make-up. Harar is a highly cross-pollinated species and hence, the variation noticed is as expected in such species. Singh and Singh (2012) also reported large variation in fruit weight, length and pulp

Table 1. Fresh and dry fruit-weight of improved landraces and seedling variety of *Terminalia chebula* Retz

Landraces	Fresh weight (gm)			Dry weight (gm)		
	Whole fruit	Pulp	Stone	Whole fruit	Pulp	Stone
Kallar	51.6	46.6	4.6	15.5	13.1	2.3
Tamber	50.1	45.3	4.4	20.3	17.0	3.3
Kothi	33.2	29.8	3.2	12.5	10.1	2.4
Pahlu	61.3	57.4	3.5	16.9	15.5	1.5
Paluri	51.3	47.6	3.7	16.7	14.8	1.9
Seedling tree	22.0	19.2	2.7	5.8	4.7	1.1
CD (p=0.05)	6.63	6.21	0.91	3.46	3.58	NS

Table 2. Fruit parameters of improved landraces and seedling variety of harar

Landraces	Fruit length (cm)	Fruit diameter (cm)	Stone length (cm)	Stone diameter (cm)
Kallar	7.9	4.2	3.5	1.6
Tamber	5.8	4.1	2.6	1.7
Kothi	5.6	3.5	2.9	1.4
Pahlu	5.1	4.7	3.0	1.5
Paluri	7.4	4.0	3.1	1.3
Seedling tree	5.7	2.9	2.6	1.2
CD (p=0.05)	0.66	0.27	0.38	0.18

content of jamun (*Syzygium cumini*). Malshe et al (2016) reported maximum fruit weight in Krishna variety of aonla which was at par with Kanchan variety while the minimum fruit weight was in NA-7 variety followed by NA-10 variety. Increased fruit weight might be attributed to the character of genotype. The weight and fruit size might be also related to the bearing habit and yield of that variety. Singh and Singh (2016) observed large variation with respect to fruit weight , fruit length fruit diameter, fruit girth and stone weight. The genotype T_{12} and T_{14}, were found superior in terms of their physico-chemical attributes than rest of the genotypes. Morphological variation in fruit characters of aonla was ascribed due to differences in their genetic make-up and environmental conditions. Bora et al (2017) studied the characterization of mango (*Mangifera indica* L.) genotypes based on physico-chemical quality attributes and found that “Mallika” and “Neelgoa” varieties were superior in terms of fruit weight, size , pulp weight and pulp stone ratio Chiranjeevi et al (2018) reported significant variability among the varieties of aonla for different fruit and seed traits. The variety Krishna registered maximum fruit length , fruit diameter, fruit weight and pulp weight . The maximum pulp content and pulp to stone ratio were recorded in NA-10 and Krishna cultivars, respectively. The maximum stone weight, seeds per fruit and seed length were highest in Kanchan variety. The variations in the fruit size depend upon the varietal and genetic characters of an individual variety and are highly influenced by environmental factors.

CONCLUSION

Pahlu was found statistically superior among all the landraces with the highest average green weight of fruit and pulp weight. The maximum fresh stone weight was recorded in Kallar strain. Tamber had the highest dry weight of whole fruit and was statistically better over all other landraces. The maximum dry pulp weight was in Pahlu. The maximum fruit length was in Kallar while the minimum was registered in Pahlu . Landrace, Pahlu showed the highest fruit diameter among all the landraces. Seedling origin tree showed the lowest value of all the fruit parameters and fruit weight.

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Comparison of Soil Physico-Chemical Properties and Phytochemicals in *Melissa officinalis* L. Grown in non-Cultivated and Cultivated Area of Dibrugarh, Assam

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***Abstract*:** The aim of the present study to record the differences in soil physico-chemical properties of the two study area and also to record the difference in antioxidant, antimicrobial activities of different parts of *Melissa officinalis* L.Total phenol and flavonoid content, antioxidant and antimicrobial activities of the plants recorded differences. In most of the cases the plant samples collected from non-cultivated area (DUC) recorded more phenol and flavonoid content and antioxidant and antimicrobial activities than the samples collected from cultivated area (KG). The higher NPK and organic matter content in DUC might be the reason of the presence of more phytochemicals in the plant grown in the respective area. Sample collected from DUC (non-cultivated area) show more activity than KG (cultivated area). The phytochemicals present in plants from DUC is more than the phytochemicals present in the plant cultivated from the KG. The Total phenol and flavonoid content was also higher in plants collected from DUC. Similarly, plants collected from DUC recorded more antioxidant activity against both DPPH and ABTS. The significant differences in antimicrobial activities were also observed in plants collected from DUC.

***Keywords*:** Physico-chemical, Antioxidant, Antimicrobial, Phytochemicals, NPK

Phytochemicals present in plants are mainly responsible for the medicinal properties of the plants. The soil condition and other environmental conditions influenced the medicinal properties of a plant. Synthesis and accumulation of phytochemicals in plants depends on the species, age of the species, climatic factor of the area and season of sample collection (Ezeabara and Egwuoba 2016). The different levels of phytochemicals in plants depend on the method of extraction, age of the plant, location and season of collection of the plant samples. *Melissa officinalis* L. is commonly known as lemon balm. The plant had properties like, anti-bacterial, anti-viral, antifungal and antioxidant, sedative, spasmolytic, anti-inflammatory, mnemonic improvement, reduce excitability, anxiety, stress, gastrointestinal disorders, sleep disturbance, treat fevers and colds, indigestion, hyperthyroidism, depression, mild insomnia, epilepsy, headaches, tooth-aches and treat Alzheimer's disease (Lang and Buchbauer 2012, Chaiyana and Okonogi 2012, Astani et al 2012, Aprotosoaie et al 2013, Bounihi et al 2013, Pirbalouti et al 2014). The domestication of medicinal plants needs the knowledge of natural habitats, soil physico-chemical properties and nutrient levels. The present study was an attempt to study the properties of a non-cultivated and cultivated area of *Melissa officinalis* L. The study also includes the comparative analysis of phytochemical, antioxidant and antimicrobial activities of different solvent extracts of different parts of the plant collected.

MATERIAL AND METHODS

Sample collection: Soil and plant samples were collected from the study area during 2017, from two areas of Dibrugarh District, one non-cultivated area (Dibrugarh University Campus, DUC) and another cultivated area (Khanikar Gaon, KG). DUC is considered as wild habitat of the plant and samples from KG is considered as domestic habitat of the plant. Immediately after collection soil samples were air dried at room temperature, sieved and analyzed for different soil parameters.

Plant samples were also collected at their full bloomed stage along with the soil samples. The herbarium specimen of the species was also prepared and deposited in the Department of Life Sciences, Dibrugarh University. From each area, different plant parts (young leaves, mature leaves, inflorescence and stem) were collected separately and cleaned properly and washed under running water to remove dust and other debris. The materials were air dried at room temperature. The stems were sliced before allowed to dry. After few days, the materials were wrapped with brown paper and allow sundry for complete dryness (less than 1-2% moisture content). The materials were grounded to fine powder using mortar and pestle. The fine powder was kept in air tight bottles for further analysis.

Preparation of extracts: Extracts were prepared in five solvents viz- water, methanol, ethanol, acetone and petroleum ether by cold maceration methods and are known

as cold extracts. The extracts were kept in air tight glass bottles at 5^0C for further analysis. Hot petroleum ether extract was also prepared using Soxhlet extractor and antimicrobial activity of the extract was done to observe the difference in activities of both cold and hot extract. The dried extracts were dissolved in DMSO (dimethyl sulfoxide) to obtain sample solution at 1mg/ml of concentration. Aqueous extracts were dissolved in distilled water at 1mgml^{-1} of concentration.

Qualitative phytochemical analysis: Qualitative analysis for detection of tannins, phlobatannins, flavonoids, saponins, alkaloids, cardiac glycosides, terpenoids, steroids, anthraquinone, free anthraquinone, carotenoids and reducing sugar were performed using standard laboratory methods (Trease and Evans 2002,Edeoga et al 2005, Egwaikhide and Gimba 2007, Chitravadivu 2009, Majaw and Moirangthem 2009, Aja et al 2010, De et al 2010, Ajayiet al 2011 and Ajiboye et al 2013)

Determination of total phenol content (TPC): Total phenol content (TPC) of the sample extract was estimated following the method described by Malik and Singh (1980).

Determination of total flavonoid content (TFC): The Aluminium chloride method was used for determination of total flavonoid content of the sample extracts (Mervat and Hanan 2009)

Determination of antioxidant activity assay of the sample extract: DPPH radical scavenging activity was determined by the method of Stanojevic et al (2009).

Determination of antioxidant activity assay of the sample extracts: The ABTS assay was carried out following the method of Re et al (1999).

Antimicrobial activity assay of the sample extracts: Antimicrobial activity of the bacterial strains was carried out by agar well diffusion method using 6mm borer (Nair et al 2005).

Test organisms: Gram positive and gram negative bacterial strains and fungal strains are used in this experiment to observe the antimicrobial activity of the sample extracts.

a) Gram positive bacterial strains- *Bacillus subtilis*(MTCC 441), *Bacillus cereus* (MTCC 8750), *Staphylococcus aureus* (MTCC 3160), *Staphylococcus epidermis* (MTCC 3615) and *Proteus vulgaris* (MTCC 744).
b) Gram negative bacterial strains- *Escherichia coli* (MTCC 443), *Enterococcus faecalis* (MTCC 439).
c) Fungal strains- *Candida albicans*(MTCC 3017) and *Penicillium chrysogenum* (MTCC 947).

Determination of soil physico-chemical properties: Soil physico-chemical properties were determined (Goel and Trivedy (1992).

Table 1. Qualitative phytochemical analysis of different parts of *Melissa officinalis* L. collected from two (DUC and KG) areas

Sample	Areas	Tannins	Phlobatannins	Flavonoids	Terpenoids	Steroids	Glycosides	Cardiac Glycosides	Saponins	Anthraquinones	Free Anthraquinones	Carotenoids	Alkaloids	Reducing Sugar	Phenols
Young leaf	DUC	+	-	+	+	-	+	+	+	-	-	+	-	+	+
	KG	+	-	+	-	-	+	+	+	-	-	+	-	+	+
Mature leaf	DUC	+	-	+	+	-	+	+	+	-	-	+	-	+	+
	KG	+	-	+	-	-	+	+	+	-	-	+	-	-	+
Inflorescence	DUC	+	-	+	+	-	+	+	+	-	-	+	-	+	+
	KG	+	-	-	-	-	+	-	+	-	-	+	-	-	+
Stem	DUC	+	-	+	+	-	+	+	+	-	-	+	-	+	+
	KG	-	-	-	-	-	+	-	+	-	-	+	-	-	+

(+)present, (-)absent

Table 2. Quantitative estimation for total phenol and total flavonoid content of sample extracts of different parts *Melissa officinalis* L. collected from DUC and KG

Sample (mg ml^{-1})	Areas	Total phenol content (mg catechol equivalent gm^{-1} dry extract)					Total flavonoid content (mg quercetin equivalent gm^{-1} dry extract)				
		Water extract	Methanol extract	Ethanol extract	Acetone extract	Petroleum ether extract	Water extract	Methanol extract	Ethanol extract	Acetone extract	Petroleum ether extract
Young leaf	DUC	2.00±0.11	2.01±1.00	1.98±0.90	1.61±0.00	1.01±0.99	2.05±0.22	2.61±1.00	1.90±0.11	1.64±0.14	1.30±0.10
	KG	2.36±0.01	2.13±0.11	2.06±0.00	1.01±0.21	1.23±0.01	1.03±0.10	2.65±0.00	1.11±0.21	0.69±0.01	1.01±0.19
Mature leaf	DUC	2.04±0.00	1.98±0.00	1.91±0.00	2.00±0.11	1.16±0.90	2.90±0.00	1.69±0.10	2.80±0.11	2.01±0.10	2.00±0.41
	KG	3.01±0.01	1.39±0.18	1.36±0.00	1.64±0.11	1.19±0.21	2.13±0.42	1.64±0.01	1.34±0.16	1.69±0.22	1.11±0.01
Inflorescence	DUC	2.33±0.00	2.99±0.20	1.61±1.03	1.00±0.00	2.11±1.00	2.01±0.99	2.10±0.22	1.98±0.66	1.45±1.00	0.98±0.07
	KG	2.96±0.36	1.96±0.10	1.36±0.12	1.11±0.00	1.34±0.03	2.110.04	1.46±0.22	1.45±0.12	1.21±0.11	0.64±0.01
Stem	DUC	1.88±0.10	1.45±1.00	1.41±0.10	1.01±0.22	0.98±0.02	1.99±1.00	1.90±0.01	1.21±0.10	1.20±0.99	0.96±0.01
	KG	0.64±0.14	1.13±0.02	0.78±0.15	0.25±0.06	1.40±0.01	0.94±0.12	0.99±0.11	1.10±0.01	1.11±0.01	1.01±0.01

Table 3. Antioxidant activity study of sample extracts of different parts of *M. officinalis* L. collected from DUC and KG

Sample (mg ml^{-1})	Areas	DPPH radical scavenging activity (% inhibition in mg ml^{-1})					ABTS radical scavenging activity (% inhibition in mg ml^{-1})				
		Water extract	Methanol extract	Ethanol extract	Acetone extract	Petroleum ether extract	Water extract	Methanol extract	Ethanol extract	Acetone extract	Petroleum ether extract
Young leaf	DUC	62.00±0.99	84.83±0.08	75.92±0.01	66.40±0.22	57.99±0.11	80.09 ±0.10	69.09±0.01	58.99±2.01	66.61±0.16	56.55±1.01
	KG	56.03±0.44	68.98±1.99	55.32±0.25	39.26±0.31	23.12±0.47	63.12±1.91	71.02±0.01	49.23±0.10	47.32±0.41	45.80±0.89
Mature leaf	DUC	81.12±0.00	85.30±0.40	79.32±1.00	69.02±0.03	61.00±1.99	77.24±2.00	69.00±0.22	60.00±1.01	75.00±1.56	55.40±0.08
	KG	79.25±0.24	78.48±0.31	64.02±0.45	53.12±0.45	58.10±0.05	69.21±1.36	69.98±0.08	39.25±0.00	48.56±0.78	49.22±0.11
Inflorescence	DUC	66.14±0.10	76.48±0.99	66.44±1.29	59.00±1.00	61.44±0.11	66.55±0.99	75.12±0.01	55.10±0.11	58.44±0.99	49.66±1.00
	KG	58.95±0.90	49.36±0.21	69.01±0.01	45.96±0.09	60.36±0.91	59.99±0.21	58.48±1.36	48.98±0.01	51.65±0.12	51.22±0.20
Stem	DUC	48.90±0.01	48.05±0.11	62.11±0.50	39.04±1.22	55.01±0.99	50.44±1.00	76.79±5.56	51.01±1.00	51.55±0.01	41.16±2.07
	KG	51.23±0.92	55.93±0.19	52.87±1.99	41.36±0.83	41.32±0.00	67.23±0.25	36.21±0.24	48.58±0.19	56.21±0.01	21.03±0.14
Ascorbic acid				90.28 ±0.02					89.00 ±0.00		

Table 4. Antimicrobial activity study of the sample extracts of different parts of *Melissa officinalis* L. collected from DUC and KG

Sample	Extracts ($mgml^{-1}$)	Areas	Diameter of Zone of Inhibition (mm)								
			Bacterial strains							Fungal strains	
			B. subtilis	*B. cereus*	*S. aureus*	*S. epidermis*	*E. coli*	*E. faecalis*	*P. vulgaris*	*C. albicans*	*P. chrysogenum*
Young leaf	Water extracts	DUC	-	-	-	-	-	-	-	-	-
		KG	-	-	-	-	-	-	-	-	-
	Methanol extract	DUC	11±1.01	8±0	-	-	10±1	10±2	-	-	-
		KG	-	-	-	-	-	8±0.99	-	-	-
	Ethanol extract	DUC	-	-	-	-	-	-	-	-	-
		KG	10±0.1	-	-	-	-	-	-	-	-
	Acetone extract	DUC	10±1	-	10±2	-	10±0	12±1	-	-	-
		KG	-	-	-	10±00	-	-	-	-	-
	Petroleum ether extract	DUC	11±1	-	11±2	-	10±0	14±2	-	-	-
		KG	-	-	-	-	-	-	-	-	-
	Hot petroleum ether extract	DUC	10±1	-	-	-	-	12±0	-	-	-
		KG	12±1	-	-	-	-	-	-	-	-
Mature leaf	Water extracts	DUC	-	-	-	-	-	-	-	-	-
		KG	-	-	-	-	-	-	-	-	-
	Methanol extract	DUC	8±0	-	-	8±1	-	-	-	-	-
		KG	-	-	-	-	-	-	-	-	-
	Ethanol extract	DUC	8±0	8±0	10±2	9±1	8±0	8±1	-	-	-
		KG	-	-	10±1.6	-	-	-	-	-	-
	Acetone extract	DUC	-	-	-	-	-	-	-	-	-
		KG	-	-	-	-	-	-	-	-	-
	Petroleum ether extract	DUC	-	-	-	-	-	-	-	-	-
		KG	-	-	-	-	-	-	-	-	-
	Hot petroleum ether extract	DUC	-	-	12±0	-	-	-	-	-	-
		KG	8±0	-	-	-	-	-	-	-	-
Inflorescenc	Water extracts	DUC	-	-	-	-	-	-	-	-	-
		KG	-	-	-	-	-	-	-	-	-
	Methanol extract	DUC	10±1	8±0	-	-	-	-	-	-	-
		KG	-	-	-	10±1	-	-	-	-	-
	Ethanol extract	DUC	8±0	10±1	8±0	-	-	8±1	-	-	-
		KG	-	-	-	-	-	-	-	-	-
	Acetone extract	DUC	-	-	12±0	-	-	-	-	-	-
		KG	-	-	-	-	-	-	-	-	-
	Petroleum ether extract	DUC	-	-	-	-	-	8±1	-	-	-
		KG	-	-	-	-	-	-	-	-	-
	Hot petroleum ether extract	DUC	10±0	12±2	-	-	-	-	8±0	-	-
		KG	10±2	-	-	-	-	-	-	-	-
Stem	Water extracts	DUC	-	-	-	-	-	-	-	-	-
		KG	-	-	-	-	-	-	-	-	-
	Methanol extract	DUC	-	8±1	9±1	-	-	8±0	-	-	-
		KG	-	-	-	-	10±2	-	-	-	-
	Ethanol extract	DUC	-	-	-	-	-	-	-	-	-
		KG	-	-	-	-	-	-	-	-	-
	Acetone extract	DUC	9±1	8±1	8±1	-	-	-	-	-	-
		KG	-	-	-	-	-	-	-	-	-
	Petroleum ether extract	DUC	-	-	-	-	-	-	-	-	-
		KG	-	-	-	-	-	-	-	-	-
	Hot petroleum Ether extract	DUC	-	10±0	-	-	-	-	8±0	-	-
		KG	-	-	-	-	10±1	-	-	-	-
Erythromycin (E)15mcg		-	32±2	30±1	28±0	30±0	12±2	48±6	12±2	-	-
Clotrimazole (CC) 10mcg		-	-	-	-	-	-	-	-	11±2	32±0

Diameter of the cork borer=6mm, '-' indicates no inhibition

Table 5. Soil parameters

Sample	Areas	Organic carbon (%)	Organic matter (%)	pH	Moisture (%)	Ash (%)	Total nitrogen content (%)	Phosphorus content (%)	Potassium content (%)
Soil	DUC	2.0±0.02	3.448±0.01	5.85±0.34	4.80±0.01	1.2±0.01	0.005±0.10	0.008±0.002	0.009±0.001
	KG	1.21±0.01	2.08±0.01	6.96±0.10	6.20±0.13	1.11±0.00	0.004±0.01	0.004±0.001	0.008±0.001

RESULTS AND DISCUSSION

Glycosides, saponins, carotenoids and phenols were present in all the parts collected from both the areas, while plantains, steroids, anthraquinone, free anthraquinone and alkaloids are not recorded in all the parts. The number of phytochemicals are more in the sample collected from DUC than KG. Similar kinds of phytochemicals present in the plant were also recorded by some earlier workers (Carvalhoet al 2011, Mutalib 2015). The difference in presence and absence of phytochemicals is might be due to the microclimate and soil condition of the study area. Water and methanol extract showed better extraction of phenolic and flavonoid content than ethanol, acetone and petroleum ether extract at 1mg/ml of concentration (Table 2). Good quantity of total flavonoid content was recorded by water extract of various parts. Total phenol and total flavonoid content was higher in leaves than inflorescence and stem. In most of the cases total phenol and total flavonoid content is higher in the sample extracts collected from DUC than KG. The amount of phenol and flavonoid content in the plant recorded by other workers seems more than the present study. The more phenol and flavonoid in the extracts might be due to the soil condition of the study area. The more organic matter present in the soil may cause the more phytochemicals present in the plant. The phytochemicals present in the plant are also depends on the extraction power of various solvents, which may cause the difference in their phenolic and flavonoid content.

Extracts from leaves recorded higher antioxidant activity against DPPH and ABTS, at 500µl of sample at a concentration of 1mg/ml (Table 3). The extracts of the plant collected from DUC recorded more antioxidant activity than the extracts collected from leaves recorded more antibacterial inhibition than the inflorescence and stem (Table 4). Petroleum ether extract of young leaves collected from DUC was recorded highest (14 mm) inhibition against *E. faecalis* than the other extracts of the plant at $1mgml^{-1}$ of concentration, while the petroleum ether extract from KG did not recorded any inhibition against the tested bacteria. All the sample extracts collected from both the areas did not recorded antifungal inhibition against *C. albicans* and *P. chrysogenum* water extract. Antimicrobial activity of the plant was recorded by other workers (Mutalib 2015, Jalal et al 2015). The difference in antimicrobial activities in different solvent extracts collected from different areas might be due to the phytochemicals responsible for the antimicrobial properties of the plant. Cold extraction in water may be the reason of inactiveness of the water extracts against the tested bacterial strains.

The soil from DUC recorded good physico-chemical properties than the soil collected from KG. Study recorded high soil pH in KG (6.96%) than DUC (5.85%). Sharma et al (2013), Abad (2014) and Maqbool et al (2017) also reported that pH of cultivated land more than the forest land. This difference is might be due to the acidic nature of litter in forest area. The soil moisture of cultivated land from KG (6.20) is higher than the forest area DUC (4.80%), which might be due to the fine texture of the soil and water supply into the land during cultivation. Wang et al (2012) from China reported that high moisture content in corn cultivated area than other places. Maqbool et al (2017) recorded that soil moisture is higher in agricultural land than the forest land which is due to the soil texture, water supply during cultivation.

Organic carbon content of non-cultivated land (2.002%) was higher than the cultivated land (1.21%), which might be due to the higher biomass production in that area. Yitbarek et al (2013), Yihenew et al (2015) and Maqbool et al (2017) reported higher organic carbon content in soil from forest area than agricultural lands.

The percentage of NPK in the cultivated area is lower than the NPK of the non-cultivated area. The good physico-chemical properties of the non-cultivated soil may cause the more phytochemicals in the plant grown in that area. Stanton-Geddes et al (2012) reported the interaction of soil habitat and plant in that area. The positive effect of environmental factors on the bio-synthesis and accumulation of phytochemicals in various plant was studied by various workers (Ibrahim et al 2013, Roux et al 2017, Shaaban et al 2018, Goldo 2019).

CONCLUSION

Sample collected from DUC (the non-cultivated area) show more activity than KG (cultivated area). The phytochemicals present in plants collected from Dibrugarh University campus soil was more than the phytochemicals present in the plant cultivated from the Khanikar Gaon. The

total phenol and flavonoid content was also higher in plants collected from Dibrugarh University campus. Similarly, plants collected from Dibrugarh University campus recorded more antioxidant activity against both DPPH and ABTS. Significant antimicrobial activities were also recorded by the plants collected from Dibrugarh University campus.

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Effect of Potassium and Zinc on Yield, Nutrient Content and Uptake in Green Gram (*Vigna Radiata* L.) in Course Textured Soil of South-West Haryana

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***Abstract*:** The study was conducted during *kharif* 2018 at Regional Research Station Bawal of CCS Haryana Agricultural University, on green gram cultivar MH 421. The total nitrogen, phosphorous and potassium content and uptake in seed and straw were significantly influenced by different potassium and zinc levels. Green gram seed and straw yield increased by increasing potassium and zinc application over control but significantly up to the levels 20 and 25 kg $ZnSO_4$ ha^{-1}. Both K_{20} and K_{30} treatments recorded significantly higher N, P, K content, and uptake of N, P, K in seed as compared to control. Similarly in straw, K_{20} and K_{30} treatments significantly higher N, P and K content and uptake in straw over the control was observed. The availability of particular nutrient increased by application to the soil and also promotes the uptake of other nutrients as well.

***Keywords*:** Green gram, Nutrient content, Potassium, Uptake, Yield, Zinc

Green gram (*Vigna radiata* L. Wikzek) belongs to the family Fabaceae and is grown all around the globe including Southern and Eastern Asia, South and North America, Central Africa, Australia and some parts of China particularly for its protein enriched grains (Dahiya et al 2015). India stands first in both area and production of green gram all around the globe with production of 2.02 million tonnes. In Haryana, during 2019-20 green gram was grown in an area of 20.17 thousand ha producing 12.00 thousand tonnes with an average productivity of 595 kg ha^{-1} (Anonymous 2020). In Haryana, can be grown in *kharif* as well as in summer season due to wide adaptability and short duration. Green gram has wider adaptability, better palatability, higher market price and easy digestibility, so it can play a key role in increasing the economy of farmers (Reddy 2009). Fertilizers are one of the vital inputs required in production of any crop. The supply of nutrients in adequate amount by the chemical fertilizers is intimately linked to the plant growth and development. Potassium is widely regarded as the "quality element" for crop production and plays a dynamic role in plant growth and sustained production of crops. Potassium (K^+) has significant effect on stomatal movement, photosynthesis, synthesis of proteins, and water-relations (osmotic adjustment and turgor regulation) in plants (Marschner 2002), activation of about 60 enzymes, in grain development, plant metabolism as well as pest and disease and drought resistance (Egila et al 2001). Application of Potassium (K^+) is very rarely done in pulses despite field studies that suggested the application of K_2O to the tune of 20-40 kg ha^{-1} is helpful in attaining higher levels of pulse production. Among various micronutrients, Zinc is one also of the seven important elements for plant growth and activates many enzymes which are involved in metabolic processes and biochemical pathways. It acts as a functional, structural or regulatory co-factor for many enzymes and has key role in DNA transcription. Nijra and Nabwami (2015) reported that Zn influences the formation of chlorophyll and auxins which resulted in formation of the growth promoting compounds. About 43 % of the soil samples collected from different parts of India was deficient in zinc. By the year 2025, it is assumed that the zinc deficiency is likely to increase from 49 to 63 % as most of the cultivated soils are showing the symptoms of zinc deficiency (Arunachalam et al 2013). The current studies were planned to decipher the effects of different levels of potassium and zinc application on content and uptake of nutrients in green gram.

MATERIAL AND METHODS

The field experiment was conducted at research area of Regional Research Station, Bawal which is situated in district Rewari in the south-west Haryana. The research station is located at latitude 28°4' N, longitude 76°35' E and 266 m above mean sea level in South-Western area of Haryana.

The soils in the Bawal region are typically of Aridisols and Entisols orders and Ustochrept group. The initial soil properties of the experiment site were analyzed Soil was loamy sand in texture containing low (102.37), medium (11.18) and medium (170.10) kg ha^{-1} available nitrogen, phosphorus and potassium, respectively The macronutrients available zinc, iron, manganese and copper were 0.97, 7.64, 6.24 and 0.52 mg kg^{-1} at 0-15 cm depth with pH 8.17, EC 0.16 dS m^{-1} and organic carbon 0.17 per cent.

Experiment layout: The experiment was laid out in split plot design in triplications on green gram cultivar MH 421 with plot size of 4.0 m x 3.6 m. Sixteen treatments were assigned consisting of four potassium application levels (0, 10, 20 and 30 kg K_2O ha^{-1}) and four zinc application levels (0, 12.5, 25.0 and 37.5 kg $ZnSO_4$). The treatments were allotted to various plots with the help of random table. The recommended dose of fertilizer (RDF) was 6-8:16:8 kg for N and P_2O_5 ha^{-1} (Anonymous 2021). The fertilizers (RDF, K_2O and $ZnSo_4$) were applied at the time of sowing through soil application. Diammonium phosphate (DAP), muriate of potash (MOP) and zinc sulphate were used to provide desired levels of nutrients to the crops. The crop was raised with all the standard package of practices (Anonymous 2018) and protection measures also timely carried out as they required.

Collection and analysis of soil samples: The soil samples were collected at random from the experiment area up to the depth of 0-15 cm before overlaying the treatments and after harvesting the crop and were analyzed for its various chemical properties. The standard methods adopted for analysis of physico-chemical properties of soil. Electrical conductivity (EC) and soil reaction (pH) were determined in (1:2) Soil: Water suspension using digital pH meter and direct read type conductivity meter (Jackson 1973). Soil organic carbon content was determined by Walkley and Black (1934) method. Available Nitrogen (N) was determined by alkaline permanganate method (Subbaiah and Asija 1956) and available P content by extracting the soil samples using 0.5 $NaHCO_3$ (pH 8.5) and analyzed by spectrophotometer at 420 nm (Olsen et al 1954). Available K was extracted by using 1N ammonium acetate (pH 7.0) using a flame photometer (Jackson 1973).

Collection and analysis of plant samples: Samples of seed and straw were collected at the time of harvesting and dried (65±2 °C for 48 hr). The dried samples thus obtained were ground to a fine powder and processed further for estimation of various macronutrients (N, P and K). Total Nitrogen content in the digested plant material was determined by colorimetric method using Nessler's reagent as described by Lindner (1944). Total phosphorus in plant sample was determined by Vanado-molybdophosphoric acid yellow colour method as proposed by Koenig and Johnson (1942). Potassium in the acid digest of plant samples was determined by using flame photometer. The Zn, Fe, Cu and Mn of plant samples were determined by using plant digestion obtained from digestion by HNO3 and HClO4 with the help of Atomic Absorption Spectrophotometer (AAS) (Lindsay and Norvell 1978).

The data on concentration of NPK, grain yield and straw yield was used to determine the uptake of nitrogen (N), phosphorus (P) and potassium (K) using the following formula:

$$\text{Nutrient uptake (kg ha}^{-1}) = \frac{\text{Nutrient concentration in grain/straw (\%)} \times \text{grain/straw yield (kg ha}^{-1})}{100}$$

Statistical analysis: The data was statistical analysis by using online statistical package OPSTAT (Sheoran et al 1998).

RESULTS AND DISCUSSION

Effect of Potassium

Seed and straw yield: The seed yield was significantly increased due to application of potassium up to K_{20} treatment. Significantly, the highest seed yield (1142 kg ha^{-1}) was with K_{30}, followed by K_{20} (1090 kg ha^{-1}). Both the treatments were statistically at par with each other but significantly superior over control. The increase in seed yield of green gram was 9.53, 34.90 and 41.34 % due to application of K_{10}, K_{20} and K_{30} treatment, respectively over control (Table 1). Application of various levels of potassium increased the straw yield of green gram (Table 2). The straw yield of green gram ranged from 1005 to 1336 kg ha^{-1}. The straw yield was significantly increased due to application of potassium up to K_{20} treatment. The significantly, highest straw yield (1336 kg ha^{-1}) was with K_{30} treatment, followed by (K_{20}) and both the treatments were statistically at par. The lowest straw yield (1005 kg ha^{-1}) was recorded with control (K_0). The K_{30} and K_{20} produced 32.94 and 22.01 % higher straw yield over control respectively. The positive effect of potassium in photosynthesis and cell elongation and more over higher nutrients uptake resulted in higher plant height and number of branches per plant and ultimately helped in realization of higher crop yield. Potassium enhances the plant vigour and strengthens the stalk. The above results found are in conformity with the results of Jadeja et al (2016) and Kumar et al (2018).

Nutrient content and uptake by seed and straw: Potassium application caused significant increase in the nutrient content (N, P, K and Fe) in seed of green gram. The content of nutrients increased with the highest level i.e. 30 kg K_2O ha^{-1} but it was at par with 20 kg K_2O ha^{-1}. The total nitrogen, phosphorous and potassium uptake and content in

seed was significantly influenced by different potassium levels. Both K_{20} and K_{30} treatments recorded significantly higher N (3.55 and 3.67 %), P (0.48 and 0.50 %) and K (1.14 and 1.16 %) t and uptake of N (38.70 and 41.91 kg ha^{-1}), P (5.23 and 5.71 kg ha^{-1}) and K (12.43 and 13.25 kg ha^{-1}) in seed as compared to control.

In case of straw, K_{20} and K_{30} treatments recorded significantly higher N (1.33 and 1.43 %), P (0.22 and 0.24 %) and K (1.40 and 1.47 %) content and uptake of N (16.32 and 19.10 kg ha^{-1}), P (2.70 and 3.21 kg ha^{-1}) and K (17.17 and 19.69 kg ha^{-1}) in straw over the control. The increase in concentration of N and P might be due to the synergistic effect of potassium on these nutrients and the increase in K concentration and uptake is due to the direct application of potassium which increases its availability to plants. Comparable results were also reported by earlier researchers (Khurhade et al 2015, Ranpariya and Polara 2018, Adsure et al 2018, Chaudhari et al 2018).

Effect of Zinc

Seed and straw yield: Application of zinc also significantly affected the seed yield of green gram. Significantly, the highest seed yield (1038 kg ha^{-1}) was recorded with $Zn_{37.5}$ treatment followed by Zn_{25} (1009 kg ha^{-1}), both treatments were statistically at par. The lowest seed yield (921 kg ha^{-1}) was recorded with control (Zn_0). The $Zn_{37.5}$ and Zn_{25} treatment produced 12.70 and 9.55 % higher seed yield over control. The interacting effect between potassium and zinc was found non-significant. Similarly, in straw yield, significantly highest straw yield (1189 kg ha^{-1}) was with $Zn_{37.5}$ treatment, followed by (1183 kg ha^{-1}) with Zn_{25} treatment. However, treatments $Zn_{37.5}$ and Zn_{25} were statistically at par with each other but superior over the control. The lowest straw yield (1121 kg ha^{-1}) was with control (Zn_0). The increase in straw yield was 0.71, 5.53 and 6.07 % due to application of $Zn_{12.5}$, Zn_{25} and $Zn_{37.5}$ treatments, respectively over control. Zinc plays a vital role in plant nutrition, which is clear from its involvement in process of photosynthesis and sugar translocation. The increase in seed yield might be due to role of zinc in biosynthesis of indole acetic acid and especially due to role in primordial for reproductive parts and partitioning of photosynthesis towards them which resulted in better flowering and fruiting. Similar trend was observed by Buriro et al (2015), Sangapa and Angadi (2016), Islam et al (2017), and Ranpariya and Polara (2018). The interaction effect of potassium and zinc levels was found non-significant on straw yield of green gram.

Nutrient content and uptake by seed and straw: Results showed that nutrients content was not found to be influenced significantly by the zinc application but their uptakes were influenced significantly by graded levels of zinc except P because application of Zn did not show any particular trend with respect to P uptake and content. Phosphorus and zinc have antagonistic effect on each other. The application of zinc @ 25 kg $ZnSO_4$ ha^{-1} showed significantly higher values of

Table 1. Effect of potassium and zinc application on seed yield (kg ha^{-1}) of green gram

Potassium levels (kg K_2O ha^{-1})	Zinc levels (kg $ZnSO_4$ ha^{-1})				Mean
	Zn_0	$Zn_{12.5}$	Zn_{25}	$Zn_{37.5}$	
K_0	775	798	820	840	808
K_{10}	852	870	887	930	885
K_{20}	995	1019	1159	1188	1090
K_{30}	1062	1146	1170	1192	1142
Mean	921	958	1009	1038	
CD (p=0.05)	Potassium (K)= 102; Zinc (Zn)=49; K × Zn=NS				

Table 2. Effect of potassium and zinc application on straw yield (kg ha^{-1}) of green gram

Potassium levels (kg K_2O ha^{-1})	Zinc levels (kg $ZnSO_4$ ha^{-1})				Mean
	Zn_0	$Zn_{12.5}$	Zn_{25}	$Zn_{37.5}$	
K_0	987	998	1013	1023	1005
K_{10}	1028	1034	1073	1077	1053
K_{20}	1149	1153	1300	1305	1227
K_{30}	1318	1329	1345	1351	1336
Mean	1121	1129	1183	1189	
CD (p=0.05)	Potassium (K)= 133; Zinc (Zn)=51; K × Zn=NS				

Table 3. Effect of potassium and zinc application on content and uptake of macro nutrients in seed of green gram

Treatments (kg ha^{-1})	Seed NPK content (%)			Seed NPK uptake (kg ha^{-1})		
	N	P	K	N	P	K
Potassium levels (kg K_2O ha^{-1})						
K_0	3.21	0.43	0.93	25.94	3.47	7.51
K_{10}	3.36	0.44	1.03	29.74	3.89	8.76
K_{20}	3.55	0.48	1.14	38.70	5.23	12.43
K_{30}	3.67	0.50	1.16	41.91	5.71	13.25
CD (p=0.05)	0.17	0.03	0.11	3.93	0.59	1.68
Zinc levels (kg $ZnSO_4$ ha^{-1})						
Zn_0	3.37	0.47	1.06	31.04	4.33	9.76
$Zn_{12.5}$	3.42	0.47	1.06	32.76	4.50	10.15
Zn_{25}	3.47	0.48	1.07	35.01	4.84	10.80
$Zn_{37.5}$	3.51	0.46	1.08	36.43	4.77	11.21
CD (p=0.05)	NS	NS	NS	2.91	NS	0.61
C. V. %	8.74	10.38	9.66	11.14	14.52	8.26

Table 4. Effect of potassium and zinc application on content and uptake of macronutrients in straw of green gram

Treatments (kg ha^{-1})	Straw NPK content (%)			Straw NPK uptake (kg ha^{-1})		
	N	P	K	N	P	K
Potassium levels (kg K_2O ha^{-1})						
K_0	1.00	0.16	1.10	10.05	1.61	11.06
K_{10}	1.14	0.18	1.19	12.00	1.79	12.53
K_{20}	1.33	0.22	1.40	16.32	2.70	17.17
K_{30}	1.43	0.24	1.47	19.10	3.21	19.64
CD (p=0.05)	0.17	0.03	0.10	2.98	0.56	2.53
Zinc levels (kg $ZnSO_4$ ha^{-1})						
Zn_0	1.13	0.20	1.26	12.67	2.24	14.12
$Zn_{12.5}$	1.22	0.20	1.28	13.77	2.26	14.45
Zn_{25}	1.26	0.21	1.30	14.91	2.48	15.38
$Zn_{37.5}$	1.30	0.20	1.31	15.46	2.38	15.58
CD (p=0.05)	NS	NS	NS	1.12	NS	0.87
C. V. %	8.52	10.33	9.82	11.66	13.65	12.32

uptake of nitrogen (35.01 kg ha^{-1} and 14.91 kg ha^{-1}) and potassium (10.80 kg ha^{-1} and 15.38 kg ha^{-1}) by seed and straw. This might be due to the increasing pattern of straw yield with graded levels of fertilizers or may be due to the dilution effect. Similar results were observed by earlier scinetists (Chavan et al 2012, Roy et al 2017, Ranpariya et al 2018, Ahmed et al 2018).

CONCLUSION

Both the seed and straw yield of green gram significantly increased with the application of potassium and zinc up to the 20 kg K_2O ha^{-1} and 25 kg $ZnSO_4$ ha^{-1}. The application of potassium @ 20 kg ha^{-1} and zinc sulphate @ 25 kg ha^{-1} significantly increase the nutrient content and uptake in seed and straw of green gram in coarse textured medium K status soil. The higher content of potassium was in straw than seed whereas higher content of nitrogen, phosphorus were observed in seed than straw. The, the higher uptake of N, P and K was observed in straw than seed.

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Consortium Biofertilizers to Economise Nutrient use and Sustain Productivity in Cassava (*Manihot esculenta* Crantz)

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***Abstract*:** Climate change events and COVID 19 pandemic have brought to focus the significance of cassava as a supplementary food crop worldwide. However, the high yield potential of the crop necessitates timely and adequate enrichment of soil with nutrient inputs. Consortium biofertilizers offer a viable option for reducing intensive fertilizer use to sustain soil heath and productivity in cassava. The efficacy of liquid consortium biofertilizer, plant growth promoting rhizobacteria (PGPR) Mix - I in economizing nutrient use in cassava were evaluated in a 4 x 3 factorial randomized block design replicated thrice. Combinations of four levels of biofertilizers and three levels of nutrients comprised the treatments. The biometric and yield observations were recorded and soil properties analyses, pre and post harvest of the crop. The liquid biofertilizer consortium at 5 % concentration + 75 % recommended dose, 37.5: 37.5: 75 kg NPK ha^{-1} as chemical fertilizers recorded the ssignificantly superior tuber yield in cassava. Considering the economics, application of PGPR liquid formulation (5 %) thrice (basal, 2 and 4 MAP), along with 37.5: 37.5: 75 kg NPK ha^{-1} or at 2 % concentration with 50:50:100 kg NPK ha^{-1} realized higher benefit-cost ratios and can be recommended in cassava.

***Keywords*:** Biofertilizer, Cassava, Nutrient, PGPR, Tuber yield

Cassava (*Manihot esculenta* Crantz), belonging to the family Euphorbiaceae, is a benchmark of food security being an affordable crop for the poor. The crop has proved to be life sustaining in times of natural calamities and famine. Compared to other food crops, cassava is bestowed with the ability to grow on marginal lands and are climate resilient. The yield potential ranges from 25 to 43.4 Mg ha^{-1} (John et al 2007) which is suggestive of its high potent bioconversion efficiency. This implies that the crop is a heavy feeder of nutrients. The nutrient removal by cassava is estimated to be 180, 22 and 160 kg N, P and K ha^{-1} respectively for 30 t ha^{-1} tuber yield (John et al 2019), necessitating a regular supply of nutrient inputs and the recommendations converges to an NPK dose of 100 kg each of N, P and K (KAU 2016), modified based on site specific soil test data. Scientific studies illustrate the use of chemical fertilizers as the source of nutrients and the practice was enormously encouraged among the farming community. Nevertheless, over the years, soil, the fountain of life has borne the maximum impact of the indiscriminate chemical use as a result of which the biology that sustained quality has been hampered (Alori and Babalola 2018). The escalating cost of chemical fertilizers and the increasing awareness on the ill effects of imperceptive chemical use have led to the added interests on integrated nutrient management practices in which organic and bio nutrient inputs can partly substitute chemical fertilizers in satisfying the crop nutrient requirements. Bio nutrient inputs focus on the microbial formulations intended to enhance the availability of nutrients to crop plants. Howbeit, being single microbe based, the adoption and use are constrained by the specificity for nutrients and need for multiple and separate inoculation for each nutrient during crop growth. This paved way for the development of consortium biofertilizers that have the advantage of a heterogeneous population of plant growth promoting rhizobacteria in a single inoculum, assuring mineralisation and solubilisation of different nutrients with a use of single formulation amending the earlier disadvantage. Gopal (2018) have illustrated the superiority of consortium formulations over the single inoculum biofertilizer. In light of the above, talc based and liquid formulations of Plant Growth Promoting Rhizobacteria (PGPR) Mix-I were developed by the Department of Agricultural Microbiology, College of Agriculture, Vellayani under Kerala Agricultural University, India which were tested for its efficacy in different crops (Jayapal, 2012). The consortium includes nitrogen (N) fixers (*Azospirillum lipoferum, Azotobacter chroococcum*), phosphorus (P) solubiliser (*Bacillus megaterium*) and potassium (K) solubiliser (*Bacillus sporothermodurans*).

As biofertilizers add to the soil flora of beneficial microorganisms and enhance nutrient solubilisation, a field experiment was undertaken in cassava (*Manihot esculenta* Crantz), the most popular tuber crop in Kerala in the southern laterites (Agroecological Unit 8), to assess the efficacy of PGPR formulations in sustaining productivity and economising nutrient use.

MATERIA AND METHODS

Site and experimental conditions: The field experiment was conducted at Kerala Agricultural University during June to December 2019. The site falling under the agroclimatic zone and agroecological unit 8 (Southern laterites) is located at 8°30'N latitude, 76°54'E longitude and at an altitude of 29 m above mean sea level and experienced a warm humid tropical climate with a maximum and minimum temperatures ranging from 29.5 to 34.1°C and 26.1 to 23.6°C respectively, relative humidity from 87.1 to 94.6 % during the cropping period. The precipitation received during the cropping period was 267.3 mm, mainly during the months of June- July and October- November 2019. Soil was sandy clay loam in texture belonging to the order ultisol. Chemical properties assessed revealed the soil to be strongly acidic (pH, 5.23), high in organic carbon (1.25 %), medium in N (294.37 kg ha^{-1}) and K (138.32 kg ha^{-1}) and high in P (42.63 kg ha^{-1}) before cropping.

The experiment was laid out in factorial randomized block design with two factors, biofertilizers [b_1: PGPR Mix -I liquid (@ 2 % ; b_2: PGPR Mix -I liquid (@ 5 %; b_3: PGPR Mix -I powder @ 10 g of 2 % mixture per plant ; b_4: without biofertilizer] and nutrient levels [n_1: 50 %; n_2: 75 % ; n_3: 100 % of recommended dose of nutrients (RDN), 50:50:100 kg NPK ha^{-1}]. After land preparation, dried and powdered farmyard manure was incorporated with final ploughing and mounds taken at a spacing of 90 cm x 90 cm in the individual plots. Cassava setts (20 cm long cuttings of stem, 4-5 nodded) of short duration variety Vellayani Hraswa, (5-6 months) were planted with two nodes of each set below the soil and remaining nodes above. The basal dose of nutrients were given with straight fertilizers, urea, rajphos and potash as per the treatments. The biofertilizers were applied thrice, at planting (one week after fertilizer application), 2 and 4 months after planting (MAP). The 2 and 5 % concentrations of PGPR Mix -I liquid were prepared by mixing 20 mL and 50 mL of liquid consortium in 1000 mL water respectively. From the prepared solution 200mL was applied in the root zone, on each mound according to the treatments. The mixture of the powder formulation was prepared by mixing 20 g talc based PGPR Mix-I with one kg of powdered cow dung and 10 g of the mixture was applied on each mound. Full dose of P was applied basally, N and K were given in three equal splits (basal, 1 and 2 MAP) using the chemical fertilizers, urea, rajphos and muriate of potash.

Biometric and yield observations: Three plants were randomly selected from the net plot area and tagged as observational plants for recording biometric observations. Cultural operations were done as per the recommended package for cassava. The crop was ready for harvest, six months after planting (MAP). The mounds were irrigated on the previous day of harvest and on the day of harvest, soil at the plant base was loosened by light digging. The plant top was cut and the remaining stump was pulled out carefully with tubers intact. Tubers were cut from the stem and weighed to record the fresh weights. The length and girth of ten randomly selected tubers in each treatment were measured and recorded. The tubers were also weighed individually to record the mean weights. The per hectare yields were computed from the yields recorded in treatment wise the net plot area in each treatment.

Plant analysis: Samples of stem, leaves and tuber collected for chemical analysis were dried separately in an air oven at 70 ± 5°C and ground to pass through 0.5 mm mesh. The N content of the plant parts was determined separately by modified micro Kjeldahl method (Jackson, 1973). The P content was estimated calorimetrically (Jackson 1973) and K content by flame photometry method (Piper 1967). The total uptake of N, P, and K at harvest were calculated by multiplying the respective nutrient content in the stem, leaf and tuber with their corresponding dry weights and expressed in kg ha^{-1}.

Economics: The economics of cultivation of the crop was worked out in terms of the returns per rupee invested (benefit cost ratio) based on the cost of cultivation and gross income realized.

Statistical analysis: The data on the biometric and yield parameters were analysed using OP Stat software.

RESULTS AND DISCUSSION

Biometric Observations

Growth attributes: The growth attributes in cassava, plant height, number of functional leaves per plant and leaf area index (LAI) varied significantly with PGPR application and varying nutrient doses (Table 1 and 2) and indicated their favourable influence on the vegetative growth. All the parameters were found to be maximum at the harvest stage, in accordance with the growth phenology of cassava.

The plant height was maximum with PGPR (L) @ 5 % (b_2) application and among the nutrient levels, n_2 (75% RDN) at 2 and 4 MAP (66.1 cm and 99.5 cm respectively) and n_1 (50 % RDN) at harvest, were significantly superior. The interaction effects were significant and PGPR (L) 5 % in combination with 75 % RDN at 2 and 4 MAP (82.3 and 114.3 cm respectively) and with 50 % RDN at harvest, recorded the tallest plants, the latter on par with 75% RDN. The number of functional leaves and leaf area index (LAI) varied significantly with the individual effects of biofertilizers and nutrient levels. Maximum values were observed with liquid PGPR @ 5 % (b_2) and 75 % RDN (n_2) at all stages of growth. The effects were

reflected in the interaction and b_2n_2 recorded the highest number of functional leaves and LAI at all stages of growth and the lowest were in treatment b_0n_1 (50 % RDN without biofertilizer). Biofertilizer consortium as a complementary nutrient input in crop production has been studied by several workers (Dhanya 2011, Jayapal 2012, Radhakrishnan et al 2013, Suja et al 2014, Gopi 2018). Singh (2013) confers better plant growth promotion ability to PGPR as the consortium, apart from the nutrient supplying potential are able to synthesise phyto hormones, decompose organic matter, enlarge the soil flora and improve the soil structure for root development and better absorption of water and nutrients. Gautam et al (2017) observed that application of liquid based biofertilizer as well as carrier based biofertilizers reinforced the rhizosphere microbiome due to availability of carbon and energy sources in the rhizosphere. When chemical fertilizers are used solely, the chances of nutrient losses are high, and microbial activity, the key regulator of improved soil health is meagre, which would have led to its comparatively poorer performance. Ansari et al (2015) reported 21 to 50 % increase in the chick pea dry seed yields with liquid biofertilizer application in pot culture study and nearly 144 % increase in the field experiment over the uninoculated control. Kaur et al (2018) discerned the enhancement in wheat yields with liquid biofertilizers to the direct and indirect mechanisms such as biological nitrogen fixation, phosphate solubilization, phytohormone production and 1-aminocyclopropane-1-carboxylic acid (ACC 1)

Table 1. Effect of biofertilizer and levels of nutrients on growth attributes in cassava

Treatments	Plant height (cm)			Number of functional leaves			LAI		
	2 MAP	4 MAP	Harvest	2 MAP	4 MAP	Harvest	2 MAP	4 MAP	Harvest
Biofertilizer (B)									
b_1 - PGPR (L) 2 %	61.69	92.96	170.48	26.40	45.41	73.59	1.38	2.34	4.17
b_2 - PGPR (L) 5 %	68.11	107.58	188.14	28.67	47.78	79.67	1.64	2.64	4.35
b_3 - PGPR (P)	64.88	103.88	144.44	28.29	42.56	73.18	1.41	2.21	3.97
b_0 - without biofertilizer	40.00	79.47	123.81	26.40	42.44	69.96	1.34	2.18	3.74
CD (p=0.05)	1.385	1.076	1.862	1.172	0.891	0.923	0.055	0.059	0.09
Levels of nutrients (N)									
n_1 - 50 % RDN	51.19	96.19	162.19	25.80	42.41	71.52	1.33	2.13	3.64
n_2 - 75 % RDN	66.11	99.46	153.52	29.63	47.11	75.86	1.56	2.58	4.35
n_3 - 100 % RDN	58.83	92.27	154.52	26.88	43.86	74.91	1.44	2.33	4.19
CD (p=0.05)	1.190	0.932	1.614	1.014	0.775	0.803	0.031	0.052	0.080

L: Liquid P- Powder RDN- 50: 50: 100 kg NPK ha^{-1}

Table 2. Interaction effects of biofertilizer and levels of nutrients on growth attributes

Treatments	Plant height (cm)			Number of functional leaves			LAI		
	2 MAP	4 MAP	Harvest	2 MAP	4 MAP	Harvest	2 MAP	4 MAP	Harvest
b_1n_1	47.33	88.66	154.56	24.67	44.55	73.11	1.26	2.12	3.75
b_1n_2	72.23	99.77	177.33	28.56	46.55	73.00	1.47	2.55	4.45
b_1n_3	66.33	90.44	179.56	26.00	44.11	74.67	1.41	2.34	4.32
b_2n_1	69.33	108.44	193.00	26.67	45.22	76.78	1.60	2.32	3.83
b_2n_2	82.33	114.32	195.00	32.22	54.11	85.22	1.83	3.15	4.63
b_2n_3	52.66	99.91	176.44	27.11	44.00	77.00	1.50	2.46	4.17
b_3n_1	52.44	109.44	187.11	28.44	43.33	71.56	1.36	2.17	3.62
b_3n_2	67.55	108.55	124.33	30.44	43.00	74.44	1.48	2.40	4.08
b_3n_3	74.67	93.66	121.88	26.00	41.33	73.55	1.41	2.08	4.22
b_0n_1	35.66	78.21	114.11	23.44	36.55	64.67	1.10	1.92	3.38
b_0n_2	42.33	75.21	117.11	27.33	44.78	70.78	1.46	2.21	3.80
b_0n_3	41.67	84.99	140.22	28.44	46.00	74.44	1.47	2.44	4.05
CD (p=0.05)	2.394	1.862	3.223	2.028	1.544	1.606	0.09	0.114	0.160

deaminase activity and siderophore production. Vendan and Thangaraju (2006) documented the advantages of liquid formulations of biofertilizers over powder formulation to include higher microbial counts, near zero contamination, greater protection against environmental stresses and increased field efficacy. According to Hoe and Rahim (2010), liquid biofertilizers have more viable cells than carrier based biofertilizers. Apart from the desired microorganisms and their nutrient solubilising properties, liquid biofertilizers also contain special cell protectants or substances that encourage the formation of resting spores or cysts for longer shelf life (Chandra et al 2005, Hegde 2002). Glycerol amended PGPR liquid formulation used in the present study, had the advantage of enhanced tolerance of cells to desiccation, osmotic pressure and temperature stress (Gopi et al 2020) due to induced synthesis of metabolites that offer protection against the stress (Kumaresan and Reetha, 2011).

The better performance of liquid biofertilizers over carrier based formulations as observed are in accordance with the reports of Maheswari and Kalayarasi (2015), Gopal (2018) and Lakshmi et al (2019). Among the two concentrations of the liquid formulations, the 5 % concentration was found to be superior. Although Gopi et al (2018) recommended 2 % concentration of PGPR (L) + 50 % RDN to be effective in a 45 day old crop of *Amaranthus tricolor,* in the present study it is reasoned that cassava variety Vellayani Hraswa, being a crop of longer duration (180 days) required a higher concentration (cell count) to realise the benefits of the formulation.

The NPK recommendation, 50: 50: 100 kg ha^{-1} found ideal for short duration variety Vellayani Hraswa (Sekhar, 2004) was the nutrient dose (RDN) adopted for the experiment. Nevertheless, the results revealed that among the different levels, 75 % of RDN (37.5: 37.5: 75 kg NPK ha^{-1}) was superior to produce a good vegetative frame of the crop and tuber yield (42.11 Mg ha^{-1}) indicating the sufficiency of 37.5 kg each of N and P and 75 kg K ha^{-1}, a saving of 25 percent of fertilizers and costs. Tuber yield was the lowest in n_1 where 50 % of RDN (25: 25: 50 kg NPK ha^{-1}) was applied. Cassava is known to be a heavy feeder and it is reckoned that this lower dose was inadequate. The positive response of the former on growth parameters (number of functional leaves and LAI) and the yield attributes (number of tubers per plant, mean tuber weight, length of tuber and girth of tuber) that influenced the yield of the crop have contributed to the significantly highest yield over the latter. The combination of 5 % PGPR Mix-I liquid and 75 % RDN recorded the highest values for the yield attributes (Table 3). The increased uptake of nutrients from soil with the integrated application of nutrients and biofertilizers would have produced enough carbohydrates in the leaves for translocation to the sink for better tuber number and bulking, thereby favouring better tuber yield. The higher LAI and tuber parameters realised in b_2n_2 indicate a better source sink balance and hence better productivity in this treatment.

Yield attributes and yield: The superiority of the liquid formulation @ 5% in enhancing tuber production is evident in terms of the number of tubers per plant (8.7) and mean tuber weight (0.67 kg) and tuber yields (5.0 kg per plant) (Table 3). Among the nutrient levels, 75 % RDN showed maximum weight (0.59 kg), number of tubers (8.86) and tuber yield per plant (4.77 kg). The interaction, b_2n_2 was superior among treatment combinations producing the highest number of tubers per plant (10.00) and mean tuber weight (0.78 kg). Tuber yield per plant (5.12 kg) was also the highest in b_2n_2. The per hectare yields followed the same trend with the maximum yield (44.49 Mg ha^{-1}) in the combination of liquid formulation of PGPR @ 5 % + 75 % RDN followed by b_1n_3 [PGPR (L) 2 % + 100 % RDN] (Table 3). The yields were 44.49 and 42.32 Mg ha^{-1} respectively and 11 and 17 % higher than the application of chemical fertilizers at the recommended dose without biofertilizers. Harvest index in the b_2n_2 combination was the highest. Exploring the individual effects, application of biofertilizer. PGPR liquid formulation at 5 % and fertilizers at 75 % RDN showed significantly higher HI (0.79 and 0.72 respectively) but in the latter, n_2 (75 % RDN) was on par with n_3 (100 % RDN). Suja et al (2005) reported that integrated use of bioinoculants (*Azospirillum* and *Phosphobacter*), organic manures, 100 % K, 50 % of N and P produced tuber yields on par with the recommended dose of fertilizers (50: 50: 100 kg NPK ha^{-1}) for cassava implying the possibility of reducing N and P fertilizers to 50 % in cassava.

Nutrient uptake: The N, P and K uptake were higher with the inclusion of biofertilizers, (Fig. 1), the maximum in b_2 and the nutrient dose of 75% RDF (n_2). Suja et al (2010) also observed significant effect of fertility levels on the NPK uptake in cassava. Among interactions b_2n_2 was also superior for all nutrients. The highest uptakes to be 203.07, 62.81 and 188.02 kg ha^{-1} for N, P and K respectively (Fig. 2).

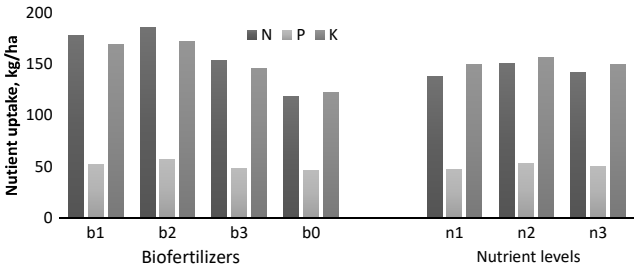

Fig. 1. Nutrient uptake in cassava as influenced by biofertilizers and different nutrient levels

Nutrient uptake by crop is a function of nutrient content in dry matter and the dry matter production and nutrient content is related to the photosynthetic activity of leaves. The uptake of N, P and K were the highest in the treatment with 5 % PGPR (L) followed by 2 % PGPR (L) followed the order N > P > K. The better plant growth observed in biofertilizer included treatments, would have resulted in improved nutrient absorption and higher biomass production that ensued the higher nutrient uptake and better yields. It is also interpreted that the addition of biofertilizers augmented the microflora and rhizospheric processes that created a conducive environment for better uptake.

Economics: The highest ratio (3.20) was in the combination of 2 % PGPR (L) consortium + 100 % RDN followed by the 5 % PGPR (L) with 75 % RDN (3.07). The benefit cost ratio with the use of 2 % concentration (3.2) was higher than that with the 5 % formulation (3.07) on account of the higher input cost, involved in the preparation of 5 % PGPR (L) and cost of fertilizers, The lowest B:C ratio recorded in the treatment combination of PGPR Mix-I powder and full dose RDN is attributed to the high cost of powder formulation and cost of FYM in applying the mixture. Thus taking into account the economics, application of 2 % formulation thrice (basal, 2 and 4 MAP) along with 50: 50: 100 kg NPK ha^{-1} or 5 % liquid formulation + 37.5: 37.5: 75 kg NPK ha^{-1} can be recommended for the cultivation of short duration cassava variety Vellayani Hraswa. The latter nutrient management practice has the added advantage of reducing the fertilizer load in soil and pollution at the same time contributing to soil health and sustenance. The improved yields in cassava with

Table 3. Effect of biofertilizer and levels of nutrients on yield and harvest index in cassava

Treatments	Number of tubers per plant	Mean tuber weight (kg)	Tuber yield per plant (kg)	Tuber yield (Mg ha^{-1})	Top yield (Mg ha^{-1})	Harvest Index
Biofertilizer (B)						
b_1 - PGPR (L) 2 %	7.85	0.62	4.61	41.31	16.36	0.72
b_2 - PGPR (L) 5 %	8.74	0.67	5.00	43.12	16.71	0.79
b_3 - PGPR (P)	7.78	0.45	4.15	39.05	14.58	0.70
b_0 - Without biofertilizer	6.59	0.49	3.47	37.60	14.38	0.67
CD (0.05)	0.260	0.048	0.237	1.013	0.858	0.062
Levels of nutrients (N)						
n_1 - 50 % RDN	6.53	0.51	4.09	38.23	15.42	0.67
n_2 - 75 % RDN	8.86	0.59	4.77	42.11	15.82	0.72
n_3 - 100 % RDN	7.83	0.52	4.36	41.07	15.29	0.70
CD (p=0.05)	0.205	0.043	0.196	0.594	NS	0.042

*See Table 1 for details

Table 4. Effect of interaction on yield and harvest index in cassava

B × N Interaction	Number of tubers per plant	Mean tuber weight (kg)	Tuber yield per plant (kg)	Tuber yield (Mg ha^{-1})	Top yield (Mg ha^{-1})	Harvest Index
b_1n_1	6.22	0.64	4.17	38.83	14.55	0.68
b_1n_2	8.78	0.58	4.28	40.16	17.09	0.70
b_1n_3	8.56	0.62	4.69	42.32	16.76	0.73
b_2n_1	5.67	0.65	4.21	39.27	17.67	0.69
b_2n_2	10.00	0.78	5.12	44.49	17.92	0.78
b_2n_3	8.67	0.44	4.16	39.54	15.23	0.72
b_3n_1	8.67	0.45	4.11	38.56	14.24	0.69
b_3n_2	9.18	0.39	4.13	39.28	14.29	0.71
b_3n_3	7.55	0.52	4.15	38.06	15.55	0.70
b_0n_1	5.55	0.55	3.42	36.01	13.56	0.67
b_0n_2	7.66	0.43	3.83	37.78	14.04	0.68
b_0n_3	6.56	0.52	3.91	38.01	15.21	0.69
CD (p=0.05)	0.450	0.066	0.393	1.752	1.475	0.042

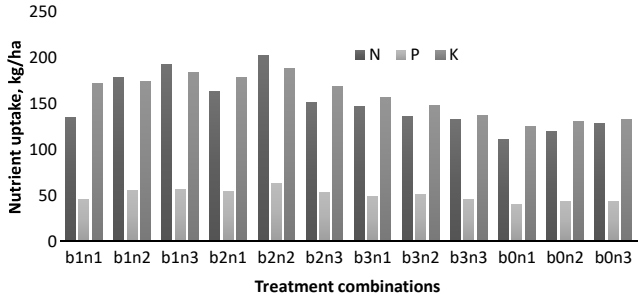

Fig. 2. Nutrient uptake in cassava as influenced by interaction of biofertilizers and different nutrient levels

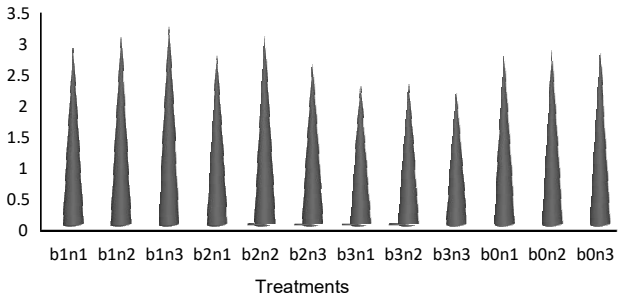

Fig. 3. Effect of biofertilizer x nutrient levels on the economics

the use of liquid formulation at 5 % concentration of the consortium biofertilizer and the 25 % saving in chemical fertilizers portray the sustainability and economic benefits of the practice in the tuber cultivation.

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Effect of Vermicompost and Fertilizer on Microbial Biomass, Carbon Pools and Hydrolyzable Carbohydrate Acid in Pot Culture Rice

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***Abstract*:** Integrated application of organic and inorganic sources elevates the soil physical, chemical and biological properties along with stabilization of soil health deterioration. Pot culture experiment was carried out at RPCAU, Pusa in *kharif,* 2019 with four levels of vermicompost (0 t $ha^{-1,}$ 1.25 t ha^{-1}, 2.5 t ha^{-1} and 3.75 t ha^{-1}) and three levels of fertilizer RDF (0 ,50 and 100 % RDF) on rice for analyzing its effect on soil microbial biomass nitrogen, phosphorus and carbon pool. The microbial biomass carbon, nitrogen, phosphorus and the different carbon pool content elevated from tillering to post harvest stage in soil with response to the higher dose of vermicompost and fertilizer RDF i.e. 3.75 t ha^{-1} vermicompost + 100 % NPK. microbial biomass carbon increased up to116%, 125% over control in tillering and post harvesting stage of crop. microbial biomass nitrogen, prosperous and carbon pool content also showed higher results in both crop growth stages due to the higher amount of organic matter addition elevated the carbon pool content and provided the substrate for microbial biomass component increase in pot culture soil.

***Keywords*:** Pot culture, Carbon pools, Rice, Vermicompost, Enriched, Fertilizer

The population growth is increasing day by day and to satisfy the need the farmers are applying large quantities of inorganic fertilizers leading to the destruction of overall soil health and decline in yield capacity of the soil. Toxicity of nutrients leads to cause imbalance of major nutrients like nitrogen, phosphorus, potassium as well as micro nutrients in soil. Rice is the major crop cultivated in the whole world and in Asia provides livelihood support to 70% of farmers. Balanced use of vermicompost and fertilizer improves the soil physical, chemical, biological, enzymatic properties (Alshehrei and Ameen 2021) as well as elevates the growth and yield of rice crops ensuring sustainability in agriculture. Vermicomposting is the process of conversion of waste materials into finely divided, enriched in nutrients and enzymes containing organic products by the use of earthworms. Prolonged application of vermicompost with fertilizers increases the buildup of carbon and nutrients pool in soil which is critical for soil fertility maintenance and enhancement (Kumar et al 2020). Enriched soil microbial diversity helps in recycling of different nutrients in soil which can be utilized by crop plants in soil which increases the yield of crops and minimizes the pollutions in environment (Prakash 2021).

MATERIAL AND METHODS

A pot culture experiment was conducted with rice crop at RPCAU, Pusa in *kharif,* 2018 with four levels of vermicompost and three levels of fertilizer with twelve treatments replicated thrice using CRD design in two factors with standard ANOVA table and OPSTAT application.

RESULTS AND DISCUSSION

Microbial biomass carbon (µg g^{-1}): The fertilizer application of 50 % and 100 % RDF with higher vermicompost dose were significantly superior over no fertilizer application (Table 2). The increase in the microbial biomass carbon in soil from tillering to post-harvest might be due to availability of more nutrients caused by enhanced rhizospheric effects, secretion of organic acids and root exudates leading to more microbial activities thus more microbial biomass carbon. The increase in the microbial biomass carbon in pot-culture experiment was slightly higher than incubation experiment. The results obtained in the incubation experiment for biomass carbon was in line with the findings of Ramachandran (2013) and Ashraf et al (2021).

Microbial biomass nitrogen (µg g^{-1}): The 50 and 100 % RDF level also gave significantly higher microbial biomass nitrogen over no fertilizer level application (Table 3). The interactions in between vermicompost and fertilizer levels were found significant. The elevated microbial biomass nitrogen was found in the treatment receiving

(vermicompost-3.75 t ha^{-1} + 100 % RDF) i.e. 105.69 which was significantly superior over control (49.56 µg g^{-1}). The increase in the microbial biomass nitrogen from tillering to post-harvest could be due to availability of more nutrients caused by enhanced rhizospheric effects, secretion of organic acids and root exudates leading to more microbial activities thus more microbial biomass nitrogen. The increase in the microbial biomass nitrogen in pot-culture experiment was slightly higher than incubation experiment. Katkar et al (2011) in the incubation experiment for biomass nitrogen indicated similar trend.

Microbial biomass phosphorus (µg g^{-1}): The integrated application of vermicompost (3.75 t ha^{-1}) + 100 % NPK showed higher amount of microbial biomass phosphorus content i.e. 4.95 µg g^{-1} and 5.29 µg g^{-1} in soil at tillering and post-harvest, respectively over the control (Table 4). The

Table 1. Standard analysis procedure

Parameters	Method	References
Microbial biomass carbon	Fumigation-extraction	Vance et al (1987)
Microbial biomass nitrogen	Fumigation-extraction, KCl extraction	Brookeset al (1985)
Microbial biomass phosphorus	Fumigation-extraction, 660nm, 0.5 M $NaHCO_3$	Brookes et al (1982)
Water soluble carbon		Ghani et al (2003)
Acid hydrolyzable carbohydrate	Standard anthrone process	Chesire and Mundie (1966)
$KMnO_4$ carbon	Permanganate extraction method	Blair et al (1995)
Organic carbon	Wet digestion technique	Walkley and Black (1934)

Table 2. Effect of vermicompost and fertilizer on microbial biomass carbon in soil of rice crop during growth period (µg g^{-1})

Treatments	Tillering stage				Post-harvest stage			
	F_0	F_{100}	F_{50}	Mean	F_0	F_{100}	F_{50}	Mean
V_0	85.62	114.66	100.79	100.36	107.80	164.74	121.72	131.42
$V_{1.25}$	92.40	164.64	137.23	131.43	135.13	193.04	201.07	176.41
$V_{2.5}$	128.38	178.05	143.84	150.09	178.17	220.47	215.08	204.57
$V_{3.75}$	136.59	185.67	172.57	164.94	200.62	243.29	222.69	222.20
Mean	110.75	160.76	138.61		155.43	205.38	190.14	
Factors	CD (5%)							
Vermicompost (V)	0.50				0.55			
Fertilizers (F)	0.43				0.48			
V X F	0.86				0.96			

V_0= Vermicompost (no manure), $V_{1.25}$= Vermicompost (1.25 t ha^{-1}), V2.5= Vermicompost (2.5 t ha^{-1}), $V_{3.75}$= Vermicompost (3.75 t ha^{-1}), F_0= Fertilizer (no fertilizer) , F_{100}= Fertilizer (100% RDF), F_{50}= Fertilizer (50 % RDF) and V_0F_0 = control (no vermicompost + no fertilizer)

Table 3. Effect of vermicompost and fertilizer on microbial biomass nitrogen in soil of rice crop during growth period (µg g^{-1})

Treatments	Tillering stage				Post-harvest stage			
	F_0	F_{100}	F_{50}	Mean	F_0	F_{100}	F_{50}	Mean
V_0	49.56	90.73	85.22	75.17	65.40	118.52	88.65	90.85
$V_{1.25}$	87.81	94.53	93.00	91.78	90.75	157.41	122.52	123.56
$V_{2.5}$	91.07	99.33	94.57	94.99	92.89	163.12	125.65	127.22
$V_{3.75}$	92.64	105.69	95.34	97.89	94.04	159.83	158.79	137.56
Mean	80.27	97.57	92.03		85.77	149.72	123.90	
Factors	CD (5%)							
Vermicompost (V)	0.44				0.46			
Fertilizers (F)	0.38				0.40			
V X F	0.77				0.80			

increase in the microbial biomass phosphorus from tillering to post-harvest might be due to availability of more nutrients caused by enhanced rhizospheric effects, secretion of organic acids and root exudates leading to more microbial activities thus more microbial biomass phosphorus. The increase in the microbial biomass phosphorus in pot-culture experiment was slightly higher than incubation experiment. Similar results were obtained in the incubation experiment for biomass phosphorus by Babu et al (2017) and Ashraf et al (2021).

Water soluble carbon (mg kg^{-1}): The water soluble carbon in soil as influenced by vermicompost and fertilizer levels was statistically significant (Table 5). The integrated application of vermicompost (3.75 t ha^{-1}) + 100 % NPK showed significant higher amount of water soluble carbon content i.e. 0.099 and 0.106 mg kg^{-1}in soil at tillering and post-harvest, respectively over the control.

Hot-water soluble carbon (mg kg^{-1}): The elevated hot-water soluble carbon was found in the treatment receiving (vermicompost-3.75 t ha^{-1} + 100 % RDF) i.e. 0.138 mg kg^{-1}which was higher over control (0.100 mg kg^{-1}) (Table 6). The higher level of vermicompost (3.75 t ha^{-1}) + 100 % RDF recorded higher hot-water soluble carbon i.e. 0.137 mg kg^{-1} over control (0.103 mg kg^{-1}) and might be due to solubilization an accumulation of carbon over a longer period of time and close to similar findings were given by Ashraf et al (2021).

Acid hydrolyzable carbohydrate (mg kg^{-1}): The effect of different levels of vermicompost and fertilizer on acid hydrolyzable carbohydrate in soil is was statistically significant (Table 7). The interactions regarding vermicompost and fertilizer levels were non-significant. However the vermicompost of 3.75 t ha^{-1} + 100 %6) RDF recorded higher acid hydrolyzable carbohydrate i.e. 133.80 mg kg^{-1}over control (94.13 mg kg^{-1}).The integrated application of vermicompost (3.75 t ha^{-1}) + 100 % NPK showed higher amount of acid hydrolyzable carbohydrate content i.e. 94.60 mg kg^{-1}in soil at post-harvest over the control (48.59 mg kg^{-1}).

$KMnO_4$-carbon (g kg^{-1}): The $KMnO_4$-carbonin soil was statistically significant as influenced by vermicompost and

Table 4. Effect of vermicompost and fertilizer on microbial biomass phosphorus in soil of rice crop during growth period (µg g^{-1})

Treatments	Tillering stage				Post-harvest stage			
	F_0	F_{100}	F_{50}	Mean	F_0	F_{100}	F_{50}	Mean
V_0	3.75	4.29	3.91	3.98	4.28	4.57	4.47	4.44
$V_{1.25}$	4.09	4.6	4.54	4.43	4.52	4.78	4.67	4.66
$V_{2.5}$	4.39	4.83	4.65	4.63	4.56	4.91	4.73	4.73
$V_{3.75}$	4.60	4.95	4.73	4.76	4.69	5.29	4.89	4.96
Mean	4.21	4.69	4.46		4.51	4.89	4.69	
Factors	CD (5%)							
Vermicompost (V)	0.11				0.10			
Fertilizers (F)	0.09				0.09			
V X F	NS				NS			

Table 5. Effect of vermicompost and fertilizer on water soluble carbon in soil of rice crop during growth period (mg kg^{-1})

Treatments	Tillering stage				Post-harvest stage			
	F_0	F_{100}	F_{50}	Mean	F_0	F_{100}	F_{50}	Mean
V_0	0.060	0.071	0.065	0.065	0.062	0.081	0.066	0.069
$V_{1.25}$	0.068	0.083	0.075	0.075	0.055	0.093	0.086	0.078
$V_{2.5}$	0.067	0.090	0.078	0.078	0.076	0.099	0.089	0.088
$V_{3.75}$	0.075	0.099	0.089	0.088	0.081	0.106	0.096	0.094
Mean	0.068	0.086	0.077		0.069	0.095	0.084	
Factors	CD (5%)							
Vermicompost (V)	0.005				0.12			
Fertilizers (F)	0.004				0.10			
V X F	NS				NS			

fertilizer levels (Table 8). The fertilizer levels of 50 % and 100 % RDF were significantly superior over no fertilizer level application. The interactions among the different levels of vermicompost and fertilizer were significant. The integrated application of vermicompost (3.75 t ha^{-1}) + 100 % NPK showed significant higher amount of $KMnO_4$-carboncontent i.e. 0.80 and 1.51 g kg^{-1}in soil at tillering and post-harvest, respectively over the control, Kumar et al (2020).also observed the same trend.

Organic carbon (g kg^{-1}):. The elevated organic carbon was

Table 6. Effect of vermicompost and fertilizer on hot-water soluble carbon in soil of rice crop during growth period (mg kg^{-1})

Treatments	Tillering stage				Post-harvest stage			
	F_0	F_{100}	F_{50}	Mean	F_0	F_{100}	F_{50}	Mean
V_0	0.100	0.115	0.107	0.107	0.103	0.118	0.109	0.110
$V_{1.25}$	0.112	0.124	0.121	0.119	0.115	0.126	0.120	0.120
$V_{2.5}$	0.115	0.128	0.120	0.121	0.115	0.131	0.120	0.122
$V_{3.75}$	0.118	0.138	0.126	0.127	0.117	0.137	0.128	0.127
Mean	0.111	0.127	0.118		0.112	0.128	0.119	
Factors	CD (5%)							
Vermicompost (V)	0.010				0.003			
Fertilizers (F)	0.009				0.003			
V X F	NS				NS			

Table 7. Effect of vermicompost and fertilizer on acid hydrolysable carbohydrate in soil of rice crop during growth period (mg kg^{-1})

Treatments	Tillering stage				Post-harvest stage			
	F_0	F_{100}	F_{50}	Mean	F_0	F_{100}	F_{50}	Mean
V_0	94.13	110.33	100.94	101.80	48.59	60.800	52.11	53.84
$V_{1.25}$	106.81	122.54	112.68	114.01	58.22	77.93	65.96	67.37
$V_{2.5}$	110.33	127.93	119.48	119.25	63.85	87.09	70.19	73.71
$V_{3.75}$	116.20	133.80	127.70	125.90	66.90	94.60	85.45	82.32
Mean	106.87	123.65	115.20		59.39	80.11	68.43	
Factors	CD (5%)							
Vermicompost (V)	1.65				2.45			
Fertilizers (F)	1.43				2.13			
V X F	NS				4.25			

Table 8. Effect of vermicompost and fertilizer on $KMnO_4$-carbon in soil of rice crop during growth period (carbon g kg^{-1})

Treatments	Tillering stage				Post-harvest stage			
	F_0	F_{100}	F_{50}	Mean	F_0	F_{100}	F_{50}	Mean
V_0	0.53	0.55	0.54	0.54	1.30	1.35	1.33	1.33
$V_{1.25}$	0.58	0.62	0.60	0.60	1.37	1.45	1.42	1.41
$V_{2.5}$	0.58	0.73	0.65	0.66	1.39	1.47	1.41	1.42
$V_{3.75}$	0.60	0.80	0.75	0.72	1.39	1.51	1.48	1.46
Mean	0.57	0.68	0.64		1.36	1.44	1.41	
Factors	CD (5%)							
Vermicompost (V)	0.02				0.01			
Fertilizers (F)	0.02				0.01			
V X F	0.03				0.02			

Table 9. Effect of vermicompost and fertilizer on organic carbon in soil of rice crop during growth period

Treatments	Tillering stage				Post-harvest stage			
	F_0	F_{100}	F_{50}	Mean	F_0	F_{100}	F_{50}	Mean
V_0	7.82	8.20	8.17	8.07	6.05	6.22	6.03	6.10
$V_{1.25}$	7.82	8.81	8.12	8.25	6.36	6.55	6.13	6.35
$V_{2.5}$	7.88	8.66	8.33	8.29	5.59	7.50	5.68	6.26
$V_{3.75}$	7.63	9.26	8.57	8.49	5.90	7.21	5.93	6.35
Mean	7.79	8.73	8.30		5.98	6.87	5.94	
Factors				CD (5%)				
Vermicompost (V)			0.19				0.14	
Fertilizers (F)			0.16				0.12	
V X F			0.32				0.24	

in the treatment receiving (vermicompost-3.75 t ha^{-1} + 100 % RDF) i.e. 9.26 g kg^{-1}which was higher over control (7.82 g kg^{-1}). The interactions among vermicompost and fertilizer levels were found significant (Table 9). Similar findings were observed by Ralebhat et at (2021). The higher level of vermicompost (2.5 t ha^{-1}) + 100 % RDF recorded higher organic carbon i.e. 7.50 g kg^{-1}over control (6.05 g kg^{-1}).

CONCLUSION

Application of higher dose of vermicompost (3.75 t ha^{-1}) and fertilizer (100%) in combined manner in soil elevated the carbon content and carbon pool with carbohydrate content, thus enhancing the soil fertility status and soil health with increasing microbial population and thus contributing higher microbial biomass carbon, nitrogen and phosphorus content during crop growth stages in pot culture rice crop.

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Effect of Residue Management Practices and Fertilizer Levels on Growth, Yield Attributes and Yield of Wheat in Rice-Wheat Cropping System

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***Abstract*:** A field investigation was carried out at *Krishi Vigyan Kendra,* Damla, Yamunanagar of CCSHAU, Hisar (Haryana) during *Rabi,* 2019-20. The experiment was conducted in split-plot design having six residue management practices in main plots ($C_0R_1T_3$: Combine harvesting of paddy without SMS + burning of crop residues + wheat sowing by conventional tillage method; $C_0R_2T_1$: Combine harvesting without SMS + residue shredding with mulcher + happy seeder sowing; $C_0R_2T_0$: Combine harvesting without SMS + happy seeder sowing; $C_1R_2T_0$: Combine harvesting with SMS + happy seeder sowing; $C_0R_2T_2$: Combine harvesting without SMS + residue shredding with straw chopper + happy seeder sowing and; $C_2R_0T_3$: Manual harvesting + residue removal + wheat sowing by conventional tillage method) and three fertilizer levels (75, 100 and 125% of recommended dose of fertilizer- RDF) in sub-plots. Among the residue management practices, the plant growth parameters, yield attributes and grain yield were the highest in $C_0R_2T_1$ which was statistically similar to other treatments with retention of paddy residue on soil surface ($C_0R_2T_0$, $C_1R_2T_0$ and $C_0R_2T_2$) but significantly higher than either burning ($C_0R_1T_3$) or removal of paddy residue ($C_2R_0T_3$). Fertilizer level with 125% RDF significantly improved the plant growth parameters, yield attributes and yield over other fertilizer levels. The interactive effects revealed that grain yield of wheat under treatments of residue retention on soil surface resulted in significantly higher grain yield at 125% RDF but in other treatments increase in fertilizer level from 100 to 125% RDF did not cause significant improvement in grain yield of wheat.

Keywords: Burning, Happy seeder, Fertilizer levels, Residue retention

Rice-wheat is the most prominent as well as exhaustive cropping system occupying around 18 mha area in Asia (Kumar et al 2019). Even in India, it is one of the most widely adopted cropping system due to favourable government policies like subsidized electricity and fertilizers, assured procurement and technological advancement like development of input responsive high yielding varieties and mechanization of both the crops (Shiferaw et al 2013). Increased mechanisation, particularly use of combine harvesters has led to generation of huge amount of crop residues. Combine harvesters finish the harvesting of paddy quickly and are easy to use as well as reduce the supervision of labour which made them popular in very short span of time. The loose residue generated during combine harvesting of paddy hampers tillage operations and sowing of subsequent wheat crop (Dutta et al 2022). For timely sowing of wheat, farmers find burning of crop residue as most economical and time saving option available to the farmers. Burning of crop residue results in loss of large volume of nutrients from the nutrient pool of soil, loss of organic matter (Sidhu et al 2015), loss of soil moisture (Moitinho et al 2021) and also enhances the degradation of soil quality and ecosystem. Scanty management of this surplus crop residue in rice-wheat cropping system is a deliberate threat to its sustainability leading to a number of problems. Instead of burning, alternate residue management practices can contribute to improved soil health, long-term sustainability and mitigation of climate change related greenhouse gases concentration in the atmosphere by reducing carbon dioxide emissions (Desrochers et al 2019).

Collection and transportation of voluminous mass of paddy residue is cumbersome, therefore, ex-situ residue management is still not an economically viable option (Lohan et al 2018). Conservation agricultural practices with reduced tillage, residue as mulch, improved crop establishment etc. are need of an hour to manage degrading soil health and to overcome yield stagnation. Soil physical (Indoria et al 2017), chemical (Jat et al 2018) and biological (Choudhary et al 2018) properties particularly those related to soil carbon sequestration (Xue et al 2018) are influenced by tillage practices. Several mechanization options in the form of combine fitted with SMS (super straw management), happy seeder, zero till drill machine, mulcher, paddy straw chopper, shrub master, reversible MB plough, baler etc. has been proposed to solve this problem of stubble burning (Gill 2018). Conservation agriculture practices like zero tillage sowing of

wheat using zero till drill or happy seeder directly into the combine harvested paddy field enables the farmer to reduce input cost, increase profitability, and conserve water, labour, energy, soil nutrients and farm chemicals along with enhanced crop growth and yield. So selection of suitable residue management practices that are eco-accommodating and also increment the farmer income is of utmost importance. Retention of crop residue returns organic matter to the soil and also affects the soil nutrient recycling (Turmel et al 2015). Nutrient supply from crop residue can affect the amount of fertilizer required for optimisation of crop yield. Crop residue left on soil surface are devoid of favourable environment for residue decomposition, thus may not contribute nutrient to the next crop and might even reduce available form of nutrients such as N by the process of immobilisation. Rejuvenation of soil health through better residue management practices along with right amount of fertilizer is a necessary imperative to maintain or increase productivity level of this most farmer adopted cropping system. Hence the present investigation was conducted to evaluate the effect of different rice residue management practices and fertilizer levels on growth and yield of wheat crop.

MATERIAL AND METHODS

A field experiment was conducted during *rabi* 2019-20 to study the effect of residue management practices and fertilizer levels on growth, yield attributes and yield of wheat in rice-wheat cropping system at Farm of KVK Damla (Yamunanagar) of CCS Haryana Agricultural University, Hisar. The soil of experimental site was sandy loam having pH (7.84), EC (0.55 dS m^{-1}), O.C. (0.34%), low in available nitrogen (132 kg ha^{-1}), medium in available phosphorus (16 kg ha^{-1}) and high in available potassium (366 kg ha^{-1}). The experiment was conducted in split-plot design keeping six treatments of residue management practices ($C_0R_1T_3$: Combine harvesting of paddy without SMS, burning of crop residues and sowing of wheat by conventional tillage method; $C_0R_2T_1$: Combine harvesting of paddy without SMS, residue shredding with mulcher followed by happy seeder sowing of wheat; $C_0R_2T_0$: Combine harvesting of paddy without SMS, direct sowing of wheat with happy seeder; $C_1R_2T_0$: Combine harvesting of paddy with SMS followed by sowing of wheat using happy seeder; $C_0R_2T_2$: Combine harvesting of paddy without SMS, residue shredding with straw chopper followed by happy seeder sowing of wheat and $C_2R_0T_3$: Manual harvesting of paddy, residue removal and sowing of wheat with conventional tillage practice) as main plots and three fertilizer levels (75% RDF: 75 per cent of recommended dose of fertilizer; 100% RDF: 100 per cent of recommended dose of fertilizer and 125% RDF: 125 per cent of recommended dose of fertilizer) as sub-plots replicated thrice. Experimental site was under rice-wheat cropping system before the establishment of the experiment for past 5 years. Previous direct seeded rice crop was harvested according to treatments in the plots followed by pre-sowing irrigation and sowing of wheat according to the treatments. Conventional tillage practice involved two harrowing followed by cultivator and planking to prepare a fine seedbed. The wheat variety HD-3086 at recommended seed rate of 100 kg ha^{-1} was sown in the first week of December, 2019 and harvested in April, 2020. Recommended dose of N-P-K was 150-60-60 kg/ha, respectively and fertilizer dose was applied as per the treatments. Entire amount of P (diammonium phosphate) and K (Muriate of potash) was applied as basal dose at the time of sowing. N dose was applied in two splits at sowing and at first irrigation. Plant population was taken 15 days after sowing by counting the number of plants from four 50 cm row length in each plot. Plant height of five plant samples from each plot was measured from ground levels to the tip of the leaf up to heading but after that it was measured from ground level to the tip of the ear. Number of tillers were counted in 50 cm row length from four randomly selected spots per plot. Five plants from each plot were sun dried followed by oven drying and averaged to dry weight per meter row length with help of number of plants per meter row length. Leaf area of plants in 0.25 m row length was measured with the help of leaf area meter and then leaf area index was calculated as

$$\text{LAI} = \frac{\text{Leaf area}}{\text{Ground area}} \times 100$$

Number of effective tillers were counted from 1 m^2 area from two spots in each plot. Ten randomly selected spikes from each plot were used to measure spike length and number of grain per spike. Weight of 1000-grains was taken to calculate the test weight in grams. At maturity each plot was harvested manually, sun dried and threshed to calculate grain yield of wheat. Statistical analysis was performed using OPSTAT analysis tool at 5% level of significance.

RESULTS AND DISCUSSION

Growth parameters: Various residue management practices posed no significant impact on plant height, dry matter accumulation and number of tillers recorded at 30 DAS (Table 1). No significant impact of various residue management practices was observed on number of tillers at 60 DAS. Highest plant height, dry matter accumulation mrl^{-1} and number of tillers were with $C_0R_2T_1$ treatment at subsequent stages which were recorded statistically similar to other residue retained treatments ($C_0R_2T_0$, $C_1R_2T_0$, $C_0R_2T_2$)

but significantly higher than either burning ($C_0R_1T_3$) or removal ($C_2R_0T_3$) of crop residue. Better soil moisture regime, less fluctuation in soil temperature, improved soil fertility, soil porosity and activity of microorganisms might have contributed to better vegetative growth in residue retained conditions (Guo et al 2015 and Gairhe et al 2021). Highest leaf area index was in $C_0R_2T_1$ (4.38) followed by other residue retained treatments ($C_0R_2T_0$, $C_1R_2T_0$, $C_0R_2T_2$) and significantly higher than treatment with residue burning ($C_0R_1T_3$) or removal ($C_2R_0T_3$) (Table 2). Meena et al (2018) also reported higher leaf area index in wheat with retention of crop residue on soil surface by virtue of lowering the water stress with increased plant available water.

Among the fertilizer levels, no significant difference in growth parameters was observed at 30 DAS (Table 1). At successive observations, 125% RDF treatment recorded significantly higher plant height, dry matter accumulation and number of tillers compared to 75% and 100% RDF treatment. This might be due to higher nutrient availability to plants particularly of nitrogen, which favored better vegetative growth (Narolia et al 2016 and Irfan et al 2018). LAI at anthesis (Table 2) with 125% RDF was 9.6 and 3.6 per cent significantly higher than 75% RDF and 100% RDF due to enhanced vegetative growth. Similar findings of higher LAI with higher NPK levels were also reported by Babar et al (2019).

Yield attributes and yield: No significant impact of various residue management practices and fertilizer levels was observed on plant population of wheat (Table 2). However, higher population was observed under treatments involving conventional tillage after removal ($C_2R_0T_3$) or burning of crop residue ($C_0R_1T_3$). This was due to better contact of seed with soil particles because of fine seed bed preparation under conventional tillage practice compared to sowing with no-tillage after rice harvest (Xue et al 2018). Yield attributes and grain yield was significantly and positively affected by various residue management practices involving retention of crop residue (Table 2). $C_0R_2T_1$ recorded significantly higher tillers, grains spike^{-1}, thousand grains weight and grain yield (332.2, 44.1, 41.9 g and 5276 kg ha^{-1}) compared to treatments with residue burning- $C_0R_1T_3$ or residue removal- $C_2R_0T_3$. Better soil moisture regime, higher availability of nutrients at later crop growth stages because of decomposition of rice residue, late maturity etc. might have contributed to increased yield attributes which in turn resulted in higher grain yield under residue retention treatments with no-till conditions (Bartaula et al 2020). Similarly, Tripathi et al (2015) also reported higher yield of wheat with retention of crop residue on soil surface.

Among the fertilizer levels, 125% RDF treatment

Table 1. Effect of residue management practices and fertilizer levels on plant height, plant dry matter and number of tillers of wheat

Treatment	30 DAS			60DAS			90 DAS			120 DAS		
	Plant height (cm)	Plant dry matter (g mrl^{-1})	No. of tillers per mrl	Plant height (cm)	Plant dry matter (g mrl^{-1})	No. of tillers per mrl	Plant height (cm)	Plant dry matter (g mrl^{-1})	No. of tillers per mrl	Plant height (cm)	Plant dry matter (g mrl^{-1})	No. of tillers per mrl
Residue management practices												
$C_0R_1T_3$	16.46	8.52	43.11	36.86	39.09	64.82	70.59	96.38	68.36	90.03	178.47	66.29
$C_0R_2T_1$	16.08	8.37	43.11	40.74	42.61	68.04	75.34	105.36	72.58	96.09	189.66	71.31
$C_0R_2T_0$	16.36	8.49	42.22	39.72	41.35	65.38	73.42	101.92	69.91	93.57	185.32	68.71
$C_1R_2T_0$	16.22	8.40	42.33	40.33	41.81	67.03	74.6	102.42	71.61	95.19	186.94	70.32
$C_0R_2T_2$	16.16	8.41	42.89	40.58	42.48	67.82	75.2	104.5	72.36	95.79	188.39	71.11
$C_2R_0T_3$	16.39	8.49	42.78	36.61	38.91	64.36	70.07	95.47	67.89	89.49	177.2	65.76
CD (p=0.05)	NS	NS	NS	1.35	1.65	NS	2.13	3.96	3.46	3.45	4.81	3.65
Fertilizer levels												
75% RDF	16.11	8.41	42.67	38.06	38.85	64.33	70.99	95.83	67.56	90.59	170.76	66.02
100% RDF	16.29	8.45	42.72	39.39	41.28	66.46	73.79	101.84	70.66	93.99	187.22	69.12
125% RDF	16.43	8.48	42.83	39.97	42.99	67.94	74.82	105.35	73.14	95.49	195.01	71.61
CD (p=0.05)	NS	NS	NS	0.63	1.46	1.54	1.09	3.71	1.88	1.76	3.88	1.98

*mrl= Meter row length; DAS= Days after sowing

Table 2. Effect of residue management practices and fertilizer levels on yield attributes, yield and LAI at anthesis of wheat

Treatments	Plant population per mrl	Effective tillers m^{-2}	Grains $spike^{-1}$	1000- grains weight (g)	LAI at anthesis	Grain yield (kg ha^{-1})
Residue management practices						
$C_0R_1T_3$	16.46	318.67	42.67	39.79	66.29	4934
$C_0R_2T_1$	16.08	332.22	44.12	41.92	71.31	5276
$C_0R_2T_0$	16.36	327.33	43.53	41.18	68.71	5153
$C_1R_2T_0$	16.22	328.67	43.68	41.35	70.32	5177
$C_0R_2T_2$	16.16	330.78	44.02	41.78	71.11	5238
$C_2R_0T_3$	16.39	317.33	42.62	39.61	65.76	4817
CD (p=0.05)	NS	8.28	0.83	1.05	3.65	131
Fertilizer levels						
75% RDF	16.11	317.28	42.10	39.83	66.02	4722
100% RDF	16.29	326.33	43.55	41.08	69.12	5190
125% RDF	16.43	333.89	44.67	41.91	71.61	5385
CD (p=0.05)	NS	4.28	0.57	0.85	1.98	66

*mrl= Meter row length; DAS= Days after sowing

Table 3. Interaction effect of residue management practices and fertilizer levels on grain yield (kg ha^{-1})

Treatments	Residue management practices						
Fertilizer levels	$C_0R_1T_3$	$C_0R_2T_1$	$C_0R_2T_0$	$C_1R_2T_0$	$C_0R_2T_2$	$C_2R_0T_3$	Mean
75% RDF	4650	4800	4747	4767	4783	4583	4722
100% RDF	5023	5377	5252	5263	5340	4887	5190
125% RDF	5130	5650	5462	5500	5590	4980	5385
Mean	4934	5276	5153	5177	5238	4817	
Factors							CD (p=0.05)
Residue management practices at same fertilizer level							186
Fertilizer levels at same level of residue management practices							168

recorded significantly higher number of effective tillers, number of grains $spike^{-1}$ and thousand grains weight (333.9, 44.7 and 41.9g) as compared to 75% RDF and 100% RDF. Grain yield was also significantly improved by 14 per cent with 125% RDF over 75% RDF treatment (4722 kg ha^{-1}). Higher yield attributes and grain yield in wheat with higher fertilizer level might have contributed by increase in availability of nutrients with increase in fertilizer levels (Safar-Noori et al 2018). Significant interaction effect was observed between various residue management practices and fertilizer levels (Table 3). Increase in grain yield was significant with 125% RDF over 100% RDF treatment only in residue retained treatments. No significant increase in grain yield was observed with increase in fertilizer level from 100% RDF to 125% RDF in treatments with no residue retention. Application of higher fertilizer dose particularly nitrogen led to faster decomposition of crop residues due to better growth of microbial population which further enhanced the nutrient availability in residue retained conditions (Roozbeh and Rajaie 2021).

CONCLUSIONS

Adoption of mechanized agricultural practice with retention of crop residue on soil surface provides a better solution for in-situ management of crop residue. Results revealed that combine harvesting of paddy without SMS, residue shredding with mulcher followed by sowing of wheat with happy seeder resulted in better growth performance, yield attributes and yield of wheat. Application of 125 per cent recommended dose of fertilizer significantly improved the grain yield in no-till residue retained conditions. This increase over the 100 per cent recommended dose of fertilizer was non-significant in conventional tillage practice having no residue retention.

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Effect of Water-Soluble Fertilizers and PGPR on Soil Microbial Population, Nodule Count and Economics of Black Gram

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***Abstract*:** Field experiment was conducted on effect of water-soluble fertilizers and PGPR application on soil microbial population, nodule count and economics were studied under rainfed condition during *Kharif*-2019 at the College of Agriculture, Shivamogga. The field experiment was laid out in randomized complete block design and replicated thrice with thirteen treatments consisting of different combinations of 19:19:19 and monopotassium phosphate with or without PGPR application. Treatments significantly differ through foliar application of water-soluble fertilizers and PGPR application. The foliar application of 19:19:19 and monopotassium phosphate (0:52:34) @ 1% each at 30 and 45 days after sowing + PGPR along with a package of practice recorded significantly higher number of nodule count (16 and 31 at 30 and 45 DAS) . The higher microbial count such as N fixers (53.73, 57.31 and 47.02), P-solubilizers (35.25, 40.32 and 35.58) and K- solubilizers (25.03, 31.18 and 26.30) $\times 10^5$ colony forming units (CFU) g^{-1} soil at 30, 60 DAS and at harvest respectively over the package of practice.

Keywords: Blackgram, Nodules, PGPR, Rhizosphere, Water soluble fertilizers

Among the grain legumes, blackgram (*Vigna mungo* L.) commonly called urdbean is an ancient and well-known leguminous crop of Asia. Though it is cultivated in every part of Asia, Africa and the West Indies, it is not much important in other countries as compared to India. Besides, it is adapted to a wide range of agro-climatic conditions because of its morphological parameters perfectly suiting for intercropping and sole cropping systems. It is extensively grown as a grain legume and guaranteed considerable importance from the point of food and nutritional security as it contains 24% protein and 60% carbohydrates with calorific value of 347 Kcal per 100 g. In India, black gram contributes about 13% of total pulse area and 10 % of their total production and is cultivated over an area of about 4.6 M ha with a production of 3.56 Mt. with a productivity of 654 kg/ha. In Karnataka, it is cultivated over an area of 1.38 lakh ha with a production of 0.47 lakh tonnes (Anonymous 2018). Nowadays soil application of mineral fertilizers is not enough to meet the nutrient demand of the crop due to various losses like leaching, volatilization, fixing on the soil and also due to soil physical and chemical properties. This result in various deficiency symptoms which effect on the plant vegetative growth and a nutrient deficiency during reproductive stages increase the flower drop and reduce the fruit set. This evidence creates preconditions to increase the importance of foliar feeding as an alternative to meet plant nutrient demand during the growing season. Foliar feeding is the most effective and economical way to overcome plant nutrient deficiency (Dixit and Elamathi 2007). Besides foliar nutrition application of PGPR to soil increases the nutrient retentive capacity, mobilizes the fixed nutrients and increases the soil biological activity by nodule formation through a symbiotic association with plant roots thus increasing the availability of nutrients. Under rainfed conditions, when the availability of moisture becomes scarce, the application of fertilizers as a foliar spray along with PGPR to soil results in efficient absorption and usage.

Soil microorganisms play an important role in nutrient cycling and are important factors of soil health and fertility. Plant growth-promoting rhizobacteria are a group of helpful soil bacteria that colonise roots and their environs (rhizosphere) (PGPR). They have a substantial impact on crop growth and development by forming symbiotic, associative or neutral relationships with plants. Plant development is stimulated by nutrient mobilisation, solubilization and transformation and plants are protected against pathogenic infections by PGPRs. At several stages of the plant life cycle, PGPR boost growth through direct or

indirect methods. Nitrogen fixation, phosphate solubilization, phytohormone synthesis and mineral availability increase are examples of direct processes. The nitrogen fixation process is aided by an oxygen-sensitive enzyme complex called nitrogenase, which converts atmospheric free nitrogen into an ammonical form (biologically fixed nitrogen) that can be used by plants or released into the soil. Phosphate solubilizers convert fixed forms of phosphorus in the soil into a form that the plant can use. Plant hormones like auxins, gibberellins and cytokinin have a role in plant growth as well. The above field experiment is carried out to determine the effect of water-soluble fertilizers and PGPR on soil microbial activity, nodule count and to work out the monetary viability of different treatments.

MATERIAL AND METHODS

Field experiment was conducted during *Kharif* season of 2019 at University of Agricultural and Horticultural Sciences, Shivamogga which is come under Southern Transition Zone (Zone-7) of Karnataka. The geographical reference point of the experimental site was 13° 58' to 14° 1' North latitude and 75° 34' to 75° 42' East longitude and at an altitude of 650 m above the mean sea level. The soil was sandy loam in texture, slightly acidic pH (6.19) and normal in electrical conductivity (0.70 dS/m), low organic carbon 4.56 g/kg and low in available nitrogen (242.22 kg/ha), high in phosphorus (P_2O_5) (75.08 kg/ha) and medium in potassium (K_2O) status (135.63 kg/ha) and Nitrogen fixers (20×10^5 CFU/g of soil), P-solubilizing bacteria (PSB) (16×10^5 CFU/g of soil), K-mobilizing bacteria (9×10^5CFU/g of soil). During the cropping period, the total actual rainfall received was 1088.8mm. Randomized complete block design was used for this experiment with thirteen treatments and three replications. Treatments consisting of different combinations of water soluble fertilizers *viz.,* 19:19:19 (N: P: K) and mono potassium phosphate (0:52:34) sprayed at 30 and 45 days after sowing with or without liquid plant growth promoting rhizomicrobial consortia application along with the package of practice (Table 1). Variety used was Rashmi (LBG-625) which matures in 85 to 90 days with average yield ranging from 8 to 9 q ha^{-1}. The plots of 3.6 m width and 3.0 m length were prepared by making bunds of 50 cm width and 30 cm height. The gross plot size was 3.60m×3.0 m and net plot size was 2.40m×2.20m. Recommended dose of fertilizers and farm yard manure @ 7.5 t/ha was applied at the time of sowing common to all the treatments. Liquid plant growth promoting rhizomicrobial consortia (*Rhizobium leguminosarum*, *Pseudomonas* sp. and *Bacillus* sp.) mixed with farm yard manure at the rate of 750 ml/haincorporated to soil as per treatments at the time of sowing. The 1% solution of water-soluble fertilizers 19:19:19 and mono potassium phosphate was used for foliar spraying at 30 and 45 days after sowing as per the treatments. The number of nodules per plant was counted manually by uprooting roots of five plants at optimum soil moisture and soaking with water to wash adhered soil. Cost of cultivation and returns of crop is

Table 1. Effect of water-soluble fertilizers and PGPR on nitrogen fixers, phosphorous solubilizers and potassium solubilizer

Treatments details	N fixers ($\times10^5$CFU/g of soil)			PSB ($\times10^5$CFU/g of soil)			KSB ($\times10^5$CFU/g ofsoil)		
	30 DAS	60 DAS	At harvest	30 DAS	60 DAS	At harvest	30 DAS	60 DAS	At harvest
T_1: Package of practices	35.83	38.59	29.36	24.56	27.95	23.02	13.65	17.68	15.35
T_2: T_1+19:19:19 @1% at 30 DAS	37.24	40.54	31.15	25.89	28.81	25.35	15.67	19.96	17.32
T_3: T_2 + PGPR	48.40	52.59	42.32	31.15	36.51	33.36	22.68	27.65	23.35
T_4: T_1 +19:19:19 @1% at 30 and 45 DAS	38.58	41.54	34.54	25.73	29.65	26.64	16.65	20.82	18.62
T_5: T_4+ PGPR	48.58	52.79	42.17	31.81	36.81	33.65	23.02	28.27	23.32
T_6: T_1+MPP @1% at 30 DAS	39.21	42.54	32.79	26.24	31.55	28.39	16.86	21.69	18.25
T_7: T_6 + PGPR	48.62	53.54	42.36	32.12	37.25	33.88	23.26	27.32	24.43
T_8: T_1+MPP @1% at 30 and 45 DAS	40.54	43.35	34.47	27.56	32.75	29.08	17.21	22.13	18.52
T_9: T_8 + PGPR	48.79	54.19	43.17	33.20	37.02	34.65	23.65	30.52	24.86
T_{10}: T_1+19:19:19@1%+ MPP @1% at 30 DAS	40.99	43.62	34.58	28.62	33.18	30.82	17.00	22.65	19.35
T_{11}: T_{10} + PGPR	51.18	55.41	44.15	34.68	38.21	34.82	23.93	30.21	25.28
T_{12}: T_1+19:19:19 @1% + MPP @1% at 30 and 45 DAS	41.92	44.56	35.31	29.72	33.99	30.91	17.50	23.05	20.65
T_{13}: T_{12} + PGPR	53.73	57.31	47.02	35.25	40.32	35.58	25.03	31.18	26.30
CD @5%	9.46	5.31	8.12	4.94	5.58	3.43	4.19	4.32	3.48

*Package of practice (6.5 t ha^{-1} FYM, 13:25:25 kg NPK ha^{-1} + 4 kg $ZnSo_4$ as basal dose)

calculated based on price of inputs during experiment and market price during prevailing year.

Enumeration of microorganism in rhizosphere: The microbial population in the soil at different growth stages and after harvest was determined by serial dilution pour plate method. Soil samples from different treatments were collected separately from each replication and analysed separately. Ten grams of pooled soil (treatment wise) weighed and mixed in 90 ml sterilized water blank to give 10^{-1} dilutions. Subsequent dilutions up to 10^{-6} were made by transferring serially 1 ml of each dilution to 9 ml sterilized water blanks. The population of N-fixers, P-solubilizer's and K- solubilizer's were estimated by pour plate serial dilution method and by taking 1ml from selected dilution of 10^{-5} were transferred aseptically to petri plates and the desired agar media was prepared by using appropriate ingredients and melted by using hot air oven then added to their respective dilutions. Plating on appropriate media *viz*., Congo red yeast extract mannitol agar (CRYEMA) media, Pikovskaya's media and Alexandrov's agar media, respectively. The inoculated plates were kept for incubation at 30°C ± 10°C for a week time and emerged colonies were enumerated by digital colony counter. Total CFU was calculated.

$$\text{CFU/g of soil} = \frac{\text{Average number of colonies} \times \text{Dilution factor}}{\text{Weight of soil sample (g)}}$$

RESULTS AND DISCUSSION

N-fixers, P-Solubilizers and K-solubilizers: The significantly higher N-fixers population (53.73, 57.31 and 47.02), P-solubilizing bacteria population (35.25, 40.32 and 35.58) and K-solubilizing bacteria population (25.03, 31.18 and 26.30) × 10^5CFU/g of soil at 30, 60 DAS and at harvest stage, respectively was recorded with the application of T_1+19:19:19 @1% + mono potassium phosphate @1% at 30 and 45 DAS + PGPR which was on par with all the treatments having PGPR application (Table 1). N-fixer's, PSB and K-solubilizing microorganisms population increased from initial observation, 30 and 60 DAS but decrease at the time of crop harvest when compared to peak period of crop growth stage. Mean population of N-fixer's, PSB and K-solubilizers was observed more in PGPR treatments when compared to without PGPR at different growth stages. This increased microbial population might be due to application of microbial consortia along with farmyard manure. Liquid consortia encourage multiplication of N-fixers, PSB and K-solubilizers in the soil up to 60 DAS later at harvest stage of the crop the population decreases due to non-availability of organic matter for their multiplication, reduced root activity and production of root exudates. Luxuriant growth of crop at vegetative stage as reflected by higher dry matter production resulted in higher microbial population. Application of liquid PGPR may increase the soil microbial population in the rhizosphere by interacting synergistically with native microorganisms (Gupta et al., 2014). Application of monopotassium phosphate and 19:19:19 each @ 1% at 30 and 45 DAS recorded higher microbial population (Bhavya et al 2020).

Number of nodules per plant: The maximum number of nodules per plant at 30 DAS (16.27) and 45 DAS (31.73) was in T_1+19:19:19 @ 1% + mono potassium phosphate @ 1% at 30 and 45 DAS + PGPR (Table 2). More number of nodules per plant was observed in the treatments that received PGPR than other treatments. Increase in the number of nodules might be due to enhanced biological nitrogen fixation due to application of plant growth promoting rhizomicrobial consortia might result in rapid multiplication of *Rhizobium sp*. in the soil. This increased population bacteria which enhance the infection to pulse roots by rhizobium bacteria might led to increased nodulation process and continuous supply of nitrogen through basal dose during initial stage along with farmyard manure and through the foliar spray at later stages of crop growth. Basim and Raghu (2015) observed that application of Farm Yard Manure and PGPR (*Bacillus sp., Pseudomonas sp. and Rhizobium* sp.) recorded significantly higher nodule count (72.25). Similar trend was recorded by Nithukumari et al (2019), Yadav and Choudhary (2012) and Gupta et al (2014). Application of PGPR along with foliar

Table 2. Effect of water-soluble fertilizers and PGPR on Seed yield, haulm yield and number of nodules per plant in blackgram

Treatments details	Seed yield (kg/ha)	Haulm yield (kg/ha)	No. of nodules per	
			30 DAS	45 DAS
T_1	802	1603	10.47	17.47
T_2	869	1640	11.07	18.13
T_3	876	1653	14.60	27.34
T_4	883	1708	11.73	17.90
T_5	965	1767	15.13	27.40
T_6	868	1624	11.73	17.27
T_7	877	1657	15.63	27.53
T_8	886	1715	11.33	17.73
T_9	967	1753	15.40	28.27
T_{10}	955	1733	12.33	18.20
T_{11}	1085	1861	15.63	28.47
T_{12}	994	1706	13.30	22.53
T_{13}	1167	2019	16.27	31.73
CD (p=0.05)	130.46	210.81	2.62	4.67

*See table 1 for treatment details

Table 3. Economics of black gram as influenced by different treatments of water-soluble fertilizers and PGPR

Treatments details	COC (₹/ha)	Gross returns (₹/ha)	Net returns (₹/ha)	B:C
T_1	24863	47366	22502	1.91
T_2	25988	51204	25215	1.97
T_3	26138	51623	25484	1.97
T_4	27113	52087	24973	1.92
T_5	27263	56808	29544	2.08
T_6	25708	51140	25431	1.99
T_7	25858	51699	25840	2.00
T_8	26703	52257	25553	1.96
T_9	26853	56899	30045	2.12
T_{10}	26433	56216	29782	2.13
T_{11}	26583	63736	37152	2.40
T_{12}	28153	58417	30264	2.07
T_{13}	28283	68573	40289	2.42

*See table 1 for treatment details

nutrition of 19:19:19 @ 1% and mono potassium phosphate @ 1% at 30 and 45 DAS resulted in the formation of active and a greater number of nodules (Hamayun and Chaudhary 2014, Hiwale 2015, Kachlam 2017).

Seed yield and haulm yield : The application of 19:19:19 and Mono potassium phosphate each @ 1 % at 30 and 45 DAS + PGPR along with a package of practice treatment recorded significantly higher seed yield (1167 kg/ha) and haulm yield (2019 kg/ha) when compared to other treatments. The increase in yield due to foliar application of fertilizers at 30 and 45 DAS enhances the absorption a rate of photosynthesis which supplies the photosynthates during critical stages such as flowering and pod formation stages and also PGPR application leads to colonization of microorganisms around the roots, it increases the nutrient availability and nutrient uptake results higher yield.

Comparative economics: Different treatments caused variation in cost of cultivation due to water soluble fertilizers and PGPR. However, foliar application of 19:19:19 and mono potassium phosphate each @ 1% at 30 and 45 DAS+PGPR along with package of practice recorded the higher gross returns (68573 ₹/ha) and net returns (40289 ₹/ha) as compared to other treatments. This is attributed by the greater grain and haulm yield in that treatment. The lower gross returns (47366 ₹/ha) and net returns (22502 ₹/ha) were obtained with Package of practice. The maximum benefit cost ratio (2.42) was with foliar application of 19:19:19 and mono potassium phosphate each @ 1% at 30 and 45 DAS + PGPR along with Package of practice and lower benefit cost ratio (1.91) was in treatment applied with package of practice (Table 3). Jadhav and Kulkarni (2016) also reported that foliar spray of 19:19:19 recorded significantly higher net return (35838 ₹/ha), similar results are also concluded by Mallesha et al (2014); Mudalagiriyappa et al (2016).

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CONCLUSION

Foliar application of 19:19:19 and Mono potassium phosphate and soil application of liquid PGPR by mixing with farm yard manure improves the soil microbial population *viz*., N-fixers, P-solubilizer's and K-solubilizer's in the soil during cropping period which ameliorates the nutrient mobility and availability to the crop. It facilitates better nutrient uptake results proper pod setting enhanced the final economical yield of crop fetches more cost benefit ratio.

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Response of Summer Sweetcorn to Drip Irrigation and Crop Growth Based Fertigation Levels under High Density Planting

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***Abstract*:** Enhancing nutrient use efficiency (NUE) and water use efficiency (WUE) are most critical and turn daunting research issues. A number of approaches are to be used in order to enhance water and nutrient use efficiency by reducing losses and to minimize the detrimental effect of water and nutrient stress in crop plants. This can be achieved through adoption of efficient methods of irrigation and fertilizer application. Field experiments were carried in, College of Agriculture, PJTSAU, Hyderabad, to determine the impact of drip irrigation and fertigation levels on the growth, yield and output of sweetcorn under high density planting during consecutive summer seasons of 2020 and 2021 respectively. Twelve treatment combinations (three levels of irrigation and four levels of fertigation through drip) with factorial concept. The results revealed that based on mean of two years, among irrigation levels, 1.0 Epan registered considerably higher growth parameters (plant height, leaf area and dry matter production), yield attributes (no. of cobs plant^{-1}, cob length, no. of kernels, cob weight, cob girth) and yield over 0.8 and 0.6 E pan. Among the fertigation levels, 125% RDNK in differential dosage (225-75-62.5 kg N, P_2O_5, K_2O ha^{-1}) in accordance with the crop coefficient curve resulted in significantly greater growth parameters, yield characteristics and yield.

***Keywords*:** Fertigation, Irrigation, Sweetcorn, Yield

Zea mays L. cv. Saccharata, also known as sweet corn, is a variety of maize whose immature grains contain 13 to 15% sugar. A medium-sized plant called sweet corn produces green ears 65 to 85 days after planting. In the recent past the demand for sweet corn is increasing in cities as a snack. In the summer, sweet corn is a great additional source of green feed to keep the cow herd afloat. Being a shorter duration crop, it can be grown as a summer crop, when irrigation is scarce, after long-lasting *kharif* crops like cotton, red gram and rice. Among the agronomic practices, optimum plant stand is most crucial one, which helps to harness optimum growth and yield. With the increasing demographic pressure and dwindling natural resources. Best management practices are the need of the hour, to meet the increasing food demand apart from enhancing resource use efficiency. Traditional methods of nutrient and water application result in application losses apart poor use efficiency and high cost of cultivation. Drip fertigation is an effective method wherein, water soluble fertilisers are applied to the active plant root zone. Plant nutritional requirements vary depending on stage of development and drip fertigation, facilitates to apply them in accordance to plant requirement and limit fertiliser loss through leaching and. Fertigation delivered in varied doses produced higher cob yield over equal splits/ doses throughout the crop growth cycle, (Jha et al 2015). Hence, there is a need to revalidated fertiliser schedule of sweetcorn as the planting density has grown by 50% (from 83,333 to 1,60,000). Further, impact of drip irrigation and N fertigation levels on grain maize and sweetcorn, exact water and nutrient scheduling based on scientific evidence such as Kc values is not available in sweetcorn. In light of this, the present experiment was conducted to determine the impact of various irrigation and fertigation levels, as well as their interaction, on the growth and yield of summer sweet corn under high-density planting.

MATERIAL AND METHODS

Present field experiment was conducted at College Farm, PJTSAU, Hyderabad, Telangana State. The farm is classified as semi-arid tropics (SAT) by Troll's categorization and is situated at an elevation of 542.3 meters above mean sea level in the Southern Telangana agro-climatic zone of Telangana, at 17°19' N latitude and 78°23' E longitude. In 2019-20, the mean weekly maximum temperature ranged from 31.00 to 39.00 °C with an average of 35.0 °C, while, in 2020-21, ranged from 37.14 to 35.50 °C with an average of

36.32 °C. In 2019-20, the weekly mean minimum temperature ranged from 10.64 to 24.29 °C with an average of 17.46 °C, while in 2020-21, it ranged from 11.21 to 16.21 °C with an average of 13.71 °C. Rainfall totaled 21.00 mm in five rainy days in 2019-20 and 4.6 mm in one rainy day in 2020-21 during the crop growth period. In 2019-20, mean weekly pan evaporation (PE) ranged from 3.74 to 7.90 mm, while in 2020-21, ranged from 2.49 to 5.96 mm. During the crop research, total evaporation was 440.7 mm in 2019-20 and 340.7 mm in 2020-21. Due to the lack of rainfall during both years of the study, the crop was mostly grown under irrigation.

The experimental soil was sandy clay loam in texture (75.24 % sand, 10.4 % silt, and 14.06 % clay) with an average bulk density of 1.59 Mg m^3 for 0-60 cm depth and was slightly alkaline (pH) 7.5 and Ec (0.27 ds m^{-1}). The available N, P and K in the experimental soil were 187.5, 64.3, and 334.2 kg ha^{-1}. The experiment consisted of twelve treatments that were replicated thrice and laid out in randomized block design with factorial concept (FRBD). Treatments consisted of the three irrigation levels *viz*; (0.6 [I_1], 0.8 [I_2], and 1.0 Epan [I_3]) and four fertigation levels were 100 per cent recommended nitrogen and potassium as per recommendation [F_1], 100 percent RDNK in differential dosage as per crop coefficient curve [F_2], 125 percent RDNK in differential dosage as per recommendation [F_3], and 125 per cent RDNK in differential dosage as per crop coefficient curve [F_4]). Sweet corn (variety Madhuri) was seeded at 30cm x 20 cm spacing on February 5th, 2020 during first season and December 11th, 2020 during second season. The recommended fertilizer doses of 180, 60, and 50 kg N, P_2O_5, K_2O ha^{-1} were supplied through urea, single super phosphate (SSP) and sulphate of potash (SOP) respectively. As a baseline, the treatments received a standard amount of phosphorus. As per the treatments, nitrogen and potassium were applied in splits through fertigation.

Irrigation was scheduled every three days. The irrigation water was applied based on data collected from a USWB open pan evaporimeter located at the Meteorological observatory at Agro-climatic Research Centre, ARI, Rajendranagar, Hyderabad. On rainy days, the amount of water applied to each treatment was modified to account for the amount of rain fall received. The laterals (16 mm diameter) were spaced 0.6 m apart, with a 0.2 m interval between two inline emitters. The discharge rate of the emitter was 2.0 liters per hour. Application rate in drip irrigation treatments was arrived.

$$\text{Application rate (mmhr}^{-1}) \frac{Q}{DL \times DE}$$

Q = Dripper discharge (liters h^{-1}), D_L = space between laterals (m), D_E = spacing between emitters (m)

Irrigation duration for each treatment was calculated:

$$\text{Irrigation time (minutes)} = \frac{E_{pan}\,(\text{mm}) \times 60}{\text{Application rate (mmhr}^{-1})}$$

Fertigation in 10 splits once in 6 days interval in differential dosage as per crop growth was done from 10 days after sowing (DAS) to 70 DAS. For the treatments F_1 and F_3, fertigation was done in differential dosages as per recommendation as 100% and 125% RDF (Table 1). F_2 and F_4 fertigation was given in differential dosages as per crop coefficient curve as 100 and 125 % RDF respectively (Table 2). The crop was harvested on 24th April, 2020 and 12th March, 2021 during 1st and 2nd seasons respectively.

RESULTS AND DISCUSSION

Growth parameters: The drip irrigation scheduled at 1.0 Epan (I_3) registered statistically higher plant height, dry matter accumulation at 30, 60 DAS and at harvest and leaf area over 0.8 Epan (I_2) and 0.6 Epan (I_1). The minimum plant height, dry matter production and leaf area were recorded in drip irrigation at 0.6 Epan (I_1). Improved growth parameters in (I3) may be attributed to the presence of ideal moisture in the root zone, which encouraged higher nutrient uptake and, in turn, more photosynthesis, which encouraged better photosynthate accumulation. These findings are in agreement with the conclusions of earlier scientist (Sharanabasava 2012, Kadasiddappa 2015, Bibe et al 2017).

Among four fertigation levels, statistically higher plant height, dry matter production and leaf area were with (F_4) and on par with (F_3) during 2020 and 2021. F_3 was also on par with F_2. Significantly lowest plant height, dry matter production and leaf area were with (F_1) and it was on par with (F_2) during 2020 and 2021. Higher growth parameters under F_3 and F_4 might be due to better crop growth and higher leaf area as a

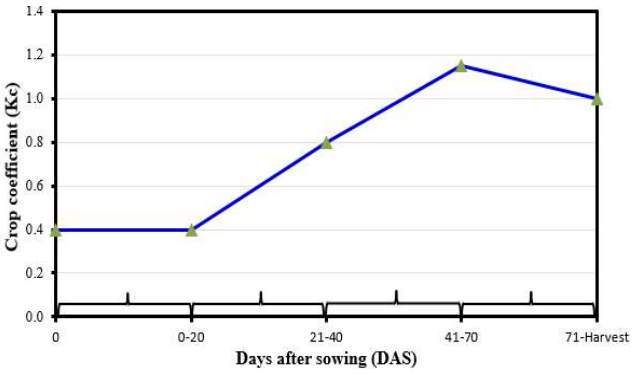

Fig. 1. Crop coefficient (Kc) values at different stages of sweetcorn crop according to FAO

Table 1. Differential dosage of fertilizer application based on growth stage of sweet corn crop as per recommendation

Crop stage	Nutrient dose (kg ha^{-1} day^{-1})	
	N	K_2O
After sowing 20 days (10-30 DAS)	1.31	0.56
Grand growth period 20 days (30-50 DAS)	4.39	1.18
Reproductive stage 20 days (50-70 DAS)	3.30	0.75

Table 2. Differential dosage of fertilizer application based on growth stage of sweet corn as per crop coefficient curve

Crop stage (DAS)	Kc values	Nutrient dose (kg ha^{-1} day^{-1})	
		N	K_2O
10-20	0.4	1.54	0.42
21-26	0.51	2	0.53
27-31	0.62	2.4	0.65
32-37	0.74	2.8	0.77
38-43	0.84	3.2	0.88
44-49	0.90	3.5	0.95
50-55	0.98	3.8	1.03
56-61	1.05	4.03	1.10
62-67	1.13	4.3	1.18
68-70	1.15	4.4	1.20
Average = 0.83			

result of nutrient supply (N and K) in higher doses than the recommended level under high density planting. Lack of nitrogen, necessary component of chlorophyll and a building block of protein, may have contributed to the slower dynamics of dry matter accumulation and resultant lower growth parameter at lower nitrogen dosages. Similar results were also reported by Fanish et al (2011). The supplying nutrients through fertigation in more scientific way under F_4 and F_2 treatments, lower fertiliser dose were applied during early, late growth stage and the crop's nutrient requirement was met by applying more fertiliser during growth period and reproductive stages, which led to greater uptake and accumulation of biomass over F_3 and F_1 treatments. These results are in line with the findings of Shekar et al (2016) and Jha et al (2015).

Time taken to attain 6th leaf stage, 50 % tasselling and silking were significantly less under drip irrigation scheduled at 1.0 Epan (I_3) over 0.6 Epan (I_1) and was at par with irrigation scheduled at 0.8 Epan (I_2) during 2020 and 2021. The reduction in days to attain 6th leaf stage, 50 per cent tasselling and silking under 1.0 Epan during both the years might be due to favourable soil moisture status throughout the crop growth. As sweetcorn crop is particularly sensitive to water deficits, onset of 6th leaf stage, emergence of tassels and silking were delayed under 0.6 Epan. Similar results were reported by Sharanabasava (2012) and Brar *et al* (2016). Number of days to attain 6th leaf stage was not significant among fertigation levels during both the years. While, the days 50 % tasselling and silking stages were lesser with (F_4) and it was comparable with (F_3). The days to attain 50%

Table 3. Plant height (cm) of sweet corn as influenced by drip irrigation and fertigation levels

Treatments	Days after sowing								
	30			60			Harvest		
	2020	2021	Mean	2020	2021	Mean	2020	2021	Mean
Irrigation levels (I)									
I_1	35.1	31.8	33.5	145.1	136.4	140.8	152.6	143.7	148.1
I_2	37.9	34.6	36.3	171.8	163.3	167.5	179.2	173.0	176.1
I_3	39.1	37.1	38.1	189.3	181.5	185.4	196.3	189.5	192.9
CD (P=0.05%)	2.4	2.5	2.5	14.2	10.0	12.1	12.8	10.6	11.7
Fertigation levels (F)									
F_1: F_1	36.4	34.0	35.2	163.0	156.3	159.6	164.4	158.6	161.5
F_2: F_2	36.8	34.3	35.6	156.6	151.9	154.3	168.3	162.7	165.5
F_3	38.0	34.7	36.4	181.2	168.2	174.7	184.3	173.3	178.8
F_4	38.4	34.9	36.7	174.2	165.4	169.8	187.1	180.4	183.7
CD (p=0.05)	NS	NS	NS	16.4	11.5	14.0	14.8	12.3	13.5
Interaction (IXF)									
CD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS

tasselling and silking was higher with (F_1) which was statistically similar with the (F_2) . Application of nitrogen and potassium fertilisers at higher doses under F_3 and F_4 treatments has increased nutrient uptake and sparked quicker development, which has led to higher LAI. This has also improved dry matter production and accelerated the flowering and maturity time compared to lesser levels. (F_1 and F_2). Similar results were reported by Sharanabasava (2012) and Brar and Vashist (2020).

Yield attributes: Data on yield attributes revealed that, cobs number per plant, cob length, cob girth, cob weight plant^{-1}, number of kernels per cob were significantly higher under irrigation given at 1.0 Epan (I_3) over 0.8 and 0.6 Epan (I_2 and I_1). Improved yield attributes with increasing irrigation levels might be due to the higher photosynthetic area at higher irrigation regimes, which might have helped in the increased production and translocation of photosynthates from source to the sink. Eralier also similar findings were reported Karato

Table 4. Leaf area (cm^2) of sweet corn as influenced by drip irrigation and fertigation levels

Treatments	Days after sowing								
	30			60			Harvest		
	2020	2021	Mean	2020	2021	Mean	2020	2021	Mean
Irrigation levels (I)									
I_1	674.9	623.1	649.0	4605.4	4274.9	4440.1	4486.2	4174.0	4330.1
I_2	774.0	713.8	743.9	5073.7	4717.4	4895.5	4954.3	4573.2	4763.7
I_3	844.3	799.3	821.8	5445.3	5143.5	5294.4	5232.9	4946.8	5089.8
CD (P=0.05%)	47.1	45.4	-	342.1	281.9	-	278.4	312.0	-
Fertigation levels (F)									
F_1: F_1	722.8	672.8	697.8	4881.9	4545.9	4713.9	4662.2	4347.4	4504.8
F_2: F_2	746.1	697.8	721.9	4779.5	4514.6	4647.1	4721.9	4411.0	4566.5
F_3	783.9	726.7	755.3	5326.1	4945.7	5135.9	5076.6	4720.7	4898.7
F_4	804.7	751.0	777.9	5178.3	4841.5	5009.9	5103.6	4779.4	4941.5
CD (p=0.05)	54.4	52.4	-	395.1	325.5	-	321.5	360.3	-
Interaction (IXF)									
CD (p=0.05)	NS	NS	-	NS	NS	-	NS	NS	-

Table 5. Dry matter production (g m^{-2}) of sweet corn as influenced by drip irrigation and fertigation levels

Treatments	Days after sowing								
	30			60			Harvest		
	2020	2021	Mean	2020	2021	Mean	2020	2021	Mean
Irrigation levels (I)									
I_1	66.3	59.1	62.7	1761.4	1708.1	1734.8	3601.4	3404.3	3502.9
I_2	75.1	66.1	70.6	1881.1	1830.3	1856.0	4184.5	4073.2	4128.9
I_3	80.7	72.2	76.5	2025.4	1937.6	1981.5	4694.6	4521.2	4607.9
CD (p=0.05)	5.3	4.8	5.1	111.2	104.1	107.7	292.8	288.9	290.9
Fertigation levels (F)									
F_1: F_1	69.6	61.7	65.7	1835.6	1781.2	1808.4	3845.7	3706.1	3775.9
F_2: $1F_2$	72.2	63.9	68.1	1806.8	1747.1	1777.0	4007.6	3864.0	3935.8
F_3	75.8	67.9	71.6	1978.3	1904.0	1941.2	4306.9	4157.2	4232.1
F_4	78.5	69.7	74.1	1936.4	1869.0	1902.7	4480.5	4270.9	4375.7
CD (p=0.05)	6.1	5.5	5.8	128.4	120.2	124.3	338.1	333.5	335.8
Interaction (IXF)									
CD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS

(2013), Kadasiddappa (2015) and Bibe *et al* (2017). Among, fertigation levels, higher yield attributes were recorded under (F_4) which were on par with (F_3). The application of 100 % RDNK differential dosage as per recommendation (F_1) recorded lower yield attributes and was on par with application of 100 % RDNK as crop coefficient curve (F_2) during 2020 and 2021. The increased physiological processes in crop plants resulted in higher growth and more photosynthates translocation the cobs with increased nitrogen and potassium fertiliser application. Further, the yield attributes produced under the treatment F_2 were comparable with F_3 as the nutrients were given more precisely based on growth needs under F_2 and F_4 fertigation pattern as compared to F_3 and F_1 fertigation treatments. These outcomes were consistent with Richa Khanna (2013). Yash pal (2016) and Bibe et al (2017).

Fresh cob yield and fodder yield (kg ha^{-1}): The drip irrigation at 1.0 Epan (I_3) has resulted significantly higher cob yield and fodder yield over other two irrigation levels (I_2 and I_1) and the lowest cob yield and green fodder yield was under drip irrigation at 0.6 Epan (I_1).

The favourable soil moisture conditions maintained

Table 6. Onset of different phenophases (number of days) of sweet corn as influenced by drip irrigation and fertigation levels

Treatments	Days after sowing								
	30			60			Harvest		
	2020	2021	Mean	2020	2021	Mean	2020	2021	Mean
Irrigation levels (I)									
I_1	26.4	27.6	27.0	53.6	59.3	56.4	59.7	65.5	62.6
I_2	25.3	26.6	26.0	50.3	56.4	53.3	56.1	62.6	59.3
I_3	24.7	25.8	25.2	49.8	54.5	52.2	54.3	60.1	57.2
CD (p=0.05)	1.3	1.2	-	2.2	2.5	-	2.4	2.7	-
Fertigation levels (F)									
F_1: F_1	26.1	27.1	26.6	52.6	58.2	55.4	58.4	64.3	61.4
F_2: $1F_2$	25.9	27.3	26.6	52.6	58.3	55.5	58.1	64.4	61.3
F_3	25.1	26.1	25.6	50.0	55.2	52.6	55.2	61.2	58.2
F_4	24.8	26.0	25.4	49.8	55.1	52.5	55.0	61.1	58.1
CD (p=0.05)	NS	NS	-	2.5	2.8	-	2.8	3.1	-
Interaction (IXF)									
CD (p=0.05)	NS	NS	-	NS	NS	-	NS	NS	-

Table. 7a. Yield attributes of sweet corn as influenced by drip irrigation and fertigation levels

Treatments	No. of cobs plant^{-1}			Cob weight plant^{-1} (g)			Cob length (cm)		
	2020	2021	Mean	2020	2021	Mean	2020	2021	Mean
Irrigation levels (I)									
I_1	1.2	1.1	1.2	128.7	120.1	124.4	17.2	16.6	16.9
I_2	1.3	1.2	1.3	156.6	150.7	153.7	18.8	18.6	18.7
I_3	1.5	1.3	1.4	181.8	171.1	176.5	20.2	20.0	20.1
CD (p=0.05)	0.08	0.09	-	11.6	15.5	-	1.1	1.0	-
Fertigation levels (F)									
F_1: F_1	1.2	1.1	1.2	141.8	133.3	137.5	17.8	17.6	17.7
F_2: $1F_2$	1.3	1.2	1.3	152.5	139.1	145.8	18.1	17.8	18.0
F_3	1.4	1.3	1.3	160.4	155.2	157.8	19.2	18.9	19.1
F_4	1.4	1.3	1.4	168.1	161.6	164.9	19.6	19.2	19.4
CD (p=0.05)	0.10	0.10	-	13.3	17.9	-	1.2	1.2	-
Interaction (IXF)									
CD (p=0.05)	NS	NS	-	NS	NS	-	NS	NS	-

during the crop growth period, might have improved the photosynthetic rate, biomass accumulation and partitioning to economic components, that have reflected in greater cob and fodder output in I3 (1.0 Epan). The crop's inability to absorb nutrients due to lack of moisture that resulted in lowest yield under I1 (0.6 Epan), as water is a medium for nutrient absorption (Islam et al 2012, Kadasiddappa et al 2013, Brar et al 2018).

Among fertigation levels, (F_4) registered significantly higher cob yield and green fodder yield over (F_1) and (F_2) during both the years. However, was statistically at par with (F_3) during 2020 and 2021. The lower fresh cob yield and fodder yield was recorded with (F_1) during both the years. Cob yield and fodder yield obtained in F_1 and F_2 were at par with each other and cob yield obtained with F_3 was also comparable with F_2 but, was statistically higher over F_1. Thus 25 % of the nutrients can be saved with recommendation based sustainable approach like crop coefficient curve. The higher yield recorded with (F_4) might be due to lower rates of fertiliser application during initial stages and higher rate at grand growth period and reproductive stages that coincided with the crop growth needs which helped towards higher

Table 7b. Yield attributes of sweet corn as influenced by drip irrigation and fertigation levels

Treatments	Cob girth (cm)			No. of kernels cob^{-1}		
	2020	2021	Mean	2020	2021	Mean
Irrigation levels (I)						
I_1	12.0	11.8	11.9	467	450	459
I_2	13.5	13.3	13.4	505	491	498
I_3	14.4	14.4	14.4	537	524	531
CD (p=0.05)	0.9	0.9	-	27	28	-
Fertigation levels (F)						
F_1: F_1	12.6	12.5	12.6	484	468	476
F_2: F_2	12.9	12.7	12.8	488	474	481
F_3	13.7	13.7	13.7	518	502	510
F_4	13.9	13.8	13.9	523	509	516
CD (p=0.05)	1.0	1.0	-	31	32	-
Interaction (IXF)						
CD (p=0.05)	NS	NS	-	NS	NS	-

Table 8. Nutrient uptake (kg ha^{-1}) by sweet corn at harvest as influenced by drip irrigation and fertigation levels

Treatments	Nutrient uptake (kg ha^{-1})								
	Nitrogen			Phosphorus			Potassium		
	2020	2021	Mean	2020	2021	Mean	2020	2021	Mean
Irrigation levels (I)									
I_1	321.4	300.9	311.2	123.9	115.4	119.7	449.7	401.2	425.5
I_2	414.6	409.8	412.2	160.2	156.3	158.3	585.7	537.3	561.5
I_3	525.6	477.4	501.5	189.8	182.6	186.2	691.9	662.5	677.2
CD (p=0.05)	56.7	52.1	-	16.7	16.6	-	66.5	73.5	-
Fertigation levels (F)									
F_1: F_1	364.5	335.0	349.8	137.6	132.1	134.9	494.9	464.8	479.9
F_2: F_2	385.7	368.3	377.0	146.9	142.4	144.6	536.1	494.3	515.2
F_3	450.5	429.4	440.0	168.1	162.2	165.2	614.4	574.6	594.5
F_4	481.3	451.5	466.4	179.3	169.0	174.2	657.8	600.9	629.4
CD (p=0.05)	65.4	60.2	-	19.3	19.2	-	76.8	84.9	-
Interaction (IXF)									
CD (p=0.05)	NS	NS	-	NS	NS	-	NS	NS	-

nutrient uptake. Nutrients were supplied more precisely and scientifically under F_4 and F_2 treatments when compared to F_1 and F_3 treatments. Improved growth and yield attributes under F_3 and F_4 fertigation levels over F_1 and F_2 was also due to increased nutrient levels (N and K) that attributed to sufficient intake of nutrients under higher density planting, which in turn improved cob yield. Similar findings on higher yields with the increase in the fertiliser rate were also reported by Sharanabasava (2012) and Richa Khanna (2013).

Water applied and water productivity: Total water applied including effective rainfall was 499.1 and 405.6 mm in case of the treatment drip at 1.0 Epan (I_3) followed by drip at 0.8 Epan (423.5 and 346.0 mm) (I_2), and drip at 0.6 Epan (348.5 and 286.5 mm) (I_1) (Table 10). Significantly higher water productivity were recorded with drip irrigation scheduled at 0.6 Epan. The, drip irrigation scheduled at 1.0 Epan produced lowest water productivity during 2020 and 2021 (Table 9). Although irrigation scheduled at 1.0 Epan produced the highest cob yield, it could not translate this yield into higher water productivity. Increase in the water productivity with the decrease in the amount of water applied were also

Table 9. Green cob yield, green fodder yield (kg ha^{-1}) and water productivity of sweet corn as influenced by drip irrigation and fertigation levels

Treatments	Green cob yield (kg ha^{-1})			Green fodder yield (kg ha^{-1})			Water productivity (kg m^3)		
	2020	2021	Mean	2020	2021	Mean	2020	2021	Mean
Irrigation levels (I)									
I_1	9967	9332	9650	27878	26551	27215	2.9	3.3	3.1
I_2	11734	11195	11465	33149	31559	32354	2.8	3.2	3.0
I_3	12870	12337	12604	36409	35044	35727	2.6	3.0	2.8
CD (p=0.05)	941	899	920	2461	2485	2473	0.2	0.2	-
Fertigation levels (F)									
F_1: F_1	10724	10156	10440	30117	28879	29498	2.5	2.9	2.7
F_2: F_2	11131	10593	10862	31423	29937	30680	2.6	3.1	2.9
F_3	11891	11300	11596	33600	32294	32947	2.8	3.3	3.1
F_4	12349	11769	12059	34776	33096	33936	2.9	3.4	3.2
CD (p=0.05)	1087	1039	1063	2842	2870	2856	0.3	0.2	-
Interaction (IXF)									
CD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	-

Table 10. Irrigation water, total water applied, effective and ineffective rainfall of *Summer* sweetcorn as influenced by drip irrigation and fertigation levels

Treatments	Irrigation water applied (mm)		Effective rainfall (mm)		Ineffective rainfall (mm)		Total water applied (mm)	
	2020	2021	2020	2021	2020	2021	2020	2021
I_1F_1	327.5	281.9	21.0	4.6	0.0	0.0	348.5	286.5
I_1F_2	327.5	281.9	21.0	4.6	0.0	0.0	348.5	286.5
I_1F_3	327.5	281.9	21.0	4.6	0.0	0.0	348.5	286.5
I_1F_4	327.5	281.9	21.0	4.6	0.0	0.0	348.5	286.5
I_2F_1	402.5	341.4	21.0	4.6	0.0	0.0	423.5	346.0
I_2F_2	402.5	341.4	21.0	4.6	0.0	0.0	423.5	346.0
I_2F_3	402.5	341.4	21.0	4.6	0.0	0.0	423.5	346.0
I_2F_4	402.5	341.4	21.0	4.6	0.0	0.0	423.5	346.0
I_3F_1	478.1	401.0	21.0	4.6	0.0	0.0	499.1	405.6
I_3F_2	478.1	401.0	21.0	4.6	0.0	0.0	499.1	405.6
I_3F_3	478.1	401.0	21.0	4.6	0.0	0.0	499.1	405.6
I_3F_4	478.1	401.0	21.0	4.6	0.0	0.0	499.1	405.6

Table 11. Economics of sweet corn as influenced by different drip irrigation and fertigation levels

Treatments	Net returns (Rs ha^{-1})			Benefit cost ratio		
	2020	2021	Mean	2020	2021	Mean
Irrigation levels (I)						
I_1	82803	71745	77274	2.9	2.5	2.7
I_2	104721	94733	99727	3.3	2.9	3.1
I_3	118327	108829	113578	3.5	3.2	3.4
CD (p=0.05)	9736	9725	-	0.12	0.10	-
Fertigation levels (F)						
F_1: F_1	93262	83505	88383	3.1	2.8	3.0
F_2: F_2	98638	88925	93782	3.2	2.9	3.1
F_3	105072	94580	99826	3.2	2.9	3.1
F_4	110830	100066	105448	3.3	3.0	3.2
CD (p=0.05)	11242	11230	-	0.14	0.12	-
Interaction (IXF)						
CD (p=0.05)	NS	NS	-	NS	NS	-

reported by Kadasiddappa *et al.* (2013) and Satish (2015). Among four fertigation levels, application of 125 % RDNK in differential dosage as per crop coefficient curve (F_4) recorded significantly higher water productivity over application of 100 % RDNK in differential dosage as per crop coefficient curve (F_2) and application of 100 % RDNK in differential dosage as per recommendation (F_1) and was on par with application of 125 % RDNK in differential dosage as per recommendation (F_3). This increase in WP with under F_4 and F_3 over F_1 and F_2 might be due to increased yield with the application of 125 % RDNK over 100% RDNK. Satish (2015) also observed improvement in water productivity with elevated levels of nitrogen. However, on growth metrics, yield attributes, sweet corn yield and water productivity there was no discernible interaction effect of drip irrigation and fertigation levels.

Economics: The irrigation scheduled at 1.0 Epan recorded significantly higher net returns and benefit cost ratio over 0.8 and 0.6 Epan. Lowest gross returns, net returns and B:C ratio were under 0.6 Epan (Table 11). Increased net returns and B:C ratio with (I_3) was mainly due to high cob and fodder yield obtained when compared to other treatments (I_2 and I_1). These results were in similarity with the Yash Pal (2016) and Brar et al (2018) with regard to higher gross and net returns with higher drip irrigation levels. Among the four fertigation levels, application of 125 % RDNK in differential dosage as per crop coefficient curve (F_4) recorded significantly higher net returns and B:C ratio on par with application of 125 % RDNK in differential dosage as per recommendation (F_3). The lower gross returns, net returns and B:C ratio were obtained with the application of 100 % RDNK in differential dosage per recommendation (F_1) which were on par with 100 % RDNK in differential dosage as per crop coefficient curve (F_2) (Table 11). The higher gross returns, net returns and B:C ratio under F_3, F_4 was due to higher fresh cob and green fodder yield obtained over other fertigation levels. Richa Khanna (2013) and Shruthi et al (2018) and also recorded higher gross returns, net returns and B:C ratio under higher fertigation levels.

CONCLUSION

Drip irrigation scheduled to summer sweet corn at 1.0 Epan throughout the crop growth period recorded higher growth, yield attributes, yield and returns over 0.6 and 0.8 Epan. Among fertigation levels, application of 125 % RDNK in differential dosage as per crop coefficient curve and application of 125 % RDNK in differential dosage as per recommendation curve recorded higher growth, yield and returns.

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Integrated Nutrient Management Practice for Maize-Wheat Cropping System in Chhotanagpur Plateau Region

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***Abstract*:** The experiment was carried out during two consecutive years of 2020-22 under the ongoing permanent manurial trial with different nutrient management practices under maize-wheat system since 1983-84 at Birsa Agricultural University, Kanke, Ranchi to study the productivity and profitability under maize-wheat cropping system. The application of 50%N through FYM along with 50% RDF through chemical fertilizer to maize fb 100% RDF through chemical fertilizer to wheat produced maximum and significantly higher grain yield (46.92 and 47.96 q/ha of maize and wheat, respectively) than other nutrient management practices and statistically at par with application of 25%N through FYM along with 75% RDF through chemical fertilizer to maize fb 75% RDF through chemical fertilizer to wheat (45.04 and 45.53 q/ha of maize and wheat, respectively). Highest net return (₹. 50583.00 in maize and ₹.79643.00 in wheat) and B:C ratio (1.32 in maize and 1.87 in wheat) was with the application of 50%N through FYM along with 50% RDF in *kharif* and 100% RDF in *rabi* which was statistically at par with the application of 25%N through FYM along with 75% RDF in *kharif* and 75% RDF in *rabi* (₹.47900.00 and ₹. 75237.00) & B:C ratio (1.28 and 1.83) in maize and wheat respectively.

***Keywords*:** Integrated nutrient management, Productivity, Profitability and Maize-wheat cropping system

Maize (*Zea mays* L.) - wheat (*Triticum aestivum* L.) is the third most important cropping system after rice (*Oryza sativa* L.)-wheat and rice-rice in India, and is grown on about 1.80 million ha each year (Jat et al 2013). Maize and wheat are the main source of world's food energy and also contain significant amounts of proteins, vitamins and minerals, which are essential nutrients for human health. Maize, a crop with high yield and market potential, fits well into rice-wheat systems by replacing rice. It is the third most important food grain crop in India, considered as a most important option for diversifying agriculture in upland areas of India which has high production potential compared to any other cereal crop. Wheat, is another major important staple cereal, supplies the bulk of calories and nutrients in the diets of a large proportion of the world population (Chatzav et al 2010). Globally, India is the second largest wheat-producing country and contributes about 11.9% to the world wheat production from about 12% of world area (Singh et al 2010). The continuous rice–wheat cropping has led to the exhaustion of natural resources and deteriorated soil fertility, producing agricultural outcomes (Hashim et al 2017). Thus, a paradigm shift in cropping systems with different crops is required to enhance profitability. Alternate systems management practices may prove beneficial to improve soil fertility and maintain environmental health. For crop diversification, maize-wheat cropping system has been identified as a suitable alternative to rice–wheat system (Brankov et al 2021). Insufficient application of nutrients and poor soil management, along with harsh climatic conditions and other factors, have contributed to the degradation of soils including soil fertility depletion. To replenish the soil nutrient depletion, application of chemical fertilizers is essential but the efficiency of applied chemical fertilizers is increased when applied along with organic manures. The current energy crisis prevailing higher prices and lack of proper supply system of inorganic fertilizers calls for more efficient use of organic manure, green manure, crop residues and other organic sources along with the inorganic fertilizers to sustain the yield levels (Sathish et al 2011). Organic manures supply nutrients to the current crop and also leave a substantial residual effect on the succeeding crops in different sequential cropping systems. The efficiency of applied chemical fertilizers is also increased when applied along with organic manures. Therefore, better management of soil nutrients is required that delivers sustainable agriculture and maintains the necessary increases in food production while minimizing waste, economic loss and environmental impacts. Integrated nutrient management (INM) is the feasible solution for sustaining the crop productivities, as nutrient requirements of both the crops are high and have shown superior response towards higher

levels of nutrient application (Sharma et al 2020). The balanced use of nutrients is the key to improving the sustainable production of crops (Mani et al 2011). The inorganic fertilizers, through soil or foliar application, have shown tremendous results in terms of agricultural productivity (Brankov et al 2020, Ferrari et al 2021). Furthermore, the use of inorganic nutrient sources coupled with organic sources is a feasible approach for higher agricultural productivity and monitoring soil health (Kumar et al 2021). The utilization of well-decomposed farmyard manure (FYM) in soil management practices is a well-known practice for enhancing crop yield, enhancing SOM, promoting microbial activities, promoting friendly soil environmental management (Blair et al 2005 and Kundu et al 2006), increasing the total organic sources supply, and increasing the plant-available macro and micronutrients in soil. Keeping these points in view, an investigation on suitable INM practice for maize-wheat cropping system in Chhotanagpur Plateau Region has been undertaken with the objective to study the productivity and profitability under maize-wheat cropping system.

MATERIAL AND METHODS

A field experiment was conducted at Birsa Agricultural University, Kanke, Ranchi during *kharif* and *rabi* seasons of two consecutive years, 2020-22. The present experiment is a long term being conducted since *Kharif* 1983 with maize-wheat cropping system. The experimental soil was loam in texture (42.4 % sand, 23.4 % silt and 34.2% clay) with slightly acidic (6.5) in reaction having low organic carbon (4.1 g/kg soil) and available nitrogen (255.0 kg/ha), medium in available phosphorous (12.50 kg/ha) and available potash (195.0 kg/ha) consisting 11.13 18.65 and 3.85 ppm available iron, manganese and zinc. Experiment was laid out in RBD with 12 treatments replicated thrice (Table 1). RDF for both component crop was @ 100:50:25 N: P_2O_5: K_2O kg/ha. "Suwan Composit-1" maize and "K 9107" wheat was the test crop variety. Recommended dose of fertilizer for both component crops were @ 100 kg N, 50 Kg P_2O_5 and 25 kg K_2O /ha. Integrated use of manure (FYM, cut paddy straw and green karanj leaf) along with chemicals at different rate of substitution, farmers' practice of fertilizer use and the control. Residual effect of organic manure application was tested in *rabi* wheat crop along with different levels of inorganic fertilizers. FYM, paddy straw and *karanj* green leaves having 0.5, 0.5 and 2.0 per cent N on oven dry basis were 3 organic sources of nutrients (50%N through FYM @10 ton, 50%N through cut paddy straw @10 ton and 50%N through green karanj leaves @2.5 ton). For substitution of inorganic fertilizers by organic sources, the calculation was done on the basis of N-concentration in organic manure and contents of P and K were ignored. Organic manure was incorporated in the soil well in advance prior to sowing of *kharif* maize only. Application of fertilizers at the time of sowing of crops were followed. The optimum dose of fertilizers for both the crops was N, P_2O_5 and k_20:100:50:25 kg/ha. Wheat was grown as test crop at 50, 75 and 100%)of chemical fertilizers only after harvest of maize. In farmers' practice urea @ 50kg/ha is applied which is equivalent to 23 kg N/ha.

Table 1. Long term effect of integrated nutrient management on yield of maize under maize-wheat cropping system (Pooled data of 2020-21 and 2021-22)

Treatment details		Maize yield (q/ha)			Wheat yield (q/ha)	
Kharif	*Rabi*	Grain	Stover	Stone	Grain	Straw
$T_1 N_0P_0K_0$	$N_0P_0K_0$	9.01	20.42	3.37	10.10	29.91
T_2 50%RDF	50%RDF	25.81	48.77	5.77	24.46	61.93
T_3 50%RDF	100%RDF	26.90	51.08	5.83	32.37	67.91
T_4 75%RDF	75%RDF	28.53	53.32	6.14	35.26	69.70
T_5 100%RDF	100%RDF	43.33	63.83	8.64	43.69	78.03
T_6 50%-N (FYM) + 50% RDF	100%RDF	46.92	70.53	8.81	47.96	82.28
T_7 5%N (FYM) + 75% RDF	75%RDF	45.04	65.19	8.75	45.53	78.73
T_8 50%N (CPS) + 50% RDF	100%RDF	38.78	60.66	8.10	41.69	76.28
T_9 25%N (CPS) + 75% RDF	75%RDF	36.30	57.85	7.98	38.41	73.25
T_{10} 50%N (GKL) + 50% RDF	100%RDF	37.64	59.04	8.05	39.38	75.71
T_{11} 25%N (GKL) + 75% RDF	75%RDF	30.65	55.15	6.24	36.69	72.49
T_{12} Farmer's practice (urea @ 50kg/ha)	Farmer's practice (urea @ 50kg/ha)	11.74	21.61	3.83	14.76	47.11
CD (p=0.05)		6.10	8.97	1.20	6.39	12.27
CV (%)		11.36	10.13	10.41	11.04	10.69

DAS: Days after sowing; CPS:- Cut paddy straw; GKL:- Green karanj leaves FYM- Farm yard manure

RESULTS AND DISCUSSION

Maize

Grain yield: Grain yield of maize in maize-wheat system influenced significantly by nutrient management practices (Table1). Grain yield of maize with application of 50% N through FYM+50% RDF through chemical fertilizer to maize fb 100% RDF through chemical fertilizer to wheat (46.9 q/ha) being like 100% RDF through chemical fertilizer to both crops (43.3 q/ha) and 25% N through FYM+75% RDF through chemical fertilizer to maize fb 75% RDF through chemical fertilizer to wheat (45.4 q/ha) recorded higher yield than all other nutrient management practices owing to more grains/cob, grins/row and heavier grain.

Stover yield: Pooled data on stover yield of maize revealed that nutrient management practices (inorganic fertilizer and in combination with organic sources) significantly influenced the stover yield of maize (Table 1). The highest stover yield (70.53 q/ha) was obtained in treatment receiving 50% N through FYM+50% RDF through chemical fertilizer to maize fb 100% RDF through chemical fertilizer to wheat. It was significantly higher than all other nutrient management practices except 100% RDF through chemical fertilizer to both crops (63.8 q/ha) and 25% N through FYM+75% RDF through chemical fertilizer to maize fb 75% RDF through chemical fertilizer to wheat 100%RDF (65.2q/ha).

Stone yield: INM practices (inorganic fertilizer and in combination with organic sources) significantly influenced the stone yield of maize (Table 1). The highest stone yield (8.81 q/ha) was in $T_6$50% N through FYM+50% RDF through chemical fertilizer to maize fb 100% RDF through chemical fertilizer to wheat. It was significantly higher than T_7 (25% N through FYM+75% RDF) recorded (8.75 q/ha) and all other INM practices (ranging from 6.24 to 8.81). T_5 and T_7 were *at par* in stone yield. The lowest stone yield (3.37 q/ha) was recorded in control T_1 ($N_0P_0K_0$). Stone yield of maize varied from 3.37 to 8.81 q/ha.

Grain, stover and stone yields were highest in T_6 receiving substitution of 50 per cent inorganic NPK by FYM on N basis which was significantly superior to rest of the treatments. The increment in yield due to combined application of chemical fertilizer along with FYM (50% substitution on the basis of N) was 8, 10 and 2% for grain, stover and stone yield of maize, respectively, as compared to balanced application of 100% RDF (chemical fertilizers). Application of other organic sources viz., paddy straw and green karanj leaves resulted in 11 and 13 % reduction in grain yield of maize as compared to balanced application of 100% RDF (as chemical fertilizers) and the level of reduction was higher with *karanj* leaves than cut paddy straw depending upon the mineralization potential and nutrient content. Application of different levels of NPK indicated a gradual and significant rise in yield levels with successive increment well up to the highest dose, which was the recommended dose of NPK for this agro climatic condition. The increase in nutritional dose from 50%RDF to 100%RDF and from 75%RDF to 100%RDF through FYM was instrumental in increasing grain yield by 80.0 and 41.0

Table 2. Long term effect of integrated nutrient management on economics of maize and wheat under maize-wheat cropping system (Pooled data of 2020-21 and 2021-22)

Treatment details		Net return (₹. /ha)		B:C Ratio	
Kharif	*Rabi*	Maize	Wheat	Maize	Wheat
T_1 $N_0P_0K_0$	$N_0P_0K_0$	-13033	-6200	-0.43	-0.17
T_2 50%RDF	50%RDF	15735	29860	0.48	0.76
$T_3$50%RDF	100%RDF	17816	44200	0.54	1.04
T_4 75%RDF	75%RDF	19327	52040	0.55	1.27
T_5 100%RDF	100%RDF	45613	69838	1.25	1.64
T_6 50%N (FYM) + 50% RDF	100%RDF	50583	79643	1.32	1.87
T_7 25%N (FYM) + 75% RDF	75%RDF	47900	75237	1.28	1.83
T_8 50%N (CPS) + 50% RDF	100%RDF	20204	65305	0.38	1.53
T_9 25%N (CPS) + 75% RDF	75%RDF	23951	59445	0.53	1.45
T_{10}50%N (GKL) + 50% RDF	100%RDF	37065	60611	1.08	1.42
T_{11}25%N (GKL) + 75% RDF	75%RDF	22830	55826	0.64	1.36
T_{12} Farmer's practice (urea @ 50kg/ha)	Farmer's practice (urea @ 50kg/ha)	-8992	7854	-0.29	0.21
CD at 5%		4539	9818	0.12	0.23
CV (%)		11.53	11.72	11.49	11.18

DAS: Days after sowing; CPS:- Cut paddy straw; GKL:- Green karanj leaves FYM- Farm yard manure

per cent, respectively, as against the increase of 58.0 and 41.0 per cent by giving the same increment through inorganic fertilizers. This increase in grain yield of maize may be attributed to the application of nutrient through organic and inorganic sources causing synchronized availability of plant nutrients in soil as well as more availability and absorption of nutrients by the plants resulting in cell elongation, root development and ultimately growth and yield of maize. The increase in yield of stover and stone also exhibited more or less a same trend. Significantly higher green fodder yield (452.5 q/ha) were achieved in Maize + Ricebean (1:1 ratio) with 100% RDF +PGPR application as reported by Rundan (2021). The findings in respect of yield of maize with integrated nutrient management practices are in close agreement earlier researchers (Kumar and Dhar 2010, Singh and Wanjari 2013, Kakraliya et al 2017, Singh et al 2017, Jain et al 2018, Lakum et al 2020, Chandra et al 2021).

Wheat

Grain yield: Grain yield of wheat in maize-wheat system influenced significantly by nutrient management practices (Table 1). Grain yield of wheat with application of 50% N through FYM+50% RDF through chemical fertilizer to maize fb 100% RDF through chemical fertilizer to wheat (47.96 q/ha) was at par with 100% RDF through chemical fertilizer to both crops (43.69 q/ha) and 25% N through FYM+75% RDF through chemical fertilizer to maize fb 75% RDF through chemical fertilizer to wheat (45.53 q/ha) recorded higher yield than all other nutrient management practices owing to more effective tillers/m^2 (362.2), grains/spike (45.8) and heavier grain (41 g).

Straw yield: The of application of inorganic fertilizers and organic sources in *kharif* crop, significantly influenced straw yield in *rabi* crop of wheat (Table 1). Straw yield varied from 29.91 to 82.28 q/ha. The highest straw yield was in 50% N through FYM+50% RDF through chemical fertilizer to maize fb 100% RDF through chemical fertilizer to wheat. It was similar to all other integrated nutrient management practices as well as 100% RDF to both crops but significantly higher than rest of the treatments. The findings in respect to yield of wheat are in line with the findings of Bannu et al (2008), Ali et al (2019a) and Kandil et al (2020).

Economics

Net Return

Maize: Perusal of data revealed that effect of continuous fertilizer & manure application (i.e. INM practices) in continuous cropping system of maize-wheat was significant on net return of the maize. Significantly highest net return of Rs. 50583 per hectare was recorded in treatment T_6 (getting 50 per cent of N through FYM+ 50 per cent recommended dose of nutrients through chemical fertilizers in maize and 100 percent RDF in succeeding wheat crop) over rest of the treatments. Farmers' practice (@ 50 kg urea/ha in both crops) gave the lowest & loss in net return i.e. Rs.8992 while control ($N_0P_0K_0$) gave further loss i.e. Rs.13033. Application of inorganic fertilizers T_5 (100% RDF in both crops) gave net return Rs.45613. Application of INM practice-T_6 (getting 50 per cent of N through FYM+ 50 per cent recommended dose of nutrients through chemical fertilizers in maize and 100 percent RDF in succeeding wheat crop) gave significantly highest net return of Rs. 50583 per hectare over rest of the treatments and it was 11% higher than that of T_5 (100% RDF in both crops). Other INM practices - T_8-½ N through CPS +½ RDF (inorganic) gave reduction in net return up to 60% compared to T_5(100% RDF in both crops) and it was significantly lower and T_{10}- ½ N through GKL +½ RDF (inorganic) resulted reduction in net return up to the tune of 27% compared to RDF and was significantly inferior to it. Net returns in T_8 and T_{10} were at par in maize-wheat system. Therefore, substitution up to ½ N through FYM was effective as one component along with ½ RDF (inorganic) in INM practices followed by T_7 -25% N through FYM+75% RDF (inorganic).

Wheat: Continuous cropping system of maize-wheat was significant on net return of the wheat. Significantly highest net return of Rs. 79643 per hectare was recorded in T_6 (getting 50 per cent of N through FYM+ 50 per cent recommended dose of nutrients through chemical fertilizers in maize and 100 percent RDF in succeeding wheat crop) over rest of the treatments. Farmers' practice (@ 50 kg urea/ha in both crops gave the net return i.e. Rs.7854 while control ($N_0P_0K_0$) gave lowest of Rs.6200. Application of inorganic fertilizers T_5 gave net return Rs. 69838. Application of INM practice-T_6 (getting 50 per cent of N through FYM+ 50 per cent recommended dose of nutrients through chemical fertilizers in maize and 100 percent RDF in succeeding wheat crop) gave significantly highest net return of Rs. 79643 per hectare over rest of the treatments and it was 14% higher than that of T_5 (100% RDF in both crops). Other INM practices - T_8-½ N through CPS +½ RDF (inorganic) gave reduction in net return up to 6% (Rs. 65305.00/) compared to T_5(100% RDF in both crops) and was significantly lower. T_{10}- ½ N through GKL +½ RDF (inorganic) resulted reduction in net return up to the tune of 13% compared to RDF and was significantly inferior to it. Net returns in T_8 (Rs. 65305.00/-) and T_{10} (Rs.60611.00/-) were *at par* in maize-wheat system. Therefore, substitution up to ½ N through FYM was found to be effective as one component along with ½ RDF (inorganic) in INM practices followed by T_7 -25% N through FYM+75% RDF (inorganic) (Rs.75237).

Benefit: cost ratio

Maize: Effect of continuous fertilizer & manure application

(i.e. INM practices) in continuous cropping system of maize-wheat was significant on benefit: cost ratio of the system. Significantly highest B:C ratio of 1.32 was in treatment T_6 over rest of the treatments and it was 6% higher than that of T_5. Farmers' practice (@ 50 kg urea/ha in both crops) produced lowest B:C ratio i.e.-0.43 while control ($N_0P_0K_0$) gave further loss. Application of inorganic fertilizers in T_5 gave B:C ratio of 1.25. INM practices - T_8 gave significant reduction in B:C ratio up to 71% (0.38) compared to T_5. T_{10} resulted significant reduction in B:C ratio up to 18% (1.08) compared to RDF. Therefore, substitution up to ½ N through FYM was effective as one component along with ½ RDF (inorganic) in INM practices followed by T_7.

Wheat: The effect of continuous fertilizer & manure application (i.e. INM practices) in continuous cropping system of maize-wheat was significant on benefit: cost ratio of the system. Significantly highest B:C ratio of 1.87 was in treatment T_6 over rest of the treatments and it was 8% higher than that of T_5. Farmers' practice (@ 50 kg urea/ha in both crops) produced lowest B:C ratio i.e.-0.17 while control ($N_0P_0K_0$) gave further loss i.e. 0.21. Application of inorganic fertilizers T_8 gave significant reduction in B:C ratio up to 39% (1.53) compared to T_5. The T_{10} resulted significant reduction in B:C ratio up to 13% (1.42) compared to RDF. Therefore, substitution up to ½ N through FYM was effective as one component along with ½ RDF (inorganic) in INM practices, followed by T_7. This is in agreement with the findings of Pathak et al (2002), Manjhi et al (2014), Hashim et al (2015) and Verma (2018).

CONCLUSION

Substitution up to 50% N through FYM + 50% RDF in *kharif* and 100% RDF in *rabi* in maize-wheat cropping system was best for higher productivity and profitability of maize and wheat cultivation in Chhota Nagpur plateau region of Jharkhand.

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Effects of Bioabsorption of Chromium and Lead on Cyanobacterial Species (*Spirulina subsalsa* Oersted ex Gomont and *Calothrix marchica* Lemmermann)

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***Abstract*:** The bioabsorption potential of two cyanobacterial species namely, *Spirulina subsalsa* and *Calothrix marchica* were tested against chromium and lead toxicity under laboratory conditions. Both the cyanobacterial species were grown in culture condition and treated with various concentrations (2, 4, 6, 8 mg/l) of chromium and lead. Parameters like - specific growth rate, chlorophyll, carotenoids and protein contents were also analyzed to study the effects of these heavy metals. Specific growth rate, chlorophyll, carotenoids and protein contents gradually decreased with increasing concentration of these two heavy metals. Atomic absorption spectroscopy analysis also revealed that both the species had bioabsorption potential, but *Calothrix marchica* had better absorption ability than *Spirulina subsalsa* against both chromium and lead salt treatment.

***Keywords*:** Bioabsorption, Cyanobacteria, Heavy metals

Many of the cyanobacteria are well adopted in extreme natural conditions like hot spring, hyper saline lake and cold desert. Extreme conditions are also created by environmental pollution of which heavy metal contamination is one. Several studies have been undertaken to find out the effect of heavy metals on cyanobacterial metabolism and the ability of cyanobacteria in absorbing heavy metals. Approximately 40 different types of heavy metals of the periodic table are potentially toxic to microorganisms, plant, animal and also human beings. These heavy metals at higher concentrations accumulate in soil, air and water integrating in the nutrient chains. Heavy metals (metals with relatively high densities, high atomic weights or atomic numbers) released from industrial and agricultural processes have adverse effects on environment and living organisms. Particularly when they affect agricultural fields and cyanobacteria are exposed to them. Precipitation, oxidation or reduction, evaporation, filtration, ion exchange or membrane technologies - these physico-chemical methods were used to remove heavy metal from industrial waste water (Volesky 2007). However, biological methods such as - biosorption or bioaccumulation have advantages over physico-chemical methods to reduce the heavy metal contaminant from polluted environment. These are less expensive and highly efficient even if the heavy metal concentration is high. Phytoremediation (use of algae to remediate environmental pollution) was found suitable for ready availability, minimum sludge, low operational cost and no need of supplementary nutrition (Chojnacka 2009). Cyanobacteria, in particular, provide one of the biological systems that have high potential for removing heavy metals from wastewater (Gani et al 2015). Some metal binding compound like metallothionein and phytochelatins were found in cyanobacterial cells (Cobbett and Goldsbrough 2002). At high concentrations, some heavy metals like – copper and zinc are toxic but their small amounts are not toxic rather than required for normal life of microorganisms. Banvalvi (2001) has shown that Heavy metal at a density of ≥ 3 g/cm^3 is required by organism as trace element and will be toxic in higher concentration than physiological concentration. Occurrences of several functional groups like - carboxyl, carbonyl, hydroxyl and sulphate on extracellular polysaccharides and proteins which cover the cell surface can interact with metals and play a major role in heavy metal removal (Ledin 2000). Cyanobacteria act as valuable model for study of physiological and biochemical mechanisms involved in tolerance to environmental stresses including heavy metal stress. Considering the outcome of different research works on the effect of stress on cyanobacteria, present work was undertaken to find out efficient organisms for bio absorption of heavy metals and to study the effect of heavy metals on the biochemical composition of cyanobacterial cells including pigment composition.

MATERIAL AND METHODS

Organisms and culture conditions: *Spirulina subsalsa* was isolated from the rice field soils of Sonajhuri, (23.6919°N, 87.6753°E), Bolpur, Birbhum and *Calothrix marchica* was isolated from rice field soils of Surul (23.6653°N, 87.6556°E), Birbhum. These organisms were identified by following monographs of Desikachary (1959) and Komárek (2013). After identification these organisms were established in their respective culture medium for further study. *Spirulina subsalsa* was cultured in Zarrouk medium (pH 10) and *Calothrix marchica* were cultured in BG 11 N- medium (pH 7) and were maintained at 25 ± 5ºC under 14/10 hours light-dark cycle.

Heavy metal salts used: Two heavy metals chromium sulphate [$Cr_2(SO_4)_3$] and lead nitrate [$Pb(NO_3)_2$] were selected for this experiment at 2, 4, 6 and 8 mg/l for 5 and 10 days under cultured conditions. Culture medium without heavy metals set as control.

Estimation of pigment content (chlorophyll, carotenoids), specific growth rate and protein content: Chlorophyll and carotenoids content were determined by methodology of Mackinney (1941) and Davis (1976), respectively. The specific growth rate was calculated on the basis of chlorophyll content by using formula of Guillard (1973), $k = \ln (N_t/N_0) / \Delta t$; where k = specific growth rate, N_0 and N_t are chlorophyll amount at the beginning and end of the experiments, Δt is the time interval (days). Estimation of protein content was done by following the method of Lowry et al (1951).

Atomic absorption spectrophotometry: For atomic absorption spectrophotometric study metal treated cyanobacterial biomass was collected by centrifugation of culture media. Collected biomass was taken for cellular digestion with concentrated HNO_3 (2 ml) followed by concentrated HCl (1 ml) to clear the content. Digested materials were filtered through Whatman filter paper and the volume of filtrate was adjusted to 10 ml with double distilled water to store for absorption analysis. The analysis of heavy metals absorption was done by flame atomic absorption spectrophotometer (Perkin Elmer's Pinaacle 900F).

RESULTS AND DISCUSSION

Specific growth rate: The specific growth rate of both the species were maximum in 2 mg/l chromium salt concentration and gradually decreased with increased salt concentrations (both after 5 and 10 days treatment) [Fig. 1 (A, B) and 2 (A, B)]. On the other hand, specific growth rates of both the species were maximum in control conditions compare to different concentrations of lead salt treatment. The decreased of growth rate could be due to the stress caused by heavy metals present in the culture media. Soeprobowati and Hariyati (2014) also reported that growth rate reduced with increasing concentration of heavy metals. The current study indicates that both the species tolerate Cr at low concentration (2 mg/l) and decrease of growth rates was observed when concentration increased. The specific growth rate was higher in *Spirulina subsalsa* than *Calothrix marchica* in all the concentrations of both Cr and Pb treatment.

Chlorophyll content: The chlorophyll content of both the species were maximum in 2 mg/l chromium salt concentration (27.50 µg/gm for *Spirulina subsalsa* and 9.43 µg/gm for *Calothrix marchica* after 10 days treatment) and gradually decreased with increased salt concentrations (Table 1). The chlorophyll content of both the species were maximum in control conditions (26.08 µg/gm for *Spirulina subsalsa* and 8.78 µg/gm for *Calothrix marchica* after 10 days treatment) under lead salt treatment (Table 2). Balaji et al (2014) also reported that with increasing concentration of heavy metals (like lead, chromium and cadmium) significant decrease in the amount of pigment content occurred. The present study indicates a gradual decrease in chlorophyll content associated with heavy metal stress.

Carotenoids content: The results indicated that the carotenoids content of both the species was maximum in 4

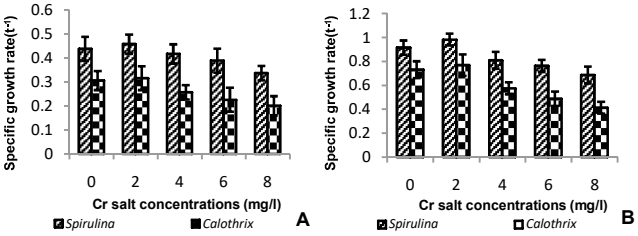

Fig. 1. (A) Effect of different concentrations of Cr on specific growth rate of *S. subsalsa* and *C. marchica* after 5 days and (B) effect of different concentrations of Cr on specific growth rate of *S. subsalsa* and *C. marchica* after 10 days

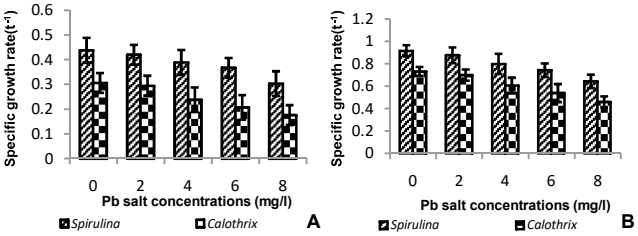

Fig. 2. (A) Effect of different concentrations of Pb on specific growth rate of *S. subsalsa* and *C. marchica* after 5 days and (B) effect of different concentrations of Pb on specific growth rate of *S. subsalsa* and *C. marchica* after 10 days

mg/l chromium salt concentration (18.29 µg/gm for *Spirulina subsalsa* and 5.91 µg/gm for *Calothrix marchica* after 10 days treatment) and gradually decreased in the growth rate against an increased salt concentration (Table 3). The, carotenoid content was maximum in 2 mg/l lead salt concentration (15.08 µg/gm) in *Spirulina subsalsa* and 4 mg/l lead salt concentration (5.05 µg/gm) in *Calothrix marchica* (Table 4).

Protein content: The protein content of *Spirulina subsalsa* was maximum in control condition in both chromium and lead salt treatment (164.53 µg/gm after 10 days treatment) whereas, in *Calothrix marchica*, protein content was maximum in 2 mg/l in both chromium (51.504 µg/gm after 10 days treatment) and lead salt treatment (48.93µg/gm after 10 days treatment) (Table 5 and 6). Balaji et al (2014) reported that significant decrease in the amount protein with increasing concentration of heavy metals. The present study indicates a gradual decrease in protein content with increasing concentration of heavy metals.

In chromium salt treatment (Table 7), maximum absorption took place by *Calothrix marchica* with 8 mg/l of salt than *Spirulina subsalsa* after 10 days (4.227 mg Cr g^{-1} for *C. marchica* and 2.137 mg Cr g^{-1} for *S. subsalsa*). In case of lead salt treatment (Table 8). The maximum absorption took place by *Calothrix marchica* with 8 mg/l of salt than *Spirulina subsalsa* after 10 days (6.312 mg Pb g^{-1} for *C. marchica* and 3.749 mg Pb g^{-1} for *S. subsalsa*). In explaining heavy metal absorption by cyanobacteria, Ledin (2000) reported that, several functional groups commonly exist on extracellular polysaccharides in addition to some proteins on the microbial

Table 1. Effect of Cr on chlorophyll content of *S. subsalsa and C. marchica*

Salt concentrations (mg/l)	Chlorophyll content (µg/gm)			
	After 5 days		After 10 days	
	S. subsalsa	*C. marchica*	*S. subsalsa*	*C. marchica*
0	14.94±1.09	5.08±0.27	26.08±2.05	8.78±0.24
2	16.22±1.71	5.82±0.65	27.50±2.36	9.43±0.67
4	13.55±1.68	4.31±0.25	25.42±2.17	6.78±0.38
6	11.07±1.94	3.9±0.32	22.94±1.07	6.42±0.49
8	9.76±1.38	3.36±0.17	17.73±2.29	5.31±0.43

Table 2. Effect of Pb on chlorophyll content of *S. subsalsa and C. marchica*

Salt concentrations (mg/l)	Chlorophyll content (µg/gm)			
	After 5 days		After 10 days	
	S. subsalsa	*C. marchica*	*S. subsalsa*	*C. marchica*
0	14.94±1.09	5.08±0.27	26.08±2.05	8.78±0.24
2	13.97±1.18	4.87±0.46	25.18±2.16	7.11±0.48
4	10.27±1.04	4.03±0.52	24.25±1.78	6.76±0.65
6	9.21±0.92	3.61±0.43	19.87±1.46	5.87±0.31
8	7.89±0.894	2.59±0.42	16.81±0.96	5.01±0.78

Table 3. Effect of Cr on carotenoids content of *S. subsalsa and C. marchica*

Salt concentrations (mg/l)	Carotenoids content (µg/gm)			
	After 5 days		After 10 days	
	S. subsalsa	*C. marchica*	*S. subsalsa*	*C. marchica*
0	8.47±1.20	2.46±0.28	14.03±2.18	4.07±0.44
2	8.93±1.43	3.12±0.39	15.84±1.25	5.55±0.48
4	10.62±1.01	3.69±0.47	18.29±2.16	5.91±0.32
6	9.84±1.21	2.81±0.22	15.32±1.03	4.48±0.34
8	8.05±1.07	2.13±0.22	12.01±1.21	3.57±0.40

Table 4. Effect of Pb on carotenoids content of *S. subsalsa and C. marchica*

Salt concentrations (mg/l)	Carotenoids content (µg/gm)			
	After 5 days		After 10 days	
	S. subsalsa	*C. marchica*	*S. subsalsa*	*C. marchica*
0	8.47±1.20	2.46±0.28	14.03±2.18	4.07±0.44
2	9.23±1.18	2.893±0.17	15.08±1.24	4.69±0.18
4	9.05±1.37	3.278±0.13	14.78±1.68	5.05±0.309
6	8.89±1.21	2.632±0.12	13.19±1.31	4.46±0.31
8	7.46±1.04	2.16±0.12	10.16±1.83	3.32±0.46

Table 5. Effect of Cr on protein content of *S. subsalsa and C. marchica*

Salt concentrations (mg/l)	Protein content (µg/gm)			
	After 5 days		After 10 days	
	S. subsalsa	*C. marchica*	*S. subsalsa*	*C. marchica*
0	81.81±7.18	23.495±3.10	164.53±13.05	36.753±4.68
2	78.27±5.54	26.308±3.54	157.98±11.53	51.504±5.82
4	73.34±6.44	16.675±1.67	153.78±9.18	43.388±3.43
6	69.46±3.88	13.439±2.27	138.74±±7.54	33.806±2.19
8	63.36±4.92	11.01±1.07	110.82±5.44	30.173±2.89

Table 6. Effect of Pb on protein content of *S. subsalsa and C. marchica*

Salt concentrations (mg/l)	Protein content (µg/gm)			
	After 5 days		After 10 days	
	S. subsalsa	*C. marchica*	*S. subsalsa*	*C. marchica*
0	81.81±7.18	23.495±3.10	164.53±13.05	36.753±4.68
2	72.37±6.44	28.17±2.45	145.62±14.18	48.93±3.37
4	67.39±7.38	21.379±1.28	129.27±8.89	38.855±2.13
6	66.47±5.17	18.124±2.13	111.74±7.15	31.813±3.04
8	61.23±4.64	16.17±1.01	103.74±5.28	24.69±2.92

Table 7. Amount of chromium absorption after 5 and 10 days by *S. subsalsa and C. marchica*

Salt concentrations (mg/l)	Metal absorption (mg /gm of cell)			
	After 5 days (mg /gm of cell)		After 10 days (mg /gm of cell)	
	S. subsalsa	*C. marchica*	*S. subsalsa*	*C. marchica*
2	0.491	0.723	0.858	1.237
4	0.803	1.760	1.33	3.082
6	1.081	2.175	1.923	3.971
8	1.380	2.826	2.137	4.227

Table 8. Amount of lead absorption after 5 and 10 days by *S. subsalsa and C. marchica*

Salt concentrations (mg/l)	Metal absorption (mg /gm of cell)			
	After 5 days (mg /gm of cell)		After 10 days (mg /gm of cell)	
	S. subsalsa	*C. marchica*	*S. subsalsa*	*C. marchica*
2	0.736	1.088	1.285	1.671
4	1.261	2.162	2.062	3.408
6	1.723	3.217	3.226	5.082
8	1.926	3.845	3.749	6.312

cell surface which can interact with metals and play a major role in heavy metal removal. The *Calothrix marchica* absorbed more amounts of both the heavy metals (both chromium and lead) than *Spirulina subsalsa*. Presence of a prominent sheath in *Calothrix marchica* could be a reason for absorbing higher amount of heavy metals as compared to *Spirulina subsalsa*.

Both the heavy metals (Cr and Pb) were absorbed more after 10 days treatment as compared to 5 days treatment. This was observed in case of all the concentrations. Our observation are in conformity with Ruangsomboon et al (2006) who reported that, with increase the age of cyanobacterial culture, the amount of polysaccharides and proteins on the cell surface were also assumed to increase, resulting in the existence of more functional groups. Thus, when the age of the culture of cyanobacteria increased, the amount of heavy metals uptake increased. So, the absorption was time dependent. It was also observed that out of these two heavy metals (Cr and Pb) amount of lead uptake was higher than chromium by both the cyanobacterial species. This indicates that lead polluted environment can be easily cleaned by the application of cyanobacterial species.

CONCLUSIONS

The aim of this work was to study the bio absorptive capacities of two cyanobacterial species-*Spirulina subsalsa* and *Calothrix marchica* for the removal of chromium and lead under *in vitro* condition. Both the species were able to tolerate low concentration of Cr and Pb contaminants. This was reflected in specific growth rate, chlorophyll, carotenoids and protein contents. The removal of heavy metal by conventional method has several disadvantages like-high cost, toxicity, inputs of chemicals. In this regards, *Calothrix marchica* was found to be the best bio absorbent among the two species investigated. So it is recommended that for phytoremediation process, use of *Calothrix marchica* will be more appropriate in both Cr and Pb polluted environment.

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Economic Analysis of Natural Farming based Apple Orchards in Himachal Pradesh

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***Abstract*:** The apple production was evaluated under two farming systems i.e., Subhash Palekar's Natural Farming (SPNF) and conventional chemical based farming (CF) systems in Himachal Pradesh, India. The data was collated from 110 apple orchards spread all across the state during the cropping season of 2020. The total variable cost of apple cultivation under natural farming was 56.53 % lower than conventional farming (CF), thereby clearly highlighting the advantage of natural farming over CF. Net returns of apples under natural farming were better (27.41%) than CF apples mainly due to sizable reduction in cultivation costs. The success of progressive SPNF adopters proves that natural farming has viable potential to sustain apple farming and enhance mountainous farmers' income in the backdrop of the looming threat of climate change.

***Keywords*:** ZBNF, SPNF, Apple cultivation, Economic impact, Net returns

The Green Revolution induced modern agricultural production technologies completely changed the Indian food production scenario towards self-sufficiency. But its untenable dependence on chemical inputs set off negative externalities like plummeting factor productivity, increasing cultivation cost, and exorbitant pesticide residues in farm products (Kotschi 2015, Supriya et al 2018, NAAS 2019, IPBES 2019). Rising awareness about nutritional quality and safety of food has led the consumer's quest for safer and chemical-free foods produced ecologically and authentically by local systems. At the same time, increasing cultivation cost and unstable commodity prices have strained the livelihood of farmers and has resulted in agrarian distress (Mishra 2008). Climate change induced catastrophes have shaken the socio-economic foundations of the hill farming systems across hilly regions in India. The rate of warming in the Himalayas is greater than the global average, confirming that the Himalayas are among the most vulnerable regions to climate change (Shrestha et al 2012). Coordinated efforts are required for adaptation and mitigation as the vulnerable mountain ecosystems are likely to face a greater risk of climate change impacts than other ecosystems (Negi et al 2012). Climate change impacts have also brought back attention to agro-ecology, its stability, and its resilience. The need of the hour is to promote farming systems and technologies which enhance income and also prove ecologically sound.

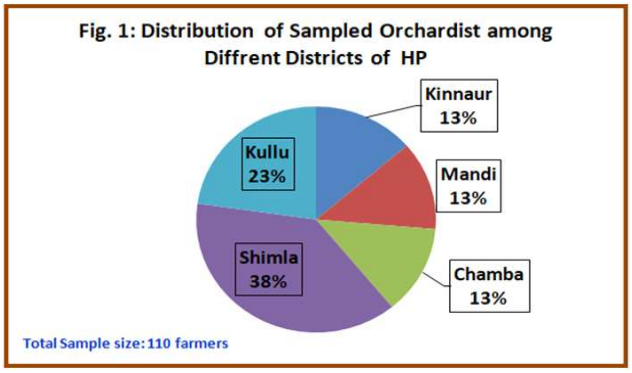
Himalayan state of Himachal Pradesh has accomplished a magnificent revolution in fruit production and the horticultural sector of the state has crossed agriculture in terms of value addition by contributing about 40 percent of the agricultural GDP (Anonymous 2021, Negi 2020). But Himalayan horticultural production is equally vulnerable to climate change and is facing threats like increasing incidences of pest and disease attacks, declining productivity, and replacement of apples with other crops. (Basannagari et al 2013, Asghar et al 2013). The temperature in apple growing regions of the mountain state of Himachal Pradesh, India showed increasing trends whereas precipitation showed decreasing trends (Rana et al 2011). This apple centric horticultural development, achieved through the use of modern production technologies, is significantly dependent upon the use of synthetic chemical inputs particularly fertilizers and pesticides (Randev 2015, Chand et al 2017). Typically considered a chemical free farming state, pesticide presence and levels are high in the produce of Himachal Pradesh (Anonymous 2015). Pesticide residues are always an important issue in food exports as several shipments of Indian agricultural produce have been rejected after the identification of residues. The quest for alternate methods, especially those which are low on chemical use, is being recognised by every section of society. In such a scenario, farmers' financial opulence can be improved by spreading the agro-ecologically compatible technologies and innovations capable of minimising the cost and improving crop productivity and quality. Such alternative

natural farming practices with ecological and social benefits have emerged and been promoted in pockets (Park and DuPonte 2008, Brown 2018). These practices are sustainable alternatives to chemical farming as they seek to optimise the use of locally available resources in place of external inputs.

Subhash Palekar Natural Farming (SPNF) is rooted in Indian tradition and is a holistic alternative based on the latest scientific discoveries in Agriculture. The F.A.O. has urged all countries to move towards the adoption of Agroecology to meet the twin goals of global food security and conservation of the environment and SPNF principles are in sync with the principles of Agroecology. It has been widely accepted by the farmers (Neelam and Kadian 2016, Tripathi et al 2018, Khadse and Rosset 2019). The viability of the organic and natural farming systems is well contested among the scientific fraternity with arguments both in favour and against (Seufert et al 2012, Strohbach et al 2015, Kuruganti 2019, NAAS 2019, Rao 2019). Highlighting the predominance of smallholder farmers, SPNF was adopted in Himachal Pradesh (HP), under the scheme 'Prakritik Kheti Khushhal Kisan' (PK3) Yojna launched in 2018, with the objective to make agriculture economically viable and profitable and reduce agrarian distress and risk through cost reduction and sustainable climate-resilient agricultural practices. SPNF practices have been adopted by a substantial number of farmers across the state for growing a diversified basket of agricultural crops like cereals, pulses, oilseed, vegetables, and fruits. Apple orchardists of the state have also embraced natural farming methods for apple cultivation in a big way. However, not much scientific data pertaining to various aspects of the cost of cultivation and performance of various crops including apple under the SPNF system is available. Keeping in view the above background, the present study was an attempt to evaluate the economic impact of apple cultivation under SPNF practices.

MATERIAL AND METHODS

This study on SPNF Apple cultivation in Himachal Pradesh was conducted during the year 2020 and data for this study was collected from randomly sampled 110 best apple orchardists practising SPNF and conventional farming (CF) methods. These orchardists were spread across apple producing districts namely Shimla (42), Kullu (25), Kinnaur (15), Mandi (14) and Chamba (14). The proportional distribution of sampled apple growers has been shown in Figure 1. These districts were selected because of their significant contribution with respect to the area and production of apples in the state. Data for this study was collocated by the field staff of ATMA i.e. BTMs and ATMs. Primary data were analysed using standard tabular and statistical methods.



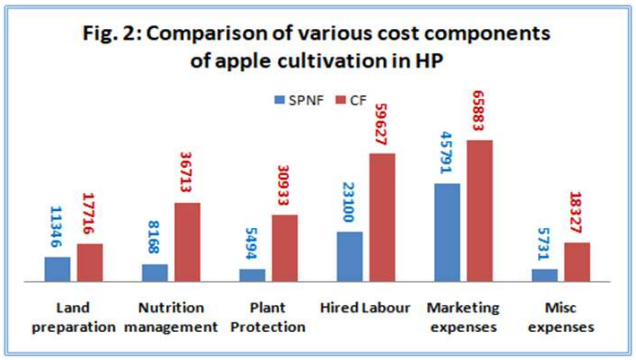
RESULTS AND DISCUSSION

Status of SPNF Apple cultivation in Himachal Pradesh): In a short span of 3 years, SPNF practices have become very popular among the apple orchardists of the state. Presently, SPNF practices usage for apple cultivation is being carried out in all the apple producing districts of the state. SPNF apple cultivation practices have been adopted by 12928 apple orchardists on 760.19 ha area spread across 47 blocks. Conventional farming of apples is highly reliant on capital and chemical inputs, therefore, farmers had expressed confidence in SPNF practices and given it a chance on a sizable part of their apple orchard. Most of the sampled orchardists have started converting their orchards under natural farming practices and the average area under SPNF and CF orchards was 0.42 and 0.33 ha, respectively. Per hectare planting density under SPNF (614) was higher than CF (506), respectively. The proportion of plants in the bearing stage under SPNF (63.7 %) was lower than CF (81.8 %).

Comparative economics: In order to assess the economic viability of Apple under Natural Farming, the study has conducted an empirical analysis on three important parameters: comparative economics, economic returns, and income. The total variable cost of apple cultivation under natural farming (₹ 99629 ha^{-1}) was 56.53 % lower than Conventional Farming (₹2,29,200 ha^{-1}), thereby clearly highlighting the advantage of natural farming over CF (Appendix 1). A significant reduction in the cost of cultivation of all the crops under ZBNF has already been documented by ABZBNF (2018), Kumar et al (2019) and Chandel et al (2021).

Land preparation cost (₹ 11346) under SPNF was 35.96 % lower than CF. The presence of companion crops under SPNF apple orchards leads to minimal weed growth, thereby, reducing the expenses on land management (Fig. 2).

Expenses on nutrition and pest management under SPNF were nearly 80 % lower than CF and it was the major attraction for orchardists to convert to SPNF practices in their apple orchards. Plant protection operation under CF involves exposure to many health hazards, therefore, alert and health conscious orchardists evaded these hazards by executing these operations by hiring the labour. But these operations under SPNF were happily carried out by the farmers themselves, leading to lower (23.19 %) dependence on hired labour. Increased awareness among consumers about the health hazards of chemical based farming has also helped the orchardist in the marketing of SPNF apples as in some cases buyers had started to lift their produce from the farm itself and it helped them save 45.96 % expense on marketing. Therefore, SPNF based apple farming is a win-win situation for the orchardists as well as society.



Productivity and returns: Productivity of apple orchards under natural farming was at par with CF and was rather marginally higher (2.10 per cent) than CF (152.6 q ha^{-1}). Price and gross profit received by SPNF apples were also 2.46 and 4.61% higher than CF apples. Net returns of apples under natural farming were better (27.41%) than CF mainly due to a sizable reduction in cultivation costs (Fig. 3). Chandel et al (2021) also reported that Fruit-pulse-vegetable based crop rotations generated 21.55% higher net returns under natural farming in Himachal Pradesh. Therefore, it can be asserted with authority that apple cultivation with SPNF practices is an economically viable option and it defies the concerns raised by the proponents of conventional apple farming. Besides this, economic returns of SPNF farming will be much higher if we take into account the positive externalities in the form of its beneficial impact on the environment and health of the apple producers and consumers.

Companion crops: Intercropping of leguminous companion crops for natural nitrogen fixing in soils is the most advocated practice in the natural farming of apple orchards. Rabi pea, kharif pea, French bean and kidney bean were the most preferred companion crops in the study area. Non-leguminous crops like tomato, garlic and cabbage/cauliflower, were also grown by farmers owing to remunerative prices fetched by these crops. Net returns from the cultivation of companion crops in apple orchards resulted in excellent financial gains for the farmers and made this farming really zero budget farming. Net returns under crops such as Cabbage/Cauliflower (₹153420 ha^{-1}) followed by Kharif pea (₹113689 ha^{-1}), Rabi Pea (₹45760 ha^{-1}), Tomato (₹42565 ha^{-1}), French bean (₹ 41251 ha^{-1}), Garlic (₹39442 ha^{-1}), Rajmah (₹ 31785 ha^{-1}) were sufficient enough to meet the working capital requirement of the main crop.

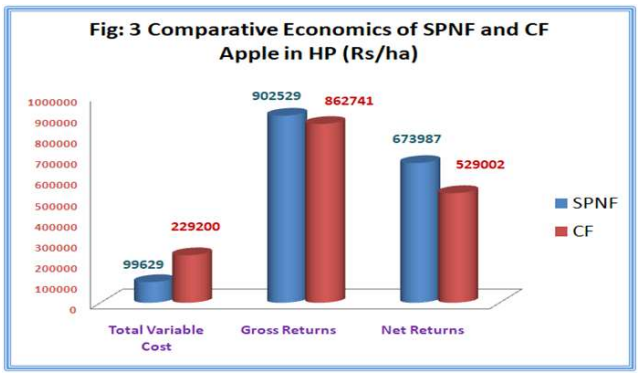
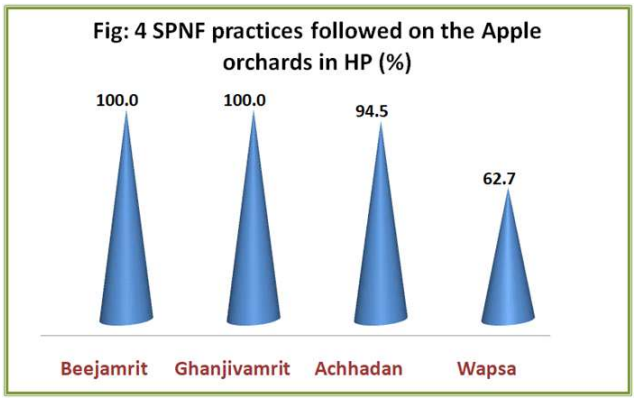


Table 1. Comparative economics of SPNF and CF Apple orchards in HP (per ha)

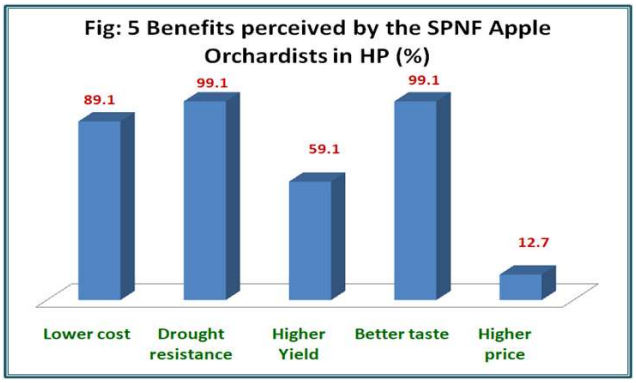
Particulars	SPNF	CF	% Change in SPNF over CF
Land preparation	11346	17716	-35.96
	(11.39)	(7.73)	
Nutrition management	8168	36713	-77.75
	(8.20)	(16.02)	
Plant protection	5494	30933	-82.24
	(5.51)	(13.50)	
Hired Labour	23100	59627	-61.26
	(23.19)	(26.02)	
Marketing expenses	45791	65883	-30.50
	(45.96)	(28.74)	
Misc expenses	5731	18327	-68.73
	(5.75)	(8.00)	
Total variable cost (Rs/ha)	99629	229200	-56.53
	(100.00)	(100.00)	
No. of plant protection sprays	6.39	7.10	-10.08
No. of nutritional sprays	8.13	5.00	62.50
Gross yield (q/ha)	155.8	152.6	2.10
Average price (Rs/q)	5791	5652	2.46
Gross returns (Rs/ha)	902529	862741	4.61
Net returns (Rs/ha)	673987	529002	27.41

Note: Figures in parenthesis are per cent of total variable cost

Natural farming practices: *Beejamrit, Ghanjivamrit and Jeevamrit, Achhadan* and *Wapsa* are the four pillars of Subhash Palekar Natural Farming. *Beejamrit, Ghanjivamrit* and *Jeevamrit* are various decoctions based on indigenous cows' urine and dung and ensure optimal nutrition and protection from diseases and infections during the various stages of plant growth. These practices were followed by 100 percent apple orchardists but *Achhadan* (94.5%) and *Wapsa* (62.7%) practices were slightly lacking because of the shortage of mulching material and labour intensive nature of these practices (Fig. 4). Partial adoption of SPNF technology pillars is resulting in lower productivity of apples under natural farming. It is expected that complete adoption of SPNF practices in the cultivation of apples can lead to better and sustainable productivity and quality of fruits in apple orchards in Himachal Pradesh.



Benefits of natural farming practices: There is no doubt about the social, economic, and environmental superiority of natural farming over conventional farming practices, yet effort was made to assess the preparation of the farmers on these aspects. According to our survey, farmers adopted SPNF for a number of reasons, including family wellbeing, food self-sufficiency, environmental issues, and cost-cutting. 99.1 per cent of the people said that they are getting better drought resistance in crops and the quality of products by using natural farming practices (Fig. 5). This shows that natural farming can be an effective tool in the hands of farmers against otherwise inevitable ill impacts of climate change. Critics have expressed major apprehension about windfall decline in crop productivity under natural farming. But 59.1 per cent of respondents reported improvements in yield levels by turning to SPNF. Saving on account of reduced cultivation cost under SPNF was confirmed by 89.1 per cent of apple orchardists but only 12.7 per cent of farmers received a better price for SPNF produce in the market. The major problems associated with natural farming in the state was the non-availability of a specialized market followed by labour-intensive farming and unfair price for products in the market (Vashisht 2021).



CONCLUSION

Orchardists of Himachal Pradesh have successfully demonstrated the worth of SPNF practices for producing good quality apples. They proved that apprehensions cast against the efficacy of natural farming practices in sustaining apple productivity were overhyped. This study based on the preliminary years of SPNF implementation in the apple orchards of the state shown that the adoption of a complete package of natural farming practices can go a long way in sustaining the long-term production and income of apple orchards in the state. These low cost and sustainable climate-resilient agricultural practices can be a boon for maintaining the agroecology of the state besides ensuring the minimal impact of climate change on the apple industry of the state, yet long-term studies are required.

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Fruit Morpho-Physical and Biochemical Characteristics of some Guava (*Psidium guajava* L.) Cultivars and Hybrids Under Subtropical Conditions of Himachal Pradesh

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Abstract: The evaluation of seven guava cultivars and hybrids for cultivation in the subtropical region of Himachal Pradesh as compared to predominantly grown cultivars Allahabad Safeda and Luckhnow-49 was done at RHR&TS, Dhaulakuan (Sirmour, HP) during the years 2016-17 and 2017-18. Fruit size (60.57 x 69.70 mm) and weight (171.71 g) were maximum in Lalit with highest fruit yield (16.45 kg/tree) but lowest yield efficiency (7.43 g/cm^2 TCSA). The fruit shape was ovate with pointed shape at the stalk end in H-1, Shweta and Lucknow-49, whereas others were round in shape. Flesh colour varied from yellow-white to greyed-yellow to orange-white to greyed-red. Maximum TSS (10.98 ºB), total sugars (7.30 %) and reducing sugars (4.84 %) were in Hisar Safeda with minimum acidity (0.19 %). Lucknow-49 has the minimum number of seeds per fruit (123.50). Fruits were soft seeded in Hisar Surkha, whereas, CISH-G-1, Lalit and Allahabada Safeda were medium seeded and the rest were hard seeded. Among nine cultivars and hybrids, Lalit is suitable for table as well as processing purposes whereas among quality characteristics, Hisar Safeda performed better.

Keywords: Guava, Cultivar, Hybrid, Morpho-physical, Biochemical

Guava (*Psidium guajava* L.) is one of the important fruit crops grown in the tropics and subtropics, belonging to the family Myrtaceae. The genus *Psidium* includes some 150 species (Mitra et al 2012). The majority of guava cultivars are diploid (2n=22) that are commercially available (Shukla et al 2012). Guava is rich in vitamin C, carbohydrates, fibres and protein and can be consumed fresh or processed for juice, jam, jelly, cheese, canned segments, nectar, R.T.S, beverages etc. (Pradhan et al 2021). The leaves are used for the treatment of diarrhea as well as for the dyeing and tanning. It has anti-diarrheal, anti-hypertensive, antioxidant, anti-microbial, hypoglycemic and anti-mutagenic activities (Eze et al 2021). Cultivated guava is native to Tropical America, where it occurs wildly. It is known to have been introduced in India as early as the 17th century and at present it is widely cultivated on a commercial scale. Guava occupies an area of 3,08,000 hectares in India resulting in production of 45,82,000 metric tonnes (Anonymous 2021). It is cultivated on an area of 2,320 hectares in Himachal Pradesh with an annual production of 2,610 metric tonnes (Anonymous 2018). It requires 100-200 cm of rainfall per annum with an optimum temperature between 23°C to 26°C.

Guava cultivation is quickly expanding in the country because of its superior adaptability, resistance to diverse biotic and abiotic stresses, and high output combined with minimal input needs. However, to make guava cultivation commercially feasible, it is critical to pick cultivars that are suited to a specific climatic environment based on fruit quality (Singh et al 2013). The lower hills of Himachal Pradesh are seen as suitable for cultivation, especially in the changing climate scenario and producers are seeking for ways to diversify their fruit crops in order to increase their income. The primary commercial cultivars grown for a long period are Allahabad Safeda and Sardar (L-49). The current investigation was conducted to examine the performance of various guava cultivars and hybrids in comparison to these cultivars in the sub-tropical regions of Himachal Pradesh.

MATERIAL AND METHODS

The performance of seven guava cultivars and hybrids viz. H-1, H-2, CISH-G-1, Lalit, CISH-G-4 (Shweta), Hisar Safeda and Hisar Surkha were examined for their fruit and yield characteristics. Allahabad Safeda and Lucknow-49 were used as check varieties that are predominantly grown in the subtropical regions of Himachal Pradesh. The experiment was conducted at Regional Horticultural Research & Training Station, Dhaulakuan (Sirmour, H.P.) during the rainy season located between 35.5 °N latitude and 77.5 °E longitude at an elevation of 468 meters above mean sea level. The plant material for this study consisted of eight-

years-old uniformly growing and bearing trees. Observations were recorded on fruit morpho-physical and biochemical parameters during the rainy season on seven different guava cultivars and hybrids with two check varieties and each having four replications. The experiment was laid out on bearing guava trees in a randomized block design, planted at a distance of 5 x 5 meters. A total of five fruits were selected randomly from all directions from each individual tree and observations so recorded were averaged. Fruit length (mm), fruit width (mm), stalk length (mm), size of sepals (mm), diameter of calyx cavity (mm), core diameter (mm) and thickness of outer flesh in relation to core diameter (mm) were recorded using vernier calipers. Average weight of fruit (g) was measured. The remaining morpho-physical (non-metric) characters were observed as per the UPOV descriptor (Anonymous 1987). Total soluble solids (TSS) content was estimated by using an "Erma–Hand Refractometer" (0 to 32 ºB). The titratable acidity, total, reducing and non-reducing sugars of guava pulp were determined (Ranganna 1995). For seed characters, the fruit pulp was cut into pieces, and boiled for 15 minutes in hot water. Later, the seeds were separated by ordinary sieve (< 20 mm) and the number of seeds was counted. The seed weight per fruit was measured. The size of the seed was measured by taking the length and width of the seed with the use of vernier calipers, and the measurements made were summed. The hardness of the seed was determined by a panel of judges by an organoleptic test and presented as hard, medium and soft. At harvest time, the final fruit yield in different cultivars and hybrids was recorded by weighing the total fruits retained in a particular tree and yield was expressed in kilograms per tree (kg/tree). The yield efficiency of each cultivar and hybrid was calculated according to the Westwood (1978) method and was expressed using the formula g/cm^2 TCSA. The statistical analysis was carried out for each observed character using MS-Excel and OPSTAT (Sheoran et al 1998).

RESULTS AND DISCUSSION

Fruit (morpho-physical) characters: The 2-year pooled data indicates there was significant variation among guava cultivars and hybrids for these traits (Table 1, 2). Lucknow-49 recorded maximum mean fruit length of 71.35 mm which is statistically at par with Allahabad Safeda and CISH-G-4 and the lowest fruit length was in CISH-G-1(48.82 mm). The maximum fruit width (69.70 mm) was in Lalit, followed by Lucknow-49, Hisar Safeda and Allahabad Safeda. Minimum pooled fruit width (57.90 mm) was observed in Hisar Surkha. Deshmukh et al (2013) also observed mean fruit length and width of the different cultivars and hybrids ranging from 59.8 mm to 65.4 mm and 61.5 mm to 69.9 mm, respectively. Similar results were obtained by Dolkar et al (2014), Gupta et al (2016) and Kumari et al (2016). The variation in fruit length and width can be attributed to the genetic constitution of a cultivar (Tiwari et al 2016). There was significant variation in fruit weight between different guava cultivars and hybrids. The maximum weight of the fruit was in Lalit (171.71 g) followed by Lucknow-49. Dolkar et al (2014) reported maximum weight in Lucknow-49 under subtropical conditions. The variation in fruit weight may be due to phenotypic and genotypic influence on different cultivars (Kumari et al 2020). The thickness of the outer flesh ranged from 9.75 mm to 16.88 mm and the core diameter ranged from 46.01 mm to 56.43 mm in all guava cultivars and hybrids. Similar trend was observed by Singh (2013) with 10.17 mm to 17.48 mm and 31.11 mm to 42.35 mm, respectively.

The shape of fruit was pomi (round) and ovate while, fruit shape at stalk end varied from broadly rounded to pointed. The shape at stalk end is rounded in 'Allahabad Safeda' as per PPV&FRA guidelines (Anonymous 2016) which was equivalent to the observations recorded in the present study. Fruit shape in guava has also been described by many workers (Dubey et al 2016; Kumari et al 2016; Ran et al 2017) and similar variations were recorded. There was no significant variation in fruit peel colour, except for slight variations in color shade, but the flesh color varied from yellow-white to greyed-red and several studies conducted also reported such variations (Meena et al 2013, Dubey et al 2016, Kumari et al 2016, Singh et al 2016, Ran et al 2017, Sohi et al 2019). Although flesh colour is a varietal character, slight variation in the intensity may be attributed to the climatic factors and soil type. Relief of the fruit surface was smooth to rough (Table 2). Singh et al (2016) reported rough fruit surfaces in 'CISH-G-1' and 'Lucknow-49'. The longitudinal ridges (present in CISH-G-4, Lucknow-49 and Hisar Safeda) and grooves (present in CISH-G-1, Lalit and Allahabad Safeda) were categorized as present or absent in nine guava cultivars and hybrids. Fruit yield was maximum (16.45 kg/tree) in Lalit and statistically at par with Lucknow-49 and minimum of 12.09 kg/tree was in Hisar Surkha and it was significantly lower than all the other cultivars (Table 1). Meena et al (2013) also reported fruit yields ranging between 5.93 kg/plant to 14.91 kg/plant. Significant variation in yield characters has been observed earlier also (Deshmukh et al 2013, Jana et al 2015, Gupta et al 2016, Kumari et al 2016, Sahoo et al 2017).

Fruit (biochemical) characters: The TSS content in fruits ranged between 9.89 ºB in Allahabad Safeda to 10.98 ºB in Hisar Safeda (Table 1). However, according to Kumari et al (2016), TSS ranged from 9.66 ºB to 11.40 ºB in sub-tropical

Table 1. Fruit morpho-physical and biochemical (metric) characters of some guava cultivars and hybrids

Characters/ Cultivars/ hybrids	Fruit length (mm)	Fruit width (mm)	Weight of fruit (g)	Core diameter (mm)	Thickness of outer flesh in relation to core diameter (mm)	Yield (kg/tree)	Yield efficiency (g/cm^2 TCSA*)	Total Soluble Solids (ºB)	Acidity (%)	Total sugars (%)	Reducing sugars (%)	Non-reducing sugars (%)	Number of seeds/fruit	Seed weight (g)/fruit	Seed size (mm)
H-1	61.77	58.94	110.75	47.28	10.66	13.18	8.64	10.36	0.28	6.89	4.52	2.26	147.88	5.23	3.82
H-2	49.85	59.96	105.88	46.84	12.63	12.52	9.68	10.64	0.32	7.07	4.73	2.23	217.25	6.30	3.89
CISH-G-1	48.82	60.80	103.29	48.94	10.86	10.98	7.66	10.29	0.22	6.84	4.30	2.42	139.75	3.00	3.36
Lalit (CISH-G-3)	60.57	69.70	171.71	56.43	12.77	16.45	7.43	10.70	0.26	7.12	4.41	2.57	182.75	3.64	3.34
CISH-G-4 (Shweta)	67.51	63.04	139.24	51.79	9.75	13.84	10.41	10.84	0.19	7.21	4.67	2.41	269.75	6.44	3.37
Hisar Safeda	61.59	67.91	157.90	54.68	12.23	14.39	9.41	10.98	0.19	7.30	4.84	2.34	257.50	7.20	4.03
Hisar Surkha	58.69	57.90	105.18	46.01	11.39	12.09	8.14	9.98	0.24	6.63	4.34	2.18	161.25	3.47	3.39
Allahabad Safeda	66.16	67.76	161.91	52.74	14.03	14.77	9.86	9.89	0.29	6.57	4.79	1.70	337.63	11.65	3.50
Lucknow-49 (Sardar)	71.35	68.12	164.44	48.33	16.88	15.48	7.85	10.61	0.24	7.06	4.52	2.41	123.50	2.97	3.48
Mean	60.70	63.79	135.59	50.34	12.36	13.75	8.79	10.48	0.25	6.97	4.57	2.28	204.14	5.55	3.53
CD (p=0.05)	5.74	4.78	29.56	4.69	1.38	1.00	2.87	0.23	0.04	0.19	0.12	0.24	7.96	0.30	0.43

*Trunk Cross Section Area

Table 2. Fruit (morpho-physical) non-metric characters of some guava cultivars and hybrids

Characters/ Cultivars/ hybrids	Fruit shape	Fruit shape at stalk end	Colour of peel	Relief of fruit surface	Ridged collar around calyx cavity	Longitudinal ridges	Prominence of longitudinal ridges	Longitudinal grooves	Colour of flesh	Seed hardness
H-1	Ovate	Pointed	Yellow-Green 145 A	Smooth	Inconspicuous	Absent	-	Absent	Greyed-Red 180 C	Hard
H-2	Pomi (Round)	Broadly Rounded	Yellow-Green 151 A	Rough	Inconspicuous	Absent	-	Absent	Greyed-Red 180 C	Hard
CISH-G-1	Pomi (Round)	Broadly Rounded	Yellow-Green 151 A	Rough	Inconspicuous	Absent	-	Present	Yellow-White 158 A	Medium
Lalit (CISH-G-3)	Pomi (Round)	Broadly Rounded	Yellow-Green 151 A	Rough	Inconspicuous	Absent	-	Present	Greyed-Red 180 C	Medium
CISH-G-4 (Shweta)	Ovate	Pointed	Yellow-Green 151 D & 145 A	Rough	Inconspicuous	Present	Weak	Absent	Greyed-Yellow 160 D	Hard
Hisar Safeda	Pomi (Round)	Rounded	Yellow-Green 153 A & 145 A	Rough	Conspicuous	Present	Weak	Absent	Greyed-Yellow 160 D	Hard
Hisar Surkha	Pomi (Round)	Rounded	Yellow-Green 145 A	Smooth	Inconspicuous	Absent	-	Absent	Greyed-Red 180 B	Soft
Allahabad Safeda	Pomi (Round)	Rounded	Yellow-Green 153 D & 145 A	Smooth	Inconspicuous	Absent	-	Present	Orange-White 159 C & 159 D	Medium
Lucknow-49 (Sardar)	Ovate	Pointed	Yellow-Green 145 A	Rough	Inconspicuous	Present	Medium	Absent	Orange-White 159 C	Hard

condition of Himachal Pradesh. Kumari et al (2020) also reported a minimum TSS in Allahabad Safeda (9.9 ºB). The total sugars ranged from 6.57 per cent to 7.30 per cent, reducing sugars from 4.30 per cent to 4.84 per cent and non-reducing sugars from 1.70 per cent to 2.57 per cent in cultivars and hybrids (Table 1). Similar trend was observed by Kumari et al (2016). The physico-chemical characteristics of guava cultivars and hybrids may vary from place to place depending on climatic factors and management practices. Similar variations for physical and chemical characters like TSS, acidity, sugars were also reported by Ghosh et al (2013), Meena et al (2013), Singh et al (2016), Dubey et al (2016), Gupta et al (2016), Kumari et al (2016) and Bhalekar and Chalak (2017).

Seed characters: Fruit quality in guava also depends upon the seed content of fruit and generally guava contains higher seed content when compared to others. The number of seeds per fruit varied from cultivar to cultivar being maximum (337.63) in Allahabad Safeda with a maximum seed weight (11.65 g) and minimum (123.50) in Lucknow-49 with minimum seed weight (2.97 g) (Table 1). Although variation in the presence of less number of seeds per fruit is a desirable character. Kumari et al (2016) also recorded a low (127) seed number in Lucknow-49 under the sub-tropical conditions of Himachal Pradesh. Seed size for different guava cultivars shows variation in Lalit with a minimum mean of 3.34 mm being statistically equal to CISH-G-1, CISH-G-4, Hisar Surkha, Lucknow-49 and Allahabad Safeda. Maximum seed size was in Hisar Safeda (4.03 mm) which was statistically equal to H-2 and H-1. This trait governs the quality of the fruit in guava, as small seed size is preferred over bold seeds. The fruit was soft seeded in Hisar Surkha, while the medium hard seeded in CISH-G-1, Lalit and Allahabad Safeda and the rest were hard seeded. The present findings are in accordance with the findings of Kumari et al (2016); Bhalekar and Chalak (2017) where seed texture of guava was in the range of soft, medium and hard.

CONCLUSION

The maximum fruit size, weight and yield were observed in Lalit with medium seed hardness and greyed red flesh colour while total soluble solids and reducing sugars were maximum in Hisar Safeda with minimum acidity. So, other than Allahabad Safeda and Lucknow-49, these two varieties (Lalit and Hisar Safeda) can also be used for commercial cultivation in the subtropical regions of Himachal Pradesh to get better returns.

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Study on Marketing Performance and Constraints of Pea (*Pisum sativum*) Output in High Hills Wet Temperate Zone of Himachal Pradesh, India

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Abstract: The purpose of the study is to identify the output marketing channels of pea crop, evaluate the marketing performance and constraints in the marketing. A multi-stage random sampling procedure was employed to select 200 sample farmers. Both qualitative and quantitative data were collected from primary and secondary sources by using structured questionnaires. The data were analysed using Acharya marketing approach and descriptive statistics. The finding of this study reveals that there are five output marketing channels used by pea growers in the study area, producer-retailer-consumer (1.50%), producer-retailer-consumer (8.00%), producer-commission agent-retailer-consumer (59.50%) , local trader-wholesaler-retailer-consumer (19%) and producer-wholesaler-retailer-consumer (12%). The total gross market margin and profit margin was highest when farmers sold pea produce directly to consumers and lowest when they sold pea produce to local traders across channel. It has been observed that producer-consumer Channel was most efficient channel among all five output marketing channels. Lack of all-weather roads followed by lack of market consultancy services and high commission charges during marketing are the major constraints faced by the farmers. Therefore, policy initiatives should aim at increasing farmers access to market, strengthen government extension services, improving market infrastructure, reducing unfair profit distribution and disseminating reliable market information.

Keywords: Pea, Marketing channel, Marketing performance, Efficiency, Constraints

Vegetable plays significant role in Indian agriculture by producing higher returns per unit area and time, while providing nutritional and economic security. Vegetable cultivation is an important source of income for smallholder farmers and demand for the products is raising in both domestic and international markets thus increase smallholder farmers' participation in the market (Thakur et al 2022). Moreover, market is crucial for economic growth and sustainable development of any country. Researchers emphasized that efficient markets are the essential tool for lifting farmers out of poverty and enhancing food security in developing countries (Chand et al 2020, Ankita 2021). Additionally, literature on agriculture marketing has indicated that favorable marketing performance could encourage farmers to produce, adopt improved technologies and increase the share of prices received by the farmers (Thakur et al 2021). However, farmers with small landholding face various problems to participate in markets. The significant increase in productivity and profitability of farms mainly depends on the marketing system. The imperfect market of agricultural products largely dominated by intermediaries and farmers are deprived of getting remunerative value of their farm produce resulting to less revenue (Mishra et al 2014). The efficient agricultural market acts as bridge between the farmers to consumer. Efficient marketing in term of both technical and pricing efficiency ensures the farmers to get better price of their farm produce and also consumers to obtain true worth of the money which maximize social welfare (Chand 2012, Balkrishna et al 2022). The marketing of vegetable crops is inflicted often with high marketing cost and low produces shares. This could be due to numerous reasons such as lots of intermediaries exist between the channels and cost of various market functions rendered by these intermediaries. The price spread is one of the important measures of market efficiency and indicates the increases in the price of a farm produce with change in control from one intermediary to another in the whole marketing system. Vegetable crops such as Pea (*Pisum sativum)* play significant role in hill farming, both in income and social spheres for improving income and nutrition (Bala et al 2011).

In Himachal Pradesh, particularly high hills zone has good potential of off seasonal vegetables production for which smallholder farming have diversified from staple food subsistence production into more market oriented and higher value produce (Arya 2001). Despite this production potential and importance of pea crop for the state as well as the study area, there has been limited study with regard to the performance and constraints in pea marketing. This study seeks to close this gap by analyzing output marketing channel in high hill wet temperate zone of Himachal Pradesh. The present study aimed at observing marketing performance and identification of constraints in market.

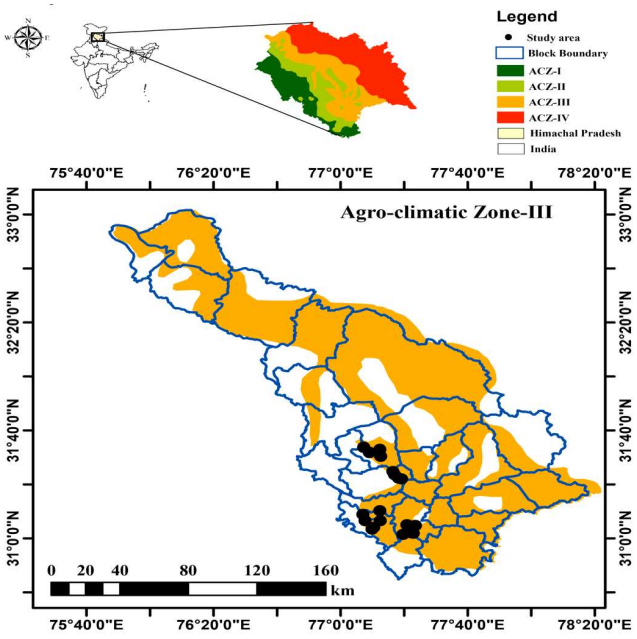


Fig. 1. Location map of h study area

MATERIAL AND METHODS

Study area: The study was conducted in high hills wet temperate zone of Himachal Pradesh, which is located in the foot hills of the North-Western Himalayas, India and lies between 30˚22' 40" to 33˚12' 20" N latitude and 75˚45' 55" to 79˚04' 20" E longitude. Its altitude ranges from 350 meters to 6,975 m above mean sea level (amsl), and is endowed with a myriad of climatic niches. The entire State of Himachal Pradesh has been divided into 4 agro-climatic zones whose elevation ranges from less than 650 to more than 2200 m amsl (Fig. 1). The high hills wet temperate zone was selected purposively because of its wider adaptability to produce off seasonal pea which is major source of income for the farmers.

Sampling design: A multistage random sampling technique was used for the selection of sample households. At the first stage of sampling, a complete list of blocks in the selected agro-climatic zone was prepared and out of which 5 blocks were selected on the basis of maximum cultivated area under vegetable cultivation. At the second stage of sampling, a complete list of Gram Panchayats in the selected blocks was prepared and out of which, 4 Gram Panchayats from each selected block were selected randomly. At the final stage of sampling, 10 farmers from each Gram Panchayats were selected randomly to constitute a sample size of 200 farmers in total (Fig. 2).

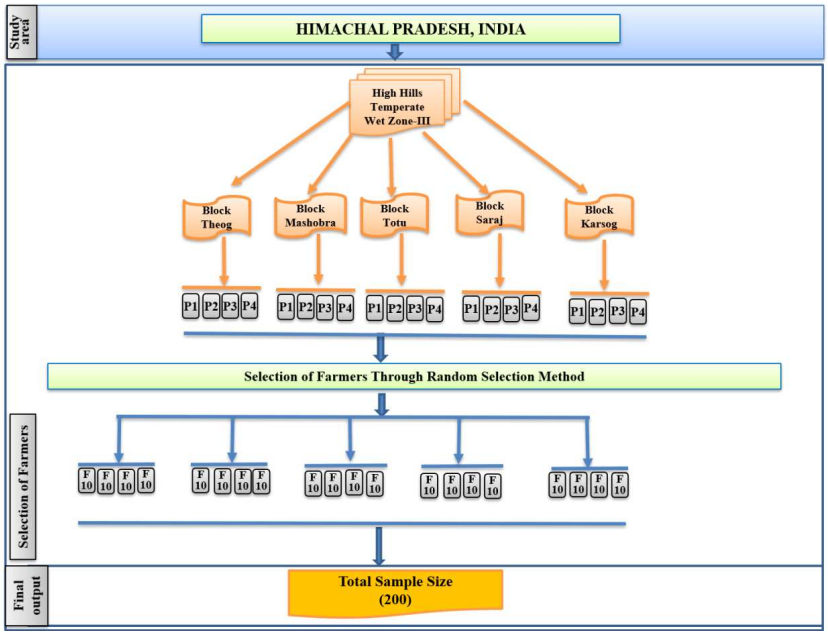


Fig. 2. Flow chart of sampling strategy

Selection of market and market intermediaries: In high hills temperate wet, Zone-III the Dhali (Shimla) and Theog market were selected to study the functioning of agricultural markets in Himachal Pradesh. Further, to examine the various aspects related to pea output marketing, a sample of 5 local traders, 5 commission agents, 5 wholesalers and 5 retailers were selected randomly from each selected markets of selected agro-climatic zone.

Data collection: Both primary and secondary data were collected to meet the objectives of the present study. Primary data were collected with the help of well-designed pre-tested schedule through survey method by interviewing the selected farmers directly pertaining to agricultural year 2020-21. Further, the market related information was also collected using per-tested schedule through personal interview method from selected local traders, commission agents, wholesalers and retailers. The required secondary data related to the present study were collected from various publications and government departments like agriculture, horticulture, directorate of economics and statistics, land Records, books, journals and university reports .

Analytical Framework

Market analysis: The total costs, incurred on marketing by the farmers were calculated as:

$$TC_m = C_g + \sum_{i=1} MC_i$$

Where,

TC_m = Total cost of vegetable marketing,

C_g = Cost paid by the grower in the marketing of his/her produce

MC_i = Marketing costs incurred by i^{th} middleman.

The following formula is used to compute percentage-marketing margins as earned by each market intermediary in the marketing of farm products:

$$A_{mi} = P_{Ri} - (P_{pi} + C_{mi})$$

Where

A_{mi} = Absolute margin of middlemen

P_{Ri} = Total value of receipts per unit (sale price)

Pp_i = Purchase value of goods per unit

C_{mi} = Cost incurred on marketing per unit.

$$TGMM = \frac{\text{Consumer's Price} - \text{Producer's Price}}{\text{Consumer's Price}} \times 100$$

Where, TGMM is the total gross marketing margin. It is useful to introduce the ides of producers' gross margin (GMM_P) which is the portion of the price paid by the consumer that goes to the producer. The producers' margin is calculated as:

$$GMM_P = \frac{\text{Consumer's Price} - \text{Gross Marketing Margin}}{\text{Consumer's Price}} \times 100$$

Where, GMMp = the producer's share in consumer price.

The net marketing margin (NMM) is the percentage of the final price earned by the intermediaries as their net income after their marketing costs are deducted.

The percentages of net income that can be classified as pure profit (i.e., return on capital), depends on the extension to such factors as the intermediaries' own (working capital) costs. The equation tells us that a higher marketing margin diminishes the producer's share and vice versa. It also provides an indication of welfare distribution among production and marketing agents.

$$NMM = \frac{\text{Gross Margin} - \text{Marketing Cost}}{\text{Consumer's Price}} \times 100$$

Where, NMM is the net marketing margin

Further, marketing efficiency of various channels in the study area has been computed by using Acharya's approach (2001).

$$\text{Marketing Efficiency} = \frac{FP}{MC + MM} - 1$$

Where

FP = Price received by the farmer

MC = Total marketing cost

MM = Net market margins.

Whereas, price spread refers to the difference between the price paid by the consumer and price received by the producer.

$$PS = \frac{PF}{PR} \times 100$$

Where

PS = Producer's share in consumer's rupee

PF and PR will be the farmer's price and retail price (consumer's price)/kg respectively.

Garrett's ranking technique: To examine the constraints experienced by farmers in pea output marketing channels in the study area, Garrett's ranking method was used (Kumar et al 2019). As per this method, the farmers were asked to assign the rank for each category of the constraints proposed to them. The per cent position for each rank was calculated.

$$\text{Per cent position} = \frac{(R_{ij} - 0.5)}{N_j}$$

Where

R_{ij} = Rank given to i^{th} position by the j^{th} individual

N_j = Numbers of problems ranked by j^{th} individual

The per cent position was converted into scores by referring to the table (Garrett ranking conversion table). The mean scores for all the factors were arranged in descending order. The-influencing factors were identified through the ranks assigned as the factors having the highest mean value score was considered to be the most severe problem faced by the farmers in the study area (Guleria et al 2022).

RESULTS AND DISCUSSION

Agricultural output marketing channels of pea: In study area there was five agricultural output marketing channels used by sampled farmers for the marketing of pea crop (Table 1). The most preferred channel for the marketing of pea crop in Zone-III was channel- C i.e., Producer → Commission Agent → Retailer → Consumer (P—CA—R—C) accounted for 59.5 per cent of the total quantity transected among the channels followed by 19.5, 12 and 1.5 t per cent in Channel-D, B and A, respectively.

Marketing costs and margins of different functionaries: The total marketing cost incurred by producer among output marketing channels were Rs. 65 per quintal in Channel-A where the produce was directly sold to the consumer followed by Channel-B (Rs. 75 per quintal) where produce sold to the retailer, Channel-C (Rs. 120 per quintal) where produce sell to commission agents (Table 2). The marketing cost incurred by the farmers in the study includes the packaging, loading/unloading and transportation cost. In the study area, the retailers appeared in four marketing channels i.e., Channel-B, Channel- C, Channel- D and Channel- E. The retailer was the only market functionary apart from the producer who was selling the produce directly to the consumer. The commission charges, transportation cost, loading/unloading cost, *Mandi* tax, were the important marketing cost incurred by them. The total cost incurred by retailer in Channel-B, Channel-C, Channel–D and Channel - E was worked out to be Rs 516.14, Rs. 554.24, Rs. 601.06 and Rs. 560.23 per quintal respectively. Further, retailer margin per quintal in Channel B Rs. 158.5 followed by Channel-C (, Channel-E, and highest in Channel-D (Rs. 180).The commission agent was important market functionary in the marketing Channel-C. The total marketing cost incurred by commission agent in Channel- C was Rs. 446.2 whereas margin was Rs. 135.5 per quintal.

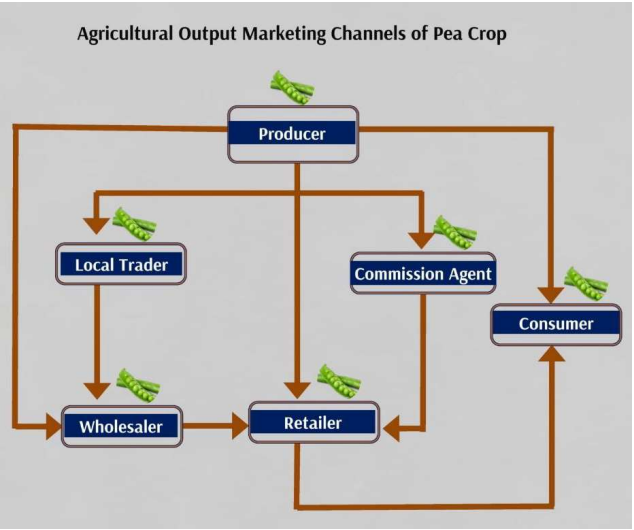
The local trader was found in only one output marketing channel i.e., Channel-D. The major components of this marketing cost were found to be the commission charges, transportation cost, loading/unloading and *Mandi* tax. The total marketing cost incurred by local trader was Rs 476.74 per quintal. The local trader further sold the produce to the wholesaler. In present study, no local trader was found to be dealing with the consumer directly. These results are in conformity with the findings of Ankita (2021). The wholesaler was one of the important market functionaries which was found in output marketing Channel-D and Channel-E. In marketing Channel-D and Channel-E, the marketing cost incurred by wholesaler was Rs. 539.78 and Rs. 501.60 per quintal respectively out of which commission charges, *Mandi* tax, and transportation constituted the important components of marketing cost. The Channel-D was operate through the local trader whereas in Channel-E wholesaler receives produce from the farmers. But in both the channels the produce was sold to the retailer.

Price spread and marketing efficiency of pea crop : In pea crop the producer's price received varied from Rs. 5175.50 in Channel-D to Rs. 5495 in Channel-A among different output marketing channels (Table 3). The total gross marketing margin was maximum in Channel-D (29.52%) and least in Channel-A (1.17%). Further, per cent share of producer in consumer's rupee was maximum in Channel-A (98.83%), when producer acted as a retailer in the sale of produce to consumers and lowest 70.48 per cent in Channel-D i.e., Producer → Local Trader→ Wholesaler→ Retailer → Consumer. Marketing margins varied from 0.00 per cent in Channel- A to11.33 per cent in channel D. The marketing cost varied from 1.17 per cent in Channel-A to 23.25 per cent in Channel- D.

Table 1. Output marketing channels of pea crop in the study area (%)

Particulars	Channels	High hill region
Channel-A	P—C	1.50
Channel-B	P—R—C	8.00
Channel-C	P—CA—R—C	59.50
Channel- D	P—LT—W—R—C	19.00
Channel-E	P—W—R—C	12.00
		100

Source: Field Survey, 2020-21
C- Consumer; CA- Commission Agents; LT- Local Trader; P- Producer; R-Retailer; W- Wholesaler



Source: Author

Fig. 3. Output marketing channels of pea crop in Himachal Pradesh

Table 2. Marketing costs and margins of different functionaries in the output marketing channels of pea crop (Rs./Qtl)

Particulars	Output marketing channels of pea crop				
Marketing cost incurred by producers	A	B	C	D	E
Net price received by farmer	5495.00	5420.50	5270.00	5175.5	5275
Transportation cost	15.00	25.00	70.00	40.00	45.00
Packing material cost	35.00	35.00	35.00	35.00	35.00
Loading / unloading	15.00	15.00	15.00	15.00	15.00
Commission charge	-	-	-	-	-
Mandi tax	-	-	-	-	-
Total	65.00	75.00	120.00	90.00	95.00
Farmer's selling price	5560.00	5495.50	5390.00	5265.50	5370
Marketing cost incurred by local trader					
Gross price paid by local trader	-	-	-	5265.50	-
Loading / unloading	-	-	-	15.50	-
Transportation cost	-	-	-	40.00	-
Mandi tax	-	-	-	105.31	-
Commission charge	-	-	-	315.93	-
Total	-	-	-	476.74	-
Local trader margin	-	-	-	105.00	-
Wholesaler purchase price	-	-	-	5847.24	-
Marketing cost incurred by commission agent					
Gross price paid by commission agent	-	-	5390.00	-	-
Loading / unloading	-	-	15.00	-	-
Transportation cost	-	-	0.00	-	-
Mandi tax	-	-	107.80	-	-
Commission charge	-	-	323.40	-	-
Total	-	-	446.20	-	-
Commission agent margin	-	-	135.50	-	-
Commission agent selling price	-	-	5971.70	-	-
Marketing cost incurred by wholesaler					
Gross price paid by wholesaler	-	-	-	5847.24	5370.00
Loading / unloading	-	-	-	17.00	17.00
Transportation cost	-	-	-	55.00	55.00
Mandi tax	-	-	-	116.94	107.40
Commission charge	-	-	-	350.83	322.20
Total	-	-	-	539.78	501.60
Wholesaler margin	-	-	-	170.00	175.00
Wholesaler selling price	-	-	-	6557.02	6046.60
Marketing cost incurred by retailer					
Gross price paid by retailer	-	5495.50	5971.70	6557.02	6046.60
Loading / unloading	-	18.00	18.00	18.00	18.00
Transportation cost	-	58.50	58.50	58.50	58.50
Mandi tax	-	109.91	119.43	131.14	120.93
Commission charge	-	329.73	358.30	393.42	362.80
Total	-	516.14	554.24	601.06	560.23
Retailer margin	-	158.50	170.00	185.50	180.00
Retailer selling price	-	6170.14	6695.94	7343.58	6786.83
Consumer purchase price	5560.00	6170.14	6695.94	7343.58	6786.83

Table 3. Price spread and marketing efficiency of pea crop

Particulars	Output marketing channels of pea crop				
Price spread	A	B	C	D	E
Producer price (Rs./quintal)	5495.00	5420.50	5270.00	5175.50	5275.00
Consumer's price (Rs./quintal)	5560.00	6170.14	6695.94	7343.58	6786.83
Gross marketing margin (GMM) (Rs./quintal)	65.00	749.64	1425.94	2168.08	1511.83
Net marketing cost (Rs./quintal)	65.00	591.14	1120.44	1707.58	1156.83
Net market margin (Rs./quintal)	0.00	158.5	305.5	832.24	450.00
Total gross marketing margin (%)	1.17	12.15	21.30	29.52	22.28
Marketing cost (%)	1.17	9.58	16.73	23.25	17.05
Marketing margin (%)	0.00	2.57	4.56	11.33	6.63
Producer's shares (%)	98.83	87.85	78.70	70.48	77.72
Marketing efficiency	A	B	C	D	E
Net marketing cost (Rs./quintal)	65.00	591.14	1120.44	1707.58	1156.83
Consumer's price (Rs./quintal)	5560.00	6170.14	6695.94	7343.58	6786.83
Net marketing margin (Rs./quintal)	0.00	158.5	305.5	832.24	450
Marketing efficiency	84.54	7.23	3.70	1.89	3.22

The Channel-A (84.54%) was most efficient channel and Channel-D least efficient (1.89%). But this channel was not preferred as the quantity of produce sold was less as compared to others channel. Furthermore, Channel-B was most efficient from the remaining four channels as the price paid by the consumer (Rs. 6170.14) were the least and the prices received by the farmers (Rs. 5420.50) were the maximum.

Table 4. Constraints faced by farmers in output marketing channels of pea

Constraints	High hills region	
	Average per cent score	Rank
Delay in payment	54.84	VI
Lack of market consultancy service	62.40	II
Distant Market	51.37	VII
Lack of technical knowledge	44.94	X
Shortage of packing material	31.20	XV
Exploitative practices by intermediaries	49.90	VIII
High commission charges	59.29	III
Non remunerative price for the produce	57.93	V
Inadequate storage facility	49.19	IX
Vehicle not available in time	58.79	IV
Lack of all-weather roads	62.75	I
High transportation charges	39.23	XIV
Price Instability	43.50	XI
Inadequate market information	42.60	XII
Inaccurate weighing instruments	41.12	XIII

Constraints faced by farmers in output marketing channels in pea crop: The constraints faced by the farmers in marketing of pea crop in high hill temperate wet zone was mainly lack of all-weather road with 62.75 score of Garrett ranking followed by market consultancy services (62.40), high commission charges (59.29) (Table 4). These results are in line with the findings of Devi et al (2020).

CONCLUSIONS

The farmers of study area were using five majors output marketing channels for marketing their pea produce. These channels were Channel-A (P—C), Channel-B (P—R—C), Channel-C (P—CA—R—C), Channel- D (P—LT—W—R—C) and Channel-E (P—W—R—C) in high hills temperate wet zone of Himachal Pradesh. In pea crops farmers mostly used the output marketing Channel-C to market their crop produce. The Channel-A was most efficient among five marketing channels. Among all the five output marketing channels Channel D was most efficient and Channel E was least. This was due to various marketing participants included in these channels and price profit are distributed till it reaches to the consumer eventually increase the price to a great extent. Therefore performance of any marketing channel depends upon the marketing efficiency at large. Channel-A was efficient but the volume transacted was very less. Since, farmers larger focus on remains on the production thereby in spite of having this channel a higher market efficiency, the farmers may not able to use this channel on account of lacking of requisite market infrastructure. The lack of all-weather roads followed by lack of market consultancy services and high commission

charges levied upon the farmers through various were the major constraints faced by the farmers during marketing of pea.

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Effect of Nitrogen and Phosphorus on Flowering, Bulb Yield and Nutrient Contents in Leaves of Tuberose (*Polianthes Tuberose* L.) cv. Prajwal

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Abstract: The present investigation was conducted to find out the optimum dose of nitrogen and phosphorus for flowering and yield of tuberose. Fertilizer requirement of tuberose was studied with 12 treatments comprising four levels of nitrogen (0, 10, 15 and 20 g/m^2) and three levels of phosphorus (0, 5 and 10 g/m^2). This field experiment was carried out as randomized block design in all possible combinations. All flowering, spike and bulb yield and NPK analysis parameters were significantly influenced with every increase in nitrogen and phosphorus dose. Flowering and spike yield in terms of spike length, rachis length, stem diameter, number of florets per spike, number of spike per plot, number of spike per hectare significantly increased with increasing nitrogen level up to 20 g/m^2 with phosphorus up to 10 g/m^2 during both the years of study, respectively. Similar trend was observed in various bulb yield parameters viz. number of bulbs per clump, diameter of bulb, weight of bulb, bulb yield and nutrient content in plant.

Keywords: Tuberose, Nitrogen, Phosphorus, Flowering, Yield and nutrients

Tuberose, which is a half-hardy bulbous summer flowering perennial ornamental plant, is best suited for cultivation in tropical to subtropical and temperate climates. It prefers to grow in an open sunny location since shady or semi-shady conditions drastically reduce the flower yield. A temperature range from 20 to 30°C is considered ideal for the cultivation of this crop (Safeena et al 2015). Flowering takes place profusely throughout the year under mild climatic conditions. The length of the spike and quality of the flowers are severely affected at temperature 40°C or above. Tuberose although not strictly photosensitive but long-day exposure promotes vegetative growth as well as early emergence of the first flower spike and increases the flower spike length. Tuberose can be grown on varieties of soil ranging from sandy loam to a clay loam but it cannot tolerate waterlogging conditions even for a short period (Safeena et al 2015). It can also be successfully grown in saline and alkaline soils. However, the soils having a pH range of 6.5 to 7.5 with good aeration and drainage are ideal for its cultivation. The area under tuberose is about 7.77 thousand hectares with a production of 40.22 thousand tonnes of loose flowers and 13.90 thousand tonnes of cut flowers (NHB 2014). The total area under tuberose in Haryana is 103 hectares with a production of 13850000 cut spikes (Saxena et al 2014). Being constituent of proteins, nucleic acids, chlorophyll, nitrogen is essential nutrient required by the plants for their growth and development. Patel et al (2017) studied that increase in flowers numbers and yield with application of nitrogen might be due significantly increased the growth parameters, which might have synthesized more plant metabolites and ultimately lead to increase in flower production. Similarly, an adequate supply of phosphorus is associated with rapid and vigorous start to plant, helping to initiate bulbs quickly, stimulates flowering of plant. Phosphorus enhances the symbiotic nitrogen fixation in flower crops and ultimately improved the uptake of nutrients. These findings were coincided with the results of Dishaben et al (2017) in bird of paradise. Amin et al (2012) observed that spike length, spike diameter, rachis length and number of flowers per spike were found maximum with the application of phosphorus 155 kg/ha in tuberose. Some aspects of the production technology of tuberose in agro-climatic conditions of Haryana have not been standardized so far. So present investigation was conducted to find out the optimum dose of nitrogen and phosphorus for flowering and yield of tuberose.

MATERIAL AND METHODS

The study effect of nitrogen and phosphorus on flowering and spike yield of tuberose (*Polianthes tuberose* L.) cv. Prajwal was carried out at CCS Haryana Agricultural University, Hisar during 2016-17 and 2017-18 to find out the optimum dose of nitrogen and phosphorus for flowering and yield of tuberose. Twelve treatment combinations comprising four levels of nitrogen (0, 10, 15 and 20 g/m^2) and three levels

of phosphorus (0, 5 and 10g/m^2) were tried in randomized block design with three replications. Thus 36 plots were used for 12 treatment combinations. The soil of the experimental field was sandy loam and clayey in texture, pH 8.10, 7.05, E.C. 1.13, 1.40 dSm^{-1}, organic carbon 0.35, 0.37 % and 118.23, 140.23 kg/ha available N, 23.00, 19.00 kg/ha available P_2O_5, 270.00, 280.00 kg/ha available K_2O_5 during both the years of experiment. Experimental field was prepared by repeated ploughing and harrowing well in advance. First year planting was done on 16th March at 2016-17 and in second year the planting was done on 25 February during 2017-18. For the first experiment, the bulbs of 3.5 cm in diameter were planted and for second experiment, the different bulbs size viz. 0.5-1.5, 1.5-2.5 and 2.5-3.5 cm in diameter were planted. Before planting the bulbs, one third dose of nitrogen as per the treatment along with full dose of FYM @ 5 kg/m^2 and phosphorus and potassium each @ 10 g/m^2 was applied and mixed thoroughly into the soil. The remaining two-third dose of nitrogen was applied in two splits first one third at 30 days after planting and second one third at 60 days after planting. Observations were recorded for various flowering, yield, bulb yield and nutrient analysis parameters in Tuberose cv. Prajwal.

RESULTS AND DISCUSSION

Floral and yield parameters: Significant increase in stem diameter, spike length, rachis length, number of florets per spike, number of spikes per plot and number of spikes per hectare with successive increase in nitrogen levels from 0 to 20 g/m^2 was observed (Table 1). Maximum spike length, rachis length, stem diameter, number of florets per spike, number of spike per plot and number of spikes per hectare was recorded when nitrogen was applied @ 20g N/m^2 and minimum spike length, rachis length, stem diameter, number of florets per spike, number of spike per plot and number of spikes per hectare was observed in control during both the years . The increase in flower yield with increasing nitrogen level might be due to the fact that increasing nitrogen level enhanced the chlorophyll formation thereby increased photosynthesis and synthesis of reserve food material, which promoted vegetative growth and increased flower yield (Maske et al 2015). Nitrogen plays an important role for improvement in yield parameters and in efficient metabolic activity which increased the rate of photosynthesis as it generates an important role in synthesis of proteins, amino acid and chlorophyll enhances the flower and bulb yield (Sendhilnathan et al 2019). Dahal et al (2014) also observed maximum spike length, rachis length, spike weight and number of florets were obtained when nitrogen was applied in splits. Similarly, Pal et al (2020) studied that nitrogen applied at 200 kg/ha recorded significantly the higher value of spike yield/ha in tuberose. The response of phosphorus was non-significant on vegetative characters while floral characters viz., rachis length and number of florets per spike were significant. Stem diameter, spike length, rachis length, number of florets per spike, number of spikes per plot and number of spikes per hectare increased significantly with gradual increase in phosphorus level from 0 to 10 g/m^2 as (Table 1). Maximum spike length, rachis length, stem diameter, number of florets per spike, number of spike per plot and number of spikes per hectare was recorded with 10 g $P_2O_5m^{-2}$ while minimum spike length, rachis length, stem diameter, number of florets per spike, number of spike per plot and number of spikes per hectare was in control during both the years, respectively. Similar results were reported in gladiolus by Sabastian et al (2017) and Sendhilnathan et al (2019) in tuberose. The interaction effect between nitrogen and phosphorus levels on Stem diameter, spike length, number of florets per spike was found significant while it was found non-significant for rachis length, number of spikes per plot and number of spikes per hectare. Maximum spike length, rachis length, stem diameter , number of florets per spike, number of spike per plot and number of spikes per hectare was recorded when nitrogen was applied @ 20 g/m^2 in combination with phosphorus @ 10 g/m^2over control.

Bulb yield parameters: Maximum Number of bulbs per clump, diameter of bulb weight of bulb and bulb yield of tuberose was recorded with 20g Nm^{-2} while minimum number of bulbs per clump, diameter of bulb, weight of bulb and bulb yield was in control during both the years of experiment. Increase in number of bulbs per clump and weight and diameter of bulb might be due to more nitrogen supply to the plant to stimulate the production and export of cytokinins to the shoots (Kejkar et al 2015). Rathore et al (2013) also observed the increase in bulb yield might be due to better availability of nutrients to tuberose plants, which resulted in better vegetative growth of plant and more accumulation of food in bulbs. Maximum number of bulbs per clump, diameter of bulb, weight of bulb and bulb yield of tuberose was recorded with 10 g $P_2O_5m^{-2}$ while minimum number of bulbs per clump, diameter of bulb, weight of bulb and bulb yield was in control during both the years. Amin et al (2012) observed in number of side bulbs, bulb diameter and bulb yield increased at higher dose of phosphorus, which might be due to the production of maximum number of side bulbs with larger size in tuberose. The interactional effect between nitrogen and phosphorus levels on bulb yield parameters was found significant. Maximum number of bulbs per clump, diameter of bulb, weight of bulb and bulb yield of tuberose was recorded when nitrogen was applied @ 20g/m^2 in

Table 1. Effect of nitrogen and phosphorus on stem diameter, length of spike, length of Rachis and number of florets per spike in tuberose

Treatment	Stem diameter (cm)		Length of spike (cm)		Number of spikes per plot		Length of Rachis (cm)		No. of florets per spike		Number of spikes per ha	
Effect of Nitrogen Levels												
N (g/m^2)	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18
0	0.857	0.649	65.96	78.24	24.67	22.78	26.09	20.50	35.80	32.56	109630	101235
10	0.873	0.692	71.87	81.60	26.11	24.89	27.91	23.29	36.58	35.47	116049	110632
15	0.881	0.765	75.76	85.04	28.44	27.00	28.96	25.53	39.31	37.93	126420	120000
20	0.978	0.800	80.31	91.64	30.33	31.11	30.07	27.71	43.60	39.22	134815	138272
CD (5%)	0.016	0.007	0.42	0.21	0.96	1.24	0.47	0.59	1.02	0.47	4266.92	5662.42
Effect of Phosphorus Levels												
P (g/m^2)												
0	0.884	0.703	72.27	81.45	26.58	24.64	27.82	23.79	37.98	34.98	118148	109511
5	0.892	0.733	73.38	83.97	27.42	26.81	28.23	24.34	38.45	36.40	121852	119137
10	0.916	0.744	74.77	86.98	28.17	27.89	28.72	24.65	40.03	37.50	125185	123956
CD (5%)	0.014	0.006	0.36	0.70	0.31	1.07	0.41	0.51	0.88	0.40	3695.26	4903.80
Interaction effect between different levels of nitrogen and phosphorus												
N× P (g/m^2)												
N_0P_0	0.840	0.632	65.33	76.00	24.33	21.00	25.73	20.47	35.20	31.13	108148	93333
N_0P_1	0.861	0.657	65.73	77.80	24.67	23.00	26.13	20.23	36.00	33.07	109630	102222
N_0P_2	0.869	0.660	66.80	80.93	25.00	24.33	26.40	20.79	36.20	33.47	111111	108148
N_1P_0	0.865	0.673	70.13	79.13	25.00	22.23	27.40	22.40	36.07	34.00	111111	98785
N_1P_1	0.875	0.698	72.20	81.07	26.00	25.56	27.87	23.53	36.73	36.07	115556	113585
N_1P_2	0.877	0.706	73.27	84.60	27.33	26.89	28.47	23.93	36.93	36.33	121481	119526
N_2P_0	0.878	0.727	74.67	80.47	27.67	25.67	28.80	25.20	37.60	37.07	122963	114074
N_2P_1	0.882	0.770	75.40	85.67	28.67	27.00	28.87	25.60	37.67	37.73	127407	120000
N_2P_2	0.884	0.798	77.20	89.00	29.00	28.33	29.20	25.80	42.67	39.00	128889	125926
N_3P_0	0.953	0.778	78.93	90.20	29.33	29.67	29.33	27.07	43.07	37.73	130370	131852
N_3P_1	0.950	0.808	80.20	91.33	30.33	31.67	30.07	28.00	43.40	38.73	134815	140741
N_3P_2	1.032	0.814	81.80	93.40	31.33	32.00	30.80	28.07	44.33	41.20	139259	142222
CD at 5%	0.027	0.012	0.72	1.40	NS	NS	NS	NS	1.76	0.81	NS	NS

Table 2. Effect of nitrogen and phosphorus on number of bulbs per clump, diameter of bulb, weight of bulb, bulb yield, Nitrogen, Phosphorus and potassium content in tuberose

Treatment	Number of bulbs per clump		Diameter of bulb (cm)		Weight of bulb (g)		Bulb yield (t/ha)		Nitrogen content (%)		Phosphorus content (%)		Potassium content (%)	
N (g/m^2)	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18
0	14.98	15.78	1.92	2.17	14.98	15.78	24.58	25.69	1.49	1.44	0.68	0.64	0.84	0.86
10	18.07	18.11	2.43	2.24	18.07	18.11	36.88	36.30	1.54	1.51	0.74	0.72	0.86	0.87
15	21.20	21.09	2.64	2.43	21.20	21.09	50.72	48.52	1.58	1.55	0.79	0.75	0.87	0.88
20	24.64	24.47	3.03	2.57	24.64	24.47	66.12	62.68	1.70	1.66	0.88	0.91	0.89	0.90
CD (5%)	0.27	0.57	0.07	0.03	0.39	0.42	1.04	2.39	0.01	0.01	0.01	0.01	0.001	0.005
P (g/m^2)														
0	18.67	18.68	2.28	2.26	18.67	18.68	39.53	38.72	1.55	1.50	0.76	0.72	0.87	0.87
5	19.83	19.77	2.56	2.36	19.83	19.77	43.53	41.72	1.57	1.53	0.77	0.76	0.86	0.88
10	20.67	21.13	2.68	2.42	20.67	21.13	50.67	49.46	1.60	1.59	0.79	0.79	0.87	0.88
CD (5%)	0.23	0.50	0.06	0.02	0.34	0.37	0.90	2.07	0.01	0.01	0.01	0.01	0.004	0.004
N× P (g/m^2)														
N_0P_0	13.13	14.20	1.41	2.07	12.94	13.93	18.88	20.94	1.48	1.41	0.65	0.58	0.85	0.86
N_0P_1	15.60	16.07	2.14	2.20	14.51	14.42	25.14	26.36	1.49	1.44	0.67	0.64	0.84	0.86
N_0P_2	16.20	17.07	2.22	2.24	16.50	15.70	29.71	29.78	1.50	1.46	0.72	0.70	0.84	0.86
N_1P_0	17.40	16.47	2.36	2.21	17.25	15.78	33.33	30.80	1.53	1.49	0.72	0.71	0.87	0.87
N_1P_1	18.33	18.47	2.45	2.22	17.31	17.61	35.27	35.44	1.54	1.51	0.73	0.72	0.86	0.87
N_1P_2	18.47	19.40	2.49	2.29	20.48	19.14	42.04	42.67	1.54	1.53	0.76	0.73	0.87	0.87
N_2P_0	20.53	20.07	2.59	2.31	20.83	20.26	47.51	44.46	1.55	1.53	0.79	0.74	0.87	0.88
N_2P_1	20.73	20.13	2.62	2.46	21.27	20.34	49.01	45.27	1.58	1.56	0.79	0.75	0.87	0.88
N_2P_2	22.33	23.07	2.71	2.52	22.43	20.91	55.65	55.82	1.60	1.57	0.80	0.77	0.88	0.89
N_3P_0	23.60	24.00	2.77	2.55	22.27	21.37	58.38	58.67	1.64	1.57	0.89	0.84	0.88	0.89
N_3P_1	24.67	24.40	3.01	2.56	23.60	22.06	64.70	59.80	1.68	1.61	0.87	0.94	0.88	0.90
N_3P_2	25.67	25.00	3.31	2.61	26.40	24.70	75.29	69.57	1.77	1.79	0.88	0.96	0.92	0.91
CD (5%)	0.47	0.99	0.13	0.05	0.68	0.73	1.80	NS	0.02	0.02	0.02	0.01	0.008	0.008

combination with phosphorus @ 10 g/m^2 while minimum number of bulbs per clump, diameter of bulb, weight of bulb and bulb yield was in control. Similar results were reported by Kejkar et al (2015) in Ratoon Spider lily and Sendhilnathan et al (2019) in tuberose.

Nutrient Content in Leaves

Nitrogen content (%): Maximum nitrogen content in leaves was observed when nitrogen was applied @ 20 g/m^2 over the control where no nitrogen was applied during both the years of study, respectively. Similarly, maximum nitrogen content was observed in leaves of tuberose supplied with phosphorus @ 10 g/m^2 and the minimum nitrogen content was without the application of phosphorus. The interaction between nitrogen and phosphorus significantly affected the nitrogen content in leaves of tuberose. Maximum nitrogen content was observed in the leaves of tuberose when plants were fertilized with nitrogen at 20 g/m^{2}along with phosphorus at 10 g/m^2 as compare to control , which was not fertilized with nitrogen and phosphorus.

Phosphorus content (%): Application of nitrogen at 20 g/m^2 significantly increased the phosphorus content in leaves of tuberose and minimum content was with no nitrogen application. The maximum phosphorus content was observed when phosphorus level was increased up to 10 g/m^2 and the minimum phosphorus content in leaves of tuberose was estimated in leaves of tuberose grown without phosphorus during both the years, respectively. The maximum phosphorus content was observed in leaves when nitrogen and phosphorus were applied @ 20 and 10 g/m^2 to the plants, whereas, the minimum phosphorus content was neither nitrogen nor phosphorus was given to the plants during both the years of study, respectively.

Potassium content (%): The maximum potassium content in leaves (0.89 and 0.90%) was observed when the nitrogen was applied @ 20 g/m^2, while the minimum (0.84 and 0.86%) was estimated where no nitrogen was applied during both the years.

The application of phosphorus @ 10 g/m^2 significantly increased the potassium content in leaves (0.87 and 0.88%) of tuberose, whereas, minimum (0.87 and 0.87%) was found in plots unfertilized with phosphorus. Phosphorus level 0 and 5 g/m^2 were statistically similar in respect of potassium content in leaves of tuberose during both the years, respectively. The maximum potassium content in leaves of tuberose (0.92 and 0.91%) was estimated when the plants were supplied with nitrogen @ 20 g and phosphorus @ 10 g/m^2, while the minimum potassium content (0.85 and 0.86%) was found in tuberose leaves taken from the plots not supplied with nitrogen and phosphorus during both the years. The results of present experiment corroborate the findings of Kejkar et al (2015) in spider lily. The soil of experimental field was medium in available phosphorus. Higher application of phosphorus led to show significant response and an increase in phosphorus content in leaves of tuberose.

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CONCLUSION

From the experiment it is concluded that nitrogen 20 g/m^2 along with phosphorus 10 g/m^2 seemed to be optimum for better flowering, yield, bulb yield and NPK content in tuberose in terms of spike length, rachis length, stem diameter, number of florets per spike, number of spike per plot and number of spikes per hectare, number of bulbs per clump, diameter of bulb, weight of bulb, bulb yield, NPK content in leaves of tuberose cv. Prajwal.

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Qualitative Analysis of Pectin Extracted Ultrasonically from Sweet Lime Peel

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***Abstract*:** In this study, ultrasound-assisted extraction of pectin from sweet lime peel was optimized by Box-Behnken design using a response surface methodology (RSM). The effect of independent factors such as solid-solvent ratio (1:10-1:30 g/ml), sonication time (15-35 min) and ultrasonic power intensity (48-80 W/cm^2) on the anhydrouronic acid concentration and degree of esterification was studied. The process factors had a significant effect on all responses of pectin extracted from the sweet lime peel. Under optimum conditions (solid-solvent ratio of 1:24.3 g/ml, sonication time of 18.4 min, ultrasonic power intensity of 80 W/cm^2), the highest degree of esterification (62.9%) and anhydrouronic acid content (64%) were obtained. The current study indicate that extracting pectin from sweet lime peel was an efficient strategy to minimize the current waste disposal problems.

Keywords: Pectin, Ultrasound assisted extraction, Anhydrouronic acid, Esterification

Agricultural waste management for economic, social, environmental and sustainable development is one of the major concerns. Agricultural waste comprises residues of raw agricultural products such as fruits and vegetable wastes (peels, pulp, seed, etc.), crop wastes (stalks, bagasse, prunings), dairy products, and meat and poultry wastes (bones, skin, feathers). From the agriculture phase up to industrial manufacturing, processing and distribution, and household consumption, each phase of the food life cycle involves food waste production. The required actions must be done to lessen agricultural waste and find the best viable approach to manage the trash that remains. Usually, agro wastes are thrown in landfills or discarded as animal feed. However, these methods provide a low economic and environmental value. The aim of the right waste management is not just to reduce the waste volumes, yet to utilize it in different effective ways. Waste valorization, the method of turning waste into more useful commodities is an efficient method for managing waste materials. The high-value products like antioxidants, pigments, protein, sugar, enzymes, films, nutraceuticals, etc can be extracted from food waste (Sindhu et al 2019). Bioactive compounds from fruits and vegetables can also be used as dietary supplements which can be responsible for most health impacts (Walia et al 2022).

Citrus is the most abundant horticultural produce, popularly grown worldwide. The citrus processing industries are responsible for the production of a significant amount of citrus waste, primarily in the form of citrus peel waste, which alone accounts for more than 50% of the wet fruit mass which has negative impacts on the environment. The citrus waste consists of valuable byproducts that can be utilized for the extraction of valuable compounds like carotenoids, flavonoids, dietary fibre, polyphenols, sugars, essential oils and ascorbic acid, along with some trace elements (Sharma et al 2017). Thus, the valorization of citrus processing waste for some value-added products has become a necessity. Sweet lime (*Citrus limetta*) is a citrus species belonging to the family of “Rutaceau”. Sweet lime is India's third most popular fruit. Sweet lime juice in India is a popular citrus drink, but the peel is discarded after the juice is extracted. Citrus peel is a good source of natural antioxidants, food flavours, dietary fibre and colorants, therefore the discarded peel can be used to extract a variety of value-added products. Peels of various citrus fruits can be extracted for phenols, flavonoids, and pectin, which can then be used in the food sector and give both economic and environmental benefits. Pectin is a heterogeneous carbohydrate consisting linear chain of α-(1,4) galacturonic acid residues that are partially esterified with methyl alcohol or acetic acid in a carboxylic acid, and is present in the intermediate lamellae and cell walls of many fruits and vegetables. It is used as stabilizing, gelling and thickening agent in food items such as confectionery, jams and jellies and fruit juice (de Oliveria et al 2016). Pectin might

be extracted from a variety of citrus peels. It can also be utilised as a biodegradable surfactant and emulsifier, a chelating agent in detergents, a rheological modifier, and edible packaging (Liew et al 2019). It was observed that edible coating prepared from the polysaccharide extracted by food waste is a cost effective solution of waste management (Gupta et al 2020). Valorisation of citrus waste for pectin production is an efficient solution for waste management. Citrus waste, mainly in the form of citrus peels is one of the common problems faced in the juice processing sector and if these peels are left untreated, they may cause environmental pollution. The utilization of citrus waste to extract bioactive compounds and pectin is the best solution for this problem. Extraction is a crucial step in the separation of value-added products from raw materials. The development of novel extraction methods offer safer, compact, efficient, energy-saving and viable extraction processes. Green technologies such as supercritical fluid extraction, ultrasound extraction, microwave extraction, controlled pressure drop process and subcritical water extraction are typically employed in extraction methods that consume less solvent and energy (Sharma et al 2017). Ultrasound-assisted extraction is an effective non-thermal process than conventional heating methods for extraction due to low energy consumption, shortened treatment time, less solvent usage, increased safety of the operators, and increased yield (Chemat et al 2017). Ultrasound waves at a frequency of more than 20 kHz move through matter, generating cycles of shearing and compression that lead to the development of cavitation bubbles. The ultrasonic then cause cell rupture by collapsing the cavitation bubbles near cell walls, resulting in stronger and improved solvent entry into the cells and intensification of mass transfer (Tiwari 2015, Siddiqui and Chand 2022). The purpose of the present study is currently focused on implementing effective extraction technique which helps in the valorization of citrus peel, improving processing efficiency, increasing profitability and turning it into valuable products.

MATERIAL AND METHODS

Sweet lime peels (10 kg) were acquired from the local market of Pantnagar, Uttarakhand. Sweet lime peels were separated, rinsed, and dried for 48 hours in a tray dryer at 60°C. 2-3 kg of dried sweet peel was ground and sieved into a fine powder of 300 microns. Prior to the studies, the obtained sweet lime peel powder was stored in zip-lock plastic bags. Chemicals of analytical grade were used in the research. To generate an acidic medium with a pH of 1.8 for pectin extraction, citric acid was combined with deionized water.

Extraction of pectin by ultrasonication: The ultrasonication procedure was done for pectin extraction from the peels of sweet lime as mentioned by Siddiqui et al (2021).The extracted pectin in powdered form was then further used for the qualitative analysis (Table 1).

Qualitative Analysis of Pectin

Estimation of total anhydrouronic acid content: The anhydrouronic acid content of pectin was calculated by using equivalent weight and methoxyl content values. Anhydrouronic acid content was calculated (Ranganna 1951):

$$\mathrm{AUA}(\%) = \frac{176 \times 0.1\mathrm{z} \times 100}{\mathrm{w} \times 1000} + \frac{176 \times 0.1\mathrm{y} \times 100}{\mathrm{w} \times 1000}$$

Where, Molecular unit of AUA (1 unit) = 176 g, z = ml (titre) of NaOH from equivalent weight determination, y = ml (titre) of NaOH from methoxyl content determination and w = weight of the sample

Estimation of degree of esterification: Degree of esterification of pectin was measured on the basis of methoxyl and AUA content (Owens et al 1952) and calculated by the expressiongiven below:

$$\mathrm{DE}(\%) = \frac{176 \times \%\mathrm{MeO}}{31 \times \%\mathrm{AUA}} \times 100$$

Where, %MeO = % Methoxyl content and %AUA = Anhydrouronic Acid Content

Statistical analysis: Box Behnken Design of Response Surface Methodology having three factors, three levels (-1, 0, 1) was used to evaluate and optimize the effect of independent variables on the quality of extracted pectin (Table 1). The independent variables considered were solid-solvent ratio (1:10, 1:20 and 1:30 g/ml), sonication time (15, 25 and 35 min) and ultrasound power intensity (48, 64 and 80 W/cm^2). Design Expert software 10.0.1 was used that designed a total of seventeen experiments with five centre points. It was used for the regression analysis of collected data to ascertain how independent and dependent variables relate to one another. The second-order polynomial regression model was applied and the equation is presented as:

$$\mathrm{Y} = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^n + \sum_{i=1}^{n} \sum_{i=1}^{n} \beta_{ii} X_i X_j \quad (1)$$

Where, β_0, β_i, β_{ii} = regression coefficients, X_i and X_j = independent parameters (where, i = 1, 2, …n and j = 1,2 …n), n = number of independent parameters (n=3) and Y = predicted response

The model developed for each determination was then assessed for significance and lack-of-fit, the response surface graphs were plotted after removal of non-significant variables.

RESULTS AND DISCUSSION

Degree of esterification and anhydrouronic acid content: The higher anhydrouronic acid content indicates the maximum purity of pectin, which is recommended to be at least 65% (Ramachandran et al 2017). However, the anhydrouronic acid content of the pectin obtained under all of the extraction conditions ranged from 62.4 to 70.3%. The maximum anhydrouronic acid content of 70.3% for the pectin was in the experiment 17. It was due to the presence of more galacturonic acid residues which is the major uronic acid in the pectin structure (Kurita et al 2008). However, the minimum anhydrouronic acid of 62.4% was obtained in the experiment 4. This decrease in anhydrouronic acid content was due to the higher sonication time. The prolonged ultrasound treatment leads to lower anhydrouronic acid content. The result was in accordance with the previous study (Grassino et al 2016). The degree of esterification of the pectin ranged from 55.8 to 64.6%. The highest degree of esterification of 64.6% for the pectin was obtained in experiment 4. This might be because of the higher levels of ultrasound power intensity and sonication time due to which enhanced cavitation effects yield the maximum amount of pectin that have a higher amount of methyl ester groups. However, the minimum degree of esterification of 55.8% for the pectin was in experiment 17. This decrease might be due to depolymerization of pectin chains at a higher solid-solvent ratio which in turn decreased degree of esterification. The result showed that degree of esterification was higher than 50% which showed the presence of high methyl ester pectin.

Optimization of degree of esterification and anhydrouronic acid content: The second-order polynomial regression model was statistically significant in both the responses showing that the extraction factors had a considerable impact on the responses (Table 2). Furthermore, the non-significant lack of fit value for regression models demonstrated that the regression model equation was acceptable to reflect the anhydrouronic acid content and esterification degree. In addition, the coefficient of determination (R^2) for a degree of esterification and anhydrouronic acid content was 0.9899 and 0.9916 respectively, thus the regression model could account for 99.16 and 98.99% of data respectively. The gap between the predicted and adjusted coefficient of determination should be less than 0.2, the appropriate precision should be larger than 4, and the coefficient of variation should not exceed 10% to improve the model's appropriateness. In this situation, the "Pred R^{2}" of 0.9368 and 0.8861 for anhydrouronic acid concentration and degree of esterification were in reasonable agreement with the Adj R^2 of 0.9808 and 0.9768, respectively. For anhydrouronic acid concentration and

Table 1. Box-Behnken design (BBD) with the observed values for anhydrouronic acid content and degree of esterification

Factors	Unit	Level of factors		
		-1	0	1
Solid-solvent ratio (X_1)	g/ml	1:18	1:24	1:30
Ultrasound power intensity (X_2)	W/cm^2	48	64	80
Sonication time (X_3)	min	15	25	35

Exp. No.	Solid-solvent ratio (X_1, g/ml)	Ultrasound power intensity (X_2, W/cm^2)	Sonication time (X_3, min)	Anhydrouronic acid content (%)	Degree of esterification (%)
1	1:24	48	35	63.5	61.7
2	1:30	64	35	62.6	62.6
3	1:24	64	25	66.0	60.2
4	1:24	80	35	62.4	64.6
5	1:24	64	25	65.2	60.9
6	1:24	48	15	65.2	60.1
7	1:24	64	25	66.0	60.3
8	1:24	80	15	63.2	63.8
9	1:24	64	25	65.9	60.3
10	1:18	80	25	70.0	56.8
11	1:30	64	15	68.8	56.1
12	1:24	64	25	66.0	60.2
13	1:18	64	35	68.0	56.8
14	1:30	80	25	63.8	62.3
15	1:18	64	15	64.8	59.6
16	1:18	48	25	66.9	56.8
17	1:30	48	25	70.3	55.8

degree of esterification, adequate precision values of 31.843 and 29.521, as well as a coefficient of variation of 0.68 per cent, were determined, confirming the accuracy adequacy of the model. The observed and projected values were strongly correlated, as indicated by the coefficient of determination (R^2) and adjusted determination coefficient (Adj R^2) values that were both near 1.

The surface models for the degree of esterification and anhydrouronic acid content of extracted pectin using ultrasonic aided extraction technique were developedbased on these findings and were given as below:

Anhydrouronic acid content (%) =
$65.69 - 0.53\ X_1 - 0.82\ X_2 - 0.67\ X_3 - 2.38\ X_1X_2 - 2.32\ X_1X_3 + 2.18\ X1^2 - 1.98X3^2$ (2)

Degree of Esterification (%) =
$65.39 - 0.84\ X_1 - 1.64\ X_2 - 0.74\ X_3 - 1.65\ X_1X_2 - 2.29\ X_1X_3 + 3.12\ X1^2 + 0.65X2^2 + 1.49X3^2$ (3)

All the independent variables viz solid-solvent ratio (X_1), ultrasonic power intensity (X_2) and sonication time (X_3) had a significant effect on both responses. Linear coefficients (X_1, X_2 and X_3) of the model were significant at a 1% level of significance ($p < 0.01$). Quadratic coefficients (X_1^2 and X_3^2) and interaction coefficients (X_1X_2 and X_1X_3) of the regression model of anhydrouronic acid content were significant. However, the quadratic coefficient (X_2^2) and interaction coefficient (X_2X_3) of the regression model of anhydrouronic acid content were non-significant. Similarly, for the regression model of degree of esterification, quadratic coefficient (X_1^2, X_2^2, X_3^2) and interaction coefficients (X_1X_2 and X_1X_3) were significant. However, the interaction coefficient (X_2X_3) of the regression model of the degree of esterification was non-significant.

Response surface graphs of anhydrouronic acid content and degree of esterification: The effects of all three processes on the degree of esterification and anhydrouronic acid content were examined and illustrated by three-dimensional (3D) response surface plots (Fig. 1). Figure 1A depicts the interactive effects of ultrasound power intensity and solid-solvent ratio on anhydrouronic acid content at an optimum level of sonication time (18.4 min) and with the increment in solid-solvent ratio, anhydrouronic acid content increased. However, higher anhydrouronic acid content was observed at the lower level of ultrasound power intensity and a higher level of solid-solvent ratio. This increase in anhydrouronic acid content has been attributed to the hydrolysis of protopectin (Nazaruddin 2011). It was observed that with higher solid-solvent ratio more protopectin gets hydrolyzed to soluble pectic substances and thus increased galacturonic acid residues. At lower level of solid-solvent ratio (1:18 g/ml), with the increase in sonication time from 15 to 35 min, the anhydrouronic acid content increased and reached the maximum at the higher levels of sonication time (Fig. 1B). With the further increase in solid-solvent ratio, there was a slight increase in the anhydrouronic acid content over the entire range of sonication time. The higher levels of both the variables resulted in decrease of anhydrouronic acid content. This decrease may be because of some other constituents that might be extracted along with pectin at the higher levels of both the variables which reduced the purity of pectin and hence decreased the anhydrouronic acid content.

Degree of esterification is also another crucial consideration to determine the quality characteristics of the pectin extracted from sweet lime peel powder. The percentage degree of esterification (% DE) of pectin indicates whether the pectin extracted has high methyl ester pectin (HM pectin- DE>50%) or low methyl ester pectin (LM pectin- DE<50%). This is important in determining the type of gel. The combined effects of solid-solvent ratio and ultrasound power intensity on degree of esterification at the optimum level of sonication time (18.4 min) (Fig. 1C). Degree of esterification increased with the increase in ultrasound power intensity (from 48 to 80 W/cm^2) and solid-solvent ratio (from 1:18 to 1:26 g/ml), overall higher degree of esterification is obtained at the central level of solid-solvent

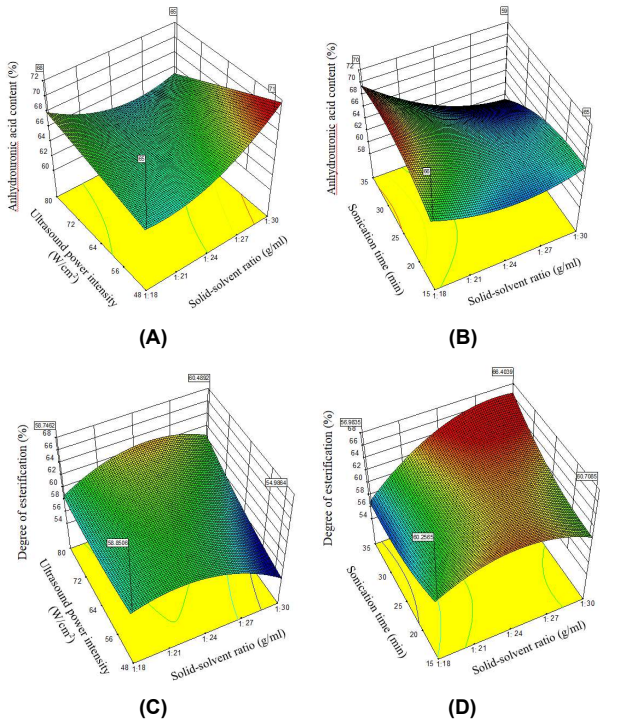

Fig. 1. Three dimensional plots (3D) showing the effect of processing variables on anhydrouronic acid content and degree of esterification

Table 2. Statistical analysis of regression model of responses

Source	Responses	
	Anhydrouronic acid content	Degree of esterification
Model	Significant	Significant
R^2	0.9916	0.9899
Adj R^2	0.9808	0.9768
Pred R^2	0.9368	0.8861
Adeq Precision	31.843	29.521
C.V. %	0.50	0.68

ratio and higher levels of ultrasound power intensity. The increase in degree of esterification was due to the sufficient solubilization of pectin at this level of solid-solvent ratio. However, at the higher solid-solvent ratio degree of esterification was decreased. This decrease was due to the dissolution of pectic substances at a higher level of solid-solvent ratio which in turn reduced the amount of esterified carboxyl group. Thus, degree of esterification was decreased.At the interactive level, the 3D surface plot shows the combined effects of sonication time and solid-solvent ratio on the degree of esterification at optimum level of ultrasound power intensity (80 W/cm^2) (Fig. 1D). The degree of esterification increases with increment in both solid-solvent ratio and sonication time. A higher degree of esterification was observed at the central level of solid-solvent ratio when the sonication time was increased from 15 to 35 min. This shows the presence of a more esterified carboxyl group under these conditions. When the sonication time increased it offers more reaction time opportunities to solvent to extract more and more pectin.

CONCLUSION

The range of anhydrouronic acid content of extracted pectin (62.4-70.3%) is close to the commercial standards (>65%). The degree of esterification varies from 55.8 to 64.6%, which shows the presence of high methyl ester (HM) pectin (DE>50%). The anhydrouronic acid content decreases with increasing values of all the independent variables. However, with the increment in the values of independent variables, the degree of esterification increases. The maximal anhydrouronic acid concentration and degree of esterification under ideal conditions were 64 percent and 62.9 percent, respectively. The present study led to the valorization of sweet lime peel for pectin as an efficient way of reducing the present waste and waste disposal problems encountered. This could provide an additional boost to the economic sector through valorization of food wastes. This research has therefore, been proved to be an efficient approach to extract valuable product from agro waste.

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Distribution and Prevalence of Downy Mildew Disease on Cucurbits in Punjab

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***Abstract*:** Cucurbit downy mildew caused by *Pseudoperonospora cubensis* is a major destructive disease affecting wide range of cucurbits which causes significant losses to the growers in terms of yield and fruit quality. The present study was conducted with an objective to assess the disease incidence and severity in Punjab under different agro-climatic zones. The survey conducted in various cucurbit growing regions of Punjab revealed that highest disease incidence was observed in Western Zone (36.41%) followed by Central Plain Zone (26.92%). The disease severity was highest in Western Zone (20.42%) succeeded by Central Plain Zone (15.62%), sub-mountain Undulating Zone (14.30%) and Western Plain Zone (12.79%). The overall mean disease incidence in Punjab state was 31.21 and 36.06 per cent during the year 2018-19 and 2019-20, whereas disease severity was 16.78 and 21.65 per cent in the respective years. Ludhiana and Sangrur emerged as the hot spot areas for the occurrence of downy mildew with disease incidence of 43.88 and 34.69 per cent, respectively. Among the different crops surveyed, the disease prevalence was maximum in cucumber (37.61%) and least in watermelon (22.00%). The hybrids cultivated in each cucurbit host reported to be more affected by the downy mildew as compared to the composites and land races.

Keywords: *Pseudoperonospora cubensis*, Disease incidence, Disease severity, Cucurbits, Survey

Vegetables are grown extensively all around the world and India holds second position in vegetable production while it ranked 25th in the production of cucumber and gherkins (1.96 lac tonnes) in the world during 2018 (Anonymous 2018). Punjab is the leading producer of vegetables with an area of 2.73 lac ha under vegetable cultivation, producing 54.42 lac tonnes and average productivity of 19.91 tonne per ha. Among vegetables, cucurbits are among the economically important vegetable crops. The family *Cucurbitaceae* includes more than 118 genera and 825 species grown worldwide in various temperate and tropical regions (Lebeda and Cohen 2011). During 2018-19, the cucurbits covered an area of 17.01 thousand ha having productivity of 159.00 q/ha and a total production of 270.45 thousand tonnes in Punjab (Anonymous 2019). Cucurbits are important crops in terms of its use in both culinary as well as non-culinary items.Among diseases, downy mildew of cucurbits caused by *Pseudoperonospora cubensis* poses a continuous threat to the cultivation of cucurbits worldwide and is known to occur annually under Punjab conditions. It was first reported by Butler in Punjab (Butler 1918) and thereafter several reports have indicated the prevalence of disease in state. The loss due to downy mildew is directly proportional to disease intensity and adversely affects the yield and quality of produce. The survey and sampling of the pathogen help in studying the occurrence and distribution of the pathogen in a particular region. It also helps in timely forecasting the disease epidemic depending upon the presence of inoculum. Samples collected from the infected fields can be further used for the identification, characterization and virulence studies, etc. Therefore, the current investigation was aimed at assessing the disease incidence and its severity of downy mildew of cucurbits under Punjab conditions.

MATERIAL AND METHODS

Extensive surveys were conducted in different cucurbit growing regions of Punjab such as Amritsar (31.6340°N, 74.8723°E), Faridkot (30.6774°N, 74.7539°E), Hoshiarpur (31.5123°N, 75.9115°E), Jalandhar (31.3260°N, 75.5762°E), Kapurthala (31.3723°N, 75.4018°E), Ludhiana (30.9010°N, 75.8573°E) and Sangrur (30.2458°N, 75.8421°E during April-October for 2018-19 and 2019-20. In order to assess the disease incidence and disease severity of downy mildew among various cucurbit hosts, different fields were inspected and the diseased samples were collected. Random plot sampling was done to record the percent disease incidence and disease severity. The diseased leaf samples were properly labelled inscribing the information of name of district, village/location and cultivar on the collection bags and these bags were brought to the laboratory for the further studies. Fifty plants were assessed randomly across the field and the percent disease incidence was calculated using formula used by Shrestha et al (2019):

$$\text{Percent Disease Incidence (PDI)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants assessed}} \times 100$$

Grade	Description
0	No symptoms/ damage
1	1-10% area chlorotic and/or necrotic
2	11-20% area chlorotic and/or necrotic
3	21-30% area chlorotic and/or necrotic
4	31-40% area chlorotic and/or necrotic
5	41-50% area chlorotic and/or necrotic
6	51-60% area chlorotic and/or necrotic
7	61-70% area chlorotic and/or necrotic
8	71-80% area chlorotic and/or necrotic
9	81-100% area chlorotic and/or necrotic or dead

Based on the percentage of leaf area affected, per cent disease severity was calculated using the following formula (Ketta et al 2016):

Percent Disease Severity (PDS) = [Σ(a×b)/ (N×K)] × 100

Where a= number of infected leaves rated; b= numerical value of each grade; N= total number of examined plants; K= highest degree of infection on scale.

RESULTS AND DISCUSSION

The prevalence of cucurbit downy mildew varied to different extents in various areas surveyed in the different agroclimatic zones of Punjab. Among the different districts surveyed during 2018-19, the highest mean disease incidence was from Ludhiana (43.88%) followed by Sangrur, Kapurthala, Faridkotwhereas minimum disease incidence was observed in Jalandhar (10.00%). Dehlon region of Ludhiana and Sunam tehsil of Sangrur recorded the maximum incidence of the disease *i.e.,* 66.00 and 76.00 percent in the respective districts with minimum disease incidence in village Dalelgarh, Sangrur (9.25%). Similarly, the highest per cent disease severity was recorded in districts Ludhiana and Sangrur which was 24.15 and 17.95 per cent, respectively. The lowest disease severity (3.36%) was recorded from Phillaur in district Jalandhar. The close scrutiny of the disease condition in the following year of 2019-20 expressed the similar trend as of the previous year with maximum disease incidence from district Ludhiana (50.02%) followed by district Sangrur and minimum in district Jalandhar (12.00%). District-wise maximum disease incidence was at Dehlon (74.35%) in Ludhiana and Sunam tehsil of Sangrur (84.00%). The per cent disease severity during this period was highest in district Ludhiana (29.26%) followed by Sangrur and the minimum was in district Jalandhar (7.86%) followed by Amritsar. The overall mean disease incidence in Punjab was31.21 and 36.06 per cent during the year 2018-19 and 2019-20, respectively where the disease severity was recorded to be 16.78 and 21.65 per cent respectively for both the years. The pooled data for overall mean disease incidence and disease severity across the state was 33.62 and 19.22 per cent, respectively for the same duration under consideration. Similar studies done by Sharma et al (2003) revealed that the downy mildew disease was prevalent in Solan, Sirmour, Shimla and Bilaspur districts of Himachal Pradesh in moderate to severe form on cucumber crop. The disease incidence and severity were ranged from 50-100 per cent and 20-78 per cent, respectively. The average disease severity was comparatively lesser in Shimla and Bilaspur than in Solan and Sirmour. Similarly, Paul and Thakur (2003) surveyed Indore area of Kangra district in Himachal Pradesh and reported the disease incidence due to downy mildew was 15-20 per cent. Earlier, during March-April 2007-08, an extensive survey was conducted in cucumber and muskmelon fields in various districts of Punjab and collected 31 isolates of *P. cubensis* in the form of infected leaves with sporulating lesions (Thind et al 2010). Thereafter, Gupta et al(2014) reported the disease incidence and intensity of downy mildew as 70% and 56%, respectively, in cucumber. Haveri et al (2019) observed disease severity up to 27.43 per cent in Karnataka during 2018-19.

Among the different agro-climatic zones surveyed during both the years, disease incidence was highest in Western Zone (36.41%) followed by Central Plain Zone and Western Plain Zone . The data for disease severity was highest in Western Zone (20.42%) succeeded by Central Plain Zone, sub-mountain Undulating Zone and Western Plain Zone (Table 1).

In terms of disease incidence and disease severity among different cucurbit crops, disease incidence was more in cucumber (37.61%) followed by summer squash, muskmelon, pumpkin, and watermelon (Table 2). Lebeda et al (2011) reporting disease prevalence of 60-100% and cucumber being most frequently affected host. Different varieties of cucumber and muskmelon cultivated in the state get infected by downy mildew to different extents. Among the cucumber cultivars, highest mean disease incidence over the period of two years was on hybrids *i.e.,* Hybrid Ziya (80.00%) followed by Radhika and Multistar. Among the summer squash incidence of downy mildew was more in cultivar PCK1 (52.62%) than the Local Landrace (19.33%). In muskmelon incidence was much higher in MH27 (56.87%) as compared to HB Raseela (41.00%) and Bobby (26.00%). In pumpkin, highest disease incidence was found in hybrid Nirvana (45.00%) followed by PPH1 (42.00%). The least disease was observed in watermelon with incidence of

Table 1. Prevalence of downy mildew disease in different cucurbit growing areas of Punjab during 2018-19 and 2019-20

Agroclimatic zone	District	Location/ Village	Crop	Variety	Disease incidence (%)			Disease severity (%)		
					2018-19	2019-20	Mean	2018-19	2019-20	Mean
Sub-mountain Undulating Zone	Hoshiarpur	Mahalpur	Cucumber	Punjab Naveen	36.00	40.00	38.00	16.46	26.68	21.57
		Sikri	Cucumber	Punjab Hybrid	20.00	28.00	24.00	15.24	20.34	17.79
		Hariana	Cucumber	Local Landrace	16.00	24.00	20.00	12.34	18.67	15.51
		Khanpur	Cucumber	Multistar	12.00	16.00	14.00	5.01	8.28	6.65
		Hariana	Muskmelon	Hara Madhu	14.00	18.00	16.00	8.69	11.25	9.97
	Mean				19.60	25.20	22.40	11.55	17.04	14.30
Central Plain Zone	Amritsar	Gheri Mandi	Cucumber	Punjab Naveen	12.25	20.55	16.40	7.85	12.32	10.09
		Hamja	Cucumber	Local Landrace	17.25	21.35	19.30	10.20	10.86	10.53
		Dashmesh Nagar	Cucumber	Local Landrace	15.00	20.00	17.50	9.34	15.28	12.31
	Mean				13.63	18.48	16.05	7.69	11.58	9.63
	Jalandhar	Phillaur	Pumpkin	Hybrid Bheema	10.00	12.00	11.00	3.36	7.86	5.61
	Kapurthala	Phagwara	Cucumber	Local Landrace	40.00	42.00	41.00	28.64	30.86	29.75
		Mustafabad	Cucumber	Radhika	42.00	48.00	45.00	16.32	28.64	22.48
		Barindpur	Muskmelon	Bobby	12.00	18.00	15.00	8.20	10.46	9.33
	Mean				31.33	36.00	33.67	17.72	23.32	20.52
Central Plain Zone	Ludhiana	Dehlon	Cucumber	Multistar	66.00	74.35	70.18	46.35	52.22	49.29
		Ladhowal	Pumpkin	Nirvana	42.00	48.00	45.00	18.43	22.58	20.51
		Research farm, Dept. of Plant Pathology, PAU	Cucumber	Punjab Naveen	55.00	62.00	58.50	23.62	35.46	29.54
			Muskmelon	MH 27	65.20	72.25	68.73	42.35	45.29	43.82
			Pumpkin	PPH 1	40.00	44.00	42.00	22.50	26.56	24.53
			Summer Squash	PCK 1	48.65	56.59	52.62	20.25	22.56	21.41
			Watermelon	Sugar 6	20.00	24.00	22.00	10.46	14.44	12.45
		Research farm, Dept. of Vegetable Science, PAU	Cucumber	Punjab Naveen	30.00	37.00	33.50	12.36	14.68	13.52
			Muskmelon	MH 27	42.00	48.00	45.00	25.06	32.64	28.85
			Pumpkin	Punjab Samrat	30.00	34.00	32.00	20.13	26.16	23.15
	Mean				43.88	50.02	46.95	24.15	29.26	26.71
Western Zone	Sangrur	Malerkotla	Cucumber	Nazia F1	70.00	74.00	72.00	36.86	44.28	40.57
		Mandiala	Cucumber	Multistar	46.00	50.00	48.00	31.28	40.34	35.81
		Badshahpur	Cucumber	Local Landrace	22.00	24.00	23.00	10.36	14.76	12.56
		Ubhawal	Cucumber	Hybrid Bella	10.00	14.00	12.00	6.34	9.69	8.02
		Sangrur	Cucumber	Local Landrace	20.00	24.00	22.00	4.60	10.26	7.43
		Sunam	Cucumber	Hybrid Ziya	76.00	84.00	80.00	38.96	42.12	40.54
		Dalelgarh	Cucumber	Pooja 20	9.25	12.50	10.88	5.55	8.66	7.11
		Bhasaur	Cucumber	NCH 840	44.00	38.00	41.00	23.46	26.82	25.14
		Madevi	Cucumber	NCH 840	15.00	18.00	16.50	7.35	10.25	8.80
		Dugni	Muskmelon	Bobby	32.00	42.00	37.00	19.36	25.04	22.20
		Amargarh	Muskmelon	HB Raseela	40.00	42.00	41.00	15.03	18.26	16.65
		Badrukhan	Pumpkin	Hybrid Bheema	32.00	35.00	33.50	16.25	24.31	20.28
	Mean				34.69	38.13	36.41	17.95	22.90	20.42
Western Plain Zone	Faridkot	Pipli	Cucumber	Punjab Naveen	29.00	34.00	31.50	9.38	11.76	10.57
		Sarawan	Pumpkin	Local Landrace	26.00	32.00	29.00	12.45	16.42	14.44
		Bargari	Summer Squash	Local Landrace	17.85	22.00	19.33	11.25	15.46	13.35
	Mean				24.28	29.33	26.81	11.02	14.55	12.79
Overall Mean					31.21	36.06	33.62	16.78	21.65	19.22

*Observations were recorded between April to October in the year 2018-19 and 2019-20

Table 2. Prevalence of downy mildew disease on different cucurbits

Crop	Variety	Disease incidence (%)			Disease severity (%)		
		2018-19	2019-20	Mean	2018-19	2019-20	Mean
Cucumber	Punjab Naveen	32.45	38.71	35.58	13.934	20.18	17.058
	Punjab Hybrid	20.00	28.00	24.00	15.24	20.34	17.79
	Multistar	41.33	46.78	44.06	27.55	33.61	30.58
	Radhika	42.00	48.00	45.00	16.32	28.64	22.48
	Nazia F1	70.00	74.00	72.00	36.86	44.28	40.57
	Hybrid Bella	10.00	14.00	12.00	6.34	9.69	8.02
	Pooja 20	9.25	12.50	10.88	5.55	8.66	7.11
	Hybrid Ziya	76.00	84.00	80.00	38.96	42.12	40.54
	NCH 840	29.50	28.00	28.75	15.41	18.54	16.97
	Local Landrace	21.71	25.89	23.80	12.58	16.78	14.68
Mean		35.22	39.98	37.61	18.87	24.28	21.58
Muskmelon	Hara Madhu	14.00	18.00	16.00	8.69	11.25	9.97
	MH27	53.6	60.13	56.87	33.71	38.97	36.34
	Bobby	22.00	30.00	26.00	13.78	17.75	15.77
	HB Raseela	40.00	42.00	41.00	15.03	18.26	16.65
Mean		32.40	37.53	34.97	17.80	21.56	19.68
Pumpkin	Hybrid Bheema	21.00	23.50	22.25	9.81	16.09	12.95
	PPH1	40.00	44.00	42.00	22.50	26.56	24.53
	Nirvana	42.00	48.00	45.00	18.43	22.58	20.51
	Punjab Samrat	30.00	34.00	32.00	20.13	26.16	23.15
	Local Landrace	26.00	32.00	29.00	12.45	16.42	14.44
Mean		31.80	36.30	34.05	16.66	21.56	19.12
Summer Squash	PCK 1	48.65	56.59	52.62	20.25	22.56	21.41
	Local Landrace	17.85	22.00	19.33	11.25	15.46	13.35
Mean		33.25	39.29	35.97	15.75	19.01	17.38
Watermelon	Sugar 6	20.00	24.00	22.00	10.46	14.44	12.45

*Observations were recorded between April to October in the year 2018-19 and 2019-20

22.00%. Hence, cucumber was most frequently affected host followed by muskmelon and the hybrids in each crop were observed to be more susceptible to the disease.

CONCLUSION

Ludhiana and Sangrur districts are the hotspot areas for downy mildew of cucurbits and was maximum on cucumber and minimum on watermelon. The hybrids cultivated in each cucurbit host were more affected by the downy mildew as compared to the composites and land races. Overall, the disease was more prevalent in major cucurbit growing pockets of Western zone in Punjab. Therefore, can be inferred that downy mildew disease occurs annually in all cucurbit growing areas in varying extents and on all the different types of cucurbit host cultivated in the state. Downy mildew is one of the main reasons of major crop and yield losses in cucurbits so, there is need to devise a careful management practice in order to discourage the increasing threat of this disease. Therefore, survey and monitoring of the prevailing condition of the disease act as a guiding step for further action to be taken.

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Influence of Physiological Parameters on Mycelial Growth and Cultural Characteristics of Blue Oyster Mushroom [*Hypsizygus ulmarius* (Bull.: Fr.) Redhead]

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Abstract: Study was conducted on physiological and cultural characteristics of blue oyster mushroom [*Hypsizygus ulmarius* (Bull.: Fr.) Redhead], which is of immense use to identify the most suitable growing conditions for this mushroom. Six level of temperature (10, 15, 20, 25, 30 and 35°C) and five level of pH (4.0, 5.0, 6.0, 7.0 and 8.0) were evaluated to find out the best temperature and pH for the mycelial growth and cultural characteristics of *H. ulmarius*. Maximum mycelial growth (84.6 mm) was at pH 8.0 followed by pH 7.0 (77.0 mm). The temperature of 25°C was optimum at which maximum diametric growth (77.7 mm) was recorded in potato dextrose agar medium after 5 days of incubation. The growth of *H. ulmarius* decreased drastically as the temperature increased above or below 25°C. The colour of the mycelium varied from absolutely white to snow white and type of growth varied from thin, sturdy, fluffy and sparse.

Keywords: *Hypsizygus ulmarius*, Oyster mushroom, Temperature, pH, Mycelial growth

Oyster mushrooms are one of the edible mushrooms having the potential of converting different agricultural wastes into valuable proteins. Among the commercially cultivated species of oyster, *Hypsizygus ulmarius* (Bull.: Fr.) Redhead also called as elm oyster or blue oyster mushroom is a high yielding mushroom gaining popularity nowadays (Aditya et al 2022a, Aditya et al 2022b). This is a novel species of oyster mushroom with very large fruiting bodies, blue colored pinheads, becoming light bluish white on maturing, palatable with meaty flavor and also has attractive keeping quality with therapeutic and medicinal values(Mane et al 2007, Aditya et al 2022c). Taxonomically, it is placed in Phylum- Basidiomycota, Class- Agricomycetes, Subclass- Agaricomycetidae, Order- Agaricales, Family- Lyophyllaceae, Genus- *Hypsizygus*, Species- *ulmarius* (Schoch 2000, Aditya 2021). Mushroom survival and multiplication are influenced by a number of factors that may act independently or in concert (Aditya et al 2022d, Aditya et al 2022e). Temperature and hydrogen ion concentration are the most important physical factors affecting the growth and development of oyster mushroom. Despite these attractive qualities, its production in subtropical zone of Himachal Pradesh, India has not yet explored. Moreover, variability studies on physiological aspects are of immense use to identify the most favorable growing conditions of the mushroom. Hence, the present investigation was therefore aimed out to find out the influence of physical factors on mycelial growth and cultural characteristics of blue oyster mushroom under sub tropical zone of Himachal Pradesh, India.

MATERIAL AND METHODS

During the academic year 2019-21, the current study was conducted at Dr. Y.S. Parmar University of Horticulture and Forestry, College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh, India.

Procurement, maintenance and preservation of culture: The Directorate of Mushroom Research, ICAR complex, Chambhaghat, Solan, Himachal Pradesh provided a pure culture of blue oyster mushroom (*Hypsizygus ulmarius*). The culture was then maintained on PDA (potato dextrose agar) medium (sub cultured periodically at an interval of 30-45 days). The fully grown culture was kept in the refrigerator at 2-4°C until it was used for the complete project.

Sterilization: In an autoclave, all media were sterilized at 15 psi pressure for 20 min. All glassware were sterilized for 2 h in a hot air oven at 180°C. After dipping the cork borer and inoculating needle in ethyl alcohol, they were flame sterilized and utilized only after complete cooling.

Cultural studies: *In vitro* investigations were carried out to determine the optimal physiological conditions for the growth of *H. ulmarius*. These investigations followed the conventional protocol established by Lilly and Barnett (1951) and Tuite (1969), with some modifications as needed.

Effect of different pH levels on mycelial growth of *H. ulmarius*: The best basal medium i.e. potato dextrose agar was adjusted to different pH levels *viz.,* 4.0, 5.0, 6.0, 7.0 and 8.0 by using 0.1 N, HCl and 0.1 N, NaOH solutions. The Petriplates containing 40 ml of medium was inoculated with mycelial bit (5.0 mm dia.) of actively growing culture (10 days old) of *H. ulmarius.* These Petriplates were incubated at 25±1^{0}C in a BOD incubator. The test fungus was evaluated for its tolerance to low and high pH range and also to determine the optimum pH level. The data were recorded in terms of diametric growth (mm/day), growth rate (mm/h) and cultural characteristics of the test fungus. The observations were recorded from 24 h of incubation up to 120 h of incubation.

Effect of different temperature regimes on mycelial growth of *H. ulmarius:* The influence of different temperatures *viz.,* 10, 15, 20, 25, 30 and 35^{0}C on mycelial growth of *H. ulmarius* was studied to find out the optimum temperature for its growth. The Petriplates containing 40 ml of basal medium adjusted to best pH were inoculated with mycelial bit (5.0 mm dia.) of actively growing 10 days old culture of *H. ulmarius.* Thereafter, these Petriplates were incubated at different test temperature regimes in the BOD incubator. The data were recorded in terms of diametric growth (mm/day), growth rate (mm/h) and cultural characteristics of the test fungus up to 5 days of incubation.

Data analysis: The experiments were conducted in completely randomized design having four replications in each treatment. The observations regarding influences of different temperatures and pH were recorded in *in vitro* conditions by culturing the fungus in Petriplates having potato dextrose agar medium. The average diametric growth (mm/day), growth rate (mm/h) and cultural characteristics were recorded after 24 h of interval up to five days of incubation. The data thus obtained were statistically analyzed by using statistically package of program OPSTAT (Sheoran 2006).

RESULTS AND DISCUSSION

Effect of different pH levels on the growth of *H. ulmarius:* Studies on different level of pH revealed significant differences in diametric growth of *H. ulmarius* (Table 1). Significantly higher mean diametric growth (45.08 mm) was at pH 8.0, followed 7.0 and at 6.0 pH levels, irrespective of different durations of incubation. However, mean minimum diametric growth (16.74 mm) was at pH 4.0. The maximum diametric growth (59.79 mm) was after 120 h of incubation followed 96 h and 72 h of incubation, while significantly mean minimum diametric growth (9.59 mm) was recorded after 24 h of incubation, irrespective of the different pH levels.

The significantly higher diametric growth (84.62 mm) was after 120 h of incubation at pH 8.0 followed by 7.0 and 6.0 pH levels after same duration. The significantly minimum diametric growth (5.06 mm) was at pH 4.0. The slightly alkaline pH supported the excellent growth of *H. ulmarius* comparative to acidic pH levels. The mycelial growth was observed to be white with circinate pattern and margins almost regular in pH 8.0 but, at pH 4.0, 5.0 and 6.0 mycelial growth was snow white with ray patterns and having irregular margins. In case of pH 7.0 mycelial growth was normal white with circinate pattern having irregular margins (Table 2, Plate 1). Growth rate was significantly maximum (0.66 mm) at pH 8.0 followed by 7.0 and 6.0 pH levels. The significantly minimum (0.20 mm) growth rate was at pH 4.0 level, irrespective of different duration of incubation. Significantly maximum growth rate was between 96-120 h of incubation (0.62 mm) followed after 72-96 and 48-72 h of incubation. However, minimum diametric growth (0.19 mm) was between 0-24 h of incubation, irrespective of different pH levels (Table 3). Interaction studies revealed that growth of *H. ulmarius* was maximum (0.93 mm) at pH 8.0 between 96-120 h of incubation under study while, minimum (0.002 mm) was at pH 4.0 between 0-24 h of incubation and with increased the pH growth rate of *H. ulmarius* was also increased with progression of incubation. It has been a well-established that

Table 1. Effect of different pH levels on diametric growth of *Hypsizygus ulmarius*

pH levels	Average diametric growth (mm) after duration of incubation (h)					Overall Mean
	24	48	72	96	120	
4.0	5.06	10.34	16.36	22.59	29.33	16.74
5.0	7.65	17.18	27.35	38.28	50.11	28.11
6.0	9.80	20.69	32.54	44.57	57.87	33.09
7.0	12.69	23.92	38.68	56.86	77.05	41.84
8.0	12.74	25.31	40.39	62.31	84.62	45.08
Overall Mean	9.59	19.49	31.07	44.92	59.79	
CD (p=0.05)	pH levels 0.14		Duration 0.14		Interaction 0.31	

hydrogen ion concentration (pH) in the growing media influence the growth and metabolism of oyster mushroom (Zervakis et al 2004, Shim at al 2005, Sardar et al 2015). The results indicate maximum mean diametric growth (45.08) at pH 8.0 and findings are in agreement with Chandravanshi (2007) and Baghel et al. (2019) where maximum radial growth and biomass of *H. ulmarius* was at pH 8.0. Singh and Kushwaha (2007), Sumi and Geetha (2016) and Sharma et al (2018) also reported maximum mycelial growth and biomass of *H. ulmarius* at pH 7.0. The reduction in mycelial growth of *H. ulmarius* at acidic pH could be attributed to the reason that the strong acidic pH might have disintegrated the cell wall and impaired the selective permeability junction of the cell wall.

Effect of different temperature regimes on the growth of *H. ulmarius*: The variation in temperature regimes significantly influenced the growth of *H. ulmarius* (Table 4). The fungus could grow at all temperatures i.e. 10 to 35^{0}C, but as the temperature increased beyond 25^{0}C, the diametric growth of the test fungus decreased drastically. Significantly mean maximum diametric growth was at 25^{0}C (41.90 mm) followed by 30 and 20^{0}C. The latter two treatments were statistically at par with each other. However, significantly mean minimum mycelial growth (13.51 mm) was at 10^{0}C followed by 35 and 15^{0}C. Similar trend in terms of diametric growth was recorded with the progression of incubation and significantly maximum (39.51 mm) diametric growth was recorded after 120 h of incubation followed by 96 and 72 h,

Table 2. Cultural characteristics of *Hypsizygus ulmarius* on different pH levels

pH levels	Colour of mycelium	Type of growth
4.0	Snow white	Fluffy mycelial growth having ray pattern with irregular margins
5.0	Snow white	Normal mycelial growth having ray pattern with concentric rings and irregular margins
6.0	Snow white	Normal mycelial growth having ray pattern with concentric rings and irregular margins
7.0	White	White mycelial growth with circinate pattern having irregular margins
8.0	White	White mycelial growth with circinate pattern having regular margins

Table 3. Effect of different pH levels on growth rate of *Hypsizygus ulmarius*

pH levels	Average growth rate (mm/h) between duration of incubation (h)					Overall Mean
	0-24	24-48	48-72	72-96	96-120	
4.0	0.002	0.22	0.25	0.26	0.28	0.20
5.0	0.10	0.39	0.42	0.45	0.49	0.37
6.0	0.19	0.49	0.49	0.49	0.55	0.43
7.0	0.32	0.46	0.61	0.75	0.84	0.59
8.0	0.32	0.52	0.62	0.91	0.93	0.66
Overall Mean	0.19	0.40	0.47	0.57	0.62	
CD (p=0.05)	pH levels 0.01		Duration 0.01		Interaction 0.02	

Table 4. Effect of different temperature regimes on the growth of *Hypsizygus ulmarius*

Temperature (°C)	Average diametric growth (mm) after duration of incubation (h)					Overall Mean
	24	48	72	96	120	
10	5.06	8.93	12.93	17.71	22.93	13.51
15	5.72	11.49	17.46	23.84	30.50	17.80
20	6.79	12.99	20.16	28.98	38.32	21.45
25	12.45	23.86	38.56	56.86	77.76	41.90
30	6.96	14.01	21.64	30.51	39.77	22.58
35	5.42	10.07	15.59	21.59	27.75	16.08
Overall Mean	7.07	13.56	21.06	29.91	39.51	
CD (p=0.05)	pH levels 0.19		Duration 0.21		Interaction 0.47	

while, significantly minimum diametric growth (7.07 mm) was after 24 h of incubation. Maximum diametric growth (77.76 mm) was at 25^{0}C after 120 h of incubation which was followed by 96 h of incubation at same temperature level. However, minimum (5.06 mm) diametric growth was observed at 10^{0}C after 24 h of incubation. An intermediate diametric growth was recorded at rest of the temperature after different duration of incubation. Thus, a temperature of 25^{0}C was most suitable for mycelial growth of the test fungus. The interaction between temperature and time was significant in all combinations.

The culture characteristics of the fungus also varied with respect to incubation temperature in media. White, thin irregular and very slow mycelial growth was observed at 10^{0}C while, snow white, thin, fluffy, irregular as well as slow growth was recorded at 15^{0}C. At 20^{0}C, white, normal irregular and moderate mycelial growth was observed whereas, growth was sturdy white and faster having prominent ray pattern with concentric rings at 25^{0}C. Above 25^{0}C, the growth of the fungus decreased and white normal irregular and moderate mycelial growth with concentric rings was observed at temperature 30^{0}C whereas, at 35^{0}C white sparse fluffy irregular and slow mycelial growth was noticed (Table 5, Plate 2).

Growth rate of *H. ulmarius* in different temperature regimes was also calculated after different durations of incubation (Table 6). The results indicate similar trend in growth rate was also recorded as in diametric growth. The average growth rate was also significantly higher at 25^{0}C (0.60 mm) which decreased drastically at 30 and 20^{0}C. Significantly lowest growth rate was at 35^{0}C (0.18 mm) followed by that at 10 and 15^{0}C. Irrespective of temperature regimes, the average growth rate of *H. ulmarius* was maximum (0.39 mm) between 96-120 h of incubation which was statistically at par with 72-96 and 48-72 h of incubation. However, the average growth rate was significantly minimum (0.08 mm) between 0-24 h of incubation.

Interaction of temperature with incubation hours revealed that the growth rate was maximum (0.87 mm) at 25^{0}C between 96-120 h of incubation which was statistically at par with same temperature between 72-96 h of incubation (0.76 mm). However, minimum growth rate (0.01 mm) was recorded at 10 and 35^{0}C temperature between 0-24 h of incubation which was statistically at par with growth rate at rest of all the temperatures between same duration except at 25^{0}C (0.30 mm).

In the present investigation maximum diametric growth of *H. ulmarius* was recorded at 25^{0}C which was in accordance with the findings of Wang and Patil (2007); Singh and Kushwaha (2007); Sumi and Geetha (2016) and Sharma et al (2018), reported the same temperature to be optimum for the

Table 5. Cultural characteristics of *Hypsizygus ulmarius* at different temperature regimes

Temperature regimes (°C)	Colour of mycelium	Type of growth
10	White	Thin, irregular very slow mycelial growth
15	Snow white	Thin, fluffy and irregular slow mycelial growth
20	White	Normal irregular and moderate mycelial growth
25	White	Sturdy mycelium, faster growth with prominent concentric rings having ray pattern
30	White	Normal irregular and moderate mycelial growth with concentric rings
35	White	Sparse, fluffy irregular slow mycelial growth

Table 6. Effect of different temperature regimes on growth rate of *Hypsizygus ulmarius*

Temperature (°C)	Average growth rate (mm/h) between duration of incubation (h)					Overall Mean
	0-24	24-48	48-72	72-96	96-120	
10	0.01	0.16	0.17	0.19	0.21	0.20
15	0.03	0.23	0.24	0.26	0.27	0.21
20	0.07	0.25	0.29	0.36	0.39	0.27
25	0.30	0.47	0.61	0.76	0.87	0.60
30	0.08	0.29	0.32	0.38	0.38	0.29
35	0.01	0.19	0.22	0.25	0.26	0.18
Overall Mean	0.08	0.26	0.36	0.36	0.39	
CD (p=0.05)	pH levels 0.06		Duration 0.07		Interaction 0.15	

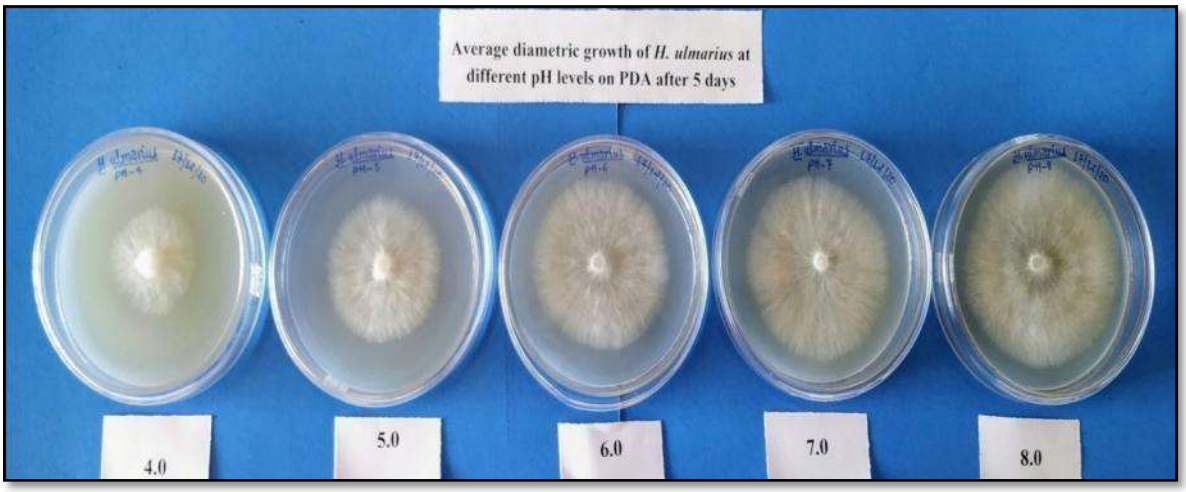

Plate 1. Petri plates showing effect of different levels of pH on mycelial growth of *Hypsizygus ulmarius*

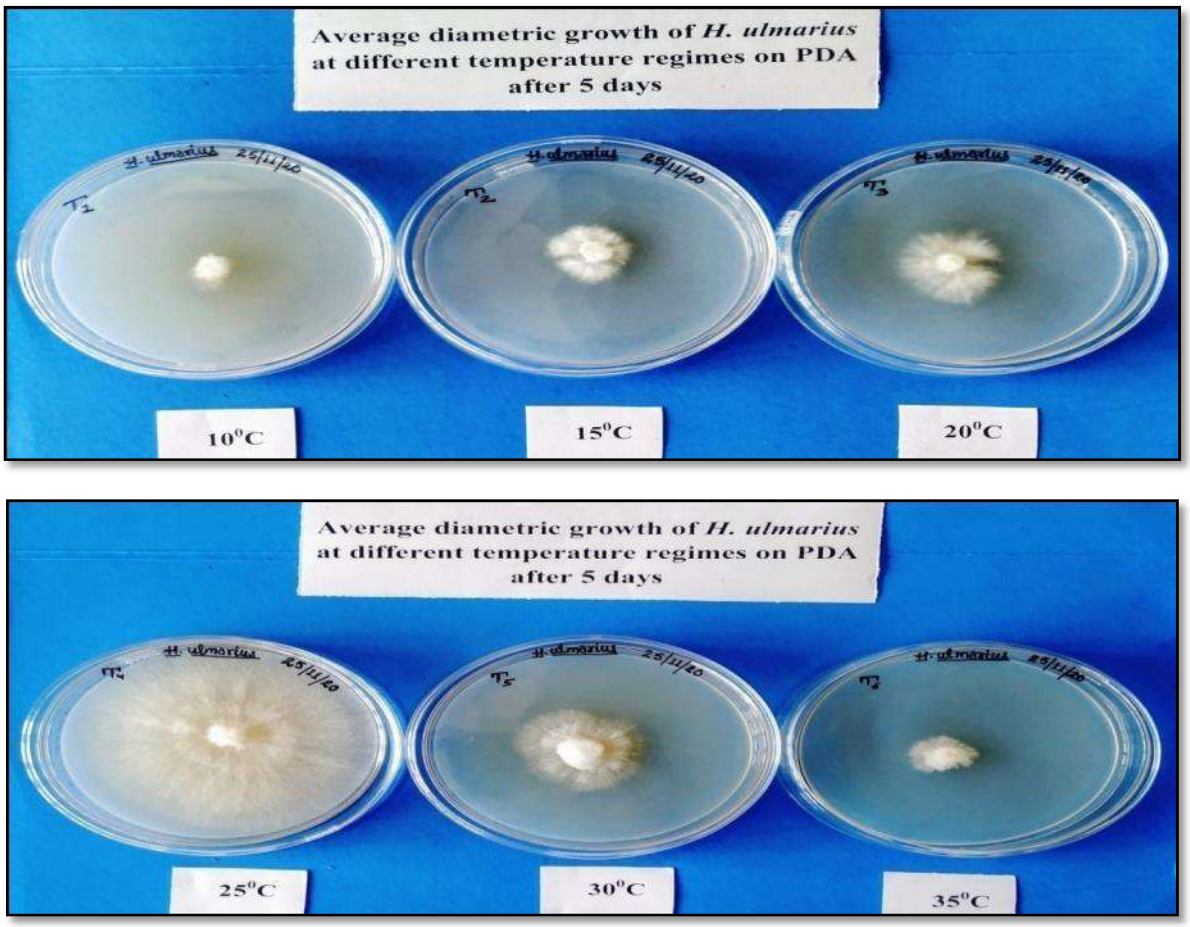

Plate 2. Petri plates showing effect of different temperature regimes on mycelial growth of *Hypsizygus ulmarius*

growth of *H. ulmarius*. Rout et al. (2015) observed that *H. ulmarius* exhibited better growth at 25^{0}C and the extent of mycelial growth was reduced drastically above or below the incubation temperature of 25^{0}C which also supports our findings. Similarly Sutha and Eshwaran (2016) also observed that growth decreased with the increase or decrease in temperature beyond 20-30^{0}C. Baghel et al. (2019) reported 26^{0}C temperature to be most favourable for the growth of *H. ulmarius*.

CONCLUSION

The pH levels affected *H. ulmarius* mycelial growth

development significantly. Mean maximum diametric growth and growth rate were recorded at pH 8.0 while, minimum at pH 4.0 after 120 h of incubation. Colour of the mycelium varied from snow white to white and type of growth was fluffy, normal mycelial growth with ray or circinate pattern having regular or irregular margins. Among various temperature regimes (10-35^{0}C) evaluated, mean maximum diametric growth and growth rate was at 25^{0}C while minimum at 10^{0}C. *H. ulmarius* could grow at all the temperatures but as the temperature increased or decreased beyond 25^{0}C, the diametric growth and growth rate of the fungus reduced drastically. Colour of the mycelium varied from absolutely white to snow white and type of growth varied from thin, sturdy, fluffy and sparse while, growing behaviour of the fungus was observed from very slow to faster growth.

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In vitro Evaluation of Different Chemicals and Plant Extract against *Xanthomonas campestris* pv*. mangiferae indicae*

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***Abstract*:** Different chemicals, plant extracts and two natural products were evaluated *in-vitro* against the *Xanthomans campestris* pv. *mangiferaeindicae* at different concentrations by using paper disc method. Seven different chemicals were evaluated at different concentrations of 500, 1000, 1500 and 2000 ppm including chitosan at 125, 250, 375 and 500 ppm with low, medium and high molecular weight. Chitosan with high molecular weight gave the maximum inhibition zone (51.44 mm) at 500 ppm followed by medium molecular weight chitosan at same concentration and high molecular weight chitosan at 375 ppm concentration whereas, minimum inhibition zone was in captan at 2000 ppm. Various plant extracts and natural products were also evaluated at different concentration of 5,10,15 and 20 per cent. Out of which, only *Eucalyptus hybrida* proved to be effective in inhibiting the growth of bacterium. Maximum diametric inhibition zone (1.85 mm) was at 20 per cent concentration and minimum was at 5 per cent concentration.

***Keywords*:** *Xanthomonas campestris* pv. *mangiferaeindicae*, Chemicals, Plant extracts, Chitosan, *Euclyptus hybrida*

Mango (*Mangifera indica* L.) belonging to family Anacardiaceae is commercially the most important fruit crop of India and consists of around 30 species of tropical fruit tree (Shah et al 2010). Mango is produced throughout the country with states in all regions contributing significantly to total output. Mango is grown worldwide with production of 55.4 MT (Anonymous 2019). In India, area under cultivation for mango production is 2291 thousand ha with production of 20444 thousand MT (Anonymous 2020). From seedling through fruiting in storage or transit, mango is susceptible to a variety of diseases. Anthracnose, black tip, bacterial leaf spot, dieback, mildew, sooty mould and phoma blight are among the diseases that growers in India are concerned about (Prakash 2007). In 1909, first documented the *Bacillus mangiferae* caused bacterial black spot of mango in South Africa (Doidge 1915). In India, Patel et al (1948) identified the disease as bacterial leaf spot of mango in Poona and Dharwar, and named the pathogenic bacterium as *Pseudomonas mangiferaeindicae*. Robbs et al (1974) proposed the current name of *Xanthomonas campestris* pv. *mangiferaeindicae*. *X. campestris* pv. *mangiferaeindicae* causes mango bacterial leaf spot disease, also known as mango canker, bacterial spot, bacterial canker, black spot, mango blight, or bacterial black spot (Gupta and Sharma 2000).

Mango bacterial black spot is very difficult to control and it usually becomes a limiting factor for mango industries when fungal diseases and other pests can be managed at acceptable levels. This is one of the most destructive bacterial disease of mango worldwide (Gagnevin and Pruvost 2001). Leaves of mango showed typical symptoms of bacterial leaf spot, formed lesions which were black, slightly raised, angular and sometimes produced a chlorotic halo. Later in the season, fruit symptoms consisted of small water-soaked spots around lenticels that later changed into black star shaped erumpent lesions. Moreover, cankers on twig were also observed sporadically (Zombre et al 2017). It results in 10 to 70 per cent fruit drop, 10 to 85 per cent loss in fresh yield and 5 to 100 per cent losses in storage all over the world (Haggag 2010).

The most effective strategy to control the disease is to prevent it from spreading to new places by enforcing tight quarantine restrictions. Various chemicals including an antibiotic, different fungicides and plant extracts were evaluated against *X. campestris* pv. *mangiferaeindicae* to manage the pathogen. Streptocycline was reported effective against the disease (Thirumalesh et al 2012, Tejaswini 2019). Chitosan is a new natural polymer which is nowadays become effective against various bacterial disease and having efficient antibacterial agent (Coqueiro and Di Piero 2011) which control different strains of *Xanthomonas.* There is no literature cited for chitosan and *Euclyaptus hybrida* against *X. campestris* pv. *mangiferaeindicae.* Hence, so the recent studies on various chemicals and plant extract was conducted.

MATERIAL AND METHODS

The present investigation was carried out in the research laboratory, Department of Plant Pathology, College of Horticulture and Forestry Neri, Hamirpur during the year 2019-2021.

***In vitro* evaluation**: In all, seven chemicals, seven plant extracts and two natural products were evaluated *in-vitro* against the pathogen isolates by paper disc method (Loo et al 1945). For this, 1 ml of 72 h old bacterial suspension was mixed with molten NSA (20 ml) in sterile Petriplate. Paper discs (5mm) soaked in each concentration of chemical was placed in Petriplate already plated with NSA and bacterial suspension. These petriplates were then incubated at 28±2ºC after 48 h and data on diametric inhibition zone (mm) were recorded.

Evaluation of chemicals against *X. campestris* pv. *mangiferaeindicae:* In all, seven chemicals including natural polymer (Chitosan) were evaluated for their efficacy against *X. campestris* pv. *mangiferaeindicae* under *in vitro* conditions by paper disc method. All chemicals were evaluated at four different concentrations (Table 1).

Evaluation of plant extracts/ natural products against *Xanthomonas campestris* pv. *mangiferaeindicae*: Extracts of dried leaves of *Azadirachta indica* (Neem), *Eucalyptus hybrida* (Safeda), *Murraya koenigii* (Curry Leaf), *Lantana camera, Justicia adhatoda* (Basuti), *Cannabis sativus* (Bhang) and *Calotropis gigantean* (Aak) and natural product (10 days old sour butter milk and cow urine) were evaluated for their inhibitory activity against *X. campestris* pv. *mangiferaeindicae* by paper disc inhibition zone method. In order to obtain various concentrations such as 5, 10, 15 and 20 per cent weight/volume of extracts, 100 g of plant material was crushed and soaked in 300 ml distilled water. The mixture was then boiled to reduce the volume to 1/3rd of original *i.e.* to get 100ml as final volume of the extract. This extract served as 100 per cent concentration. The contents were then filtered through double layered muslin cloth so as to remove debris. These extracts were then autoclaved at 15 p.s.i. pressure at 121ºC for 20 minutes. Final desirable concentration of the extract was obtained by adding desired amount of sterilized distilled water to the extracts. The paper discs were soaked in each concentration of extract as mentioned earlier. In case of natural product, 10 days old sour butter milk and cow urine itself served as 100 per cent concentration which was further adjusted to desired concentration by adding sterilized distilled water. All the plant extracts,10 days old sour butter milk and cow urine were evaluated at four concentrations *viz.,* 5, 10, 15 and 20 per

Table 1. *In vitro* effect of different chemicals against *X. campestris* pv. *mangiferaeindicae*

Chemicals	Diametric inhibition zone (mm) at different concentration (ppm)				Overall mean
	500	1000	1500	2000	
Streptocycline*	22.50 (28.30)	23.78 (29.17)	25.78 (30.50)	27.23 (31.44)	24.82 (29.85)
Copper hydroxide	8.56 (16.94)	11.83 (20.11)	14.22 (22.15)	15.78 (23.39)	12.60 (20.65)
Captan	6.45 (14.70)	8.00 (16.41)	9.89 (18.32)	10.33 (18.74)	8.67 (17.04)
Bordeaux mixture**	18.78 (25.67)	20.67 (27.03)	21.33 (27.50)	22.67 (28.42)	20.86 (27.15)
Cuperous oxide	0.00 (0.00)	7.33 (15.70)	8.00 (16.42)	9.44 (17.88)	6.12 (12.50)
Low molecular weight chitosan***	28.22 (32.08)	35.00 (36.26)	38.22 (38.17)	44.22 (41.67)	36.42 (37.04)
Medium molecular weight chitosan***	31.11 (33.89)	41.22 (39.93)	43.11 (41.02)	48.89 (44.35)	41.08 (39.79)
High molecular weight chitosan***	34.22 (35.79)	45.33 (42.30)	47.78 (43.71)	51.44 (45.81)	44.69 (41.90)
Copper oxychloride	7.33 (15.70)	7.89 (16.30)	10.11 (18.52)	11.67 (19.96)	9.25 (17.62)
Overall mean	18.13 (22.56)	22.34 (27.02)	24.27 (28.48)	26.85 (30.18)	

$C.D_{p\geq0.05}$	$S.E_{(d)}$
Treatment	0.55	0.28
Concentration	0.37	0.18
Treatment ×Concentration	1.01	0.55

Figures in parentheses are angular transformed values, * indicates the concentrations were 50, 100, 150 and 200 ppm. ** indicates the concentrations were 1500, 2000, 2500 and 3000 ppm,*** indicates the concentrations were 125, 250, 375 and 500 ppm.

cent. Simultaneously, a check treatment was maintained in which the paper discs were soaked in sterilized distilled water instead of plant extract. Data on diametric inhibition zone (mm) of the pathogen were recorded after 48 h of incubation at 28±2ºC.

Data analysis: The laboratory experiments were conducted with 3 replications while, results were statistically analyzed by using and online software OPSTAT (Sheoran 2006).

RESULTS AND DISCUSSION

Evaluation of different chemicals against *X. campestris* pv. *mangiferaeindicae:* The significantly maximum mean diametric inhibition zone (44.69 mm) was in high molecular weight chitosan treatment (Table 1, Plate 1) followed by medium and low molecular weight chitosan and streptocycline that the significantly maximum (51.44 mm) zone of inhibition was recorded when high molecular weight chitosan at 500 ppm followed by medium molecular weight chitosan (at same concentration and high molecular weight chitosan at 375 ppm concentration. Significantly minimum (6.45 mm) zone of inhibition was in captan followed by copper oxychloride and copper hydroxide at 500 ppm. However, no inhibition was observed in cuperous oxide at 500 ppm. An intermediate zone of inhibition was recorded in rest of the chemicals evaluated at different concentrations. There is no literature cited for the chitosan against *X. campestris* pv. *mangiferaeindicae*. The result proved that chitosan was the most effective chemical against the pathogen. To further support our study, different workers have reported the antibacterial nature of chitosan and chitosan nanoparticles against different species of *Xanthomonas* including *X. garderni* and *X. campestris* (Coqueiro and Di Piero 2011, OH et al 2019, Moon et al 2020, Esyanti et al 2020) which supported our results. However, streptocycline is found to be second best chemical to control the growth of *X. campestris* pv. *mangiferaeindicae*. Tejaswini (2019) also reported streptocycline as the best chemical against this bacterium. The study shows that only *Eucalyptus* leaves extract was able to inhibit the growth of the test bacterium. However, with respect to other species of *Xanthomonas*, the Earlier scientist observed the antibacterial nature of *Eucalyptus* spp. against different species of *Xanthomonas* (Yugander et al 2015, Yemanta et al 2019, Abo Elyousr et al 2020, Sharma 2020).

Evaluation of different plant extract/ bio-products against *X. campestris* pv. *mangiferaeindicae:* Among seven plant extracts, cow urine and 10 days old sour butter milk evaluated against the test pathogen, only *Eucalyptus hybrida* leaf extract was able to inhibit the growth of the

Table 2. *In vitro* evaluation of different plant extract/ bio-products against *X. campestris* pv. *mangiferaeindicae*

Plant extracts/bioproducts	Diametric inhibition zone (mm) in different concentration (%)				Overall mean
	5	10	15	20	
Calotropis gigantea	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Justicia adhatoda	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Cannabis sativa	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Murraya koeinigii	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Cow urine	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Lantana camara	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Azadirachta indica	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Eucalyptus hybrida	6.67 (2.77)	12.33 (3.56)	13.67 (3.83)	16.67 (4.20)	12.33 (3.61)
Sour buttermilk	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Overall mean	0.74 (1.12)	1.37 (1.23)	1.52 (1.31)	1.85 (1.36)	

$C.D_{p\geq0.05}$	S.E.(d)
Treatments	0.02	0.01
Concentration	0.02	0.01
Interaction	0.05	0.02

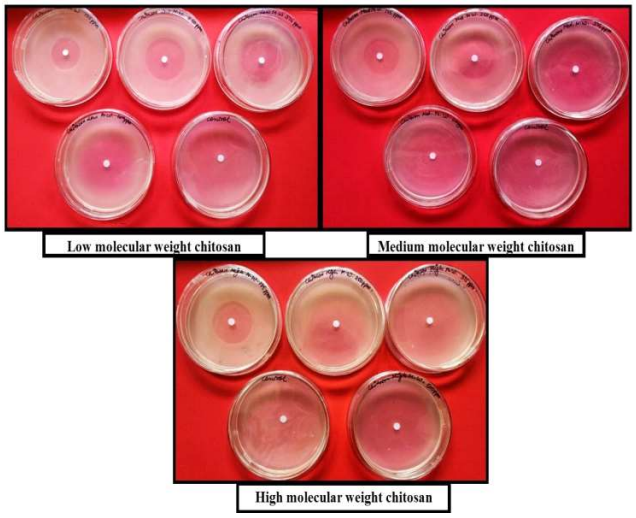

Plate 1. Effect of chitosan against *Xanthomonas campestris* pv. *mangiferaindiace*

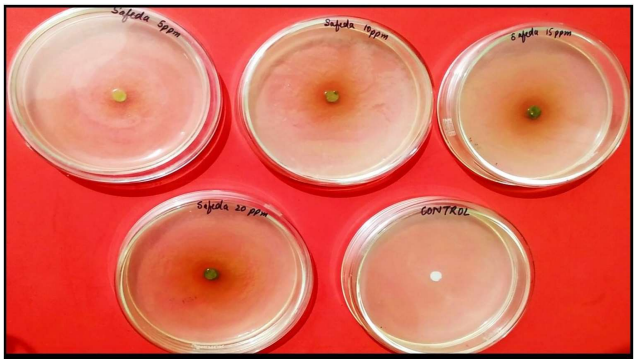

Plate 2. Effect of *Eucalyptus hybrida* extract on *Xanthomonas campestris* pv. *mangiferaeindicae*

bacterium (Table 2, Plate 2) with a mean inhibition diametric zone of 12.33 mm. Rest all the plant extracts, cow urine, and 10-day-old sour butter milk failed to inhibit the growth of test bacterium even at their highest concentrations evaluated. In *Eucalyptus hybrida* leaf extract, significantly maximum zone of inhibition (16.67 mm) was recorded at 20 per cent concentration which decreased significantly with reduction in the concentration at each level and was significantly minimum (6.67 mm) at 5 per cent concentration.

CONCLUSION

Among all various chemicals evaluated *in vitro* against the bacterium by paper disc method, chitosan (high, medium and low molecular weight), streptocycline, Bordeaux mixture, copper hydroxide and copper oxychloride proved to be effective in inhibiting the growth of bacterium as compared to control. Maximum inhibition zone was recorded in case of high molecular weight chitosan at 500 ppm. However, minimum inhibition zone was recorded in case of capstan at 2000 ppm. Among the various plant extracts and natural products evaluated *in vitro* against *X. campestris* pv. *mangiferaeindicae*, only *Eucalyptus hybrida* proved effective in inhibiting the growth of bacterium. Maximum diametric inhibition zone was at 20 per cent concentration and minimum was at 5 per cent concentration of *E. hybrid.*

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Evaluation of *Withania somnifera* Endophytic Bacterial Isolates under In-Vitro Conditions

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***Abstract*:** Endophytes are colonized with the internal tissues of their host plants and can form a range of different types of relationships including symbiotic, mutualistic and trophobiotic. Endophytic bacteria can promote plant growth, yield and can act as biocontrol agents. To explore biocontrol potential of various bacterial isolates an attempt was made to isolate endophytic bacteria and examined for hydrogen cyanide (HCN) production, siderophore production and cellulase activity. Thirty-two endophytic bacterial isolates (ARBE1-ARBE32) were retrieved from the roots of *Withania somnifera* grown at Medicinal and Aromatic Plant Section, Chaudhary Charan Singh Haryana Agricultural University , Hisar. Under *in vitro* conditions siderophore production was observed in isolates ARBE5, ARBE9, ARBE11, ARBE13, ARBE14, ARBE17, ARBE18, ARBE19, ARBE22, ARBE30, ARBE31 and ARBE32. Hydrogen cyanide production indicated by a change in color of alkaline picrate-soaked filter paper was observed in isolates ARBE5, ARBE9, ARBE11, ARBE13, ARBE14, ARBE16, ARBE18, ARBE19, ARBE23, ARBE27, ARBE28, ARBE29 and ARBE32. The isolates, ARBE13, ARBE20, ARBE26, ARBE29 and ARBE30 were positive for cellulase activity. The isolates possessing multi biocontrol traits can further be exploited for management of plant diseases in integrated manner.

***Keywords*:** Ashwagandha root bacterial endophyte, Biocontrol activities, Endophytic, Wilt, *Withania somnifera*

Pesticide residues possess harmful effects, visible since the onset of green revolution. Therefore, researchers of the present era are focusing on environment-friendly and sustainable options for managing pests (Tao et al 2022). In this sequence, endophytic microbiota catches the interest of researchers as well as environmentalists due to its sustainability and environmental friendliness. Endophytic colonization is a subsystem of the soil microbiome that plays an important role in the synthesis of biologically active compounds that protect plants from soil-borne pathogens (Cays 2021). The first report of the presence of endophytes was by Vogl (1898), who found a mycelium residing in grass seed *(Lolium temulentum* L.). Endophytic micro-biota plays an important role in plants' metabolic activities. They trigger various biochemical pathways in the host plant by producing physiologically important chemical compounds. They produce a diverse range of secondary metabolites with vital medicinal values and can be used in medicine, agriculture and industry (Siegel et al 1987). Endophytes are the microorganisms (fungi, bacteria, actinomycetes, slime-molds, archae a, viruses, phytoplasmas and other protozoan species) that colonize the host tissues and establish a relationship where both partners get benefit from their interactions which spend at least a part of their life cycle inside the plant tissues without causing any diseases (Prasad et al 2014, Esmael & Goodwin 2022, Mahnkopp-Dirks et al 2022). The specific benefits of many endophytes are, however unknown. Generally, the bacterial endophytes are linked with the plants and can perform three functionalities – biotic and abiotic stress alleviation and phytohormones production. The population of plant endophytes is generally less than the rhizosphere bacteria because roots favour the growth of several microbial communities (Samuel et al 2022). Plants mainly attract microorganisms from microbial hubs around them. Plants not only absorb water and nutrients from the soil but they also absorb a variety of microorganisms through their roots (Hardoim et al 2015).

Although, there was a misconception among the researchers to consider the presence of any bacteria within plant tissues as symptomatic of a particular pathological condition, however, this perception was changed by one famous scientist Perotti, 1926 who described the occurrence of a non-pathogenic flora in root tissues. Endophytic microorganisms may be present in all parts of a healthy plant at a specific life stage or throughout their life and they generally do not cause diseases in plants. They are mutually beneficial to their host (Shurigin et al 2022). Endophytes enhance host plants' resistance by producing valuable substances and inducing systemic resistance (Zaghloul et al

2016, Alsultan et al 2019). They also possess various powerful bioremediation effects (Miao et al 2022). In the last few decades, medicinal plants are becoming popular due to their excellent remediation characteristics and no side effects as compared to allopathic medicinal systems. Medicinal plants provide valuable therapeutic agents in traditional medicines used at the global level for human health. Ashwagandha (*Withania somnifera*) also known as Indian ginseng is one of the most important medicinal plants (Shabbir and Mohammad 2014). It is reported in literature that plant root endophytes can produce metabolites with antifungal properties to avoid pathogen infection by competing with pathogens or by direct antagonism through the production of antimicrobial compounds (Compant et al 2005). However, little information is available regarding antagonistic mechanism of bacterial root endophytes, which is needed to be fully explored (Herrera et al 2022). To accomplish this, the present study was designed to assess various biocontrol attributes *viz.,* siderophore, cellulase activity and HCN production of bacterial endophytes of ashwagandha for development of eco-friendly approach for disease management.

MATERIAL AND METHODS

Root samples: Root samples of ashwagandha were collected during crop season 2019-2020. An intact root system was dug out and the roots of ashwagandha plants were carefully taken in plastic bags for various studies.

Isolation of bacterial root endophytes from ashwagandha roots: The roots were washed with running tap water and surface sterilized sequentially in 75 % (v/v) ethanol for 2 min, 2.6 % (w/v) sodium hypochlorite solution for 5 min and 75 % (v/v) ethanol for 1 min. One gram of plant tissue was crushed in a pestle and mortar with 10 ml of sterile distilled water to get a homogenous paste and allowed to settle down for 20 minutes. The supernatant was diluted serially and approximately 10µl was placed on nutrient agar (NA) plates and incubated at 28±2°C for 3 days. The bacterial colonies appearing on the plates were considered to be endophytes. Colonies were characterized according to different visual observations, purified on plates using streak plate technique and maintained at 4 ±1°C for further studies.

Screening of Endophytic Bacterial isolates for Biocontrol Activities

Siderophore production: Siderophore production by bacterial endophytes of ashwagandha was observed by Chrome azurol S (CAS) assay (modified method of Schwyn and Neilands 1987). The presence of siderophore (iron chelator) was indicated by the decolourization of blue-coloured ferric dye complex, resulting in yellow halo zones around the bacterial colonies.

Hydrogen cyanide production: Hydrogen Cyanide production by bacterial endophytes was assessed using the method of Alstrom and Burns (1989). Active culture of different bacterial endophytes was prepared by inoculation of 48 h old culture from nutrient agar slants to freshly prepared Kings B broth. The production of cyanide was detected after 72 h of incubation at 28±2°C, using alkaline picrate-soaked filter paper fixed underside of the test tube. A change of colour from yellow to light brown, brown and reddish-brown was recorded as an indication of weak, moderate or strong cyanogenic potential.

Cellulase activity: Ashwagandha root bacterial endophytes were also screened for cellulase activity using the procedure described by Apun et al (2000). Freshly grown bacterial cultures were spot inoculated on carboxy-methyl-cellulose (CMC) agar plates, incubated at 28±2°C for 48 h and then flooded with 0.1% aqueous solution of Congo red for 15-20 minutes followed by washing with 1 M NaCl. Cellulase production was indicated by a clear zone around the colony.

RESULTS AND DISCUSSION

Screening of ashwagandha root bacterial endophytes for biocontrol activity: The biocontrol activity of ashwagandha root endophytes was assessed under laboratory conditions by examining their potential for siderophore production, HCN production and cellulase activity.

Siderophore production by endophytic bacterial isolates: The isolates ARBE5, ARBE9, ARBE11, ARBE13, ARBE14, ARBE17, ARBE18, ARBE19, ARBE22, ARBE30, ARBE31 and ARBE32 were found positive for siderophore production (Table 1, Fig. 1). Joshi et al (2018) isolated 10 bacteria from the roots, stems and leaves of *Aloe vera* and *Ocimum sanctum* and observed that only three of the isolates, TNR15, TKR 1 II and AVJR7 II were able to produce siderophore on CAS-medium. Etminani and Harighi (2018) observed that only five isolates, namely Pb1, Pb71, Pb78, Sp15 and Bp108 were able to produce siderophore out of ten isolates extracted from the leaves and stems of healthy wild pistachio trees. Arora and Singh (2016) observed ashwagandha endophytic bacteria (*Pseudomonas* sp.) as a growth-promoting agent as well as for their siderophore production activities and revealed *Pseudomonas* PSE-1 strain as potent siderophore producer.

Hydrogen cyanide production: Among 32 isolates a change in the colour of alkaline picrate-soaked filter paper was observed in isolates ARBE5, ARBE9, ARBE11, ARBE13, ARBE14, ARBE16, ARBE18, ARBE19, ARBE23, ARBE27, ARBE28, ARBE29 and ARBE32 (Table 2, Fig. 2).

Table 1. Siderophore production by ashwagandha root bacterial endophytes

Isolate	Siderophore production	Isolate	Siderophore production	Isolate	Siderophore production
ARBE1	-	ARBE12	-	ARBE23	-
ARBE2	-	ARBE13	+	ARBE24	-
ARBE3	-	ARBE14	+	ARBE25	-
ARBE4	-	ARBE15	-	ARBE26	-
ARBE5	++	ARBE16	-	ARBE27	-
ARBE6	-	ARBE17	+	ARBE28	-
ARBE7	-	ARBE18	+	ARBE29	-
ARBE8	-	ARBE19	++	ARBE30	+
ARBE9	+	ARBE20	-	ARBE31	+
ARBE10	-	ARBE21	-	ARBE32	++
ARBE11	++	ARBE22	+		

'+' Orange zone formation (light colour), '-' No zone formation'++' Orange zone formation (dark colour)

Inducing resistance and serving as a plant defence mechanism against pathogens, hydrocyanic acid works as an inducer of resistance. This volatile substance prevents electron transportation, interferes with cell energy delivery and ultimately kills pathogens. This substance indirectly makes phosphorus and iron more available to plants, which results in faster plant development. Etminani and Harighi (2018) showed that out of 10 isolates extracted from the leaves and stems of healthy wild pistachio trees only isolate Ba66, having a closer similarity to *B. anthracis*, was able to produce HCN. Etesami et al (2014) reported that amongst 200 bacterial isolates retrieved from the berseem clover plant's rhizosphere, roots and nodules only five were positive

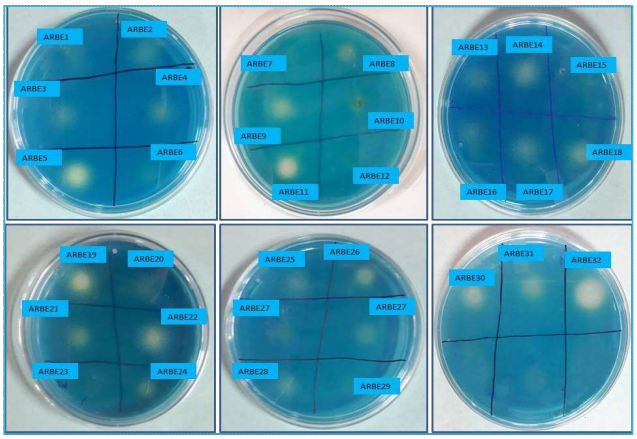

Fig. 1. Siderophore production by bacterial endophytes

Fig. 2. HCN production by bacterial root endophytic isolates

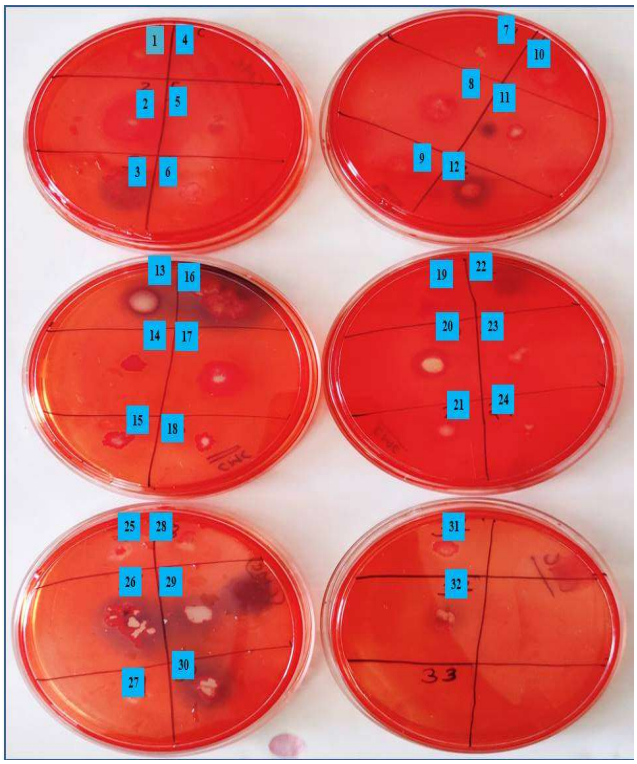

Fig. 3. Cellulase activity of ashwagandha root bacterial endophytic isolates

Table 2. Hydrogen cyanide production by endophytic bacterial isolates retrieved from ashwagandha roots

Isolate	HCN production	Isolate	HCN production	Isolate	HCN production
ARBE1	-	ARBE12	-	ARBE23	+
ARBE2	-	ARBE13	++	ARBE24	-
ARBE3	-	ARBE14	+	ARBE25	-
ARBE4	-	ARBE15	-	ARBE26	-
ARBE5	++	ARBE16	+	ARBE27	+
ARBE6	-	ARBE17	-	ARBE28	+
ARBE7	-	ARBE18	+	ARBE29	+
ARBE8	-	ARBE19	++	ARBE30	-
ARBE9	+	ARBE20	-	ARBE31	-
ARBE10	-	ARBE21	-	ARBE32	++
ARBE11	++	ARBE22	-		

'+' Orange zone formation (light colour), '-' No zone formation'++' Orange zone formation (dark colour)

for HCN production. Abdallah et al (2016) reported that the endophyte bacterium retrieved from ashwagandha was able to produce hydrogen cyanide.

Cellulase activity: Amongst 32 isolates, ARBE13, ARBE20, ARBE26, ARBE29 and ARBE30 were found positive for cellulase activity (Fig. 3). Microbes mediate nutrient cycling in soils in which extracellular enzymes *e.g.,* protease, lipase, amylase, cellulose, phosphatases, chitinase, urease, *etc.* play a significant role by mineralizing organic compounds (Das and Varma 2010). Soil enzyme activity measurements have been used as an indicator of soil quality and health (Badiane et al 2001). The results of the present study showed the diversity of culturable endophytic bacteria that reside in the interior root tissues of the ashwagandha plant. Ntabo et al (2018) isolated 42 bacteria from the leaves and roots of mangrove plants. They reported endophytes, including *Bacillus*, *Myroides, Pseudochrobactrum* and *Serratia* isolated primarily from leaves and suggested their potential role in colonising the leaf tissues and expressed cellulase activity. Kukla et al (2014) isolated twenty-nine endophytic bacteria from ryegrass and evaluated them for their cellulase enzyme, siderophore and hydrogen cyanide production.

CONCLUSION

A total of 32 endophytic bacterial isolates were retrieved from the ashwagandha roots. Present study focused on suitable screening for the selection of the promising endophytic isolates capable to produce cellulase, siderophore and hydrogen cyanide. The efficient isolates could be exploited as a biocontrol agent, for management of plant diseases and to overcome the fungicidal load in agricultural sector. The use of endophytic microorganisms as bioinoculants could also be a promising alternative to synthetic fertilizers, especially for the cultivation of medicinal plants.

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Assessment of Neem (*Azadirachta Indica*) and Lemongrass (*Cymbopogon citratus*) Extracts on *Meloidogyne incognita*

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***Abstract*:** Several Phyto-nematicides are available but none of them is capable to control *Meloidogyne* species infestation efficiently. Thus, there is a great need for an eco-friendly, highly effective sustainable control measure for this pest. In this study, to manage the *Meloidogyne* infestation, leaf extract of *Azadirachta indica* and *Cymbopogon citratus* were tested. Organic compounds present in extracts were characterized by GCMS. In vitro bioassays were conducted by applying three concentrations over 100 individuals of the 2nd juvenile stage. The mortality status of the nematode population by counting the dead and live individuals after applying 100 µl of the extract was recorded at different time durations i.e., 24, 48 and 72 hr. Results indicated significant variability toward different concentrations and durations for both extracts. GC-MS profiles of *C. citratus* and *A. indica* revealed the presence of diverse types of compounds in varying quantities. In such a way active compounds present in *A. indica* and *C. citratus* will be screened out. These compound/s-based formulations will be available for Integrated pest management programs, especially in horticultural crops to minimize infestation of *Meloidogyne incognita*.

***Keywords*:** Plant-parasitic nematodes, *Meloidogyne, Azadirachta indica, Cymbopogon citratus*

Nematode pest cause approximately 21.3% downfall in global food production annually (Kumar et al 2020). Approximately 157 billion US dollars in a global loss was reported due to Phyto-parasitic nematodes (Hassan et al 2013). More than 4100 species of plant-parasitic nematodes (PPNs) are reported, out of this *Meloidogyne* species is prominent and worldwide in distribution (Jones et al 2013). *Meloidogyne* species are obligate parasites that parasitize thousands of different plant species including monocotyledons, dicotyledons, herbaceous and woody plants. Various species of genus *Meloidogyne* such as *Meloidogyne incognita* (Kofoid & White), *M. Javanica* (Treub), *M. arenaria* (Neal), *M. acronea* Coetzee are major PPNs species of economically valuable crops that grow in tropical, subtropical, and temperate climates. Several plant products based on nematicides exist in the market but none of them can manage the PPNs population successfully at the commercial level. There is very fewer information available regarding the detailed structure of active chemical compounds, proper stability testing data, and shelf life of these plant products based on nematicides. Thus, this study was conducted to observe the effect of the leaf extract of *A. indica* (A. Juss) and *C. citratus* (DC.) Stapf on the 2nd juveniles' stage of *Meloidogyne* infestation at three-time durations i.e., 24, 48 and 72 hr (hours). Chemical profiling of compounds present in *A. indica* and *C. citratus* extract were also conducted to know the actual active compound responsible for nematicidal activity through GCMS.

MATERIAL AND METHODS

Establishment of *M. Incognita* culture and nematode extraction: Brinjal variety (Hybrid BS6-793) was grown on a nematode-free soil bed specially prepared in an isolated area in a poly house by following standard agronomical practice for pure culture establishment. The study was carried out during the brinjal crop cultivation season in the winter of 2021-22 (September – March at 28° 32' 7.8612" N, 77° 23' 27.7044" E). The nematode-infected plants were collected from the Amity Institute of Organic Agriculture, Amity University Noida Uttar Pradesh India. Females of root-knot nematodes were segregated on the basis of microscopic morphology, from the root-knot of affected plants. Later selected females were transferred to the host brinjal for obtaining pure nematode culture. The purified culture of *M. incognita* was verified, established and mass multiplied. Nematodes were extracted from soil by the modified Baermann funnel method (Cesarz et al 2019). For this, a rubber tube 10 cm in length and 3.3 cm in diameter was connected to the glass funnel stem. The distal end of the rubber tube was tightened with a clamp to regulate the water flow. This assembly was mounted on a funnel stand. Two-third length of the funnel was filled with water. A wire-mesh basket was placed over the funnel in such a manner that the lower end of the mesh was touching to water layer present in the funnel. On the top of the wire-mesh basket double layer of tissue paper was placed. such assembly was used for *M.*

incognita extraction. For *M. incognita* extraction from the brinjal plant, the nematode population containing nodes and associated soil mass of the targeted sample were spread out evenly on the tissue paper layer of the above-mentioned assembly. Adequate quantity of water was added to maintain the water level around 2-3 cm above from the wire mesh. Care was taken for the maintenance of water and soil contact throughout the extraction period. After 24hr clamp was removed and elute was collected from the rubber tube. Elute was filtered from the 400µm sieve for nematode harvesting collected nematode population were diluted in 20 ml amount of water. Nematode density was measured by counting the number of *M. incognita*. The stages of *M. incognita* were identified by microscopic examination and 2nd juvenile stages were utilized for the bioassay process.

Plant extract preparation: From each selected plant, leaves were collected, washed with water, and air dried and 30g leaves were soaked in 300ml HPLC (High-Performance Liquid Chromatography) grade distilled hexane for 24 hr then it was filtered through Whatman no. 1 filter paper. Anhydrous sodium sulfate was added to the filtrate @1/10 gm leaf samples. This was kept for 2 hr for dehydration. After dehydration, it was filtered through Whatman no. 1 filter paper for the removal of sodium sulfate (Singh et al 2019). The filtrate was run through a silica gel column (60-120 mesh size). The extract distillation was conducted at 60-70ºC in a round bottom flask. The residues left over at the bottom of the flask were collected in a glass vial by rinsing with a small quantity of HPLC grade distilled hexane. The solvent was evaporated completely, the residues were collected and diluted with 25ml HPLC grade hexane for preparing different concentrations (100, 50 and 25%) for use in bioassay and Gas Chromatography-Mass Spectrometry (GCMS) studies.

Gas chromatography-mass spectrometry analysis: GCMS analysis for the identification of compounds present in plant extracts was conducted by employing SHIMADZU-GCMSQP2010ULTRA. It was equipped with an Rtx-5 MS column measuring 30 m×0.25 mm I.d. × 0.25 µm film thickness. An electron ionization system was utilized and operated in electron impact form which produced an ionization energy of 70eV. Helium gas (99.9% pure) was adopted as carrier gas. The flow rate of carrier gas was 1 ml/min. The injection volume of the extract was 1 µl (a split ratio of 10:1). The Gas Chromatography column oven's initial temperature was 60°C. The injector temperature was 260°C and the source ion temperature was 230°C. The total run time provided for each sample was 60 min. The mass-to-charge ratio (m/z) taken was 40.00. Illumination on the mass spectrum was carried out using the database of the National Institute of Standards and Technology (NIST). Host plant Brinjal (Hybrid BS6-793) was established for pure culturing *M. incognita*. The purified culture of *M. incognita* was established and mass multiplied.

Impact assessment of plant extract on *M. incognita* population: The tissue culture plate of polystyrene consisting of 24 Wells (each of 2.5 ml capacity) was selected as a platform for in vitro assessment of three concentrations i.e.,100, 50, and 25% of lemongrass and neem on the 2nd juvenile stage of *M. incognita*. For this 500 µl of nematode-rich pure culture and a 100 µl selected concentration of the targeted extract were mixed. For each concentration of targeted extract 3 sets (each containing 10 replicas) were maintained. Along with this for both the extracts control were also maintained (each containing 10 replicas) in a similar kind of Tissue Culture Plate. In one set of control plate 500 µl nematode culture and 100 µl hexane were applied. While in another by using 500 µl nematode culture and 100 µl water was utilised. The mortality status of the nematode population was estimated for each sample by counting the number of live as well as dead individuals in the entire volume of each well of the designated set at different time durations i.e., 24, 48 and 72 hr.

Statistical analysis: All data were analysed by using Windostat software (version 8.5) developed by Indostat Services, Hyderabad, India.

RESULTS AND DISCUSSION

Effect of 100, 50 and 25% dilutions of volatile cues present in lemongrass hexane leaf extract were observed by measuring the mean value of no. of live and dead individuals of root-knot nematodes *M. incognita*. Mortality status was noticed at different time durations (24, 48 and 72 hr). In Lemongrass, mortality status after 72 hr was observed to be 31.40% in 50% concentration and 30.76% in 100 % concentration. Maximum mortality (37.01%) was observed for lemongrass extract after 48 hr at an application of 25% concentration. For Neem extract the highest mortality (29.46%) was recorded after 24 hr on use of 50% concentration followed by 27.80% (24 hr duration and 25% concentration). Least mortality was observed for neem extract.

Chemical profiling of lemongrass shows 66 fractions whereas from neem 36 fractions were obtained. The analysis of various fractions of neem and lemongrass signified the presence of alkane, alkene, alcohol, aldehydes, carboxylic, triene, terpene and ketones. Out of these all compounds having a similarity index of more than or equal to 95% were targeted. Dodecane and Neophytediene exist in both extracts. Pentadecane, heneicosane, pentacosane, tetracontane, hexacosane and hexatriacontane were

prominent alkanes present in neem extract whereas Decane, dodecane, tetradecane, octadecane, dodecylcylohexane, octacosane eicosane tetratetracontane were recorded from lemongrass extract. Izuogu et al (2015) observed that lemongrass at 75% aqueous extract gives the best results due to the presence of tannin, crude alkaloids, saponnins and crude extracts against. *Meloidogyne* species*, Pratylenchus*, *Helicotylenchus*, *Radopholus*, *Rotylenchus* and *Xiphinema*.

Table 1. Effect of lemongrass and neem leaf extracts on mortality of root-knot nematode

Concentration	24hr		48 hr		72 hr	
	Lemongrass	Neem	Lemongrass	Neem	Lemongrass	Neem
100%	18.92	25.59	19.02	26.94	30.76	19.07
50%	31.72	29.46	27.40	18.32	31.40	18.89
25%	22.98	27.80	37.01	18.45	28.20	19.42
Control	7.06	07.06	05.85	05.85	06.60	06.06
Hexane	16.23	16.23	16.88	16.88	25.25	25.25

Table 2. Chemical profile of lemongrass and neem

Name of compound ≥ 95%	Lemon grass		Neem	
	Percent area	S.I.	Percent area	S.I.
Decane	0.23	96	-	-
Dodecane	0.83	97	0.83	97
Cyclohexane, Hexyl-	0.31	97	-	-
1-Tetradecene	1.09	96	-	-
Cyclohexane, 1-Ethenyl-1-Methyl-2,4-Bis(1-M)	0.55	95	-	-
Tetradecane	2.15	97	-	-
Cyclohexane, Octyl-	0.51	96	-	-
1,6-Cyclodecadiene, 1-Methyl-5-Methylene	0.81	95	-	-
E-14-Hexadecenal	2.30	97	-	-
Hexadecane	2.28	97	-	-
Cyclohexane, Decyl-	0.45	96	-	-
Eicosene, (E)	1.86	96	-	-
Octadecane	1.75	97	-	-
Neophytadiene	0.56	95	0.19	96
Dodecylcyclohexane	0.39	97	-	-
1-Nonadecene	1.00	96	-	-
Octacosane	0.46	96	-	-
N-Heptadecylcyclohexane	0.10	95	-	-
Eicosane	0.19	95	-	-
Tetratetracontane	0.51	95	-	-
Pentadecane	-	-	0.22	97
Gamma. -Elemene	-	-	0.17	96
Germacrene B	-	-	0.17	96
Heneicosane	-	-	0.07	97
Pentacosane	-	-	0.74	98
Squalene	-	-	0.24	96
Tetracontane	-	-	15.01	97
Hexacosane	-	-	1.02	97
Hexatriacontane	-	-	1.77	96

Nile et al (2018) studied the nematotoxic potential of neem plants using in vitro and in-planta trials against *M. incognita*. Neem extracts were lethal to second-stage juvenile (J2) and egg hatching. Chavan et al (2021) observed lemongrass, basil and peppermint oil showed the greatest mortality at 96 hr. Shakya and Yadav (2020) observed that neem was the most effective against *M. javanica* in vitro. Divya et al (2021) observed *Purpureocillium lilacinum* in combination with neem cake gave the best results. In accordance with these studies, the elevated mortality rate of *M. incognita* could be ascribed due to the variation of compounds present in lemongrass, especially straight-chain alkanes.

CONCLUSION

Treatment of the host plant with a 25% concentration of lemongrass extract was most effective against *Meloidogyne* species. In further study effective root-knot nematodes repellent compound/s present in the extract may be identified by testing the efficacy of individual compounds to validate their activity. After validation, these compounds may be formulated in an inert base as a commercial eco-friendly nematicide for minimizing infestation of *Meloidogyne* species.

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Damage of Galleria mellonella in Apis mellifera Colonies and Stored Combs

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***Abstract*:** The present investigation was carried out at Chaudhary Charan Singh Haryana Agricultural University, Hisar for two years (2016 and 2017). The comb area damaged by *Galleria mellonella* varied during different months. During both the years, comb area damaged by *G. mellonella* started increasing in April and increased till July. From August, the damaged area started decreasing in different and reached to lowest limit in March. The maximum comb area damaged was d in July. During both the years, the mean comb area damaged was lower in stronger colonies as compared to weak colonies. Correlation studies between comb area damaged by larvae in 4, 6 and 8 frame strength and weather parameters showed significant positive correlation with maximum temperature and maximum RH. In stored combs, cumulative comb area damaged per frame increased with the passage of time from June to October. In October, comb area damaged per frame was maximum when ten combs were stored in a hive body followed by 9, 8 and 7 combs whereas the minimum area damaged was in 6 combs. The correlation study indicated significant positive correlation between extent of comb area damaged by wax moth larva with maximum temperature, maximum humidity and non-significant with minimum temperature and minimum RH.

Keywords: Stored combs, Damaged comb area, Bee colonies, Weather parameters

Most serious pest of honey bees, *Galleria mellonella* is cosmopolitan in nature causing serious economic damage to honey bee colonies and combs during storage (Gillard 2009 and Ellis et al 2013). Larvae feed on the wax comb in weak bee colonies or during the storage of wax combs in winter (Caron 1999). Wax moth larvae remains in beehives which feed on wax and young bees, fill the hive tunnels with silk threads and start reducing the web and debris to a mass of combs and fecal matter of the larvae, condition described as "*Galleriasis*." The larvae are especially harmful to colonies that are old or unguarded and to stored combs also. Weak colonies are much more vulnerable to wax moths infestation with especially in comparison with strong colonies (Basavarajappa 2011). The information on comb damage by greater wax moth in *Apis mellifera* colonies is little in stored combs in India. So, keeping these above facts in view, the present study comb area damaged by *Galleria mellonella* in *Apis mellifera* colonies and stored combs and its correlation with weather parameters was planned.

MATERIAL AND METHODS

Five colonies each with 4, 6, 8 and 10 frame bees strength were selected among the apiary with the same queen age and equalized w.r.t bee strength, brood (unsealed and sealed) and food, stores (pollen, nectar and honey). Each comb after brushing off the bees was held against the sunlight and observations at an interval of 21 day were recording comb damage. Extent of comb area damaged in stored combs (7, 8, 9 and 10 empty combs of without bees) was recorded at fortnightly interval. The comb area damage caused by the larvae of *G. mellonella* was recorded using brood measuring frame having wire grid squares, each measuring one inch squares in size. The number of squares covering the infested area completely or partially were counted as damaged and then converted to cm^2 by multiplying with 6.45. The experiments were conducted with five replication for recording damage during both the years. Temperature and relative humidity (%) data were collected from observatory of the Department of Agriculture Meteorology, CCS Haryana Agricultural University, Hisar and data were correlated with extent of damage.

Statistical analysis: Data were statistically analyzed with the OPSTAT software system.

RESULTS AND DISCUSSION

Comb area damaged by *G. mellonella* in *A. mellifera* colonies: During 2015-16, the comb area damaged by *G. mellonella* in 4 frame bee strength colonies started increasing in April (212.85 cm^2) and increased till July (1376.00 cm^2) and there after started decreasing and lowest in March (86.00 cm^2) (Table 1). The maximum comb area damaged was in July (1376.00 cm^2). The pattern of comb area damage was same in case of 6, 8 and 10 frame bee strength colonies. In 10 frame bee strength colonies started

increasing in April (101.05 cm^2) and increased till July (602.00 cm^2) and thereafter started decreasing and lowest in March (32.25 cm^2). The maximum comb area damaged was in July (602.00 cm^2). Irrespective of the bee strength, the maximum comb area damaged (cm^2) was recorded in July (1037.38) whereas the minimum in March (61.28). However, the mean area damaged was lowest in 10 frame bee colonies (234.14 cm^2) followed by 8, 6 and 4 frame bee colonies. The overall average number of wax moth infected combs in a colony was 1.16 combs which were higher than average infested combs (0.9) reported by Amsalu (2011).

Correlation between weather parameters and comb area damage by *G. mellonella* in *A. mellifera* colonies: During 2015-16, the mean combs damage area in all frame strength (4, 6, 8 and 10) was maximum in July (1037.38 cm^2), whereas lowest comb damage in March (61.28 cm^2). In 2016-17, maximum damaged area (995.45 cm^2) were in July and minimum was in March (54.83 cm^2). During year 2015-16, July recorded as maximum temperature (35.10°C), minimum temperature (26.10°C), maximum RH (90%), minimum RH (72%) and number of bright sunshine hour (5.9 hour). In March, maximum temperature (29.90°C), minimum temperature (13.70°C), maximum RH (88%), minimum RH (46%) and number of bright sunshine hour (8.10 hour) were recorded. In 2016-17, weather parameters in July were recorded as maximum temperature (35.10°C), minimum temperature (27.00°C), maximum RH (88%), minimum RH (67%) and number of bright sunshine hour (6.8 hour). In March, the average of weather parameters were recorded as maximum temperature (29.90°C), minimum temperature (13.70°C), maximum RH (88%), minimum RH (46%) and number of bright sunshine hour (8.10 hour). There was significant positive correlation exists between comb area damage with maximum temperature, maximum humidity and negatively non-significant with minimum temperature and minimum RH (Table 3). In 4**-** frame bee strength whereas no correlation existed between comb damage and sunshine hours. Significant positive correlation of comb area damage with maximum temperature in 6 and 8 frame bee strength maximum humidity and non-significant with minimum temperature and minimum RH, respectively in year 2015-16 while in year 2016-17, correlation of comb damage with maximum temperature in 6 and 8 frame, maximum humidity and negatively non-significant with minimum RH and positively non-significant with minimum temperature. There was a non-significant correlation between comb area damage by larvae in 10 frame bee strength with maximum temperature, minimum temperature, maximum RH, minimum RH and sunshine hours during 2015-16 and 2016-17. The significant correlation was between the comb area

Table 1. Comb area damaged by *G. mellonella* in *A. mellifera* colonies (cm^2)

Mean bee frame strength in different year	Comb area damage in									
	2015-16					2016-17				
Month	4	6	8	10	Mean	4	6	8	10	Mean
November	559.00	462.25	359.05	242.95	405.81	548.25	359.05	301.00	275.20	370.88
December	232.20	152.65	103.35	70.95	138.71	189.20	137.60	73.10	70.95	106.43
January	111.80	101.50	86.00	66.65	92.56	101.05	86.00	70.95	30.10	82.24
February	101.05	86.00	70.95	32.25	72.56	101.05	70.95	70.95	25.80	68.26
March	86.00	70.95	55.90	32.25	61.28	70.95	70.95	55.90	21.50	54.83
April	212.85	150.50	118.25	101.05	145.66	176.30	122.55	118.25	86.00	125.78
May	548.25	425.70	202.10	163.40	334.86	473.00	361.20	172.00	150.50	289.18
June	599.85	488.05	326.80	219.39	408.52	520.30	447.20	236.50	187.05	347.76
July	1376.00	1268.50	903.00	602.00	1037.38	1333.00	1204.00	885.80	559.00	995.45
August	1182.50	1098.65	774.00	464.00	879.79	1019.10	935.25	526.75	376.25	697.14
September	1014.80	922.35	623.50	451.50	753.03	857.85	771.85	457.95	359.00	611.66
October	911.60	774.00	612.75	363.35	657.92	752.50	660.05	436.45	333.25	545.56
Mean	577.99	500.09	352.97	234.14		511.87	435.55	283.8	206.21	
Factors			C.D.					C.D.		
Month			0.62					0.62		
Bee strength			0.35					0.35		
Month × Bee strength			1.24					1.24		

damage with maximum temperature, maximum humidity, and negatively non-significant with minimum RH in 4, 6 and 8 frame strength. Correlation did not exist between comb area damage and sunshine hours in 4, 6, 8 and 10 frames bee strength during both the years. Raghunandan and Basavarajappa (2014) reported infestation in semi-arid region during summer (30.8%) followed by rainy season (23.4%). However, the infestation was less 11.0 and 6.6% during summer and winter seasons respectively in Malnad.

Effects of storing different number of combs on damage by *G. mellonella* during June to October: Data on comb area damaged by *G. mellonella* by storing different number of combs during June to October in two consecutive years (Table 3). During 2016, data indicated that in case 7 combs were stored, the area damaged per comb was minimum at the time of first observation in mid-June (8.60 cm^2). In the following observations, the cumulative area damaged per frame increased till the end of September (169.85 cm^2). In October, area damaged did not increased and was almost same as in the end of September. The area damaged per comb was 170.28 and 170.32 cm^2in mid-October and end October, respectively. The pattern of damage during different

Table 2. Correlation between weather parameters and comb area damage by *G. mellonella* in *A. mellifera* colonies

Weather parameters	2015-16				2016- 17			
	Number of bee frames covered by bee							
	4	6	8	10	4	6	8	10
Max. temp. (°C)	0.852*	0.767**	0.792**	0.820**	0.793*	0.767**	0.786**	0.817**
Min. temp. (°C)	0.697	-0.635	-0.651	-0.688	-0.635	-0.565	-0.617	-0.659
Max. RH (%)	0.686**	0.222**	0.232**	0.162**	0.645**	0.191**	0.206**	0.144**
Min. RH (%)	-0.267	-0.251	-0.258	-0.240	-0.315	-0.302	-0.290	-0.267
Bright sunshine (hr)	0.130	0.043	0.006	0.023	0.134	0.036	0.003	-0.023

*, ** Significant at 5% and 1% level of significance

Table 3. Effects of storing different number of combs on damage by *G. mellonella* during June to October 2016 and 2017

Number of combs stored	Cumulative comb area damaged per frame (cm^2) in 2016										Mean
	June		July		August		September		October		
	15.6.16	30.6.16	15.7.16	30.7.16	15.8.16	30.8.16	15.9.16	30.9.16	15.10.16	30.10.16	
7 Frame	8.60	12.90	20.43	40.85	96.75	107.50	144.05	169.85	170.28	170.32	94.14
8 Frame	10.75	19.35	25.80	58.05	101.05	122.55	169.85	189.20	189.41	190.00	107.54
9 Frame	12.90	21.50	32.25	79.55	118.25	141.90	180.60	202.10	202.31	202.31	119.36
10 Frame	19.35	23.65	38.70	133.30	139.75	161.25	193.50	219.30	219.51	219.52	136.78
Mean	12.90	19.35	29.32	77.94	113.95	133.30	172.00	195.11	195.38	195.58	-
Factors						C.D.					
Month						0.18					
Comb number						0.29					
Month × Comb number						0.59					
	Cumulative comb area damaged per frame (cm^2) in 2017										
7 Frame	9.68	13.98	21.50	43.00	101.05	109.65	148.35	174.15	174.36	174.40	97.00
8 Frame	9.57	19.89	27.95	60.20	105.35	126.85	172.00	199.95	200.16	200.16	112.20
9 Frame	15.05	22.58	33.33	81.70	122.55	146.20	182.75	204.25	204.46	204.50	121.73
10 Frame	20.43	24.73	39.78	135.45	141.90	167.70	197.80	223.60	223.81	223.89	139.90
Mean	13.68	20.30	30.64	80.09	117.71	137.60	175.23	200.49	200.70	200.73	-
Factors						C.D.					
Month						0.17					
Comb number						0.28					
Month × Comb number						0.57					

Each value represents mean of five observation

Table 4. Correlation of weather parameters with comb area damaged by *G. mellonella* in stored combs

Number of combs stored in different years Weather parameters	Number of combs stored/ hive body							
	2016				2017			
	7	8	9	10	7	8	9	10
Max. temp. (°C)	0.703*	0.767**	0.792**	0.820**	0.704*	0.767**	0.786**	0.817**
Min. temp. (°C)	-0.512	-0.604	-0.651	-0.688	-0.475	-0.565	-0.617	-0.659
Max. RH (%)	0.227**	0.222**	0.232**	0.162**	0.222**	0.191**	0.206**	0.144**
Min. RH (%)	-0.267	-0.251	-0.258	-0.240	-0.315	-0.302	-0.290	-0.267
Bright sunshine (hr)	0.130	0.043	0.006	0.023	0.134	0.036	0.003	-0.023

*, **Significant at 5% and 1% level of significance

months was same when 8, 9 and 10 combs were stored. At the time of first observation (mid-June), the area damaged per comb (cm^2) was significantly higher when 10 combs were stored (19.35) than 9 (12.90), 8 (10.75) and 7 combs (8.60). On 30th October, the area damaged (cm^2) was maximum (219.52) when 10 combs were stored followed by 9, 8 and 7 combs. From June to October, the area damaged per comb (cm^2) was maximum in case 10 combs were stored followed by 9, 8 and 7 comb storage. The pattern of damage during different months was almost same in year 2017 also i.e. highest damage was recorded in 10 combs were stored followed by 9, 8 and 7.

Correlation of weather parameters with comb area damaged by *G. mellonella* in stored combs: The damage caused by *G. mellonella* in the stored combs of different frames strength (4, 6, 8 and 10 frames) was recorded from June to October 2016 and 2017 (Table 4). This indicated that the mean comb area damaged in all frame was maximum in 30th September (195.11 cm^2 and 200.49 cm^2), whereas comparatively lowest during first fortnight of June (12.90 cm^2 and 13.68 cm^2, respectively) during the year 2016 and 2017. The correlation study indicated significant positive correlation of comb area damage with maximum temperature, maximum humidity and negatively non-significant with minimum temperature and minimum RH. Significant positive correlation were in 8, 9 and 10 comb damage with maximum temperature maximum humidity and negatively non-significant with minimum temperature and minimum RH.

There was no significant relation found with the comb damage and sunshine hours during both the year in all frame strength i.e. 7, 8, 9 and 10. Sohali (2017) also reported that the minimum and maximum temperatures were strongly correlated with wax moth abundance and consequently, the percent of hives with moth damaged combs.

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CONCLUSIONS

In both the years, the mean comb area damaged was lower in stronger colonies as compared to weak colonies. Correlation between comb area damaged by larvae in 4, 6 and 8 frame strength with the weather parameters showed significant positive correlation with maximum temperature and maximum RH, whereas significant negative correlation with minimum temperature and minimum RH and number of bright sunshine hours. Cumulative comb area damaged per frame was maximum when ten combs were stored in a hive body followed by 9, 8 and 7 combs. There was significant positive correlation between extent of comb area damaged by wax moth larvae and maximum temperature, maximum humidity and non-significant with minimum temperature and minimum RH.

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Studies on External Male and Female Genitalia of Five Species of *Miresa* Walker (Lepidoptera: Limacodidae) with New Species and New Record from Western Ghats, India

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***Abstract*:** The communication deals with the examination of external male and female genitalia of five species of genus *Miresa* Walker. Out of which, four species i.e., *M. albipuncta* Herrich-Schäffer, *M. decedens* Walker, *M. argentifera* Walker, *M. nivaha* Moore were already known to literature. Besides, one species i.e., *M. chandani* sp. nov. is being described for the first time and *M. argentifera* Walker has been recorded for the first time from India. The updated information on their distribution will be helpful in the conservation of these species.

***Keywords*:** Genus, *Miresa* Walker, Species, Male & female genitalia, New species

Genus *Miresa* was erected by Walker in 1855 for its type-species *albipuncta* Herrich-Shaffer. Hampson (1892), in his fauna volume, divided this genus under two sections and studied a total number of six species with five from India and mainly relied upon external morphological characters and did not studied the external male and female genitalic characters. During present study on Limacodidae from the Western Ghats in India, authors observed that only few references are available on species under *Miresa* genus, hence the study was undertaken to observe the distribution of species under *Miresa* in area under reference for further studies for their conservation.

MATERIAL AND METHODS

The adult moths were collected with the help of portable light trap (Fig. 1) or fluorescent lights at night hours from different localities of Western Ghats of India (Fig. 2). The external morphological characters were studied for description of species. To study wing venation, permanent slides (Common (1970), Zimmerman (1978) has been followed. For the study of external male genitalia, methodology given by Robinson (1976) was followed. The diagrams of genitalia were drawn using stereo zoom binocular. The terminology given by Klots (1970) has been followed in the present studies for nomenclature purpose. All the collections were deposited in the Insect Museum, Department of Zoology & Environmental Sciences, Punjabi University, Patiala.

RESULTS AND DISCUSSION

Miresa Walker

Miresa Walker 1855. *List. Lep. Ins. Br. Mus*. 5: 1115.

Type-species: *Miresa albipuncta* Herrich-Shaffer.

Diagnosis: Antenna broadly bipectinate over the basal half; labial palpusupcurved, not reaching beyond frontal tuft, third segment incospicuous; mid and hind tibia with terminal pairs of spurs; forewing rufous brown with yellowish patches, a silvery white postmedial, associated with a central white triangle, costa nearly straight, apex rounded, termen convex, tornus rounded, R_3 and R_4 and R_5 stalked; hindwing pale yellow, rounded; male genitalia with well sclerotizeduncus, tip forming a spine; female genitalia with two signa.

Key to the studied species of genus *Miresa* Walker

1.	Male genitalia with valva furnished with few small hairs distally ….....….	*albipuncta* Herrich-Schäffer
-	Male genitalia with valva with a tuft of hairs distally …	2
2.	Male genitalia with valva with costa concave at base …	*decedens* Walker
-	Male genitalia with valva with costa convex at base	3
3.	Aedeagus with two spine-like cornuti ……..	*argentifera* Walker
-	Aedeagus without cornuti……..	4
4.	Valva with costal region relatively less sclerotized, uncus beset with short hairs ……..	*nivaha* Moore
-	Valva with costal region strongly sclerotized, uncus beset with rather long hairs	*chandani* sp. nov.

***Miresa albipuncta* Herrich-Schäffer (Plate 1, Figs.- A to H)**

Nyssia albipuncta Herrich-Schäffer, 1854. *Aussereurop. Schmett. L.f.* 179.

Miresa albipuncta Herrich-Schäffer, Samml. asussereur. Schmett. i., fig. 179; C. & S. no. 1305; Forsayeth, Trans. Ent. Soc. 1884, pl. 14, figs. 8 a-c (larva and pupa).

Miresa guttifera Walker, Cat. v, p. 1124; C. & S. no. 1312.

Male: Alar expanse 34 mm. Vertex and frons green; antennae bipectinate upto basal half, distal half serrated, brown; labial palpus porrect, light brown, second segment larger than third, third segment hardly visible; thorax fulvous yellow with a fine brown streak in middle; forewing with costa straight, apex quadrate, termen straight, tornus rounded, anal margin nearly straight, ground colour red-brown, a silvery white spot beyond the lower angle of cell, cilia red-brown; hindwing oval, ochreous, suffused with red brown towards costa, cilia yellowish; legs thickly covered with brown scales, tarsi with yellowish scales; abdomen fulvous yellows.

Wing venation : Forewing with Sc ending beyond middle of costal margin, R_1 from cell, R_2, R_3, R_4 and R_5 stalked, M_1 beyond upper angle, M_2 and M_3 on a short stalk originating from lower angle, Cu_1 before lower angle of cell, Cu_2 from cell, 1A and 2A present, 3A forked at base; hindwing with $Sc+R_1$ originating before middle of cell, Rs and M_1 stalked, M_2 towards middle of discocellulars, M_3 from lower angle, Cu_1

Fig. 1. Area surveyed

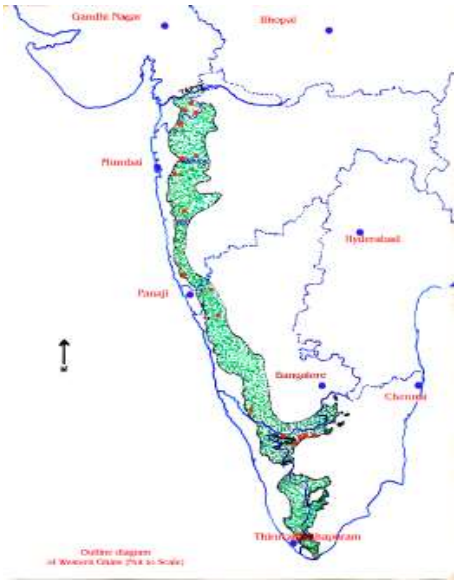

Fig. 2. Portable light trap

Table 1. Localities visited during the study and their geographical information

State	District	Locality	Geographical coordinates	Altitude
Gujarat	The Dangs	Ahwa	20.7606° N, 73.6912° E	520mASL
Karnataka	Chikmaglur	Kallathi Falls	13.5486° N, 75.7891° E	960mASL
	Dakshina Kannada	Gundya	12.8278° N, 75.5714° E	40mASL
	Kodagu	Baghamandala	12.3866° N, 75.5287° E	900mASL
		Medikeri	12.4244° N, 75.7382° E	1100mASL
	Uttar Kannada	Kulgi	15.1664° N, 74.6373° E	360mASL
		Ganeshgudi	15.2843° N, 74.5302° E	480mASL
		Jog Falls	14.2004° N, 74.7922° E	480mASL
		Kulgi	15.1664° N, 74.6373° E	360mASL
Kerala	Idukki	Marayoor	10.2762° N, 77.1615° E	960mASL
		Vallakadavu	8.4750° N, 76.9195° E	780mASL
	Kollam	Shendurney	8.8578° N, 77.2175° E	70mASL
	Palakkad	Mukkali	11.0587° N, 76.5402° E	560mASL
	Pathanamthitta	Vadaserikara	9.3307° N, 76.8209° E	30mASL

and Cu_2 from cell, 1A, 2A and 3A present.

Male genitalia: Male genitalia with uncus strongly built, highly sclerotized, much broader at base, setosed with long hair-like setae, tip blunt; gnathos biarmed at base with both the arms conjoint at second half, tip rounded; tegumen well formed, shoulder V-shaped; vinculum small, U-shaped; saccus small oval; valva well sclerotized, sparsely setosed with hair-like setae, flap-like, costa and sacculus not well marked, harpe and ampulla absent, cucullus and valvulla not differentiated; juxta flap-like; aedeagus moderately long and narrow, well sclerotized, almost straight, without spines, ductus ejaculatorius enters subapically.

Female genitalia: Not studied.

Material examined: India: Kerala: Dist. Idukki, Vallakadavu, 780mASL, 11.ix.2004, 01♂, coll. Amit Katewa; Karnataka: Dist. Chikmaglur, Kallathi Falls, 960mASL, 26.vii.2004, 01♂; Gujarat: Dist. The Dangs, Ahwa, 520mASL, 29.ix.2005, 01♂, coll. Amit Katewa.

Old distribution: Throughout India and Ceylon (Hampson, 1892).

Hampson (1892) reported the species *albipuncta* Herrich-Schäffer from throughout India and Ceylon without mentioning any specific locality from where it has earlier been collected. The present study will be helpful to mark particular areas about the availability of the species, under reference, for designing further studies on its biology and conservation. Alongwith this contribution, the male genitalia of this species is illustrated and studied in detail for the first time. The species can be easily differentiated from other species due to the presence of a silvery-white spot beyond the lower angle of cell and a faint silvery postmedial line on the forewing.

***Miresa decedens* Walker (Plate 2, Figs. A to H)**

Miresa decedens Walker, 1855. *List. Lep. Ins. Br. Mus*. **5**:1125.

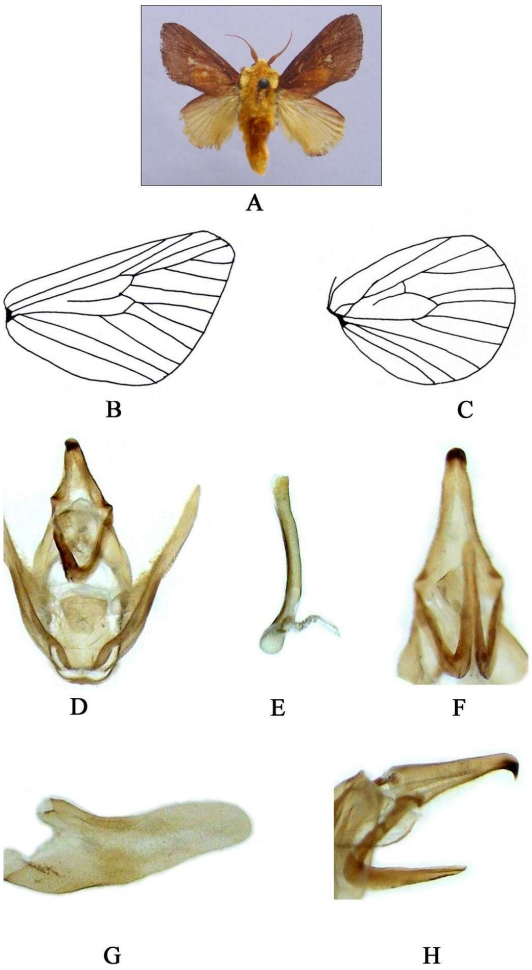

Plate-1: *Miresa albipuncta* Herrich-Schäffer

Figs.- A= Adult, B=Forewing, C= Hindwing, D= Male external genitalia, E= Aedeagus, F= Uncus and Gnathos ventral view, G= Valva, H= Uncus and Gnathos lateral view

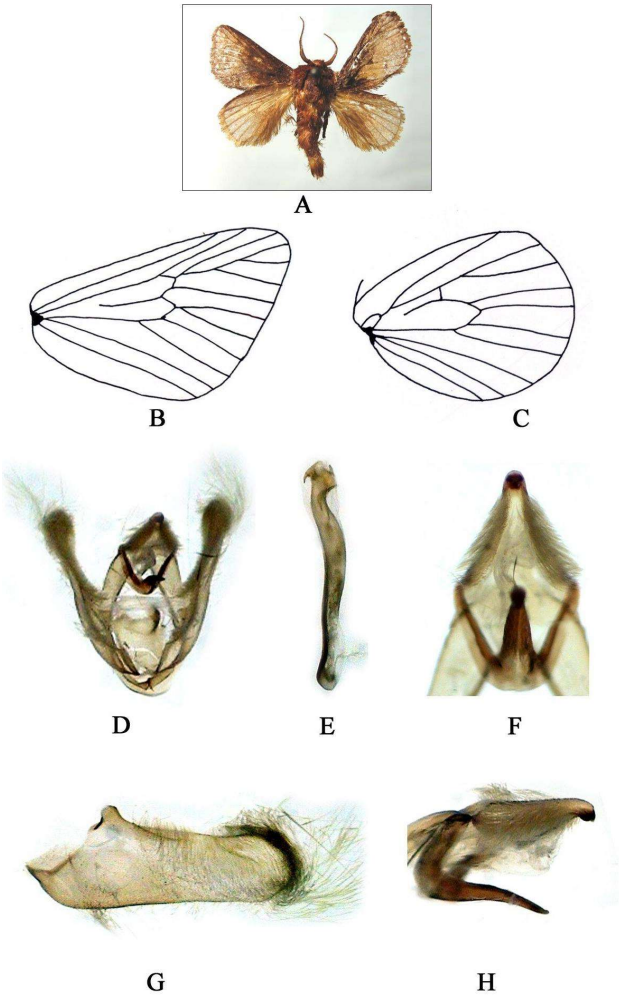

Plate-2: *Miresa decedens* Walker

Figs.- A= Adult, B=Forewing, C= Hindwing, D= Male external genitalia, E= Aedeagus, F= Uncus and Gnathos ventral view, G= Valva, H= Uncus and Gnathos lateral view

Male: Alar expanse : 31 mm. Vertex brown; frons green with a brown streak in middle; antennae bipectinate up to basal half, distal half serrated, brown; labial palpus porrect, brown, second segment larger than third, third segment hardly visible; thorax green with brown tinge, a streak in middle, brown at sides; forewing with costa straight, apex quadrate, termen slightly convex, tornus rounded, anal margin convex, ground colour dark brown, a broken ill-defined postmedial silvery line, curved from costa to M_3, some silvery scales towards outer margin; hindwing oval, dark brown, cilia dark brown at costal margin, rest dull brown; legs thickly cloathed with rusty red scales tinged with yellow; abdomen dull brown.

Wing venation : Forewing with Sc ending before posterior one-fourth of costal margin, R_1 arising from the posterior one-third of cell, R_2 arising in the posterior one-third of cell, R_3, R_4 and R_5 stalked, stalk originating from upper angle of cell, M_1 and M_2 almost straight and parallel to each other, M_3 arising from lower angle of cell, CuA_1 arising just before lower angle of cell, CuA_2 arising before posterior one-fourth of cell, CuP well developed, 1A+2A straight; hindwing with $Sc+R_1$ anastomosing with Rs basally after origin, Rs and M_1 connate at upper angle of cell, M_2 arising near M_3 than M_1, M_3 arising from lower angle of cell, CuA_1 arising well before lower angle of cell, CuA_2 arising beyond middle of cell, CuP well developed, 1A+2A and 3A present, well developed, gently diverging distally.

Male genitalia : Male genitalia with uncus strongly built, highly sclerotized, much broader at base, setosed with long hair-like setae, tip blunt; gnathos biarmed at base with both the arms conjoint at second half, tip rounded; tegumen well formed, shoulder V-shaped; vinculum small, U-shaped; saccus small oval; valva well sclerotized, densely setosed with hair-like setae, flap-like, costa and sacculus not well marked, harpe and ampulla absent, cucullus and valvulla not differentiated; juxta flap-like; aedeagus moderately long and broad, well sclerotized, slightly curved at middle, two prominent spines present near tip, ductus ejaculatorius enters subapically.

Female genitalia: Not studied.

Material examined : India: Karnataka: Dist. Dakshina Kannada, Gundya, 40mASL, 28.vii.2004, 02♂♂; Dist. Uttar Kannada Kulgi, 360mASL, 16.vii.2007, 01♂, coll. Amit Katewa.

Hampson (1892) reported the species *decendens* Walker from Nilgiris and Assam.

***Miresa argentifera* Walker(Plate 3, Figs. A to H)**

Miresaargentifera Walker, 1855. *List. Lep. Ins. Br. Mus.* **5**: 1124.

Male and Female : Alar expanse : 30 mm; 36 mm. Vertex brown, frons green with a brown streak in middle; antennae bipectinate upto basal half, distal half serrated in male, simple in female, brown; labial palpus porrect, brown, second segment larger than third, third segment hardly visible; thorax green with a fine brown streak in middle, brown at sides; forewing with costa straight, apex quadrate, termen slightly convex, tornus rounded, anal margin convex, ground colour brown, a broken ill-defined postmedial silvery line, curved from costa to Cu_2, then straighter and more prominent to inner margin, some silvery scales towards outer margin, a series of silvery marginal spots; hindwing oval, brown, cilia brown at costal margin to tornus, yellow at anal margin; legs thickly cloathed with rusty red scales tinged with yellow; abdomen yellow with some brown scales at few starting segments.

Wing venation: Forewing with Sc ending beyond middle of costal margin, R_1 from cell, R_2, R_3, R_4 and R_5 stalked, M_1 beyond upper angle, M_2 and M_3 on a short stalk originating from lower angle, Cu_1 before lower angle of cell, Cu_2 from cell,

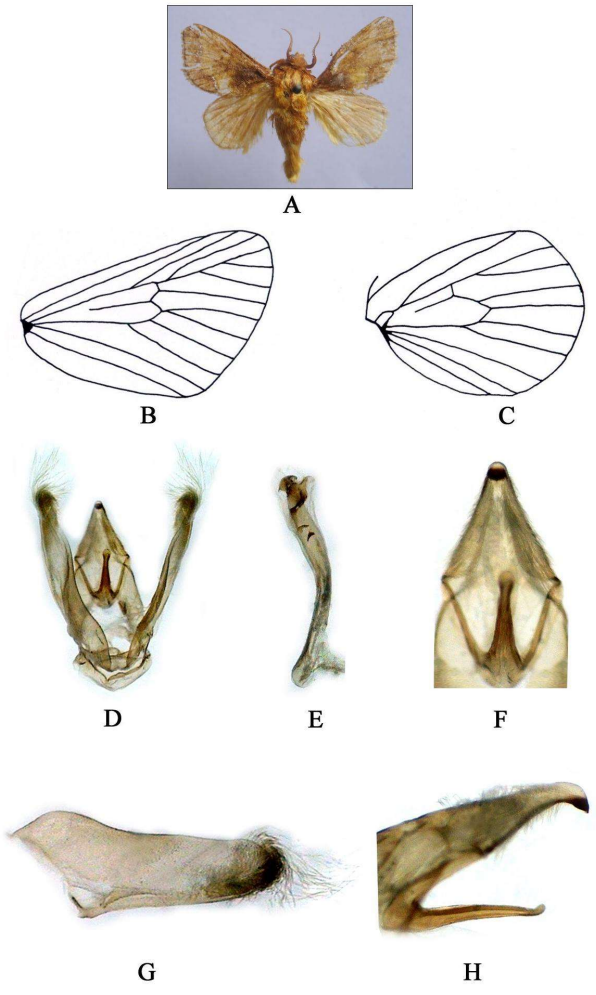

Plate-3: ***Miresa argentifera*** **Walker**

Figs.- A= Adult, B=Forewing, C= Hindwing, D= Male external genitalia, E= Aedeagus, F= Uncus and Gnathos ventral view, G= Valva, H= Uncus and Gnathos lateral view

1A and 2A present, 3A forked at base; hindwing with Sc+R_1 originating before middle of cell, Rs and M_1 stalked, M_2 towards middle of discocellulars, M_3 from lower angle, Cu_1 and Cu_2 from cell, 1A, 2A and 3A present.

Male genitalia: Male genitalia with uncus strongly built, broad at base, tapering towards tip, setosed with fine setae, tip giving the appearance of bird's beak; gnathos present; tegumen well sclerotized; vinculum U-shaped; saccus present; valva simple, without any projections, broad towards distal end, tip blunt with long hairlike setae; transtilla membranous; juxta simple, flap-like; aedeagus long and narrow, slightly curved, bulbuous at distal end, hook-like structure present at proximal end, three or four cornuti present, ductus ejaculatorious enters subapically.

Female genitalia: Not studied.

Material examined: India: Kerala: Dist. Idukki, Vallakadavu, 780mASL, 10.ix.2004, 03♂♂, 11.ix.2004, 01♂; Dist. Palakkad, Mukkali, 560mASL, 19.ix.2004, 01♂, 21.ix.2004, 01♂, 22.ix.2004, 01♂, 01♀; Dist. Idukki, Marayoor, 960mASL, 15.ix.2004, 01♂; Dist. Kollam, Shendurney, 70mASL, 03.ix.2004, 07♂♂; Dist. Pathanamthitta, Vadaserikara, 30mASL, 07.ix.2004, 02♀♀, coll. Amit Katewa; Karnataka: Dist. Uttar Kannada, Jog Falls, 480mASL, 24.vii.2004, 01♂; Dist. Kodagu, Medikeri, 1100mASL, 25.ix.2003, 01♀; Dist. Kodagu, Baghamandala, 900mASL, 31.vii.2004, 01♂; Dist. Uttar Kannada, Ganeshgudi, 480mASL, 20.vii.2004, 04♂♂; Dist. Uttar Kannada, Kulgi, 360mASL, 16.vii.2004, 01♂, coll. Amit Katewa.

Old distribution: Ceylon (Hampson, 1892).

In view of aforesaid distribution, the species *argentifera* Walker is recorded for the first time from India.

***Miresa nivaha* Moore (Plate-4, Figs. A to H)**

Miresa nivaha Moore, 1858-59. *Cat. Lep. Mus. E.I.C.* **2**: 413.

Male and Female : Alar expanse 32 mm, 40 mm. Vertex and frons dark brown; antennae bipectinate upto basal half, distal half serrated in male, simple in female, brown; labial palpus porrect, dark brown, second segment larger than third, third segment hardly visible; thorax dark brown; forewing with costa straight, apex quadrate, termen straight, tornus rounded, inner margin straight, ground colour dark brown, a broken ill-defined postmedial silvery line, more prominent in inner margin, a series of silvery marginal spots; hindwing oval, silky brown with yellowish tinge, cilia dark at tips; legs cloathed with dark brown scales; abdomen rusty red.

Wing venation : Forewing with Sc ending before posterior one-fourth of costal margin, R_1 arising from the posterior one-third of cell, R_2 arising in the posterior one-third of cell, R_3, R_4 and R_5 stalked, stalk orginating from upper angle of cell, M_1 and M_2 almost straight and parallel to each other, M_3 arising from lower angle of cell, CuA_1 arising just before lower angle of cell, CuA_2 arising before posterior one-fourth of cell, CuP well developed, 1A+2A straight; hindwing with Sc+R_1 anastomosing with Rs basally after origin, Rs and M_1 connate at upper angle of cell, M_2 arising near M_3 than M_1, M_3 arising from lower angle of cell, CuA_1 arising well before lower angle of cell, CuA_2 arising beyond middle of cell, CuP well developed, 1A+2A and 3A present, well developed, gently diverging distally.

Male genitalia : Male genitalia with uncus strongly built, highly sclerotized, much broader at base, setosed with long hair-like setae, tip blunt; gnathos biarmed at base with both the arms conjoint at second half, tip rounded; tegumen well formed, shoulder V-shaped; vinculum small, U-shaped; saccus small oval; valva well sclerotized, densely setosed with hair-like setae, flap-like, costa and sacculus not well

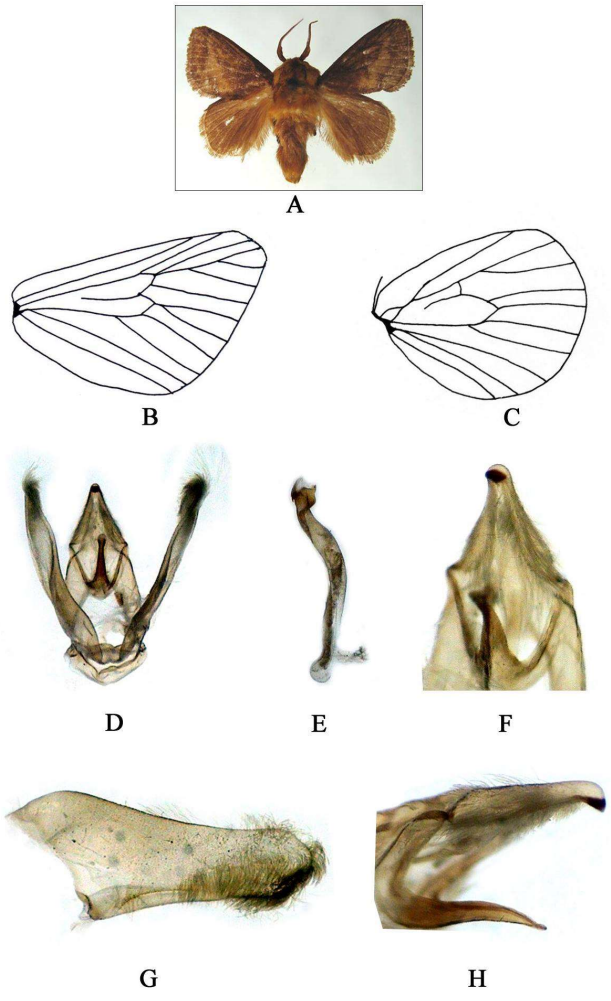

Plate-4: *Miresa nivaha* Moore

Figs.- A= Adult, B=Forewing, C= Hindwing, D= Male external genitalia, E= Aedeagus, F= Uncus and Gnathos ventral view, G= Valva, H= Uncus and Gnathos lateral view

marked, harpe and ampulla absent, cucullus and valvulla not differentiated; juxta flap-like; aedeagus moderately long and broad, well sclerotized, highly curved, two small spines present near tip, ductus ejaculatorius enters subapically.

Female genitalia: Not studied.

Material examined: India: Kerala: Dist. Idukki, Vallakadavu, 780mASL, 10.ix.2004, 01♂; Dist. Palakkad, Mukkali, 560mASL, 22.ix.2004, 02♂♂; Dist. Kollam, Shendurney, 70mASL, 03.ix.2004, 01♂, coll. Amit Katewa; Karnataka: District Uttar Kannada, Ganeshgudi, 480mASL, 21.vii.2004, 01♂; Dist. Uttar Kannada, Jog Falls, 480mASL, 29.vii.2004, 01♂; Dist. Uttar Kannada, Kulgi, 360mASL, 17.vii.2004, 02♂♂; Dist. Dakshina Kannada, Gundya, 40mASL, 28.vii.2004, 04♂♂, coll. Amit Katewa.

Distribution: The species *nivaha* Moore has earlier been reported from a single locality *i.e.,* Canara in Karnataka by Hampson (1892). However, the same has been collected from the aforesaid localities of the said state for the first time. Its collection from the state of Kerala is a new record.

***Miresa chandani* sp. nov. (Plate-5, Figs. A to H)**

Male: Alar expanse : 28 mm. Vertex and frons dark brown; antennae basal half bipectinate, distal half serrated, brown; labial palpus upturned, reaching upto frons, second larger than third, third segment hardly visible, dark brown; thorax dark silky brown; forewing with costa straight, apex quadrate, termen convex, tornus rounded, anal margin straight, ground colour dark silky brown, a broken ill-defined postmedial line from beyond middle of costa to nearly middle of anal margin, some silvery scales towards outer margin cilia silky brown; hindwing with costa convex, apex rounded, termen convex, tornus rounded, anal margin convex, ground colour light silky fuscous, cilia silky fuscous; legs covered with dark silky brown scales, hind tibia with long brown scales which darkest at tip; abdomen silky brown; anal tuft fuscous.

Wing venation: Forewing with Sc ending beyond middle of costal margin, R_1 from cell, R_2, R_3, R_4 and R_5 stalked, M_1 beyond upper angle, M_2 and M_3 on a short stalk originating from lower angle, Cu_1 before lower angle of cell, Cu_2 from cell, 1A and 2A present, 3A forked at base; hindwing with $Sc+R_1$ originating before middle of cell, Rs and M_1 stalked, M_2 towards middle of discocellulars, M_3 from lower angle, Cu_1 and Cu_2 from cell, 1A, 2A and 3A present.

Male genitalia : Male genitalia with uncus strongly built, slightly wavy, setosed with long setae, tip blunt, highly sclerotized; gnathos pointed; tegumen moderately broad, long; vinculum short, U- shaped; saccus present; valva simple, without any projection, costa and succulus well-marked, tip blunt, dressed with long hair-like setae; transtilla membranous; juxta short; aedeagus long and narrow, slightly curved, a pair of well formed spines present on distal end, vesica small, membranous, without any bifurcation, cornuti absent, ductus ejaculatorious enters subapically.

Female genitalia: Not studied.

Material examined: Holotype: India: Karnataka: Dist. Uttar Kannada, Kulgi, 360mASL, 17.vii.2004, 01♂, coll. Amit Katewa.

Larval host plant: Unknown.

Etymology: The species is named as *chandani* sp. nov. on the basis of an academician and poet.

Remarks: A phenon/complex comprising forty-four specimens has been identified into four already known species *i.e., Miresa albipuncta* Herrich-Schäffer, *Miresa decedens* Walker, *Miresa argentifera* Walker and *Miresa nivaha* Moore. One of the individuals collected from the locality Ganeshgudi is though completely congeneric to the aforesaid species, yet differ from them in respect of maculation of the wings, shape of the aedeagus and

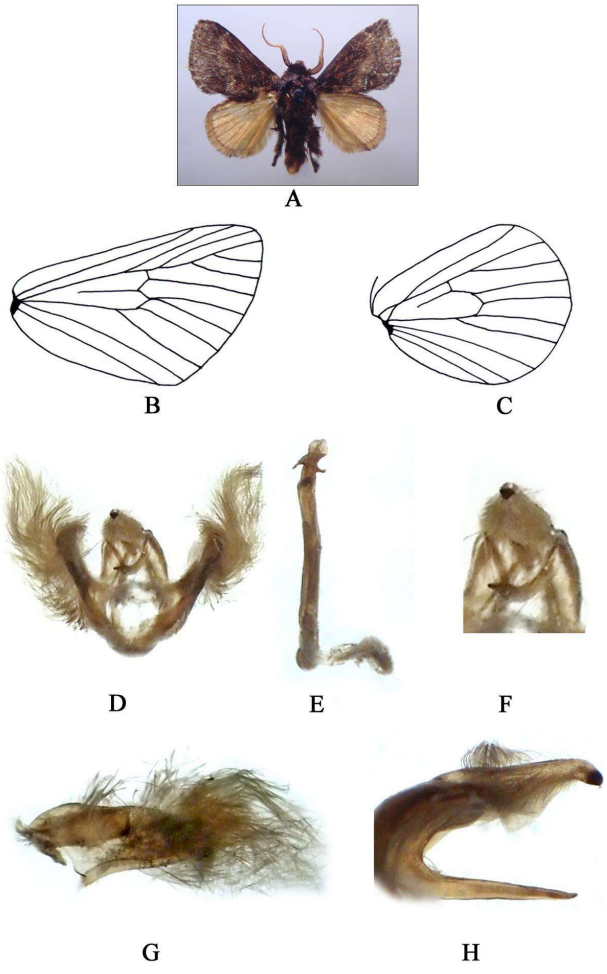

Plate-5: *Miresa chandani* sp. nov.

Figs.- A= Adult, B=Forewing, C= Hindwing, D= Male external genitalia, E= Aedeagus, F= Uncus and Gnathos ventral view, G= Valva, H= Uncus and Gnathos lateral view

structure of the valvae. This specimen could neither be identified from any of the museums nor relevant literature (Cai, 1986; Holloway, 1986; Chang, 1989; Epstein, 1996; Epstein, and Corrales, 2004 and Dubatolov and Strel'tsov, 2005) and hence is named and described as a new species *Miresa chandani* sp. nov.

During the present communication, survey-cum-collection led to the collection of as many as 45 representatives of genus *Miresa* Walker from the different localities of the Western ghats. On sorting, whole phenon was divided into five species. Out of which, four could be identified through literature and the identification was confirmed by comparison of these species with the identified collections of genus *Miresa* Walker from IARI, New Delhi. However, one species could not be identified from any source of identification, hence is described here as new to science. External male and female genitalia of all the studied species have been described and illustrated in full details for the first time. In this work, one species i.e., *M. argentifera* Walker has been reported for the first time from India.

CONCLUSIONS

The communication has information on taxonomy and distribution of five species of genus *Miresa* Walker. Out of which, four species i.e., *M. albipuncta* Herrich-Schäffer, *M. decedens* Walker, *M. argentifera* Walker, *M. nivaha* Moore were already known to literature, however, it is addition of newer localities to their distribution may help future studies on the species under reference. Besides, one species i.e., *M. chandani* sp. nov**.** is being described for the first time and *M. argentifera* Walker has been recorded for the first time from India. The communication may help in the conservation of these species.

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Abbreviations: 1A+2A= Vein representing fused first and second anal vein, 3A= Third anal vein, cua1= Fist anterior cubital vein, cua2 = Second Anterior cubital vein, cup = Posterior cubital vein, M1= First median vein, M2= Second median vein, M3= Third median vein, R1= First radial vein, R2= Second radial vein, R3= Third radial vein, R4= Fourth radial vein, R5= Fifth radial vein, Rs= Radial sector, Sc= Subcostal vein, Sc+R1= Stalk of subcostal and first radial vein, sp. nov.= New species.

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Host influenced Preference of *Callosobruchus chinensis* (L.) and *C. maculatus* (F.) towards Selected Pulses under Storage Ecosystem

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***Abstract*:** Pulse beetle, *Callosobruchus* sp. (Coleoptera: Bruchidae) has got great economic importance and is the most destructive pest on stored pulses. The host preference of pulse beetles towards selected pulses *viz*., black gram (*Vigna mungo* L.), green gram (*Vigna radiata* L.), Cowpea (*Vigna unguiculata* L.), lablab (*Lablab purpureus* L.), red gram (*Cajanus cajan* L.), broad bean (*Vicia faba* L.), bengal gram (*Cicer arietinum* L.) Kabuli and desi was studied under laboratory conditions. Egg laying, number of adults emerged, per cent adult emergence, developmental period and per cent weight loss on selected pulses were recorded. Among the various hosts tested for preference by *Callosobruchus chinensis* (L.) and *C. maculatus* (F.), the minimum egg laying and least number of adult emergence was observed on broad bean as against the maximum on green gram. The lowest adult emergence was noted for red gram as against the highest in Bengal gram. The lowest developmental period was observed for the beetles fed on red gram whereas it was the highest on Lablab. The lowest percent weight loss was observed in broad bean as against the highest in green gram.

***Keywords*:** Pulse beetle, Egg laying, Developmental period, Per cent weight loss

Many pulses such as black gram, green gram, bengal gram, red gram and play a significant role in our dietaries (Sreelekshmi et al 2011). Among all the pests of stored products, the insects act as the chief source of food grain damage (Negamo et al 2007). Mainly bruchids of the genus *Callosobruchus* are well known to inflict post-harvest loss to stored legumes primarily through consumption of the resource and secondarily through the qualitative deterioration of the commodity or reduced seed stock viability. The genus *Callosobruchus* is cosmopolitan in distribution, sometimes it causes 100% damage within 3-4 months of storage (Swamy and Wesley, 2017). Chauhan and Ghaffar (2002) reported 55-65% loss in seed weight and 45.50-66.30% loss in protein content due to its damage and the infested seeds became unfit for human consumption. Considering the damage caused by *Callosobruchus,* it is imperative to evaluate its preference towards various pulses, when stored together. Hence, host preference was evaluated by recording fecundity, percent adult emergence, mean developmental period and percent weight loss by *C. chinensis* and *C. maculatus* on certain pulses.

MATERIAL AND METHODS

Mass culturing of *Callosobruchus chinensis* and *C. maculatus*: Pulse beetles required for this study were mass reared on respective hosts *viz*., black gram, green gram, cowpea, lablab, bengal gram (Kabuli and Desi), red gram and broad bean. The mass culture was initiated by collecting the grains which are infested by the pulse beetle, from domestic storage structures. The culture was maintained in the plastic containers (6 cm dia x 11 cm high) covered with lid and such containers were staked in shelves. The adult beetles obtained from the culture were released into the plastic containers having respective seeds without any prior infestation and they are untreated. Before opening the container for transfer of adults to the new seeds, the container was tapped on the floor for preventing the escape of adults. The adults were allowed to mate and oviposit on the fresh seeds. The seeds were changed in the interval of 40 days for avoiding the fungal growth and also for reducing the competition for egg laying by the freshly emerged adults. The seeds with the eggs were maintained in separate containers for obtaining the freshly emerged adults. Sub-culturing of this beetle was done at regular intervals so as to maintain a continuous supply of insects for the experiments. The freshly emerged adults from the culture were utilized for all the following experiments.

Evaluation of preference of pulse beetle towards selected hosts: Eight legumes *viz*., black gram, green gram, cowpea, lablab, bengal gram (Kabuli and Desi), red gram and broad bean were provided to the beetles under no choice condition. One pair of freshly emerged male and female pulse beetle was released on 40 numbers of seeds (40 numbers) kept in a petri dish. Adults are separated by the most distinguishing characteristics namely the sex specific coloration of the post abdominal plate referred as “Pygidium” in female (Fatima *et al*. 2016). The preference of pulse beetle towards various hosts was evaluated based on fecundity, per cent adult emergence, mean development period and percent weight loss.

Evaluation based on fecundity: To study the fecundity of pulse beetle, each treatment was replicated three times. Eggs laid on

seeds were counted till the death of both male and female bruchids.

Evaluation based on per cent adult emergence: Per cent adult emergence was calculated (Howe 1971).

$$\text{Per cent adult emergence} = \frac{\text{Number of adults emerged}}{\text{Number of eggs laid}} \times 100$$

Evaluation based on mean developmental period: Mean developmental period (MDP) is the time taken for 50 per cent of adults to emerge (Tripathi et al 2015).

$$\text{Mean developmental period} = \frac{D_1A_1 + D_2A_2 + D_3A_3 + \ldots\ldots D_nA_n}{\text{Total number of adults emerged}}$$

Where, D_1- Day at which the adults started emerging (First day), N_1- Number of adults emerged on D_1th day.

Evaluation based on per cent weight loss: Per cent weight loss was calculated (Jat et al 2013).

Statistical analysis: The data thus obtained from evaluation of host preference were analyzed used OPSTAT software.

RESULTS AND DISCUSSION

The fecundity of *Callosobruchus* sp. differed significantly among the selected pulses. Under no choice condition, the mean number of eggs laid on the test pulses ranged from 20.33 to 49 eggs per 40 seeds (Table 1). Significantly the lowest number of eggs was recorded on broad bean which was on par with lablab indicating that these hosts were the least preferred for oviposition by the adult females. The highest numbers of eggs were on green gram indicating that this host was highly preferred for oviposition. Based on the fecundity, green gram was the most preferred host and broad bean was the least preferred host by pulse beetle. Tiwari et al (2012) also reported that green gram and cowpea as the most preferred hosts by pulse beetle. In the present study, pulse beetles laid more eggs on smooth surfaced hosts like green gram and cowpea and laid lesser number of eggs on the host having hard and wrinkled seed coat like bengal gram (Kabuli type). Shivana et al (2011) reported that the preference was high in cowpea and green gram that possess smooth skinned seed texture. Among the different hosts, the percent adult emergence ranged from 71.43 to 87.43. Red gram recorded the significantly least adult emergence which was followed by broad bean. The mean developmental period (days) of *Callosobruchus* sp. grubs on different pulses ranged from 21.66 to 37.31 days (Table 1). Red gram recorded the least developmental period which was on par with black gram whereas pulse beetle that fed on lablab recorded the highest number of days to complete their development Chakraborty et al (2015) observed that mean developmental period ranged from 26.70 to 32.20 days in different pulses. Shivanna et al (2011) reported that cowpea, green gram, bengal gram and horse gram recorded significantly the lowest developmental period. Tiwari et al (2012) revealed that the lowest developmental period (29.00 days) was recorded on cowpea. The weight loss caused due to feeding in different pulses ranged from 7.26 to 40.66 %. The per cent weight loss was the lowest in broad bean which was statistically on par with Bengal gram, weight loss was high in green gram which was on par with black gram and cowpea (Fig. 1). Hosamani et al (2016) reported that red gram variety TS-3R recorded significantly the lowest weight loss as against the highest on cowpea followed by green gram. Chakraborty et al (2014) reported that black gram recorded

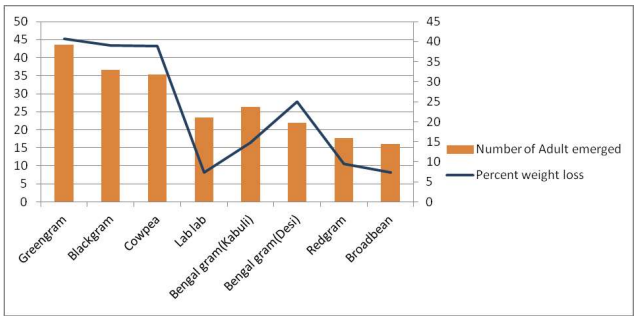

Fig. 1. Preference of pulse beetle towards selected hosts based on number of adults emerged and Percent weight loss

Table 1. Preference of pulse beetle towards selected hosts

Host	No. of. eggs laid/ 40 seeds*#	Adult emergence (%) **#	Developmental period (Days) *#	Weight loss (%) **#
Green gram	49.00 (7.07)^{e}	84.96 (67.19)^{c}	32.36 (5.77)^{d}	40.66 (39.6)^{e}
Black gram	42.33 (6.58)^{d}	86.46 (68.43)^{a}	23.75 (4.97)^{b}	39.06 (38.66)^{e}
Cowpea	41.33 (6.53)^{d}	85.20 (67.55)^{c}	26.23 (5.22)^{c}	38.86 (38.54)^{e}
Bengal gram (Kabuli)	29.00 (4.72)^{a}	81.70 (64.66)^{c}	36.41 (6.18)^{e}	7.40 (15.77)^{a}
Bengal gram (Desi)	30.66 (5.44)^{b}	87.43 (69.25)^{d}	27.24 (6.11)^{e}	14.80 (22.61)^{c}
Red gram	29.66 (5.62)^{c}	71.43 (57.70)^{a}	21.66 (5.31)^{c}	25.00 (29.98)^{d}
Lablab	21.33 (5.50)^{bc}	80.40 (63.75)^{b}	37.31 (4.75)^{a}	9.49 (17.93)^{b}
Broad bean	20.33 (4.65)^{a}	75.10 (60.06)^{a}	30.81 (5.63)^{d}	7.26 (15.62)^{a}

*,**Figures in the parentheses are square root transformed and are arc sine transformed

significantly lowest weight loss while the maximum weight loss was recorded in cowpea. Shivana et al (2011) recorded that the loss in grain weight among different pulses ranged from 1.82 to 4.02 per cent and red gram recorded significantly the lowest weight loss as against the highest weight loss recorded in cowpea.

CONCLUSIONS

The female pulse beetle's host selection behaviour and oviposition preference are influenced by a variety of factors, including host seed size, seed coat characteristics, seed morphology, seed infestation, photoperiod, and the number of copulating males, but not by the length of host deprivation period. The beetle exhibited some level of host size discrimination. Which was accompanied by other factors as stated above. The pulse beetles not only preferred larger seeds with smooth coating, but also preferred fresh healthy seeds under normal photoperiod.

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Rainfall as Correlate of Species Richness of Indian Ants (Hymenoptera: Formicidae)

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***Abstract*:** The rainfall as a key predictor for the spatial distribution of any terrestrial species and this study aimed to understand the effect of rainfall on the subfamilies of ants of India. The simple and multiple linear regression models was used to understand the effect of annual and seasonal rainfall independently and collectively on species richness of ant subfamilies. The pre-monsoon and winter rainfall independently had significant effect while monsoon and post-monsoon rainfall independently had no significant predictor effect on species richness of subfamilies of ants. However, the combining seasonal rainfall for multiple linear regressions indicated that monsoon, post-monsoon, winter and pre-monsoon rainfall have significant predictor effect on five ant subfamilies.

***Keywords*:** Ant, Species richness, Linear model, Rainfall, India

Ants are eusocial insects belong to the family Formicidae under order Hymenoptera. With more than 13000 described species worldwide, ants are one of the most abundant groups of insects in many ecosystems, play a considerable role in shaping ecosystems and participate in numerous and diverse interactions with other organisms (Guénard et al 2012). Global studies found that the Oriental region and tropical Asia have highest generic and endemic diversity of ants (Guénard et al. 2012). From India, 828 species and subspecies belonging to 100 genera are reported of which the state of West Bengal has the highest number of species (382) representing 65 genera followed by state of Sikkim with 276 species representing 69 genera (Bharti et al 2016, Fig. 1). For a large majority of taxa, including invertebrates, species richness increases with precipitation at macroecological scales (Delsinne et al. 2010). It is a general prediction that species diversity of insect increases with the increase of rainfall but, huge variation in the life history and survival strategies in the insects does not follow this trend always. In case of ants, usually rainfall has no or negative influence on diversity (Dunn et al 2009, 2010, Delsinne et al 2010).

It is a common assumption that correlative relationship model between environment and diversity wield in a similar manner across the globe but for insects these models remain largely unexplored (Jenkins et al 2011). If evolutionary history has shaped a fauna such that it responds differently to the environment in one region than fauna in other regions, then a region-specific model may be needed (Ricklefs 2007). Understanding the influence of precipitation on the diversity and composition of ant assemblages at a large geographical scale is difficult (Delsinne et al 2010). In the present work, we performed simple and multiple linear modelling to assess the effect of rainfall on species richness of ant subfamilies in India.

MATERIAL AND METHODS

The 30 administrative areas of India were selected for the present study (Fig. 1). State wise 2016 rainfall data was obtained from Kaur and Purohit (2016). For analysis considered rainfall data (in mm) of 4 seasons namely winter (January and February), pre-monsoon (March, April and May), monsoon (June, July, August and September) and post-monsoon (October, November and December) as well as total annual rainfall. The distribution data of 10 subfamilies of ants (Amblyoponinae, Dolichoderinae, Dorylinae, Ectatomminae, Formicinae, Leptanillinae, Myrmicinae, Ponerinae, Proceratiinae and Pseudomyrmecinae) of India was collected from Bharti et al (2016) and Maqbool (2018).

The two factor analysis was performed using SPSS software (v20) to observe significant difference of species richness of ant subfamilies present between the administrative areas as well as between the subfamilies. The 5 predictor variables (annual rainfall, winter rainfall, pre-monsoon rainfall, monsoon rainfall and post-monsoon rainfall) and species richness of ant subfamilies as response variables for simple linear regression modelling were

considered. The 11 possible combinations of predictors (monsoon + post-monsoon, pre-monsoon + monsoon, winter + monsoon, winter + post-monsoon, winter + pre-monsoon , pre-monsoon + post-monsoon, pre-monsoon + monsoon + post-monsoon, winter + monsoon + post-monsoon, winter + pre-monsoon + monsoon, winter + pre-monsoon + post-monsoon and winter + pre-monsoon + monsoon + post-monsoon) was considered for multiple linear modelling with response variables (species richness of 10 subfamilies and total species richness of all subfamilies). For the families with more than one model, calculated corrected Akaike Information Criterion (AIC) values to get the best predictor model.

RESULTS AND DISCUSSION

There was significant difference in species richness between different ant subfamilies and also significant difference present in species richness of ant subfamilies in different administrative areas of India. With total annual rainfall, only subfamily Ectatomminae has significant and positive simple linear relationship (Table 1, Fig. 2). Winter rainfall has great effect on species richness of subfamilies of ants (Table 2, Fig. 2). Out of 10 subfamilies, species richness of 6 subfamilies namely Amblyoponinae, Dolichoderinae, Dorylinae, Ectatomminae, Formicinae and Myrmicinae has significant and positive simple linear relationship with winter rainfall. Collectively, total species richness of all the subfamilies also has significant and positive simple linear relationship with winter rainfall. Species richness of 6 subfamilies namely Dolichoderinae, Ectatomminae and Ponerinae has significant and positive simple linear relationship with pre-monsoon rainfall (Table 3, Fig. 2). Collectively, total species richness of all the subfamilies also has significant and positive simple linear relationship with

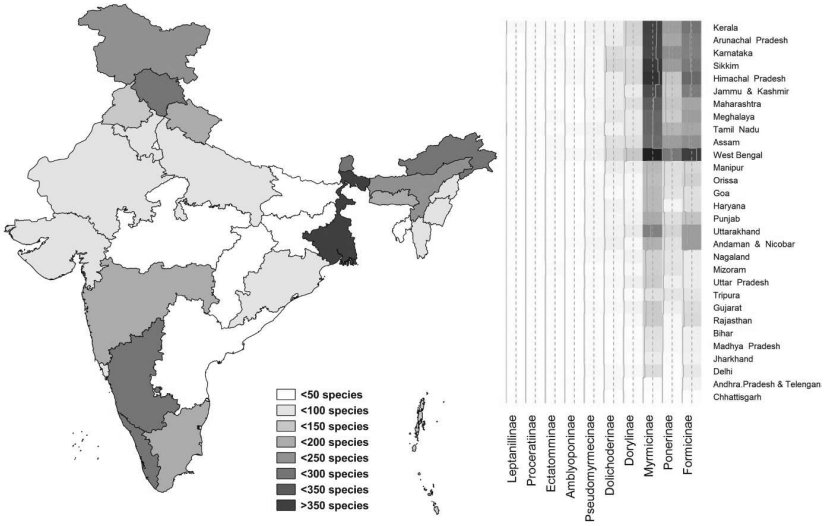

Fig. 1. Species richness of 10 ant subfamilies in different states and union territories of India

Table 1. Relationship between annual total rainfall and species richness of subfamilies of ants

	β	R^2	SE	$F_{1,28}$	p
All subfamily	0.026	0.0460	0.023	1.349	0.255
Amblyoponinae	0.0002	0.027	0.0003	0.77	0.388
Dolichoderinae	0.002	0.081	0.001	2.477	0.127
Dorylinae	0.001	0.028	0.002	0.821	0.373
Ectatomminae	0.0008	0.331	0.0002	13.84	0.0009
Formicinae	0.006	0.041	0.006	1.196	0.283
Leptanillinae	-2.176×10^{-5}	0.0009	1.375×10^{-4}	0.025	0.875
Myrmicinae	0.008	0.028	0.01	0.796	0.38
Ponerinae	0.006	0.078	0.004	2.362	0.136
Proceratiinae	0.0001	0.028	0.0002	0.804	0.378
Pseudomyrmecinae	0.0005	0.057	0.0004	1.684	0.205

The p value in boldface type signifies the linear relationship (α = 0.05)

pre-monsoon rainfall. Monson and post-monsoon rainfall has no effect on species richness of subfamilies of ants (Table 4 and 5).

Out of possible 121 multiple linear models (11 combinations of predictors and 11 response variables) only 15 models were be significant (Table 6, Fig. 3). Unlike simple linear regression models, monsoon and post-monsoon rainfall have significant predictor effect on 7 and 8 multiple linear models, respectively. Winter and pre-monsoon rainfall have significant predictor effect on 12 and 9 multiple linear models. Species richness of only 4 families namely Dolichoderinae, Ectatomminae, Formicinae and Myrmicinae as well as total species richness of the all subfamilies showed significant response to the multiple linear regressions. Rest five subfamilies (Amblyoponinae, Dorylinae, Leptanillinae, Ponerinae, Proceratiinae and Pseudomyrmecinae) have no significant response to the multiple linear regressions.

Understanding the factors that drive species richness and composition at multiple scales is of crucial importance for conservation (Pacheco et al 2012). The works by earlier

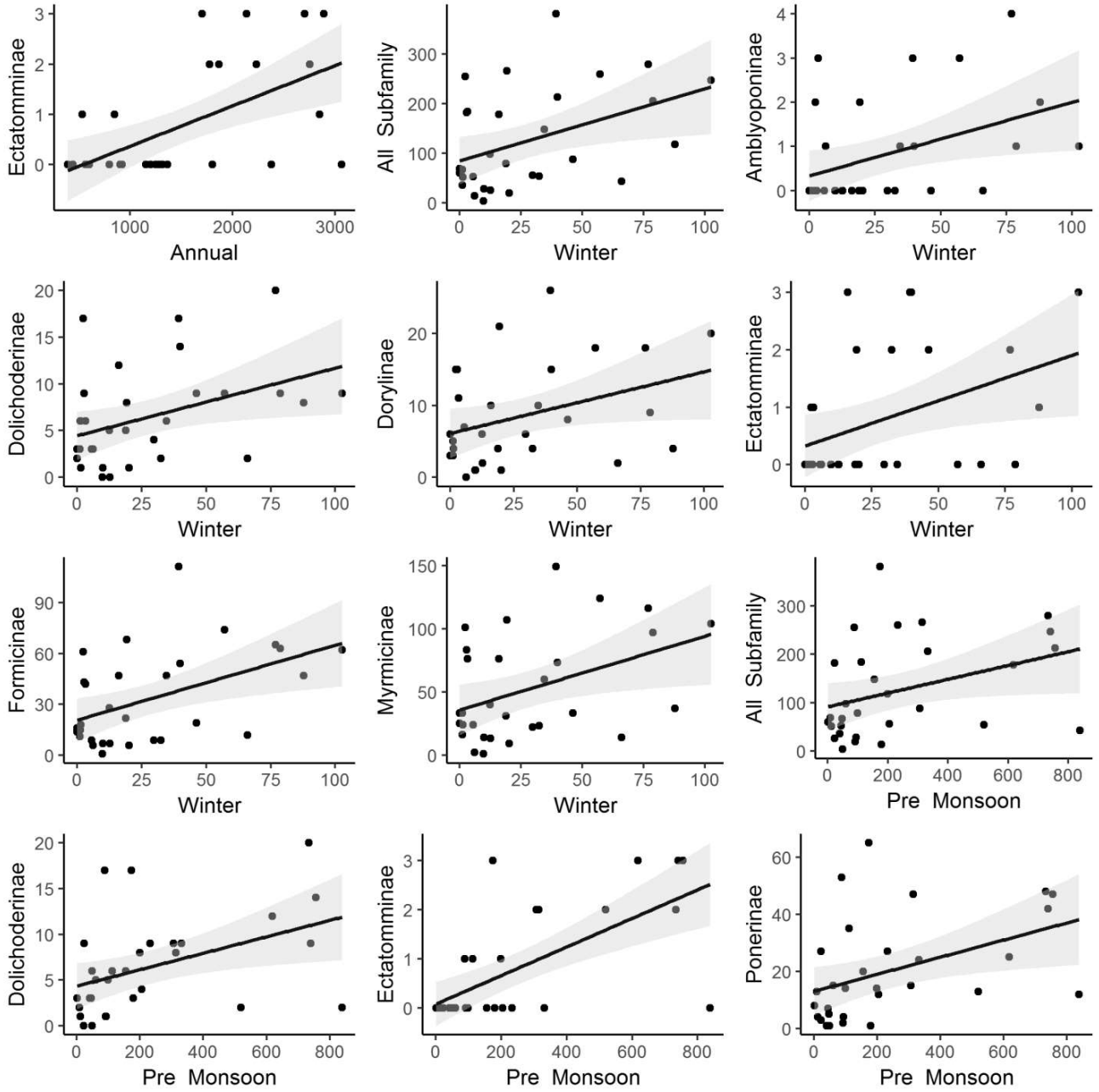

Fig. 2. Significant simple linear regression models between rainfall (in mm) (on x axis) and species richness (on y axis) of subfamily of ants ($p < 0.05$)

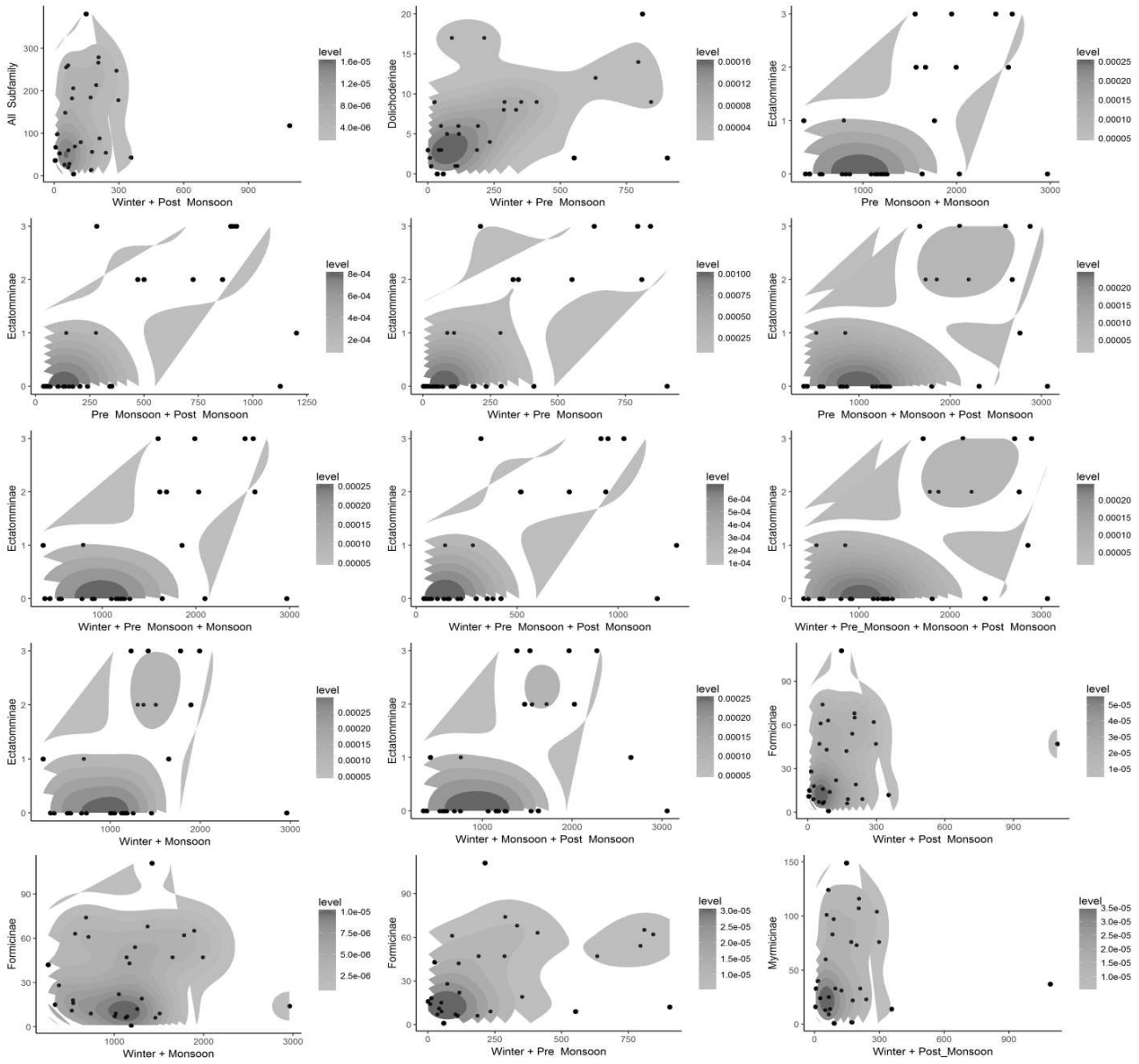

Fig. 3. Significant multiple linear regression models between seasonal rainfalls (in mm) (on x axis) and species richness (on y axis) of subfamily of ants ($p < 0.05$)

Table 2. Relationship between winter rainfall and species richness of subfamilies of ants

	β	R^2	SE	$F_{1,28}$	p
All subfamily	1.449	0.182	0.581	6.23	0.019
Amblyoponinae	0.017	0.172	0.007	5.807	0.023
Dolichoderinae	0.072	0.161	0.031	5.354	0.028
Dorylinae	0.086	0.131	0.086	4.208	0.049
Ectatomminae	0.015770	0.168	0.007	5.638	0.025
Formicinae	0.441	0.225	0.155	8.104	0.008
Leptanillinae	-0.0008	0.002	0.004	0.047	0.829
Myrmicinae	0.583	0.171	0.243	5.782	0.023
Ponerinae	0.2187	0.13	0.1071	4.167	0.051
Proceratiinae	0.004	0.034	0.004	0.978	0.331
Pseudomyrmecinae	0.014	0.055	0.011	1.637	0.211

The p value in boldface type signifies the linear relationship ($\alpha = 0.05$)

Table 3. Relationship between pre-monsoon total rainfall and species richness of subfamilies of ants

	β	R^2	SE	$F_{1,28}$	p
All subfamily	0.142	0.133	0.068	4.307	0.047
Amblyoponinae	0.001	0.060	0.0008	1.801	0.190
Dolichoderinae	0.009	0.189	0.004	6.536	0.016
Dorylinae	0.009	0.119	0.005	3.781	0.062
Ectatomminae	0.002	0.433	0.0006	21.35	7.82×10^{-05}
Formicinae	0.034	0.103	0.019	3.222	0.083
Leptanillinae	0.00005	0.0006	0.0004	0.016	0.902
Myrmicinae	0.053	0.109	0.029	3.418	0.075
Ponerinae	0.03	0.186	0.012	6.388	0.017
Proceratiinae	0.0009	0.128	0.0005	4.096	0.053
Pseudomyrmecinae	0.001	0.035	0.001	1.024	0.320

The p value in boldface type signifies the linear relationship (α = 0.05)

Table 4. Relationship between monsoon rainfall and species richness of subfamilies of ants

	β	R^2	SE	$F_{1,28}$	p
All subfamily	0.018	0.011	0.033	0.308	0.583
Amblyoponinae	7.662×10^{-5}	0.001	3.918×10^{-4}	0.038	0.8464
Dolichoderinae	0.001	0.022	0.002	0.639	0.4308
Dorylinae	0.0009	0.007	0.002	0.188	0.668
Ectatomminae	0.0008	0.165	0.0003	5.526	0.026
Formicinae	0.004	0.007	0.009	0.187	0.669
Leptanillinae	-6.124×10^{-5}	0.003	1.954×10^{-4}	0.098	0.756
Myrmicinae	0.006	0.006	0.014	0.162	0.691
Ponerinae	0.005	0.027	0.006	0.784	0.384
Proceratiinae	8.391×10^{-5}	0.005	2.247×10^{-4}	0.14	0.712
Pseudomyrmecinae	0.0005	0.024	0.0006	0.698	0.411

The p value in boldface type signifies the linear relationship (α = 0.05)

Table 5. Relationship between post-monsoon rainfall and species richness of subfamilies of ants

	β	R^2	SE	$F_{1,28}$	p
All subfamily	0.025	0.002	0.103	0.057	0.813
Amblyoponinae	0.001	0.04	0.001	1.152	0.292
Dolichoderinae	0.004	0.015	0.005	0.438	0.514
Dorylinae	-0.002	0.002	0.007	0.065	0.800
Ectatomminae	0.002	0.088	0.001	2.701	0.112
Formicinae	0.017	0.014	0.028	0.389	0.538
Leptanillinae	8.133×10^{-5}	0.0006	6.093×10^{-4}	0.018	0.895
Myrmicinae	-0.006	0.0008	0.043	0.022	0.884
Ponerinae	0.006	0.004	0.018	0.102	0.752
Proceratiinae	-3.347×10^{-5}	8.133e-05	7.014×10^{-4}	0.002	0.962
Pseudomyrmecinae	0.006	0.072	0.002	2.181	0.151

The p value in boldface type signifies the linear relationship (α = 0.05)

Table 6. Significant multiple linear regression model between species richness of ant subfamilies (Response variables) and seasonal rainfall (Predictor variables) ($p < 0.05$)

Subfamily					Predictors							
					Winter rainfall		Pre-monsoon rainfall		Monsoon rainfall		Post-monsoon rainfall	
	AICc	R^2	F	p	β	SE	β	SE	β	SE	β	SE
All subfamily		0.212	$F_{2,27} = 3.644$	0.03	1.763	0.656					-0.107	0.105
Dolichoderinae		0.209	$F_{2,27} = 3.565$	0.042	0.035	0.043	0.006	0.005				
Ectatomminae	82.587	0.465	$F_{2,27} = 11.71$	0.0002			0.003	0.0007	0.0004	0.0003		
Ectatomminae	83.665	0.445	$F_{2,27} = 10.82$	0.0004			0.003	0.0007			0.0007	0.0009
Ectatomminae	84.141	0.436	$F_{2,27} = 10.44$	0.0004	-0.003	0.008	0.003	0.0009				
Ectatomminae	85.269	0.468	$F_{3,26} = 7.636$	0.0008			0.003	0.0007	0.0003	0.0003	0.0004	0.001
Ectatomminae	85.315	0.468	$F_{3,26} = 7.611$	0.0008	-0.003	0.008	0.003	0.0009	0.0003	0.0003		
Ectatomminae	85.909	0.457	$F_{3,26} = 7.291$	0.001	-0.006	0.008	0.003	0.0009			0.001	0.001
Ectatomminae	87.978	0.476	$F_{4,25} = 5.681$	0.002	-0.005	0.008	0.003	0.0009	0.0003	0.0003	0.0007	0.001
Ectatomminae	91.948	0.268	$F_{2,26} = 4.953$	0.015	0.013	0.007			0.0006	0.0003		
Ectatomminae	94.816	0.269	$F_{3,26} = 3.192$	0.04	0.012	0.007			0.0006	0.0004	0.0002	0.001
Formicinae	284.187	0.238	$F_{2,27} = 4.227$	0.025	0.499	0.177					-0.019	0.028
Formicinae	284.691	0.225	$F_{2,27} = 3.931$	0.032	0.448	0.162			-0.002	0.008		
Formicinae	284.731	0.224	$F_{2,27} = 3.908$	0.032	0.446	0.217	-0.0008	0.025				
Myrmicinae		0.234	$F_{2,27} = 4.121$	0.027	0.77	0.269					-0.064	0.04

scientist showed lack of meaningful relationships between ant diversity and precipitation (Morton and Davidson 1988, Medel 1995, Pfeiffer et al 2003, Dunn et al 2009, Dunn et al 2010, Delaine et al 2010). Present work has similitude with those works as in general, annual rainfall has no significant effect on the species richness of subfamilies of ants. But seasonal rainfall has significant effect on the species richness of different subfamilies, both independently as well as in linear combination. Different ant families have different life history and survival strategies. The variation in the effect of rainfall to the species richness of ant subfamilies may be results from this variation of life history and survival strategies.

CONCLUSION

The total annual rainfall has no effect on the ant subfamilies other than Ectatomminae but seasonal rainfall has a major impact. The monsoon and post-monsoon rainfall has no effect on any of the ant subfamilies. The winter and pre-monsoon rainfall have major impact on the ant subfamilies. Thus, seasonal variation in rainfall can shape the ant community of a region. Any change in rainfall pattern, especially due to climate change, can be devastating to ant communities. Further studies should be conducted to find out the optimal ecological correlates of Indian ant species richness in different habitats so that appropriate conservation actions can be planned and implemented for sustainable management of natural resources.

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Population Dynamics of White Grub, *Holotrichia seticollis* (Coleoptera: Scarabaeidae) in NW Himalayan Regions of India

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***Abstract*:** Population dynamics analyses of *Holotrichia seticollis* Moser, 1912 (Coleoptera: Scarabaeidae) through the light trap, pheromone trap and in-situ sampling were conducted in Uttarakhand, Himalayas for three consecutive years (2019-2021). The three chemical synthetic attractants tested for their efficacy in attracting and trapping the adult males of *H. seticollis* showed that the most potent species-specific synthetic attractant was methoxybenzene that trapped up to 14.58 beetles/day, followed by diethyl benzene (1.29) and 1, 4- diethyl benzene (0.53). Second fortnight of June was peak period for the emergence of *H. seticollis*. The maximum trap catches were between 21 to 26 standard meteorological weeks (SMWs), while a sharp decline in population after 26 SMW in both the traps as well as in-situ collection. However, the light trap catches were extremely low in comparison to pheromone traps, thus indicating that, although *H. seticollis* is a nocturnal insect, but it was not positively phototactic. Therefore, pheromone traps can be successfully used for monitoring pest abundance of *H. seticollis* at field level and also assist in drawing accurate risk maps for designing and implementing sustainable and eco-friendly integrated pest management programs in the Indian Himalayas.

Keywords: *Holotrichia seticollis*, Para-pheromone, White grub management

Order Coleoptera represents the largest group of insects with over 4,00,000 species (Beutel and Haas 2000). Amongst the different families of Coleoptera, Scarabaeidae is the second largest family with more than 30,000 species of cosmopolitan distribution all over the world (Jameson and Ratcliffe 2001). The majorities of the beetles belonging to the family Scarabaeidae are pleurostict (phytophagous) and commonly known as scarabs, whereas, their larvae are known as white grubs and are rhizophagous. Uttarakhand Himalayas, India constitutes the vast diversity of Scarabaeid beetles with more than 100 species reported to infect agriculture fields, horticulture crops and forest trees in the state (Mittal 2005, Chatterjee 2010 and Chandra et al 2012). Among these 100 species, *Anomala dimidiata* (Hope, 1831), *Holotrichia longipennis* (Blanchard, 1851) and *Holotrichia seticollis* Moser, 1912 are the three dominant species causing more than 50% of crop damages during the Kharif season every year (Selvakumar et al 2011, Subbanna et al 2020). *H. seticollis* is one of the predominant and most destructive scarab species causing considerable economic losses in agro-ecosystems of the North-Western Indian Himalayas (Malik et al 2019). Both, the grubs and adults are pestiferous and mainly attack crop plants such as potato, ginger, upland rice and sugarcane, in different regions of the country (Abdullah 2012, Chandel et al 2012, Padala et al 2017). The demand for efficient risk assessment techniques and accurate methods for monitoring the pest population dynamics of these notorious white grubs is increasing over the years, in order to implement timely and efficient pest management programs to avoid environmental risks. However, due to the cryptic habitat of the white grubs, it becomes difficult to evaluate their population dynamics and pest control turns to be a very difficult affair, thus, leading to unsatisfying results and leaching of inputs incurred. Beetle population monitoring could be useful for understanding the potential risk of white grub damage in a particular area. Given the economic importance of *H. seticollis* in the Indian Himalayas, a simple and effective strategy for monitoring the population density and dynamics of *H. seticollis* is necessary, as it would yield us a correct and perfect timing for planning an effective and environmentally sustainable wide-area IPM program.

For many decades, light traps were primarily used to monitor the abundance and diversity of positively phototactic and nocturnally active insect species in a particular habitat (Hong et al 2021). Off late, the mechanism of chemical communication among the individuals of an insect species is exploited, by developing the synthetic analogs of the so-called “pheromones” released by insects which are species-specific, less laborious and more economical with no extra

external inputs like power as in case of light traps (Mullen and Dowdy 2001). These synthetic sex pheromones used in the traps mainly include sex or aggregation pheromones, that play an important role in the lives of many insects and are exploited as monitoring tools for estimating the pest population dynamics and ultimately in their management (Baker and Heath 2005). Although, recently the database on insect pheromones and other attractants has been developed by several authors but the studies to exploit them in insect attraction and management have mostly been confined to laboratory levels (El-Sayed 2008). Additionally, the database on pheromone/attractants specifically-related to notorious white grub species belonging to the same genera *i.e., Holotrichia consanguinea* (Leal et al 1996) and *Holotrichia reynaudi* (Ward et al 2002) are available. But, studies specifically related to detecting the *H. seticollis* population at an early stage of infestation in order to maintain low pest densities and avoid serious pest outbreaks are not available. In view of this, the present study on quantitative monitoring of *H. seticollis* populations using light traps and pheromone traps as lures has been carried out at field levels in the NW Indian Himalayan region. Further studies to identify the response of adult males of *H. seticollis* to different synthetic chemical attractants and light traps were conducted and the most efficient trap for monitoring the population dynamics and mass trapping of insect pests was identified. In addition to these studies, daily in-situ field surveys were conducted in the study area to confirm the accuracy of the catch of both light and pheromone traps installed in the field.

MATERIAL AND METHODS

Study site: The present investigation was carried out for three consecutive years (2019-2021) from the 20th to 33rd standard meteorological weeks (SMWs) in Experimental Farm, ICAR- Vivekananda Parvatiya Krishi Anushandhan Sansthan (29.64º N, 79.63º E and 1284 m above mean sea level), Hawalbagh, Almora, Uttarakhand, India. The study location comes under the Alpine and Humid subtropical climatic zone of the NW Indian Himalayas.

Mating cycle of adult *H. seticollis*: In order to understand the important timelines in the mating cycle of adult *H. seticollis,* the time of female emergence, settlement, male calling, mating, uncoupling and egg-laying were recorded from more than 100 pairs of *H. seticollis.* The current data was used for extraction of the natural pheromone from adult females through a sampling apparatus for in-situ volatile collection (Patent number: IN 373714) (Fig. 1).

Screening of synthetic attractants: The natural pheromone extracted through a handheld headspace sampling apparatus for the in-situ volatile collection was identified as 1,2 1,3 and 1,4 diethyl benzene through Gas chromatography Mass spectrometry. The three synthetic analogs, diethyl benzene, 1,4- diethyl benzene and methoxybenzene of 1,2 1,3, and 1,4 diethyl benzene were tested as lures in preliminary field trials in the year 2019. All these chemical attractants are commercially available and obtained from Himedia Laboratories Pvt. Ltd., India. The most potent species-specific synthetic attractant among the three was used for further comparative study with the light trap. In addition to this, the effect of these synthetic attractants on the non-target and beneficial insect species was also evaluated.

Light trap vs. pheromone trap: In the present study, two types of traps (light trap and pheromone trap) were used for monitoring the population dynamics of adult beetles of *H. seticollis* in the study area. The light trap (VL white grub beetle trap-1; Indian patent number: IN 290170) is specifically designed to attract and trap the scarab beetles (Fig. 2a). The trap is designed based on a simple mechanism of attracting positively phototactic and nocturnally active insects. The hitting fins fixed at an angle of 120º increase the efficiency of the trap, wherein, the scarab beetles hovering around the light source hit the fins and fall into a collection vessel fixed at the bottom through the Y-shaped vessel. The collection vessel is half-filled with water in order to avoid the escape of the trapped beetles. The pheromone trap was identical to the VL white grub beetle trap-1, except, the light source was replaced with the synthetic pheromone (Fig. 2b). The synthetic pheromone (500 µl) is filled into a 1.5 ml plastic vial with needle holes at the cap and fixed onto the trap. The scarab beetles get attracted to the pheromone and hover around this synthetic attractant. During this process, get hit by the hitting fins and fall into the collection vessel. The synthetic pheromone was replaced every 7 days.

Both the traps were installed in the first week of May, to record the information on the date of the first emergence, time of emergence, time of maximum activity, population density and population abundance of adults of *H. seticollis*. Moreover, the pheromone traps and light traps were installed at a height of 1.0 m from the ground level and at a distance of 300 m in order to minimize inter-trap interference.

In-situ sampling: In-situ sampling of adults, *H. seticollis* was also carried out simultaneously, by scouting the fields from 19:00 to 21:00 hrs on daily basis in the study area during 2019-21, in order to confirm the accuracy and reliability of light and pheromone trap catches. Apart from the monitoring study, the mating pairs per unit area (10 m^2) were also observed and the decline in the number of mating pairs over a period of three years was also assessed. In addition to this, the number of unmated females was also observed and an

increase in the number of unmated females over a period of three years was also assessed to observe the effect of pheromone traps on mating disruption.

Data collection: The emergence of adult beetles from soil commences in the evening hours immediately after receipt of the first pre-monsoon rains. They emerge in large numbers for feeding and breeding. Considering the activity of beetles, the traps were operated between 19:00-6:00 hrs daily and the in-situ sampling was done from 19:00-21:00 hrs for three consecutive years (2019-21). During this time period, the beetles were actively mating and feeding on their host plants. The beetles collected through both methods were sorted out and the numbers of males of *H. seticollis* were recorded separately and the total number of adults of *H. seticollis* captured by all means were counted and noted on daily basis.

Data analysis: The SE (m) values were calculated through SPSS software for WINDOWS version 16.0 (SPSS Inc., Chicago).

RESULTS AND DISCUSSION

Reproductive behavior and mating cycle of *H. seticollis*: The emergence of *H. seticollis* starts immediately after the first pre-monsoon showers, during the second fortnight of May and continues till the first fortnight of August. The adults emerge from the soil for feeding and mating at 19:15-19:30 hrs and settle on nearby host plants (leaves of *Rosa indica,* bark of *Cedrus deodara*, *Dalbergia sissoo* and *Thuja occidentalis*) (Fig. 3a, b). The adult female settles on the host tree, protrudes its pheromone gland and releases male attracting sex pheromone, which attracts a large number of males. The sexually active time of *H. seticollis* was observed to be between 19:10-19:40 hrs and mating occurs for 10 minutes. Immediately after mating, both the males and females uncouple themselves and the females return to the soil for egg-laying, while, the males were observed to move to their host plants for feeding.

Screening of synthetic pheromones/ parapheromones: In order to assess the effectiveness of three chemical attractants (diethyl benzene, 1,4- diethyl benzene and methoxybenzene) in attracting and trapping the adult males of *H. seticollis,* three traps lured with three different chemical attractants were installed in the field at a minimum distance of 300 m. The most potent species-specific synthetic attractant was methoxybenzene (anisole, $CH_3OC_6H_5$) that trapped up to 14.58 beetles per day (Fig. 3c), followed by diethyl benzene (1.29 beetles) and 1,4- diethyl benzene (0.53 beetles), respectively (Fig. 4). The attraction for anisole was stronger than the actual pheromone released from female of *H. seticollis* and this synthetic parapheromone masked the activity of natural pheromone, thus, disrupting the mating process of *H. seticollis*. Moreover, none of the traps lured with synthetic attractants trapped any non-target or beneficial insects. Methoxybenzene was used for further comparative study with the light trap and in-situ samplings.

Light trap v/s pheromone trap v/s in-situ sampling: Species sampling is a basis for documenting the spatial distribution of species in an ecosystem (Zhang 2011). A simple and effective method is very important to estimate the abundance and population dynamics of an insect species in a particular habitat (Southwood and Henderson 2000). A large number of trap designs are commercially available and have

Fig. 1. Natural pheromone extracted through a handheld headspace sampling apparatus for in-situ volatile collection

Fig. 2. Traps used for monitoring the population of *H. seticollis*; (a) VL white grub beetle trap-1; (IN 290170) and (b) Pheromone trap

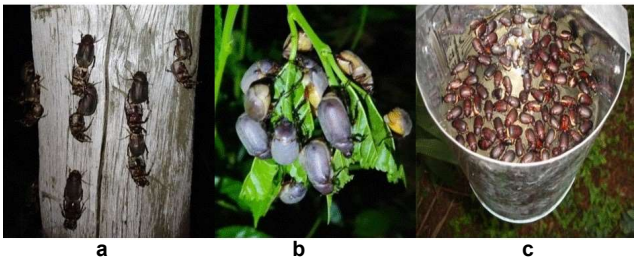

Fig. 3. *Holotrichia seticollis*; (a) Matting pairs settled on a tree trunk, (b) Feeding on a host tree and (c) Trapped in a pheromone trap with a synthetic attractant (methoxybenzene) as a lure

been tested for the detection, monitoring and control of various insect pests (Ávalos and Soto 2015, Fite et al 2020). Both light and pheromone traps were examined for their efficiency in trapping the adult beetles of *H. seticollis.* The second fortnight of June was the peak period for the emergence of *H. seticollis* (Fig. 5). Sreedevi et al (2014) also reported the peak emergence of scarab beetles during the second fortnight of June. The highest pheromone trap catches were between 21st to 26th SMWs, while, a sharp decline in population was observed after 26th SMW in both the traps as well as in-situ collection. The light traps showed the least activity in trapping the adults of *H. seticollis*, thus indicating that, although, *H. seticollis* is a nocturnal insect but it was not strongly phototactic. Although, previous studies conducted by Dhaliwal and Arora (2010), Banjar et al (2020) and Menis and Rodrigues (2021) showed that the light trap is the best sampling method for monitoring the population density of nocturnal white grub beetles which are positively heliotactic in nature. But, *H. seticollis* was not strongly phototactic and thus the use of the light trap to assess the population dynamics of *H. seticollis* in the Indian Himalayas is not the right strategy.

Pheromone traps were highly efficient & extremely species-specific and the trap catches were observed at least a week or two earlier than the light trap when the population of *H. seticollis* was supposed to be very low. The pheromone trap catches recorded activity from 20th to 32nd SMWs during the entire activity period of the beetles in all three years, whereas, in-situ collection studies carried out to confirm the activity of both traps showed that, the emergence of adult beetles was observed from 21st to 30th SMWs in 2019 and 20th to 30th SMWs in 2020 and 2021, respectively. Witzgall et al (2010) and Ahmad and Kamarudin (2011) also stated that pheromone traps are efficient even at low pest population densities with no adverse effect on non-target species and the long-term use of synthetic pheromones can lead to a reduction in pest populations. This study clearly indicates that pheromone traps are more efficient than light traps in detecting the presence of scarab beetle, *H. seticollis*. Both the traps as well as in-situ collection data indicated that the emergence of *H. seticollis* started during the second fortnight of May and the pest abundance increased till mid-June and the population density reduced from the last week of June (Fig. 5).

The number of mating pairs settled on a tree trunk of five *Cedrus deodara* trees in a 10 m^2 area in one day in three different localities were counted for three consecutive years and was observed that the number of mating pairs declined over the years (from 28.33 in 2019 to 24.33 in 2021) (Fig. 6). Moreover, the studies conducted at USDA APHIS (2011) reported that trapping of a large number of male adults through synthetic sex pheromones can result in an imbalance in the pest sex ratio and this may affect the mating pattern of the pests. Concurrently, our studies showed a continuous reduction in the mating pairs of *H. seticollis* over a period of three years, when methoxybenzene-lured pheromone traps were continuously used for trapping the adult males. Although, the decline was not drastic, but continuous use of pheromone traps may lead to population decline over the years.

Increase in number of unmated females: Number of unmated females increased over the years per unit area

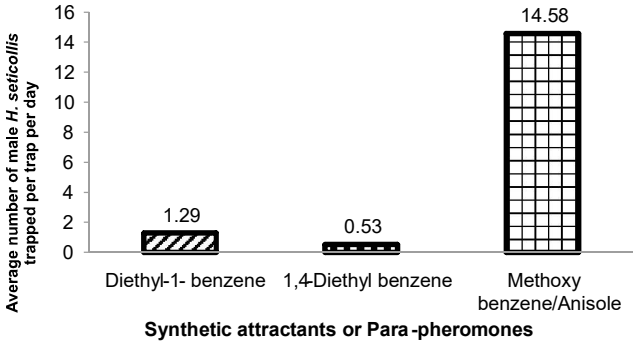

Fig. 4. Field efficacy of synthetic attractants used for trapping males of *H. seticollis*

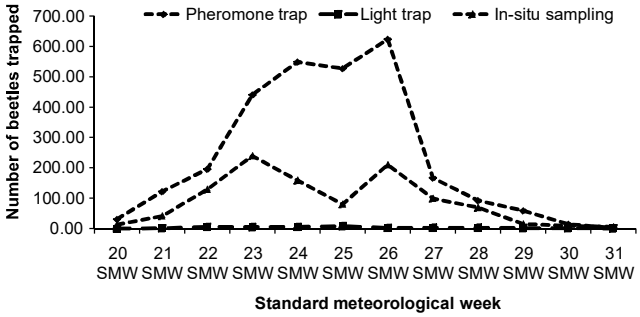

Fig. 5. Mean weekly trap catches of adult males of *H. seticollis* over a period of three years

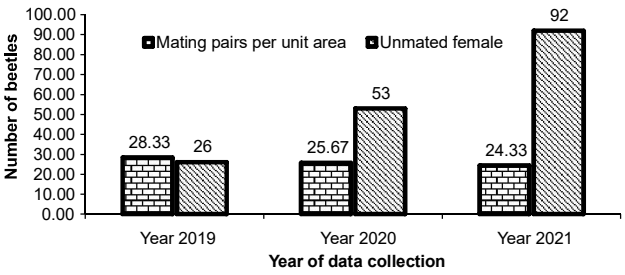

Fig. 6. Number of mating pairs and unmated females of *H. seticollis* collected during in-situ sampling during three years (2019-2021)

(Fig. 6) from 26 in 2019 to 92 in 2021. The continuous use of pheromone traps can lead to a significant increase in the number of unmated females and a decline in the number of mated females per unit area, which in turn reduces egg-laying and thus, lead to population reduction over the years. Furthermore, Kamarudin et al (2010), Muniyappa et al (2018) and Luo et al (2020) reported that mass trapping through pheromone traps, not only controlled the male population but, also efficiently reduced the larval population in subsequent generations, thus, resulting in a drastic decline in crop damage and yielding better quality products. Ward et al (2002) extracted the female pheromone from the abdominal glands of *H. reynaudi* and also tested three parapheromones; anisole, indole and phenol (singly and as binary mixtures) and recorded that no beetles were trapped in indole or phenol-baited traps and thus, concluded that, anisole is the major component of the female sex pheromone and plays a major role in attracting males of the same species. So, for all three species anisole is a sex pheromone. Moreover, the adult scarab beetle population above the ground can be positively correlated with the population of white grubs in the soil; this correlation could be utilized for pest risk assessment in a particular area. Therefore, the adult population levels assessed through pheromone traps can be utilized as a single risk assessment factor for crop plant damage by pest species (Furlan et al 2020).

CONCLUSIONS

The results of the present study provide one of the very first demonstrations of the use of pheromone traps for accurately monitoring the population dynamics of the target insect and obtaining a reliable and consistent estimate of the pest risk by *H. seticollis*. The pheromone trap with anisole as a synthetic attractant was identified as the best monitoring and trapping method against *H. seticollis*. So, this eco-friendly and cost-effective technology can easily be adapted by the farmers to monitor the pest population dynamics of *H. seticollis* on a wide area basis and take up early, timely and economic threshold-based pest management practices in the Indian Himalayas. However, few pheromones are not target-specific and attract all the insects of the same genera or sometimes beneficial insects, additionally; natural or synthetic pheromones are not available commercially for a large number of insects, which needs further research.

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Defensive Role of Fibre Fractions in Rice Genotypes against Rice Leaf Folder *Cnaphalocrocis medinalis* (Guenee)

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***Abstract*:** Rice leaf folder, *Cnaphalocrocis medinalis* (Guenee) is a major insect pest in rice and can cause serious damage under favourable conditions. Host plant resistance is an economical, feasible and eco-friendly option for management of this pest. Fibre fractions viz. cellulose, hemicellulose and lignin were quantified in seven genotypes and their effect on larval and pupal parameters was studied. The total larval duration ranged from 15.1-19.6 days in these genotypes. Prolonged duration was in TKM6 and IET22155 (19.5 and 19.6 days, respectively), while percentage pupation (54.0% and 64.0%, respectively) was significantly less on these genotypes. Final instar larval weight was less in W1263, TKM6 and IET22155 (22.7-24.9 mg) than in other genotypes (>25.5 mg). Pupal weight varied from 17.1-22.9 mg and TKM6 and IET22155 recorded lesser pupal weight (17.1 and 18.8 mg, respectively). The cellulose and hemicellulose content was higher in TKM6, W1263 and IET22155, while lignin content was higher in TKM6, W1263 and JGL21066. Positive correlations were observed between larval duration and cellulose and hemicellulose. While negative correlations of larval weight with cellulose and hemicellulose was recorded. The per cent pupation was negatively correlated to lignin. Higher amount of the fibre fractions has negatively impacted the pupal weight.

***Keywords*:** *Cnaphalocrocis medinalis*, Rice leaffolder, Fibre fraction, Resistance, Cellulose, Hemicellulose, Lignin

Rice is the staple food of more than 60 per cent of the world population. India has the largest area among rice growing countries and stands second in production following China. More than 100 species of insects attack this crop in Asia and 20 of these are of economic importance and yield loss due to these insect pests has been estimated at about 25 per cent (Sharma et al 2017). In India, losses incurred by different insect pests of rice are reported to the tune of 15,120 million rupees which is 18.60 per cent of total losses (Chandramani et al 2010). Rice leaffolder (RLF), once considered as minor pest but due to application of high doses of nitrogen fertilizers and non-judicious use of insecticides causes RLF outbreaks (Punithavalli et al 2013). Due this pest, the losses in seed yield have been reported from 20-50% at tillering and flowering stages, respectively (Padmavathi et al 2013). Various chemicals recommended for the control of RLF does not achieved the desired control and their indiscriminate use causes resistance, resurgence and residue problems (Wang et al 2009). Therefore, the use of host plant resistance is the most effective and safest measure for RLF management as it is less expensive and ecologically safe method and can be easily adopted by farmers. By understanding the mechanism of biochemical activities responsible for resistance; these can be incorporated into the cultivated plants and this will help to breed crop varieties that support lower RLF population or that can better tolerate insect infestation. Plant genotypes, either due to environmental stress or genetic makeup, possess physiological and biochemical differences which alter the nutritional value for plant feeding insects that in result make host plant unfavorable to phytophagous insects (Mitchel et al 2016). The rice genotypes have also shown such diversity in antibiosis and biochemical factors influencing the biology of the insect pests (Muduli et al 2021). At present, the information about the host plant resistance mechanism due to the crude fibres i.e. cellulose, hemicellulose and lignin in rice genotypes is very limited and there has been a large scope to work on this area. Therefore, the present studies are undertaken to estimate the effect of the fibre fractions on larval weight, pupal weight and per cent pupation of RLF.

MATERIAL AND METHODS

All the studies related to biological parameters of *C. medinalis* on seven rice genotypes was carried out at Rice Research Farm, Punjab Agricultural University (PAU), Ludhiana (30°54'N and 75°48'E, 247 m above mean sea level) during 2016-17 wet crop season. The analysis of fibre fractions at constitutive and induced levels was carried out in Animal Nutrition Laboratory, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana during 2017-18 wet crop season.

Raising of test plants and *C. medinalis* population: The

seeds of the test genotypes (TKM6, IET22155, RP4918-142, JGL21066 and W1263) were obtained from Indian Institute of Rice Research, Hyderabad and TN1 and PR121 from Rice section, PAU. These test genotypes were grown separately in earthen pots (20 cm diameter). The insect culture was multiplied in Rice Entomology Laboratory, Dept. of Plant breeding & Genetics on TN1 plants. The seeds of TN1 were sown in pots (20 cm diameter) at periodic intervals to ensure continuous supply of fresh green plants. These pots were placed in the insect rearing cages and insect larvae collected from field were introduced in the cages on 30 days old plants (DOPs). The food plants were changed regularly to maintain the insect culture. Adults emerging in the rearing cages were transferred to new cages provided with TN1 plants and 10 per cent honey solution for oviposition to maintain the insect culture.

Larval and pupal parameters of *C. medinalis*: All the experiments related to above studies were evaluated under screen house conditions in completely randomized design (CRD). The experiments were replicated five times and five potted plants of each genotype having pot size of 20 cm constituted one replication. Two neonate larvae (1st instar) from the insect culture were released on 50 DOPs of test genotypes. Plants were then covered with mylar cages and kept in screen house at 28±2°C and 75±5% RH conditions. These plants were observed daily during larval development and total time taken by the larva to pupate was recorded to calculate larval duration and last instar larvae were collected to measure the weight. Then, number of pupae formed was counted to calculate percentage pupation and newly formed pupae were collected and pupal weight was recorded.

Fibre fractions: A separate set of experiments were laid out to study various fibre fractions in leaf blades of 50 and 70 DOPs of different genotypes under constitutive and induced conditions and such studies were replicated five times. Five fourth instar larvae were used per replication to get infested plants for biochemical analysis in induced resistance conditions. Standard methods of AOAC (2000) were followed for the determination of cellulose, hemicellulose and lignin.

Determination of hemicellulose: Hemicellulose was calculated after determining neutral detergent fibre (NDF) and acid detergent fibre (ADF). To determine NDF, half gram of dried and ground rice leaves sample was taken in a spoutless beaker and 50 ml of neutral detergent solution (NDS) was added to the sample. The mixture was heated on a hot plate and the liquid content was filtered through sintered glass crucible mounted on suction flask. Vacuum was created using suction pump and residue was washed with hot distilled water and acetone. The crucible was then kept at 100^{0}C in hot air oven for overnight. The crucible was weighed after cooling in a desiccator**.** The NDF of the sample was calculated by the given formula:

NDF (%) =W_b-W_a/ W_o x 100

Where, W_a = Weight of oven dried crucible; W_o = Initial weight of sample (dried & ground leaves); W_b = Weight of oven dried sample and crucible

To determine ADF, half gram of dried and ground paddy straw sample was taken in a spoutless beaker and 50 ml of acid detergent solution (ADS) was added to the sample. The mixture was heated on a hot plate and the liquid content was filtered through sintered glass crucible mounted on suction flask. Then vacuum was allowed from a suction pump and washing with hot distilled water and acetone was done. The crucible was kept at 100^{0}C in hot air oven for overnight. The crucible was weighed after cooling in a desiccator**.** The ADF of the sample was calculated by the given formula:

ADF (%) =W_b-W_a/ W_o x 100

Where, W_a = Weight of oven dried crucible; W_o = Initial weight of sample (dried & ground leaves); W_b = Weight of oven dried sample and crucible

Then hemi-cellulose was obtained by subtracting ADF from NDF

Hemicellulose (%) = NDF(%) – ADF(%)

Determination of cellulose and lignin: Cellulose and lignin were calculated after determining acid detergent lignin (ADL).To determine ADL, cold solution of 72% H_2SO_4 (w/w) was added to the residue of ADF. The lumps were broken with a glass rod. The crucible was refilled with the solution as the acid drains. Then suction was applied to wash the contents of the crucible with hot distilled water until the washings were acid free to pH paper. The crucible was heated at 100^{0}C in hot air oven. Dried sample was cooled and weighed. Then cellulose content of the sample was calculated.

Cellulose (%) = W_b-W_c/ W_o x 100

Where, W_o = Initial weight of sample (dried & ground leaves); W_b = Weight of oven dried fibre (ADF) and crucible; W_c = Weight of 72% H_2SO_4 treated sample and silica crucible

To determine lignin content, the crucible containing 72% H_2SO_4 treated sample was ignited at 600^{0}C in muffle furnace for 3 hour and the crucible was placed in oven at 100^{0}C for 1 hour after removing it from furnace. The crucible was cooled in a desiccator and weighed. Lignin was determined as given below:

Lignin (%) = W_c-W_d/ W_o x 100

Where, W_o = Initial weight of sample (dried & ground paddy straw); W_c = Weight of 72% H_2SO_4 treated sample and crucible; W_d = Weight of furnace burnt sample and crucible

Statistical analysis: The data from different larval and pupal experiments were subjected to analysis using statistical software SPSS (IBM 2011). The data of fibre fractions was

subjected to factorial CRD. Pearson's correlation coefficients were determined to find relationship between fibre fractions and antibiosis factors.

RESULTS AND DISCUSSION

Larval duration: The total larval duration indicated significant variation in different genotypes (Table 1). The total larval duration was maximum in genotype IET22155 (19.6 days) followed by TKM6 (19.5) and W1263 (18.4). The other genotypes were in descending order for larval duration as JGL21066, RP4918-142, PR121 and TN1 (Table 1). As fibres affect the nutritional value of insect diet, so the longer total larval duration in some genotypes could be due to higher amount of cellulose and hemicellulose in these genotypes. High level of fibres increase bulk density of the diet and make it difficult to ingest adequate levels of nutrients and water to complete life cycle (Santiago et al 2013). The results are supported by study of Wang et al (2020) that suggested longer larval duration of potato tuber moth, *Phthorimaea operculella* on *Solanum tuberosum* having highest cellulose content (20%) while, shorter larval duration was observed on *S. lycopersicum* and *Physalis alkekengi* having lower cellulose amount of 11 and 8 per cent, respectively.

Larval weight and per cent pupation: The significant variation was observed in the weight of final instar of larvae feeding on different genotypes. The least larval weight was on resistant check TKM6 (22.7 mg) followed by IET22155 (23.4 mg) and W1263 (24.9 mg) and these genotypes were at par. The other genotypes were significantly at par to each other and JGL21066 had the highest larval weight among these genotypes (Table 1).

The per cent pupation varied significantly and was maximum in susceptible check TN1 (82%) followed PR121 (78%), RP4918-142, JGL21066 and W1263 and was minimum among IET22155 (64%) and TKM6 (54%) (Table 1). The variation in larval survival among genotypes could be due to the concentration of lignin in leaves of various genotypes. Lignin is the end product of the phenyl propanoid pathway that accumulates in the plant tissues after injury that triggers the pathway. Its deposition also makes plant tissues tougher and less palatable that make it difficult for the insect to feed on the plant (Armani et al 2020). Wang et al (2020) also observed higher larval survival on *S. tuberosum* (83%) having lower lignin content (4%) but larval survival was much lower on resistant *Lycium barbarum* (42%) that had higher lignin content (18%).

Pupal weight: The pupal weight in different genotypes varied significantly from 17.1 mg to 22.9 mg and minimum pupal weight was recorded in TKM6 (17.1 mg) followed by IET22155 (18.8 mg) and JGL21066 (20.6 mg). The pupal weight in other genotype viz., PR121, RP4918-142, W1263 and TN was in ascending order (Table 1). The variation in the pupal weight could also be due to the factors that were involved in affecting the larval weight. Wang et al (2020) observed that pupal weight was higher on plants having lower lignin content and vice-versa. However, present study revealed strong negative correlation of pupal weight with that of hemicellulose and cellulose and a weak negative correlation with that of lignin content Table 5).

Fibre fractions in leaves of different rice genotypes at constitutive and induced levels: Fibre fractions viz. cellulose, hemicellulose and lignin were analyzed in un-infested (constitutive) and infested (induced) leaves of 50 and 70 DOPs of selected genotypes.

Cellulose at constitutive and induced levels: The amount of cellulose in 50 days old uninfested plants varied significantly from 24.1 per cent in PR121 to 27.5 per cent in TKM6 (Table 2). In the infested plants, the cellulose content decreases after feeding and highest per cent decrease was in RP4918-142 (19.1%) followed by IET22155 (17.4%) but minimum decrease was in W1263 (10.9%). The decrease in cellulose content after infestation could be due to feeding by the larvae. In 70 DOPs, similar trend was observed and W1263 (33.4%) had highest cellulose content and PR121 (28.2%) had minimum cellulose content in uninfested plants (Table 2). After infestation, the cellulose content decreased in

Table 1. Larval duration, larval weight, per cent pupation and pupal weight of *C. medinalis* on rice genotypes

Genotype	Total larval duration (Days) (Mean±SE)	Larval weight (mg) (Mean±SE)	Per cent pupation (Mean±SE)	Pupal weight (mg) (Mean±SE)
PR121	15.5±0.5^{a}	25.9±0.4^{c}	78.0±3.7^{c}	22.0±0.7^{cd}
RP4918-142	16.5±0.5^{ab}	25.5±0.9^{bc}	76.0±5.1^{c}	22.1±0.5^{cd}
W1263	18.4±0.2^{bc}	24.9±1.0^{abc}	74.0±2.4^{c}	22.9±0.6^{d}
JGL21066	16.7±1.0^{ab}	26.2±0.8^{c}	74.0±5.0^{c}	20.6±0.4^{bc}
IET22155	19.6±0.4^{c}	23.4±0.8^{ab}	64.0±5.1^{b}	18.8±0.8^{ab}
TKM6	19.5±0.9^{c}	22.7±0.6^{a}	54.0±6.8^{a}	17.1±0.8^{a}
TN1	15.1±0.5^{a}	26.1±0.5^{c}	82.0±3.7^{c}	22.9±0.7^{d}

Means within a column followed by same letter are not significantly different at $p \leq 0.05$ according to LSD

70 DOPs. Cellulose is the main component of cell wall, insoluble in water and highly stable polymer (Taylor 2008).

Hemicellulose at constitutive and induced levels: The similar trend was followed for hemicellulose content in all the genotypes and there was reduction in the hemicellulose amount after the insect infestation and was lowest in PR121 (30.0%) followed by TN1 and highest in TKM6 (34.2%) in 50 DOPs (Table 3). The decrease in hemicellulose amount in leaves among different genotypes could be due to the consumption of the primary cell wall by the larvae during the feeding process. After infestation, there was 11.7 to 22.8 per cent decrease in hemicellulose and highest hemicellulose content after infestation was found in TKM6 (28.4%) and lowest in JGL21066 (24.5%) (Table 3). In 70 days old uninfested plants, hemicellulose content in TN1 and PR121 was significantly less (35.1 and 35.4%, respectively) than other genotypes viz., TKM6, IET22155, W1263, RP4918-142 and JGL21066 having hemicellulose amount from 40.9 to 43.3 per cent (Table 3).

In the infested plants, TN1, PR121 and JGL21066 had significantly less hemicellulose content (29.1, 30.6 and 33.9%, respectively) than W1263 (38.5%), TKM6 (38.3%), IET22155 (37.4%) and RP4918-142 (36.7%) (Table 3). Hemicellulose refers to a diverse class of polysaccharides which differ from the cellulose because this fraction is amorphous and easily soluble or hydrolysable in alkaline or acid solutions (Somerville et al 2004).

Lignin at constitutive and induced levels: The lignin content in 50 DOPs uninfested plants varied significantly and was maximum in JGL21066 (3.7%) followed by TKM6 (Table 4). Whereas, W1263, IET22155, TN1, PR121 and RP4918-142 has lower lignin content. Slight numerical increase was recorded in lignin content after insect feeding in various genotypes in both 50 and 70 DOPs. The per cent increase in lignin content in 50 DOPs of different genotypes after insect infestation varied from 5.0 to 9.4 per cent. However, in 70 DOPs there was increase in the amount of lignin in all the genotypes. The highest lignin content in uninfested plants was again in JGL21066 (4.3%) and TKM6 (4.2%) and lowest in TN1 (3.5%) and RP4918-142 (3.2%) (Table 4). Likewise, under infested conditions lignin content was highest in JGL21066 (4.6%) and TKM6 (4.5%). The genotypes

Table 2. Cellulose content (Per cent of dry weight) before and after infestation by *C. medinalis* in the leaves of 50 and 70 days old plants of selected genotypes

Genotype	50 days old plants		70 days old plants	
	Uninfested (Mean±SE)	Infested (Mean±SE)	Uninfested (Mean±SE)	Infested (Mean±SE)
PR121	24.17±0.74^{a}	20.37±0.49^{a}	28.23±0.41^{a}	24.13±0.18^{bc}
RP4918-142	27.20±1.14^{bc}	22.00±0.97^{ab}	28.30±0.87^{a}	24.13±0.52^{bc}
W1263	27.07±0.67^{bc}	24.10±1.42^{c}	33.40±0.74^{b}	28.60±0.74^{d}
JGL21066	25.60±0.35^{ab}	21.73±0.68^{ab}	29.60±0.92^{a}	23.03±1.05^{ab}
IET22155	26.77±0.82^{c}	22.10±0.56^{b}	29.63±0.97^{a}	25.20±1.19^{c}
TKM6	27.53±0.93^{c}	23.83±0.32^{c}	32.20±0.78^{b}	29.60±0.99^{d}
TN1	24.83±0.43^{a}	20.80±0.75^{ab}	28.63±0.47^{a}	22.40±0.29^{a}

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD

Table 3. Hemicellulose content (per cent of dry weight) before and after infestation by *C. medinalis* in the leaves of 50 and 70 days old plants of selected genotypes

Genotype	50 days old plants		70 days old plants	
	Uninfested (Mean±SE)	Infested (Mean±SE)	Uninfested (Mean±SE)	Infested (Mean±SE)
PR121	30.00±0.76^{a}	26.47±0.12^{bcd}	35.47±0.38^{a}	30.63±0.78^{a}
RP4918-142	31.30±0.44^{ab}	25.03±0.94^{ab}	43.37±1.36^{c}	36.77±1.01^{c}
W1263	33.20±0.71^{bc}	27.03±0.56^{c}	41.83±0.87^{bc}	38.50±0.78^{c}
JGL21066	31.80±1.36^{ab}	24.53±0.99^{ab}	40.93±0.39^{b}	33.97±0.59^{b}
IET22155	31.50±1.17^{ab}	27.80±1.00^{d}	41.67±1.11^{bc}	37.47±1.13^{c}
TKM6	34.20±1.01^{c}	28.47±1.58^{d}	42.47±0.59^{bc}	38.33±0.80^{c}
TN1	30.93±0.58^{a}	25.43±0.93^{abc}	35.17±0.48^{a}	29.16±0.84^{a}

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD

RP4918-142 (3.5%) and TN1 (3.6%) recorded the minimum lignin content after infestation (Table 4). Per cent increase in lignin content among genotypes varied from 3.7 to 9.5 per cent after insect infestation. Lignin is the most resistant polymer in nature and plays an important role in plant growth and development by providing structural support for land plants and as a resistance mechanism to biotic and abiotic stresses (Wang et al 2020). The increase in the lignin amount after insect infestation was due to the triggering of the defense mechanism via phenyl propanoid pathway that results in the formation of lignin, final product of phenyl propanoid pathway (Boerjan et al 2003). Yanni et al (2011) observed that lignin content in maize leaves increased from 34.4 to 39.3 g/kg in non-Bt variety and 31.6 to 33.6 g/kg in Bt variety after the infestation of European corn borer (ECB). Similarly, Barros-Rios et al (2011) investigated that maize line (EP39) resistant to ECB was less damaged by the pest having lignin content of 69 g/kg than susceptible line (EP47) with lignin content of 50 g/kg.

Correlation between antibiosis parameters and fibre fractions: The Pearson's correlation analysis showed a significant positive correlation of larval duration with that of cellulose (r = 0.63) (Fig. 1) and hemicellulose and lignin means with increase in the dietary fibre in the plant leaves it take more time for the insect to complete its larval phase (Table 5). However, the final larval weight has negative correlation with cellulose and hemicellulose (Fig. 2) (Table 5). Larval survival was negatively correlated to lignin content in leaves (Fig. 3) i.e. higher lignin content has fatal effect on developing larvae but cellulose and hemicellulose has no significant effect on larval survival. Pupal weight was found to have negative correlation with cellulose and hemicellulose (Table 5).

Table 5. Pearsons correlation between antibiosis parameters and biochemical factors

Characters	Cellulose	Hemi-cellulose	Lignin
Larval duration	0.63**	0.62**	0.42*
Larval weight	-0.43**	-0.41*	-0.12
Per cent pupation	-0.30	-0.27	-0.36*
Pupal weight	-0.49**	-0.50**	-0.34

*Significant at 5 and** 1per cent level of significance

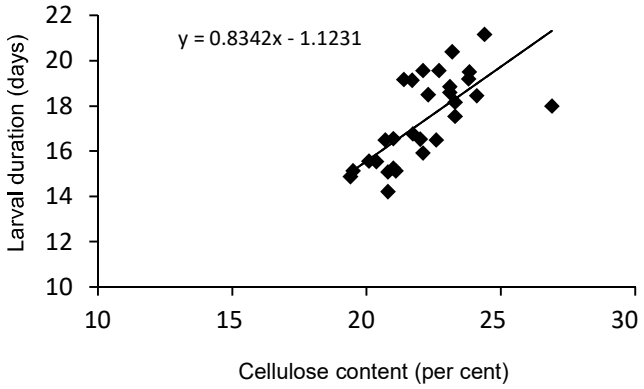

Fig. 1. Correlation between cellulose content and larval duration

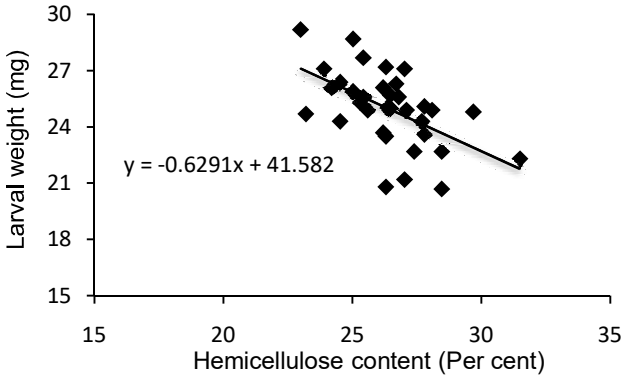

Fig. 2. Correlation between hemicellulose content and larval weight

Table 4. Lignin content (per cent of dry weight) before and after infestation by *C. medinalis* in the leaves of 50 and 70 days old plants of selected genotypes

Genotype	50 days old plants		70 days old plants	
	Uninfested (Mean±SE)	Infested (Mean±SE)	Uninfested (Mean±SE)	Infested (Mean±SE)
PR121	3.13±0.09^{a}	3.33±0.07^{a}	3.63±0.03^{bc}	3.83±0.09^{b}
RP4918-142	3.07±0.12^{a}	3.36±0.12^{a}	3.23±0.09^{a}	3.50±0.15^{a}
W1263	3.43±0.13^{b}	3.70±0.10^{b}	3.80±0.12^{c}	4.07±0.03^{c}
JGL21066	3.73±0.09^{c}	4.07±0.17^{c}	4.30±0.17^{d}	4.60±0.20^{d}
IET22155	3.17±0.12^{a}	3.43±0.12^{a}	3.44±0.09^{ab}	3.77±0.09^{b}
TKM6	3.67±0.13^{bc}	3.93±0.18^{bb}	4.26±0.09^{d}	4.53±0.09^{d}
TN1	3.17±0.12^{a}	3.33±0.09^{a}	3.50±0.11^{b}	3.63±0.07^{ab}

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD

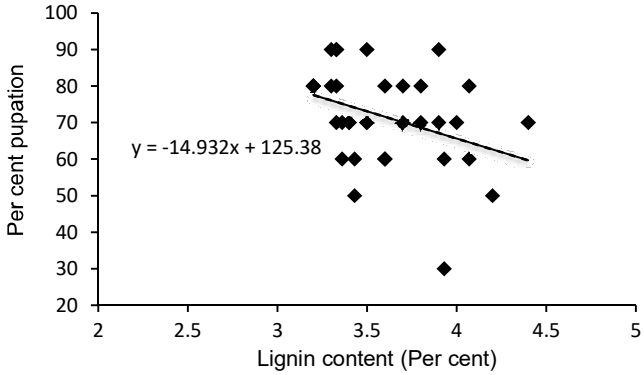

Fig. 3. Correlation between lignin content and per cent pupation

CONCLUSIONS

The genotypes that had higher amount of fibre fractions in their leaves provide some kind of resistance to the plants against RLF as the insect took more time to complete its larval stage on such genotypes. The last instar larval weight was also less on the genotypes having higher cellulose and hemicelluloses content. On the contrary, cellulose and hemicelluloses did not significantly affect larval survival but higher lignin content resulted in significantly lesser number of larvae turning into pupae. However, pupal weight was significantly reduced by greater amount of cellulose and hemicelluloses but lignin content had no significant effect on pupal weight of the RLF. These promising factors could be analyzed to screen the promising genotypes against RLF and against other rice lepidopteran insect pests.

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Phytoremediation of Secondary Treated Sewage through Constructed Wetland: Lab-Scale Study

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***Abstract*:** Due to increasing urbanization and industrialization, India freshwater resources are deteriorating. Discharge of untreated wastewater into the aquatic ecosystems is the major source of pollution to these ecosystem. Conventional methods of treatment of the wastewater are quite costly, energy-intensive and require huge investment while natural systems such as Constructed Wetlands (CW) can be a better option for the treatment of wastewater. Under the present study laboratory scale Vertical Flow Constructed Wetland (VFCW) was utilized for the further treatment of secondary treated effluent collected from the MBBR (Moving bed biofilm reactor) based sewage treatment plants (STPs). The lab scale VFCWs were planted with *Typha latifolia* and *Canna indica* plants and filled with gravel and sand which act as growth and filter medium. Experiments have been performed to evaluate the performance of VFCW at different retention times (RT) in hours (h) i.e., 0 h, 24 h, 48 h, and 72 h. The secondary treated sewage was supplied to the VFCW and a change in the physicochemical properties of the sewage with increasing time was measured. There was continuous decrease in the major parameters of the secondary treated sewage in 72 h of incubation period thereafter a little or no change was observed. The removal efficiency for biological oxygen demands (BOD), chemical oxygen demands (COD), nitrate also phosphate was more after 72 h through VFCW planted with *Typha*than with *Canna*. The VFCW planted with *Typha*also showed greater performance than VFCW planted with *Canna*. The removal efficiency of VFCW for unplanted (control) system wase less than the planted system.

Keywords: Constructed wetlands, Domestic sewage, Freshwater, Removal efficiency, Retention time, Traditional methods, Vertical flow constructed wetland

Contamination of rivers, lakes, ponds, waterways, and groundwater has become a serious concern in developing nations like India. Agricultural run-off, industrial discharges, domestic sewage, and other sources of pollution have badly contaminated the bulk of aquatic habitats (Gupta et al 2020, Yadav et al 2021). In the Indian context, agriculture discharge, industrial effluents and sewage contribute as 65, 25, and 10% towards the quantitative pollution load of the aquatic environment (Kumar et al 2015). However, industrial effluents are now regulated and controlled by the laws and agricultural runoff being the diluted source domestic sewage being the most problematic fraction for the surface water resources (Kaur et al 2012). The majority of the water consumed in the houses and residential accommodations of the industries returned as sewerage (domestic sewage), and is discharged into nearby riverine ecosystems without proper treatment (CPCB 2013, Harshvardhan and Jha 2013) and thus become the greatest nuisance to the surface water resources. Existing sewage treatment plants are insufficient to treat the total sewage generated in India (CPCB 2016, Kamyotra and Sinha 2016). According to the survey conducted by Central Pollution Control Board (CPCB) of India in 2017 out of the total 1469 sewage treatment plants (STPs) 578 were operational thus treating 26869 Million Liters per Day (MLD) of sewage and the non-functional STPs at the same time discharged 123.16 MLD untreated sewage into the aquatic environment (CPCB 2018). Despite being the fact that there had been a sharp increase in the total installed capacity of STPs the gap between total sewage produced and untreated has widened (CPCB 2013). Most of STPs are quite costly, energy intensive and complex in natures which require huge capital and running cost. Such limitations put a demand for new, ecofriendly and cost effective technology for the sewage treatment. Constructed Wetland (CWs) is an effective, environment friendly, and economic feasible option for sustainable treatment of sewage (Mishra et al 2018, Singh et al 2021, Singh et al 2022). Constructed wetlands have been used for the treatment wide variety of pollutants including sewage. However, most of the studies dealing with CWs have been done in Europe and North America. Therefore, we performed this study with an objective to treat the secondary treated sewage by using lab scale Vertical Flow Constructed Wetland (VFCW).

MATERIAL AND METHODS

Study area: This study was conducted at the Institute of Environment and Sustainable Development (IESD), Banaras

Hindu University (BHU), Varanasi. The study involved further treatment of secondary treated domestic wastewater collected from Bhagwanpur Sewage treatment plant (STP) of Varanasi, Uttar Pradesh. This STP is located between 25°0' to 25°16' N latitude and 82°5' to 83°1' E longitude. This STP receives domestic sewage from BHU and the surrounding area with an installed capacity to handle 8 MLD sewage.

Sampling and analysis of secondary treated and CW treated sewage: Sewage samples were collected from the discharge point of the Bhagwanpur STP and brought to the laboratory for analysis. The study was performed for six months during which the treatment of secondary and tertiary sewage was performed through lab-scale VFCW. The effluent was analyzed before it was batched into the laboratory-scale constructed wetlands. To each wetland set up 3.5 liters of secondary treated effluent was supplied and the effluents were analyzed at various retention times i.e., 0 h, 24 h, 48 h, and 72 h for different physicochemical properties. Samples were collected and analyzed for various physicochemical properties viz TDS, alkalinity, total hardness, BOD, COD, nitrates, phosphates, and microbial activity (total coliform, fecal coliform, and *E. coli*). The samples were analyzed following the methods of APHA (2017). Microsoft Excel (version 2013) was used for the statistical analysis. Following formula was used to calculate the removal efficiency for different parameters.

$$\text{Removal percentage (\%)} = \frac{\text{Initial concentration} - \text{Final concentration}}{\text{Initial concentration}} \times 100$$

Experimental design: A lab scale vertical flow constructed wetlands (VFCW) was built at the IESD, BHU, Varanasi. The wetland was constructed using a plastic bucket of dimensions 30 cm x 36 cm x 30 cm. The bucket was filled with sand (<0.5 mm) of 13cm length followed by gravel (20 mm – 22mm) of 10 cm of length at the bottom of the bucket. The perforated polyvinyl chloride (PVC) pipes were used for the aeration in the constructed wetland. Three different setups of VFCW in triplicates were established in the laboratory. Setup 1 was planted with *Typha*, set up 2 with *Canna,* and set up 3 with no plant (control), to evaluate the concentration of sewage with increasing time. Each Set of VFCW was irrigated with tap water for one month initially before the experiment to acclimatize the wetland plants to their new environment. The detailed study and design of experimental VFCW is presented in Table 1 and Figure 1 respectively.

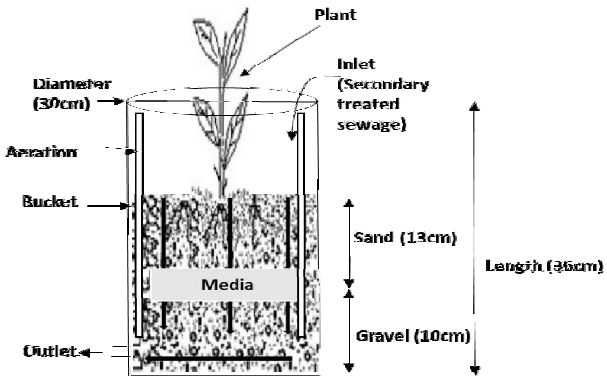

Fig. 1. Diagrammatic representation of constructed wetland set up in the laboratory

RESULTS AND DISCUSSION

General physicochemical properties of treated sewage: The secondary treated sewage collected from Bhagwanpur STP was analyzed immediately after the collection and analyzed before treatment i.e., at zero hours (Table 2).

Performance of vertical flow constructed wetland (VFCW) for sewage treatment: The sewage from Bhagwanpur STP was subjected to treatment with two different setups of VFCW, v.i.z., setup 1; wetland planted with *Typha* and set up 2 wetland planted with *Canna*.The analyses of the treated sewage samples were performed at different time intervals i.e. 0, 24, 48, and 72 h. The experiment was performed further but there were no significant changes therefore further experiments were conducted up to 72 hours of retention time (RT) (Figs. 2, 3).

Table 1. Details of experimental VFCW

Set up no.	Set up type	Container size	Media used	Media length	Plant used
1.	Set up 1	D-30 cm L-36 cm V-25 lit.	Sand & Gravel	13 cm 10 cm	*Typha* (Triplicates- plant A, B & C)
2.	Set up 2	D-30 cm L-36 cm V-25 lit.		13 cm 10 cm	*Canna* (Triplicates- plant A, B & C)
3.	Set up 3	D-30 cm L-36 cm V-25 lit.		13 cm 10 cm	Unplanted (Control)

Analysis of different parameters of effluent treated by both the experimental VFCW set up revealed a significant change in all the parameters during 72 h period. The VFCW planted with *Typha* has removed 68.65, 63.26, 67.14 and 65%, of biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrate, and phosphate respectively during 72 hours of retention time. The removal efficiencies after 72 h of incubation period through VFCW planted with *Canna* for BOD, COD, nitrate, and phosphate were 26.40, 55.90, 28.57 and 61.66%, respectively. When compared the change in the BOD, COD by both the setups it was observed that set up 1, i.e. VFCW planted with the *Typha latifolia* showed the higher removal of BOD & COD in comparison to the set up 2, i.e. VFCW planted with *Canna*. Therefore, *Typha latifolia* as better performer than *Canna indica* for the removal of BOD & COD (Fig. 4). This indicates that *Typha* has developed a dense network of roots in short span of time in comparison to *Canna*. The elimination of BOD and COD may be accelerated by the presence of dense plant roots and the filter media such as sand and gravel (Stefanakis et al 2014, Barya et al 2020, Shukla et al 2021). The BOD and COD elimination was also dependent on the retention time, increasing retention time results in higher removal efficiencies (Bakhshoodeh et al 2020). Sehar et al (2015) elucidated that the BOD and COD elimination is supported by the mutual interactions among the microbial and physical mechanism of the removal of the pollutants by involving the dissolved oxygen.

The major nutrients present in sewage i.e. nitrate and phosphate were also removed by VFCW. The highest removal of these parameters was observed at the retention time of 72 hours in both the set ups of VFCW (set up 1 & 2) (Fig. 4). The VFCW planted with *Typha* (set up 1*)*, showed the 28.3% and 61% removal of nitrate and phosphate while for the same parameters VFCW planted with *Canna* (set up 2) showed 56.4 and 57.6% removal, respectively. The removal of nitrate and phosphate might be due to physico-chemical pathways with sorption and precipitation (Reddy et

Table 2. General physicochemical composition of sewage (Mean ±SD)

Parameters	Concentration (0 h)
TDS (mg/l)	362.06±0.11
Alkalinity (mg/l)	586.03±0.06
Total hardness (mg/l)	230.006±0.01
BOD (mg/l)	47.56±0.05
COD (mg/l)	231.33±0.57
Nitrate (mg/l)	27.10±0.001
Phosphate (mg/l)	0.60±0.001

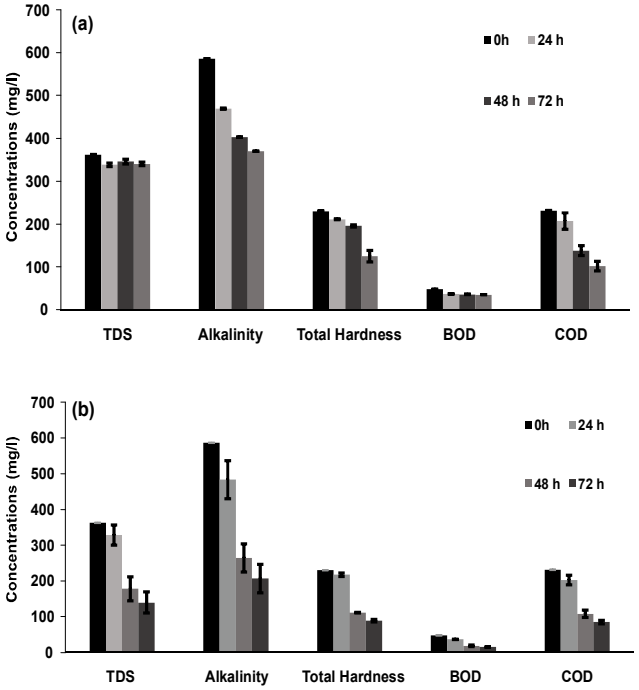

Fig. 2. Performance of (a) *Typha* sp. and (b) *Canna* sp. in reducing different physicochemical parameters at different retention times (RT) in VFCW

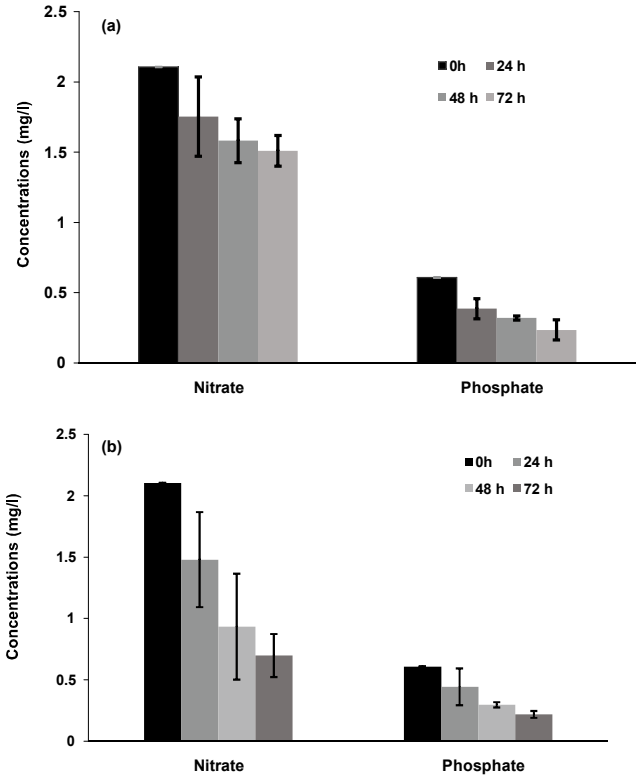

Fig. 3. Performance of (a) *Typha* (b) *Canna* in reducing nitrate and phosphate at different retention time (RT) in VFCW

al. 1999). The particulate phosphorous is removed through filtration (Kadlec and Wallace 2009) by the filter media used in the constructed wetland system. Gagnon et al (2010) have observed that the contents of the organic compound into the specific organic carbons are the factors that determine the elimination of the nitrates from the wastewater into the artificially created swampland systems. Zhang et al (2018) and Zhu et al (2014) have verified that by the escalation of the COD/NO_3 ratio into the treated wastewater, the efficiencies of the nitrate elimination may increase. Macrophytes with the CW system plays an essential role in the treatment of various parameters by the releasing oxygen through their rhizomes to the bottom treatment wetlands, as well as provides the medium underneath the water surface for the attachments of the micro-organisms to achieve the biological treatments (Batool and Seleh 2020). Zhang et al (2007) have demonstrated that the perennial plant ensures the continuous treatment. The *Typha latifolia* and *Canna indica* are resistant, fast growing and robust plant which can perform for the treatment of diverse set of pollutants.

Treatment of coliforms from secondary treated sewage through VFCW planted with *Typha:* The macrophytes such as *Typha* rooted in the filter media of sand and gravel possesses a great potential to reduce the number of coliforms. As revealed by the lab analysis the secondary treated sewage still contains significant number of total coliforms and is the source of pathogens which is the most important indicator of fecal contamination of water bodies.

Under the present investigation VFCW also performed well for the reduction of total and fecal coliforms as well as *E. coli.* from the secondary treated sewage. The initial value (MPN) of total coliformwas 1.6×10^4 before the treatment. During 72 h of experimental period there was a significant reduction in this number and after treatment it was reduced to 9.2×10^2 at 72, whereas the control shows a reduction in MPN up to 1.6×10^3. The initial value of fecal coliform was 1.7×10^2 at 0 hours which after 72 hours of treatment was reduced to 1.7×10. The control experimental set up (without plant) showed a reduction in MPN to 7.8×10 after 72 hours. The MPN value of *E. coli* at 0 hours was 5.1×10 and it was reduced to 2.2×10 after treatment in 72 h. It implies that the plants are responsible for reducing the coliform in the samples. The VFW without plant also showed the some pathogen removal in the same period.

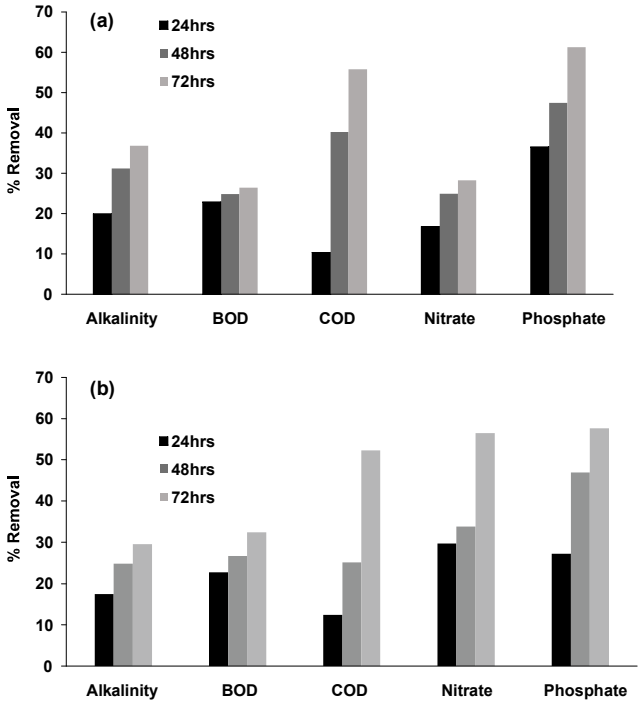

Fig. 4. Removal efficiency of VFCW planted with (a) *Typha* and (b) *Canna* for the treatment of various parameters

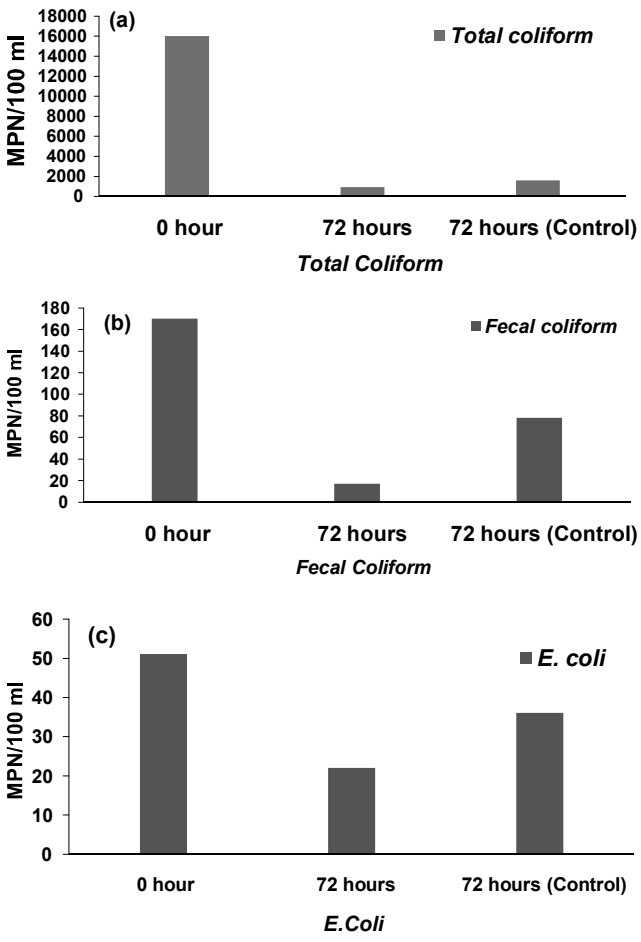

Fig. 5. Performance of (a) *Total coliforms*, (b) *Fecal coliform* & (c) *E. coli* at different retention time (RT) in VFCW

Overall, the results revealed the significant removal of various important parameters like BOD, COD, nutrients, and coliforms of the secondary treated sewage through VFCW planted with *Typha latifolia* and *Canna indica*. The performances of the planted VFCW were better for the removal of almost all the parameters of the secondary treated sewage. This may be attributed to the role of the plant's root which aerated the root zone and performed the uptake of nutrients from the root zone. This was also recognized that the elimination of almost all the parameters increased with increasing detention time till 72 hours. Figure 3 shows the behavior of nutrients and organic matters. The reduction of microbes (Coliforms and *E. coli*) with planted VFCW system showed was higher than the unplanted (control) systems. Lesser removal in unplanted (Control) constructed wetland system was due to the pore size available in the substrate (sand and gravel) (Ramprasad et al 2017) and unavailability of vegetation. Substrates like gravel and sand provide a large surface areas for the microbial growths, also high oxygen accessibility which functioned proficiently to the elimination of the pollutants (Ge et al 2015). Substrates (sand and gravel) play essential roles in percolation, adsorptions, as well as ion exchange. Hydraulic permeability and the adsorption capacities were the chief characteristic of the substrate which promoted the performance of the experimental CWs. The gravel generally increases the percolation of the swamp lands as well as minimizes clogging also increases the nitrification process (Rai et al 2015). Sand media provides support to plants' development and also provides a platform for microbial development as well as ion exchange (Arroyo et al 2013).

CONCLUSION

Constructed wetlands are gaining the attention of scientists as valid alternative for the sewage treatment in India. It is an effective as well as efficient system for removing nutrients loads from municipal sewage wastewater. VFCW have proven efficient in treating the sewage for almost all the major parameters. The activity of the substrate (sand & gravel), plants, and microbes inside the systems supports the decent environmental conditions which enhance the water quality. *Typha latifolia* as the vegetation was a better performer for the treatment of sewage VFCW. *Canna indica* remained assessed as proficient in the reduction of some parameters like nitrates. There were important differences in reducing the physicochemical parameters between the control (unplanted) and planted system. VFCW have abundant potentials to be applied for the treatment of domestic sewage. Decades of researches have shown the domestic wastewater treatment through VFCW is a promising alternative to conventional treatments. This can be applied as a means of educations to identify the individuals about the low-cost also about low maintenance wastewater treatment technology specially in Indian context.

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Estimating the Extreme Flood Height Quantiles Using Bayesian Approach

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***Abstract*:** In the classical method of estimation, the GEV distribution was identified as the most appropriate model for estimating peak flood heights at twelve study sites in the Mahanadi River Basin. This paper presents that Bayesian parametric estimates of the GEV distribution are better compared to maximum likelihood estimates in estimating peak flood heights and their return periods at these sites. To arrive at this target Markov Chain Monte Carlo Bayesian technique is utilized to acquire parameters of GEV distribution. The estimates of Bayesian approach for peak flood heights and their return periods at the sites showed better predicted flood peak return periods with the Bayesian method. These were shorter than estimates obtained using the maximum likelihood method for all the sites.

Keywords: Bayesian, MLE, Mahanadi, Flood heights

Floods are frequent visitors to the Mahanadi River Basin (MRB) in India, causing grave harm to human society in the affected area. Hydrological extremes, such as floods, can be described using extreme value theory by estimating high quantiles of extreme flood levels and their return periods. In the classical method of estimation, Generalised Extreme Value (GEV) distribution was considered to be a good model for frequency analysis in hydrology (Nagesh and Laxmi 2021). The appropriate flood frequency distributions were identified for twenty six sites of MRB, in which the GEV distribution was found to be good probability model for twelve sites. The Maximum Likelihood Estimation (MLE) is one of the most widely used methods to estimate parameters of the flood frequency distributions (Dombry 2015, Ferreira and De Haan 2015). The likelihood based procedure is alluring, however the problem is the regularity conditions that are need for the normal asymptotic properties related with the MLE to be justified (Alam et al 2019). The Bayesian procedure is a popular method of parametric estimation technique alternate to MLE method and Bayesian estimates can be considered as an improved estimates over maximum likelihood estimates (Reis and Stedinger 2005, Chandra et al 2015, Alam et al 2019). Maposa et al (2014) showed that Bayesian-Based estimates were better than Maximum Likelihood estimates for GEV distribution at two sites in the lower Limpopo river basin, Muzambique. Bayesian approach allows comparison of other source of information by means of prior and posterior distribution. To estimate the parameters of a GEV distribution and make further predictions of the return levels and their related return periods, the researcher employs Bayesian Markov Chain Monte Carlo (MCMC) inference, which has the advantage of not requiring regularity constraints. These results and forecasts are contrasted to those obtained using a frequentist technique based on maximum likelihood GEV distribution estimations in a block maxima framework. Ferreira and De Haan (2015) revealed that the block maxima approach can outperform the Peaks over Threshold method in some circumstances. In this study our key objective is to check whether Bayesian estimates are improved estimates over MLE.

MATERIAL AND METHODS

This section explains how the data in this paper was analyzed using the methodologies employed in the study. In the Bayesian and frequentist paradigms, the methods include algorithms, prior distribution methods and the likelihood of the framework of block maxima.

Data: In the present work, daily water level (metres) data of the MRB recorded thrice a day at twelve hydrometric stations related to the period 1971-2017 were obtained from Central Water Commission (CWC), Bhubaneshwar.

Generalized extreme value model: The GEV distribution is one of the important extreme value distributions to determine the occurrence of the probability of rare event in the field of hydrology, climatology finance, insurance etc. Let the values x_1, x_2, x_3, …., x_n be the annual daily maximum flood height observations of n independent and identically distributed random variable X. As n sufficiently large, the annual daily maximum flood height observations approximate to GEV distribution. The Distribution Function of the GEV distribution is given by

$$F(x;\mu,\sigma,\xi)=\exp\left\{-\left[1+\xi\left(\frac{x-\mu}{\sigma}\right)\right]^{-1/\xi}\right\};\xi\neq 0,-\infty<\mu<\infty,\sigma>0 \quad (1)$$

In which μ, σ and ξ are respectively the location, scale and shape parameters of the distribution and are estimated using MLE and MCMC Bayesian approach.

Bayesian model for flood frequency: The observation vector x= {x= x_1, x_2, x_3, …., x_n} consists of iid realizations of annual maximum flood heights and parameter vector θ={ μ, σ and ξ). The posterior distribution is computed using Bayer's Theorem

$$\pi(\theta|x)=\frac{f(x|\theta)\pi(\theta)}{\int f(x|\theta)\pi(\theta)d\theta} \quad (2)$$

which is usually written as

$$\pi(\theta|x)\propto f(x|\theta)\pi(\theta) \quad (3)$$

x is a vector of observations, θ is a parameter vector, π(θ) is the prior density function π(θvx) is the posterior distribution, F(θvx) is the density of x, interpreted as the conditional density of x given $_\theta$. The numerator is the joint density of θ and x and the denominator is the marginal density of x. The symbol θ now represents both a random variable and its value. When the parameter θ is discrete, the integral in the denominator of (2) is replaced by a sum. The conditional density π(θvx) of θ given x =x is called the posterior density, a quantification of our uncertainty about θ in the light of data (Ghosh et al 2006). A Bayesian can simply report posterior distribution, or report summary descriptive measures associated with posterior distribution. For example, for a real valued parameter$_\theta$, the posterior mean

$$E(\theta|x)=\int_{-\infty}^{\infty}\theta\pi(\theta|x)d\theta \quad (4)$$

and the posterior variance

$$Var(\theta|x)=\int_{-\infty}^{\infty}(\theta-E)(\theta|x)^2\pi(\theta|x)d\theta \quad (5)$$

Trivariate normal distribution

$$f(x)=\frac{1}{\sigma}\exp\left\{-\frac{1}{2}(\theta-\nu)^T\Sigma^{-1}(\theta-\nu)\right\}$$

where ϑ is mean vector, ∑ is symmetric positive definite covariance matrix.

Trivariate normal distribution is considered as prior distribution for MCMC Bayesian approach. All the outcomes of the present analysis were achieved through use of R software packages ismev and extRemes.

RESULTS AND DISCUSSION

The analysis for twelve hydrometric sites of MRB under the Block Maxima approach was done and estimated parameters of GEV distribution using both MLE and Bayesian approach (Table 1). The 95% credible interval for μ shows that true average of population of annual maximum flood heights fall in the range 3.80m-4.34m at the site with probability 0.95 (Table 1). According to the 95 percent confidence intervals for the ML estimates and 95 percent credible intervals for the Bayesian estimates, the shape parameter of the GEV distribution at Bamnidhi is not significantly different from zero, indicating that annual maximum flood heights can be modeled at the site by a light-tailed Gumbel family of distributions. The quantile function and the parameter estimates from Table 1 were used to construct Table 2.

Bayesian estimates of maximum flood heights are frequently greater than their corresponding ML estimates (Table 2), which is consistent with Table 1. The experimentally recorded maximum flood heights were compared to the anticipated flood heights using both Bayesian and frequentist techniques, and only 6m flood height that occurred during the disastrous floods of 1975 is greater than the 50-year flood level at Bamnidhi. In diagnostic plots displays empirical results for annual daily maximum flood heights for Bamnidhi site, reveal that annual peak flood heights are positively skewed (Fig. 1), with a maximum flood height of 6m occurring in 1975. The probability plot and probability density plot show that GEV Model is a good fit (Fig. 2). Because all of the points on the probability plot are extremely near to the fitted line, and the probability density plot shows that the GEV distribution imitates the empirical distribution form, as seen in Figure 2's histogram. Figure 3 depicts return level plot, which are calculated by Bayesian approach. Black line indicates return level for GEV distribution of observed values for Bamnidhi site. Red dotted lines indicate the interval for return levels considered different return periods. A simulation study of MCMC was also conducted to generate trace and subsequent marginal posterior densities estimated by the MLE method at Bamnidhi site (Figure 4). Fast convergence is observed in trace plots of Figure 4 and the results of posterior marginal densities indicate that posterior estimation of is very improbable to be below 3.80m and very unlikely to be above 4.34m for Bamnidhi site. Similar results are obtained for other eleven sites.

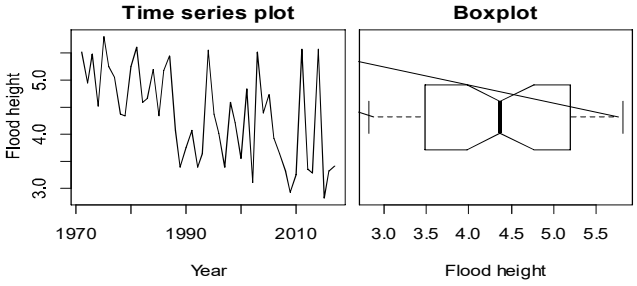

Fig. 1. Time series plot and Boxplot of Bamnidhi site

Bayesian estimates of the parameters are higher than ML estimates (Table 3). The confidence interval for the population average μ indicates that actual population average is the annual maximum heights of Dharamjaigarh, Kesinga, Kotni, Manendragarh, Mohana, Pathardhi, Rajim, Seorinarayan, Simga, Sundargarh and Alipilngal lies in the range of 4.76 - 5.53m, 6.98 – 8.76m, 6.74– 8.47m, 3.35 – 4.16m, 2.46 – 3.05m, 4.91m – 6.40m, 4.07m – 5.31m, 8.86 – 10.80m, 8.01 – 9.66m, 6.08 – 6.81m and 10.14 – 11.03m, respectively. The standard errors of parameter estimates

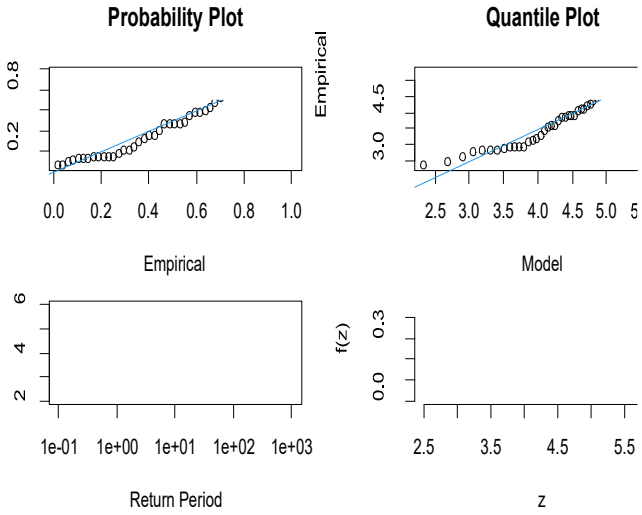

Fig. 2. Diagnostic plots using MLE for Bamnidhi site

Table 1. Parameter estimates of GEV distribution for Bamnidhi Site

ML estimates			
Parameter	Estimate	SE	95% CI
μ	4.1388	0.1607	(3.7807 4.4969)
σ	0.9319	0.1394	(0.6213, 1.2426)
ξ	-0.4917	0.1735	(-0.878, -0.1051)
Bayesian estimates			
Parameter	Estimate	SE	95% CI
μ	4.1399	0.0036	(3.8001, 4.3400)
σ	0.9900	0.0029	(0.7100, 1.1700)
ξ	-0.3541	0.0034	(-0.6300, 0.0000)

SE-Standard Error, CI-Confidence interval for ML estimates and Credible interval for Bayesian estimates

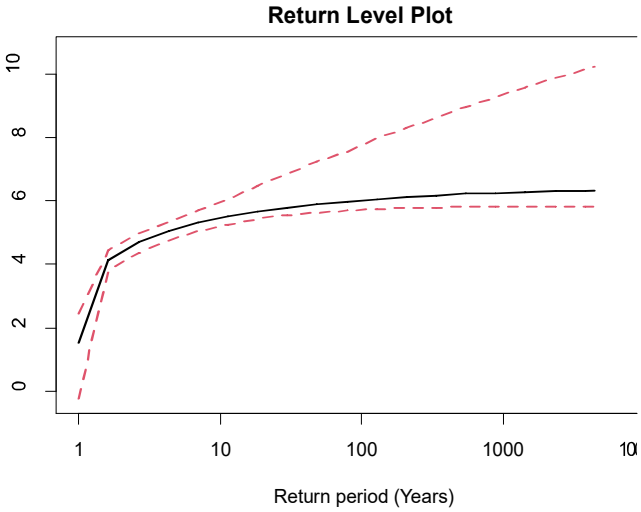

Fig. 3. Return level plot for Bamnidhi site

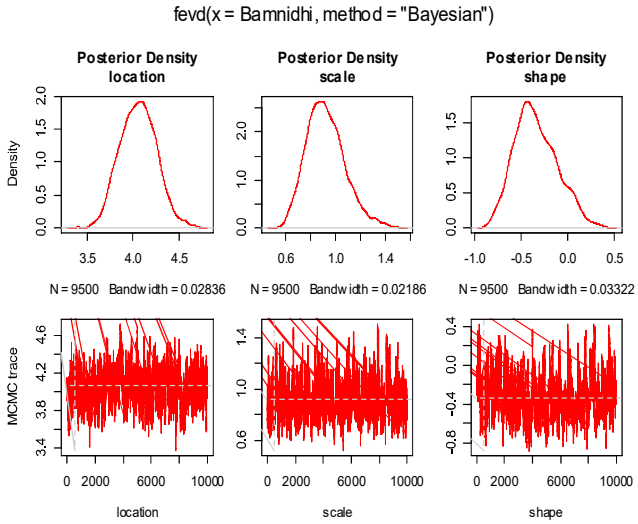

Fig. 4. Posterior density and trace plot for Bamnidhi site

Table 2. Estimation of tail quantile and expected return levels for Bamnidhi site

1-p	p	T	ML estimate (Exceedances)*	Bayesian estimate (Exceedances)*
0.9500	0.05	20	5.7422	5.9034
0.9800	0.02	50	5.8495	6.2753
0.9900	0.01	100	5.9031	6.3828
0.9950	0.005	200	5.9411	6.4665
0.9960	0.004	250	5.9508	6.4893
0.9980	0.002	500	5.9749	6.5497
0.9990	0.001	1000	5.9920	6.5968
0.9999	0.0001	10000	6.0205	6.6913

using the Bayesian technique are lower than those obtained using the MLE approach.

Expected return levels calculated using Bayesian approach is greater than expected return levels calculated using MLE (Table 4). Credible intervals are narrow than confidence interval which indicates Bayesian estimates of GEV distribution have narrow intervals than ML estimates which can be considered as one of the points to highlight the improvisation of Bayesian approach.

Due to heavy rain and cyclonic conditions, extreme flood heights occurred at twelve study sites: 6, 8, 9, 11 and 12 at Bamnidhi (1975), Rajim (1980), Manendagarh, Mohana, Sundargarh, and Pathardi (1990, 1990, 1998, and 2007, respectively); Dhrmarjgarh (1991) and Kotni, Kesinga, and Alipingal (1978, 2006, and 2011 respectively). Both methodologies in Table 2 and 4 were used to calculate the associated return periods of maximum flood heights. For the aforementioned sites, the findings of the Bayesian technique yielded return periods of 20, 10, 50, 50, 20, 200, 20, 20, 10, 2, 10 and 200 years, respectively, implying that these occurrences have a very low chance of being equalized or exceeded at least once in the above-mentioned years.

Table 3. Parameter estimates of GEV distribution for 11 sites

Site name		MLE			Bayesian		
		μ	σ	ξ	μ	σ	ξ
Dharamjaigarh	Estimates	5.150 (0.171)	0.839 (0.139)	0.224 (0.155)	5.191 (0.004)	0.939 (0.004)	0.2171 (0.0035)
	CI	(4.767, 5.532)	(0.529, 1.149)	(-0.121, 0.571)	(4.80, 5.61)	(0.66, 1.33)	(-0.07, 0.54)
Kesinga	Estimates	7.176 (0.399)	2.235 (0.311)	-0.399 (0.134)	7.873 (0.008)	2.190 (0.006)	-0.3701 (0.0031)
	CI	(6.987, 8.765)	(1.543, 2.92)	(-0.699, -0.099)	(6.93, 8.42)	(1.77, 2.82)	(-0.55, 0.00)
Kotni	Estimates	7.411 (0.386)	2.12 (0.307)	-0.384 (0.163)	7.518 (0.009)	2.183 (0.007)	-0.3252 (0.0037)
	CI	(6.749, 8.472)	(1.435, 2.805)	(-0.747, -0.021)	(6.70, 8.26)	(1.68, 2.89)	(-0.61, 0.05)
Manendragarh	Estimates	3.761 (0.182)	0.901 (0.135)	0.080 (0.129)	3.75 (0.004)	0.89 (0.003)	0.10 (0.0031)
	CI	(3.355, 4.166)	(0.599, 1.203)	(-0.209, 0.369)	(3.42, 4.13)	(0.73, 1.25)	(-0.09, 0.44)
Mohana	Estimates	2.758 (0.131)	0.637 (0.117)	0.394 (0.173)	2.777 (0.003)	0.711 (0.003)	0.4336 (0.0040)
	CI	(2.465, 3.051)	(0.376, 0.898)	(0.007, 0.780)	(2.53, 3.13)	(0.48, 1.12)	(0.14, 0.81)
Pathardhi	Estimates	5.659 (0.335)	1.702 (0.264)	-0.518 (0.131)	5.69 (0.008)	1.65 (0.007)	-0.4666 (0.0034)
	CI	(4.913, 6.406)	(1.113, 2.291)	(-0.810, -0.226)	(4.87, 6.16)	(1.35, 2.47)	(-0.77, -0.17)
Rajim	Estimates	4.498 (0.278)	1.545 (0.213)	-0.239 (0.163)	4.66 (0.006)	1.50 (0.005)	-0.21 (0.0035)
	CI	(4.078, 5.317)	(1.069, 2.021)	(-0.604, 0.124)	4.14, 5.18)	(1.22, 2.05)	(-0.47, 0.13)
Seorinarayan	Estimates	9.635 (0.437)	2.185 (0.323)	-0.391 (0.114)	9.81 (0.010)	2.25 (0.008)	-0.3121 (0.0032)
	CI	(8.861, 10.809)	(1.563, 3.006)	(-0.646,-0.135)	(8.78, 10.7)	(1.79, 3.28)	(-0.58, -0.01)
Sigma	Estimates	8.840 (0.369)	2.160 (0.279)	0.391 (0.116)	8.850 (0.008)	2.213 (0.005)	-0.3426 (0.0025)
	CI	(8.017, 9.66)	(1.637, 2.88)	(-0.651, -0.13)	(8.14, 9.55)	(1.83, 2.79)	(-0.52, -0.11)
Sundargarh	Estimates	6.147 (0.163)	0.790 (0.122)	-0.183 (0.157)	6.432 (0.004)	0.864 (0.003)	-0.1607 (0.0034)
	CI	(6.083, 6.812)	(0.617, 1.163)	(-0.533, 0.167)	(6.13, 6.78)	(0.74, 1.29)	(-0.47, 0.13)
AlipiIngal	Estimates	10.589 (0.199)	2.418 (0.189)	-0.958 (0.215)	10.65 (0.008)	2.30 (0.008)	-0.93 (0.0026)
	CI	(10.14, 11.032)	(1.974, 2.861)	(-1.438, -0.478)	(9.63, 11)	(1.89, 3.29)	(-1.14, -0.65)

Table 4. Expected return periods and return levels for 11 sites

Site name	Estimation techniques	Expected return periods						
		20	50	100	200	250	500	1000
		Expected return levels (in metre)						
Dharamjaigarh	MLE	10.196	12.242	14.082	16.225	16.989	19.619	22.691
	Bayesian	10.746	12.962	14.942	17.237	18.052	20.848	24.095
Kesinga	MLE	12.245	12.625	12.830	12.985	13.026	13.133	13.214
	Bayesian	12.342	12.765	12.998	13.178	13.226	13.354	13.453
Kotni	MLE	11.849	12.233	12.444	12.604	12.647	12.759	12.845
	Bayesian	12.284	12.794	13.086	13.318	13.382	13.554	13.691
Manendragarh	MLE	7.772	8.957	9.904	10.903	11.236	12.309	13.443
	Bayesian	7.834	9.080	10.090	11.165	11.526	12.697	13.947
Mohana	MLE	8.381	11.594	14.906	19.249	20.917	27.141	35.316
	Bayesian	7.974	10.946	14.000	17.992	19.523	25.222	32.688
Patahrdhi	MLE	8.487	8.662	8.748	8.807	8.822	8.859	8.885
	Bayesian	8.626	8.838	8.946	9.023	9.043	9.094	9.130
Rajim	MLE	8.554	9.072	9.391	9.660	9.737	9.952	10.135
	Bayesian	8.589	9.161	9.521	9.831	9.921	10.176	10.397
Seorinarayan	MLE	14.358	14.762	14.981	15.147	15.191	15.307	15.395
	Bayesian	14.820	15.375	15.696	15.955	16.026	16.220	16.375
Simga	MLE	13.313	13.712	13.928	14.092	14.136	14.251	14.338
	Bayesian	13.557	14.036	14.308	14.521	14.579	14.734	14.856
Sundargarh	MLE	8.887	9.268	9.513	9.728	9.792	9.973	10.133
	Bayesian	8.891	9.296	9.563	9.800	9.871	10.076	10.258
Alipingal	MLE	13.047	13.086	13.099	13.106	13.107	13.110	13.111
	Bayesian	13.051	13.092	13.107	13.114	13.116	13.119	13.121

CONCLUSIONS

Bayesian estimates are higher than ML estimates at all sites. For all sites, the standard errors of parameters estimated using MLE are larger than those of Bayesian estimates. The confidence intervals for MLE-estimated parameters are broader than credible intervals for Bayesian-estimated values. Non-exceedance probability is used to calculate expected return levels for various return periods. Return levels computed using the Bayesian technique is higher than those predicted using the MLE approach. If return levels high, we may take precautions right away. The, study concludes that Bayesian approach has improved results by allowing inclusion of uncertainties through priors. The outcome of Bayesian analysis gives better information than MLE.

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Quality of Tube Well and Open Well Water from Ausa Tahsil of Latur District

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Abstract: The study was carried out in Ausa tahsil of Latur district to estimate the quality of groundwater for agricultural purposes. Total 100 water sample was collected from tube well and open well in different location and analyzed for pH, EC, cations (Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) and anions (CO_3^{2-}, $HCO_3^{-}$and Cl^{-}) to assess the irrigation water quality with sodium adsorption ration (SAR) and residual sodium carbonate (RSC). From the computation of SAR and RSC values, 100 percent groundwater samples were suitable. USSL diagrams show that the samples are safe for irrigation usage. Considering overall results, the concentration of EC, cations (Na^{+}, Ca^{2+} and Mg^{2+}) and anions ($HCO_3^{-}$and Cl^{-}) in tube well were higher as compare to open well water. Thus, can concludd that open well water is suitable for irrigation as compare to tube well water for irrigation.

Keywords: pH, Electrical conductivity, SAR, RSC and USSL diagram

In India, one-third of the land area is covered by dry and semi-arid climates and rainfall is seasonal and variable, supplemental irrigation is essential. Irrigation is used in areas where rainfall is insufficient to sustain agricultural development or when the rain does not fall when the plants need it most. Irrigation's purpose is to provide plants with water when needed in order to enhance yields. The magnitude of groundwater-related environmental issues varies by region, depending on geology, hydrologic climatic conditions and geochemical factors. In India, groundwater is the single largest and most productive source of irrigation water (Subramani et al 2005). Black soil was developed from weathered alluvium of Deccan basalt or basic parent material under arid and semi-arid condition. Major crop grown in this region are soybean, sugarcane, gram, sorghum, bajara, sunflower, pigeon pea and horticultural crops like pomegranate, mango and guava. For these crops farmers are applying regular and protective irrigation. The quality of a region's groundwater is largely determined by atmospheric precipitation, surface water, host rock, lithology and subsurface geochemical processes. Similarly, the composition of the water is primarily determined by mineral dissolution in the aquifers from which it flows. The form and amount of dissolved salts in irrigation water may have a significant impact on its consistency. Irrigation water contains salts in minimal but significant concentrations. They are formed by the dissolution or weathering of rocks and soil minerals such as lime, gypsum, and other slowly dissolved soil minerals. Water transports these salts to wherever they are required. The salts are applied with water during irrigation, they stay in the soil as the water evaporates or is consumed by the crop.

The chemical quality of groundwater can influence the chemical composition of the rocks and soil through which the water flows, depending on mineral dissolution, mineral solubility, ion exchange, oxidation, reduction as well as anthropogenic activities (population explosion, poor sanitary conditions, application of fertilizers and pesticides for higher crop yields without utmost care etc.). The quality of water for irrigation is determined by the concentrations of some elements that contribute to the specific conductance of groundwater. Particularly, higher concentration of sodium causes dispersion and swelling of soil which is inevitably unfavorable thereby leading to surface crusting. The pH of water is a measure of its acidity or alkalinity. The overall concentration of ionized components in natural water is generally determined by electrical conductivity. The ratio of sodium ions to calcium and magnesium ions can be used to predict the degree to which irrigation water tends to enter the cation exchange reaction in soil. This, ratio called the sodium adsorption ratio, is used to determine the sodium hazard for irrigation waters. Chloride is considered as the most prominent hazardous ion in irrigation water. Because chloride is not absorbed by colloids, it flows freely through the soil, is absorbed by the crop, passes into the transpiration stream and accumulates in the leaves. To determine the relative effect on water quality, the research utilizes multivariate statistical techniques such as correlation matrix. Therefore present investigation was carried out on assessment of ground water quality from Ausa, Latur district, Maharashtra state.

MATERIAL AND METHODS

Latur district is located between 18^0 05' to 18^0 75' North altitude and $76^0$25' to 77^0 25' East latitude. The geographical area of the district is 7166 sq. km with annual rainfall 787 mm. The elevation is 725 to 750 from sea level which comes under Central Marathwada Plateau Agro- climatic Zone and semi-arid region. In January 2021, five underground water samples (48 water samples from well and 52 water samples from tube well) were collected from each village. Both water samples were taken for further examination. By using standard procedure water samples were collected (Richards, 1954) for chemical analysis and completed using the appropriate standard methodologies for the analysis of groundwater. Electrical conductivity (EC) and pH were both measured using digital pH meter and EC meter. By using a flame photometer, sodium (Na+) and potassium (K+) were measured. Titrimetric analysis was used to determine the concentrations of calcium and magnesium. Titration with H_2SO_4 and standard solution were used to measure carbonate and bicarbonate concentrations, chloride were estimated by titration against $AgNO_3$. Sodium adsorption ration and residual sodium carbonate computed by following formula:

a) Sodium Adsorption Ration (SAR) {Richard 1954}:

$$SAR\ (meL^{-1}) = \frac{Na^+}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}}$$

b) Residual Sodium Carbonate (RSC){Eaton, 1950}:

$$RSC\ (meL^{-1}) = (CO_3^- + HCO_3^-) - (Ca^{2+} + Mg^{2+})$$

RESULTS AND DISCUSSION

pH: pH of irrigation water from Ausa tahsil in open well and tube well was 7.73 and 7.68 respectively. The highest pH value of open well was in Chulburga 8.57 followed by tube well water in Yerandi 8.45, while lower pH value of tube well was observed in Belkund 6.9 followed by open well water was in Haldurg 7.08 respectively. The data with respect to categorization of pH, out of 48 open well water samples, 11 samples found in neutral condition, 36 samples were alkaline and 1 sample was sodic. The 75 percent of open well water alkaline and 15 samples were neutral and 37 samples were alkaline. The 71.15 percent samples of tube well water were alkaline condition. pH of open well was high as compare to tube well water, most of the samples of open well and tube well were alkaline. The high pH value was due to atmospheric precipitation, soils, topography, subsurface geochemical processes and fluctuates due to the generation of hydrogen ions in various chemical reactions with the redox potential, temperature and pressure, the pH determines the chemicals dissolved and precipitated in the groundwater regime. The number of cations and anions in solution determines the number of redox potentials in groundwater (Bhat et al 2018). The sodium potential increases when the pH of the irrigation water rises over 8.2. The higher pH of groundwater might be attributed to high concentrations of sodium, calcium, magnesium, carbonate and bicarbonate, which produce hydroxyl ions. Subbarao et al (2012) recorded that pH value of open well from Varah river basin, Visakhapatnam ranged from 7.0 to 8.2 and might be due to carbon dioxide, carbonate and bicarbonate equilibrium were used to keep the pH in balance.

Electrical conductivity: EC value of open well and tube well varied from 0.27 to 1.17dSm^{-1} and 0.34 to 2.39 dSm^{-1} with an average 0.662 and 0.736 dSm^{-1} respectively. In the overall water samples, maximum EC of tube well was in Hasala followed by open well in Belkund while minimum EC was in tube well in Sarola (0.34 dSm^{-1}). On the basis of salinity classes indicated that out of 48 samples of open well water, 31 samples fall under class C_2, which were good in condition and 17 samples falls in C_3 class which was permissible limits. From 52 tube water samples, 31 samples fall under class C_2, 20 samples categorized under class C_3 and 1 sample in C_4 class under doubtful condition. The 64.58 and 59.62 percent samples of open well and tube well water were safe for irrigation but need moderate leaching. The 35.42 and 38.46 percent samples were cannot be used on soils with restricted drainage. One sample of tube well water falls within unsuitable under ordinary condition. Salinity of tube well water was high as compare to open well water this might be due to the leaching or dissolution of the aquifer material or mixing of saline sources at the sampling site and depends on temperature, precipitation, concentration of types of salts or ions present in groundwater. Salinity alters the accessibility of water to crops. EC of irrigation water affect the soil structure, permeability and aeration which indirectly impact on the plant growth (interfere with absorption of water and nutrient from soil) (Rajeswari et al 2019). Osmotic pressure of the soil water by plant roots results in a physiological drought condition. Sharma et al (2017) from Southwest Punjab observed that the high EC in water samples could be due to leaching or dissolution of the aquifers material or mixing of saline sources or combination of these activities.

Chlorine (Cl^-): The chloride concentration of open well and tube well was ranged from 1.7 to 8.4 and 1.5 to 12.7 meL^{-1} with an average value 3.44 and 3.64 respectively. In total water samples, the highest chlorine value of tube well in Nagarsoga followed by open well water in Nagarsoga while minimum chlorine value of open well in Borfal. Chloride is usually occurs as NaCl, $CaCl_2$, $MgCl_2$ and in broadly fluctuating

concentration, in all natural water. Among the 48 samples of open well water12.5 percent were safe for all plants, 64.58 percent samples were sensitive condition and 22.92 percent were moderate tolerant. The 11.54 percent samples were safe for all plants, 67.31 percent samples were in sensitive condition, 19.23 percent were moderately tolerant to plants and 1.92 percent was not suitable for irrigation. The tube well water shows the higher concentration as compare to open well water, because chlorine in groundwater originate from various sources, including weathering, leaching of sedimentary rocks, soils and salt water intrusion. Chloride was widely distributed element in all type of rocks in one or the other form due to high affinity towards sodium. Therefore, its concentration was high in tube well water. Ramakrishnaiah et al (2009) studied water quality index of groundwater in Tumkur taluka, Karnataka and narrated that concentration of chloride was high in groundwater, temperature was high and precipitation was less and soil porosity and permeability also has a key role in building up the chlorides concentration. Subba Rao et al (2012) from Varah river basin, Visakhapatnam district revealed that chlorine content varied from 130 to 420mgL^{-1} where, chlorine anion is caused by the influence of poor sanitary condition irrigation return flows and chemical fertilizers.

Sodium adsorption ratio (SAR): The sodium concentration utilized for expressing interactions with the soil and recognizing the reduction in permeability were important aspects of water quality. Sodium adsorption ratio (SAR) of water is regarded a better measure of sodium (alkali) hazard in irrigation since it is directly connected to sodium adsorption by soil and was a valuable criteria for assessing the suitability of water for irrigation. The SAR values of open well and tube well water samples were ranged from 0.16 to 1.16 and 0.17 to 1.67 meL^{-1} with mean values was 0.493 and 0.643 meL^{-1} respectively. The maximum SAR value of tube well in Yerandi followed by open well water in Hasala while minimum value of tube well in Kalmata followed by open well water in Sarola village. As evident from the SAR values, the open well and tube water of the study area falls under the category low sodium hazard, which reveals that groundwater of the study area was free from any sodium hazard. The tube well shows the higher values as compare to open well water, due to concentration of cations present the deep water. All samples fall under, less than 10meL^{-1} category, so open well and tube well water was safe for irrigation. Jain et al (2012 reported that SAR ranged from 0.03 to 0.53 meL^{-1}in pre monsoons in bore well and ground water falls under the category of low sodium hazard, which reveals that ground water of the study area, is free from any sodium hazard. Ayisha et al (2016) assessed groundwater quality from open well from Malappuram district and recorded that SAR ranged from 0.11 to 1.36me$L^{-1}$where, all samples was suitable for irrigation purpose.

Residual sodium carbonate: The RSC values of open well and tube well water samples varied from -17.15 to -1.85 and -30.05 to -1.3 with mean value was -9.4 of both well respectively. The highest RSC value of open well in Wagholi followed by tube well water in Chulburga - while minimum was of open well in Belkund -followed by tube well water in Hasala village. In water having high concentration of bicarbonate there is a tendency for calcium and magnesium to precipitate as carbonate. The tube well water shows the higher RSC as compared to open well water. Negative value of RSC represents that magnesium and calcium precipitates of carbonates are excessively sufficient than sodium build up. All value of RSC in open well and tube well water was fall in less than 1.25meL^{-1} category, so all samples was suitable for irrigation purpose. Daxa et al (2017) collected twelve water samples from well in Ghogha taluka of Bhavnagar district, Gujarat and observed that RSC value ranged between -43.1 to 1.8 mg/L where, negative value of RSC represent that Mg^{2+} and Ca^{2+} precipitates of CO_3^{-} Kumar and Balamurugan (2018) observed that groundwater quality for irrigation purpose in Attur taluka, Salem, Tamilnadu and reported that RSC values ranged from -15.4 to 1.4 epm while, negative RSC indicate that Na^{+} buildup is unlikely since sufficient Ca^{2+} and Mg^{2+} are in excess and can be precipitated as CO_3^{2-} . Only one sample was marginal category due to occurrence of white patches of soil. Choudhary *et al*. (2020) in underground irrigation water of paddy and sugarcane growing area in Navsari district of Gujarat observed that RSC value of bore well water ranged from 0.05 to 2.23 meL^{-1} in pre monsoon, while for the post monsoon ranged between -0.10 to 2.23 meL^{-1} respectively. The higher mean value of RSC in pre monsoon might be due to dissolution salts present in the groundwater due to high rainfall during monsoon season in the study area.

Salinity and alkalinity hazard classes of irrigation water: The US Salinity Laboratory (USSL) has designed a graph to explain the combined effect of salinity and sodium hazards. The graph divides groundwater into C_1, C_2, C_3 and C_4 categories based on salinity hazard and S_1, S_2, S_3 and S_4 categories based on sodium hazard. The USSL classification was developed to investigate the suitability of groundwater for irrigation purposes. When classifying irrigation waters, it is expected that the water will be used under average conditions with respect to soil texture, infiltration rate and drainage, quantity of water used, climate and salt tolerant crop. The data classified as per village wise from Ausa tahsil based on salinity and alkalinity hazard indicated in Table 1

and narrated that the SAR and EC values of the water samples of water samples are plotted in the USSL diagram (Fig. 1 and 2). Village wise EC and SAR of open well water were ranged from 0.35 to 0.89 dSm^{-1} and 0.19 to 0.78 meL^{-1} respectively. The maximum salinity of open well in Wanwada and Belkund, alkalinity in Hasala village while minimum salinity and alkalinity were in Sarola village. Salinity and alkalinity hazard concentration of tube well water were ranged from 0.36 to 2.26 dSm^{-1} and 0.27 to 1.37 meL^{-1} respectively. The higher concentration of salinity and alkalinity observed in Hasala and Yerandi while lower concentration in Sarola and Belkund. From twenty villages, 85 percent of open well water were falls under C_2S_1 category (medium salinity with low sodium water), which are good quality water and 5 percent were categorized C_3S_1 class (high salinity with low sodium water) with medium to good quality of water and 55 percent villages tube well water were falls under class C_2S_1 (medium salinity with low sodium water), 40 percent villages categorized under class (high salinity with low sodium water) and only one village (Hasala) fall in C_4S_1 category (very high salinity with low sodium water) with

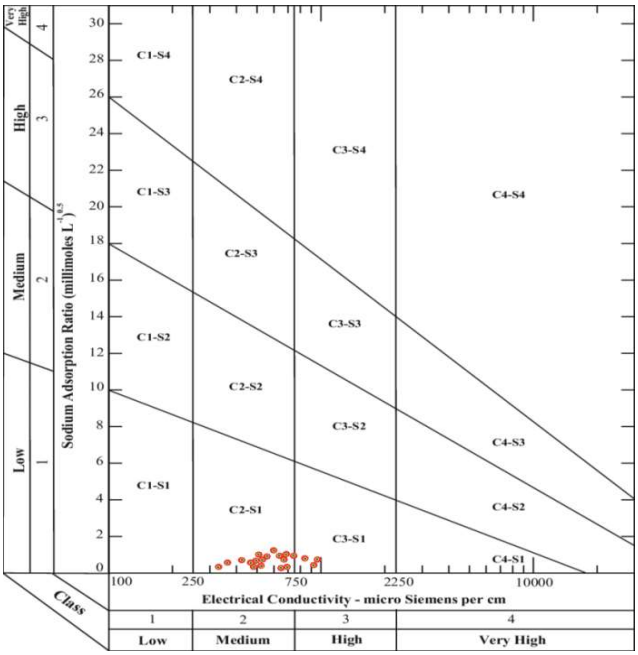

Fig. 1. USSL diagram for classification of open well irrigation water from Ausa tahsil

Table 1. Classification of water samples from Ausa tahsil based on salinity and alkalinity hazard

Village	Latitude	Longitude	Open well water samples			Tube well water samples		
			EC	SAR	Class	EC	SAR	Class
Gondri	18°24'35"	76°46'32"	0.66	0.43	C_2S_1	0.58	0.63	C_2S_1
Sarola	18°13'12"	76°35'12"	0.35	0.19	C_2S_1	0.36	0.81	C_2S_1
Yerandi	18°15'12"	76°36'42"	0.65	0.60	C_2S_1	0.87	1.37	C_3S_1
Yakatpur	18°14'32"	76°34'05"	0.67	0.58	C_2S_1	0.74	0.66	C_2S_1
Wagholi	18°11'13"	76°34'56"	0.46	0.55	C_2S_1	0.51	0.46	C_2S_1
Chulburga	18°10'78"	76°34'82"	0.66	0.61	C_2S_1	0.57	1.0	C_2S_1
Jawli	18°10'44"	76°41'06"	0.71	0.41	C_2S_1	0.62	0.48	C_2S_1
Lamjana	18°08'42"	76°42'22"	0.56	0.37	C_2S_1	0.66	0.45	C_2S_1
Chincholi	18°05'12"	76°32'12"	0.68	0.74	C_2S_1	0.83	0.58	C_3S_1
Talani	18°04'10"	76°51'52"	0.69	0.75	C_2S_1	0.80	0.46	C_3S_1
Wanwada	18°18'12"	76°46'12"	0.89	0.68	C_3S_1	0.82	0.67	C_3S_1
Malkondji	18°11'40"	76°42'03"	0.42	0.48	C_2S_1	0.47	0.46	C_2S_1
Belkund	18°15'24"	76°40'28"	0.89	0.33	C_3S_1	1.05	0.27	C_3S_1
Masurdi	18°17'04"	76°35"03	0.72	0.60	C_2S_1	0.67	0.80	C_2S_1
Borfal	18°19'03"	76°45'18"	0.56	0.44	C_2S_1	0.50	0.58	C_2S_1
Bhada	18°27'18"	76°37'08"	0.69	0.24	C_2S_1	0.81	0.52	C_3S_1
Kalmata	18°28'04"	76°38'50"	0.72	0.58	C_2S_1	0.69	0.6	C_2S_1
Haldurg	18°26'12"	76°43'06"	0.59	0.28	C_2S_1	0.91	0.7	C_3S_1
Nagarsoga	18°22'39"	76°39'17"	0.70	0.27	C_2S_1	0.95	0.45	C_3S_1
Hasala	18°19'56"	76°31'02"	0.82	0.78	C_3S_1	2.26	1.03	C_4S_1
Minimum			0.35	0.19		0.36	0.27	
Maximum			0.89	0.78		2.26	1.37	

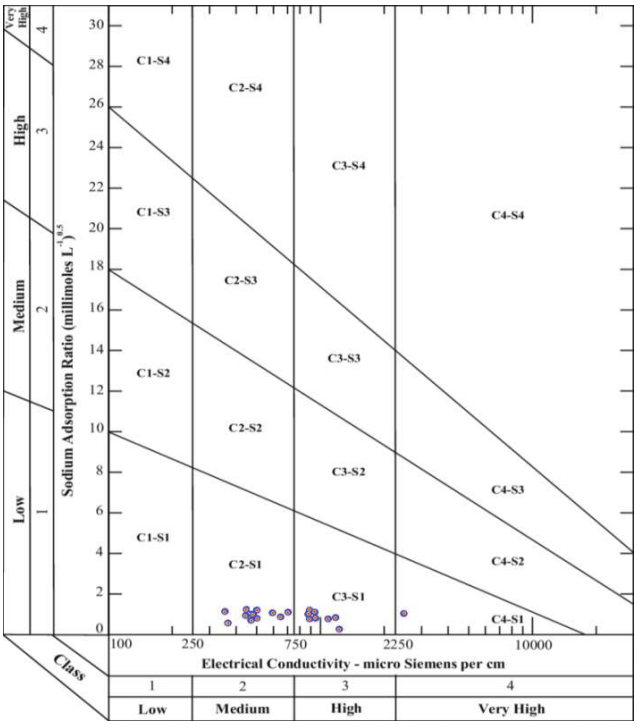

Fig. 2. USSL diagram for classification of tube well irrigation water from Ausa tahsil

medium to bad quality of water. Ahamed *et al.* (2013) studied comparative evaluation of suitability of groundwater use for irrigation in Karur district (T.N) and they reported that the SAR classification Based on USSL diagram, the water quality shows that the majority of the samples falls in the C_4-S_1 (very high salinity with low sodium), C_3-S_1 (high salinity with low sodium) categories, a single sample fall in the field of C_2-S_1 (medium salinity with low sodium), which can be used for irrigation on all types of soil without danger of exchangeable sodium. Adimalla et al (2018) evaluated groundwater suitability for domestic and agricultural utility in semi-arid region of Basara, Telangana and reported that 64.70 percent fall in category of C_2S_1, 12 percent samples fall in C_2S_2 class, 5.88 percent in C_3S_1 class and 5.88 percent into C_3S_2 category.

CONCLUSION

Most of water samples were alkaline condition. According to salinity classes 64.58 percent and 59.61 percent of open well and tube well water falls in C_2 class, while 35.2 percent and 38.46 percent of open well and tube well water categorized in C_3 class. In sodicity classes (SAR) all samples of open well and tube well water fall were suitable for irrigation purpose. Residual sodium carbonate was safe and suitable for irrigation. On the basis of salinity and alkalinity categorization, 85 percent of open well water samples were categorize under C_2S_2 class which was good quality and 5 percent in C_2S_3 class (medium to good quality of water). In tube well water, 55 percent fall under category C_2S_2 classes which are good quality of water, 40 percent in C_2S_3 category which are medium to good quality of water. The groundwater samples collected from Ausa tahsil was medium to good quality for irrigation. It can be concluded that open well water was suitable for irrigation as compared to tube well water.

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Impact of Water Management Options on Groundwater Draft, Energy Consumption and Carbon Emission in Different Districts of Bihar

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***Abstract*:** The impact of different efficient water management options like sprinkler irrigation, drip irrigation, laser land levelling and raised bed planting for rice-wheat cropping system on ground water draft, energy consumption and carbon emission was studied for different districts of Bihar. The reduction in the total energy requirement under sprinkler irrigation system water management options in case-1(a), 1(b) and 1(c) (10 % , 20 % and 30 % of tube wellcomm and is converted from surface to sprinkler irrigation) were 5, 10 and 14%, respectively. The reduction in the total energy requirement under drip irrigation system water management options in case-2(a), 2(b) and 2(c) (10% 20 and 30 % of total tube well command is converted from surface to drip irrigation) were 8, 14.6 and 23.1% , respectively. The total carbon emission reduction under drip irrigation system water management options in case-2(a), 2(b) and 2(c) were 7.5, 15 and 22.5%, respectively. The reduction in the total energy requirement and carbon emission under laser land levelling water management options in case-3(a), 3(b) and 3(c) (If 10%, 20 % and 30 % of total irrigated area is levelled with laser land leveller) were 1.25, 2.5 and 3% respectively. The reduction in the total energy requirement under bed planting system water management options in case-4(a), 4(b) and 4(c) were 3.6, 8.5 and 12%, respectively. Among the different water management options, drip irrigation was most efficient for reduction in energy requirement and carbon emission for ground water pumping. The different water management options which included sprinkler, drip, laser land levelling and raised bed planting for rice-wheat cropping system can also be used as an alternative for a reduction in energy requirement and carbon emission for groundwater pumping.

***Keywords*:** Carbon emission, Laser land levelling, Drip Irrigation, Sprinkler Irrigation

Groundwater is the country's most extracted resource with withdrawal rates currently in the estimated range of about 250 back per year, thereby putting India on the top of the World's biggest groundwater exploiting nations, being responsible for 25 % of total global abstraction (Sinha 2021). Groundwater irrigation plays an important role in increasing agricultural production and food security in India; however, declining groundwater results in an increase in energy consumption and carbon emission for groundwater lifting. About 88% of the total groundwater withdrawal in India is used for irrigation. Groundwater pumping for irrigation combined with the weakening of the Indian monsoon has resulted in widespread groundwater depletion in India in the last 20 years. The rate of abstraction in many regions is higher than groundwater recharge (Siebert et al 2010), causing recurrent water stress (Hanasaki et al 2008), persistent groundwater depletion (Gleeson et al 2010), and long-lasting impacts on stream flow, lakes, and wetlands (Wada et al 2010). The Indo-Gangetic plain and northwest India have experienced a severe decline in groundwater storage (Asoka et al 2017, Rodell et al 2009) and corresponds to one of the largest groundwater footprints in the world (Gleeson et al 2012).

Bihar state of eastern India has an area of 94163 km^2 with 60.5 per cent (5.696 m ha) area under crop cultivation at 144.7 per cent crop intensity. Irrigation potential through groundwater resources is a 3.48 million-hectare area. This may cover about 61 percent net sown area of state. Total availability of groundwater resources is 3.373-million-hectare meter with good quality for irrigation. Only 38 per cent of groundwater has been utilized. This shows high scope for further development. Sustainable groundwater management requires the practice of efficient water management options for reducing groundwater withdrawal. In state of Bihar most of the tube well commands are under surface irrigation. Water use efficiency in the case of surface irrigation is considerably low compared to pressurized irrigation systems such as sprinkler and drip irrigation (Chandra and Singh 2018, Koech and Langat 2018). Pressurized irrigation systems have the potential to avoid the water loss related to surface irrigation increasing the open irrigation application efficiency from 45-60% to pressurized irrigation with efficiency in the range of 75 -95%. Laser land levelling is an effective water saving tool in the new context of land use and ownership on smaller private

plots. Abdulaev (2007) observed that laser land levelling can reduce the water application by 593m^3/ha, in 2005 by 1509m^3/ha in 2004 and in 2006 by 333m^3/ha in comparison with the unlevelled field, located in the similar agro-ecological conditions. The deep percolation was 8% lower and run off 24% less than in non-levelled field.

Irrigation is crucial for agricultural activities; however, if not managed well, it entails high water losses and can be inefficient in its application. The raised bed system is an improved surface irrigation strategy, which enhances water productivity and makes the application of water in irrigated systems more efficient (FAO 2016). Raised bed planting registered 40 to 50% saving in irrigation water (Chandra et al 2007). India is the world's largest groundwater user, withdraws about 230-billion-m^3 groundwater annually for irrigation (Mishra et al 2018). Excessive groundwater pumping in India leads to rapid groundwater depletion and carbon emissions. The estimates show that groundwater irrigation emits 45.3–62.3 MMT of carbon annually, contributing 8–11% of India's total carbon emission (Rajan et al 2020). Food energy and water nexus is now a well-established fact and state like Bihar is energy deficient state. There are various viable water management technologies which can be useful in reducing the energy requirement and carbon emission. The objectives of this paper are to estimate (i) annual groundwater draft (ii) energy requirement associated with ground water pumping (iii) carbon emission associated with ground water pumping. The article also explains the impact of different efficient water management options on groundwater draft, energy requirement and carbon emission associated with groundwater pumping.

MATERIAL AND METHODS

General description of study area: Bihar is located in the eastern region of India between latitude 24°-20'-10" N ~ 27°-31'-15" N and longitude 83°-19'-50" E ~ 88°-17'-40" E. It is an entirely landlocked state, in a subtropical region of the temperate zone. Bihar lies between the humid West Bengal in the east and the sub humid Uttar Pradesh in the west, which provides it with a transitional position in respect of climate, economy and culture. It is bounded by Nepal in the north and by Jharkhand in the south. Bihar plain is divided into two unequal halves (North Bihar and South Bihar) by the river Ganges which flows through the middle from west to east.

GEC methodology: The method used for resource assessment is known as groundwater resource estimation methodology-1997(GEC-97). Fifth Minor Irrigation Census (reference year 2013–14) data were used for the calculation of energy consumption and carbon emission.

Fig. 1. Map of Bihar state

Estimation of annual draft (ha-m): The amount of ground water extracted from the tube well with the help of pumping unit is called ground water draft. The ground water draft was calculated by using the norms of GEC-1997 using unit draft as 0.6, 1.0, and 30.0 ha-m for dug wells, shallow tube wells and deep tube wells respectively. For medium tube wells, 4.0 ha m was considered as unit draft based upon the findings of sample tube well at RPCAU, Pusa. The annual draft was calculated by multiplying number of tube wells and unit draft.

Estimation of energy (kWh): The energy required for groundwater abstraction will be estimated as per the methodology provided by Rothausen and Conway(2011) which prescribes the energy required to lift 1 m^3 of water (with a density 1000 kg-m^{-3}) up 1m at 100% efficiency is 0.0027 kWh,

$$Energy(kWh) = \frac{9.8ms^{-2} \times lift(m) \times mass(kg)}{3.6 \times 10^6 \times efficiency(\%)} \qquad (1)$$

In practice, the efficiency of this process is closer to 20 to 30 percent of theoretical maximum. Here 30 percent efficiency rate has been considered, the effective energy use in 9.080 kWh per thousand cubic meters of water lifted one meter vertically. All the district of Bihar selected for study and the standard lift for dug wells, shallow tube wells, medium and deep tube wells are 15, 30, 50 and 80 m respectively.

Estimation of carbon emission: The amount of carbon released to lift 1000 m^3 of water one meter depends on source of energy. Diesel does not have a unique chemical formulation so the mass and carbon vary by mixture. A litre of standard diesel fuel contains approximately 0.85 kg carbon and energy content of approximately 10.01 kWh. Therefore, with diesel pump the amount of carbon released to lift 1000 m^3 of water one meter is 0.665 kg C (0.732*9.080/10.01). The ratio of carbon emission to energy content for diesel is 0.0732 kg C per kWh (Nelson and Robertson, 2008). The all India average value of 1.4894 kg of CO_2 per kWh at the station (0.4062 kg C per kWh) was used to estimate the release of CO_2 from electric pumps. With five percent transmission

losses an effective carbon emission rate of 0.4265 kg C per kWh at the generating facility or 3.873 kg C to lift 1000 m^3upto one meter was used. The emission from coal based electricity is about 5.82 (3.873/0.665) times higher than the rate of emission with diesel pumps (Nelson et al 2009).

Efficient water management option for reduction in energy consumption and carbon emission: Different efficient water management options were considered for energy conservation and carbon emission reduction in Ground Water Pumping. The following water management scenarios were considered.

Case -1

If 10%, 20% and 30% of total tube well command area is converted from surface to sprinkler irrigation system and designated as 1a, 1b and1c

Case-2

If 10, 20 and 30% of total tube well command area is converted from surface to drip irrigation system and designated as 2a,2b and2c.

Case -3

If 10, 20 and 30% of total irrigated command area is leveled with laser land leveler and designated as 3a,3b and3c

Case-4

If 10, 20 and 30% of total irrigated command area is converted into bed planting system and designated as 4a,4b and5cImportant considerations

Important consideration:

- The total annual draft was reduced by 10, 20 and 30% respectively according to the case.
- The range of efficiency for the sprinkler irrigation system is 50-60%, but for this calculation, it was taken as 50%.
- The range of efficiency of drip irrigation system is 70-80%, but for this calculation, it was taken as 75%.
- Irrigation water savings under Precision Land Levelling versus traditional land leveling were 12–14% in rice and 10–13% in wheat (Jat et al 2006), but for this calculation, it was taken as 10%.
- 40 to 50% saving in irrigation water was recorded with Raised bed planting in comparison with flood irrigation of controlled plots (Ahmad and Mahmood 2005), but for this calculation, it was taken as 40%.

RESULTS AND DISCUSSION

Energy requirement and carbon emission due to pumping of groundwater from different irrigation structures: Estimated energy requirement in. The total energy requirement in ground water pumping was estimated to be 8256394 MWh for state of Bihar (Table 1). The lowest energy requirement for groundwater pumping was 44397 MWh for Banka district and highest energy requirement for

Table 1. Energy ('000' kWh) requirement and carbon emission (tonne) due to pumping of groundwater from different irrigation structures in different districts of Bihar

Districts	Energy ('000' kWh)	Carbon emission (Tonne)
Araria	93407	9145
Arwal	79286	7762
Aurangabad	190405	18641
Banka	44397	4346
Begusarai	266925	26132
Bhagalpur	74501	7294
Bhojpur	464755	45499
Buxar	52743	5164
Darbhanga	543512	53210
E.champaran	299943	29364
Gaya	303358	29699
Gopalganj	200461	19625
Jamui	58474	5725
Jehanabad	171842	16823
Kaimur	393017	38476
Katihar	196359	19224
Khagaria	332823	32583
Kishanganj	76124	7453
Lakhisarai	132086	12931
Madhepura	151960	14877
Madhubani	181101	17730
Munger	118017	11554
Muzaffarpur	399866	39147
Nalanda	845720	82796
Nawada	220079	21546
Patna	375224	36734
Purnia	79909	7823
Rohtas	86136	8433
Saharsa	51631	5055
Samastipur	541268	52990
Saran	314639	30803
Shekhpura	86729	8491
Sheohar	51609	5053
Sitamarhi	102705	10055
Siwan	106954	10471
Supaul	71141	6965
Vaishali	177612	17388
West Champaran	319676	31296
Total	8256394	808303

Table 2. Energy ('000' kWh) requirement for pumping of groundwater under efficient water management options of case 1a to case 1c (Sprinkler Irrigation)

Districts	Prevailing energy requirement	Case -1 (a)	Case -1 (b)	Case -1 (c)
Araria	93407	88736	84066	79396
Arwal	79286	75322	71358	67393
Aurangabad	190405	180884	171364	161844
Banka	44397	42177	39957	37737
Begusarai	266925	253579	240233	226887
Bhagalpur	74501	70776	67051	63326
Bhojpur	464755	441517	418279	395041
Buxar	52743	50106	47468	44831
Darbhanga	543512	516336	489160	461985
E.champaran	299943	284946	269949	254952
Gaya	303358	288190	273022	257854
Gopalganj	200461	190438	180415	170392
Jamui	58474	55550	52627	49703
Jehanabad	171842	163250	154658	146066
Kaimur	393017	373366	353715	334064
Katihar	196359	186541	176724	166906
Khagaria	332823	316182	299541	282900
Kishanganj	76124	72318	68511	64705
Lakhisarai	132086	125482	118877	112273
Madhepura	151960	144362	136764	129166
Madhubani	181101	172046	162991	153936
Munger	118017	112117	106216	100315
Muzaffarpur	399866	379873	359880	339887
Nalanda	845720	803434	761148	718862
Nawada	220079	209075	198071	187067
Patna	375224	356463	337702	318940
Purnia	79909	75914	71919	67923
Rohtas	86136	81829	77523	73216
Saharsa	51631	49049	46468	43886
Samastipur	541268	514205	487141	460078
Saran	314639	298907	283175	267443
Shekhpura	86729	82392	78056	73720
Sheohar	51609	49029	46448	43868
Sitamarhi	102705	97569	92434	87299
Siwan	106954	101606	96258	90910
Supaul	71141	67584	64027	60470
Vaishali	177612	168732	159851	150970
West Champaran	319676	303693	287709	271725
Total	8256394	7843575	7430756	7017936

Table 3. Energy ('000' kWh) requirement for pumping of groundwater under efficient water management options of Case- 2(a) to Case-2(c) (drip Irrigation)

Districts	Prevailing energy requirement	Case -1 (a)	Case -1 (b)	Case -1 (c)
Araria	93407	86401	79396	72390
Arwal	79286	73340	67393	61447
Aurangabad	190405	176124	161844	147563
Banka	44397	41067	37737	34408
Begusarai	266925	246906	226887	206867
Bhagalpur	74501	68914	63326	57739
Bhojpur	464755	429898	395041	360185
Buxar	52743	48787	44831	40876
Darbhanga	543512	502748	461985	421222
E.champaran	299943	277448	254952	232456
Gaya	303358	280606	257854	235102
Gopalganj	200461	185426	170392	155357
Jamui	58474	54088	49703	45317
Jehanabad	171842	158954	146066	133178
Kaimur	393017	363540	334064	304588
Katihar	196359	181632	166906	152179
Khagaria	332823	307861	282900	257938
Kishanganj	76124	70414	64705	58996
Lakhisarai	132086	122179	112273	102367
Madhepura	151960	140563	129166	117769
Madhubani	181101	167518	153936	140353
Munger	118017	109166	100315	91464
Muzaffarpur	399866	369877	339887	309897
Nalanda	845720	782291	718862	655433
Nawada	220079	203573	187067	170561
Patna	375224	347082	318940	290799
Purnia	79909	73916	67923	61930
Rohtas	86136	79676	73216	66756
Saharsa	51631	47758	43886	40014
Samastipur	541268	500673	460078	419483
Saran	314639	291041	267443	243845
Shekhpura	86729	80224	73720	67215
Sheohar	51609	47739	43868	39997
Sitamarhi	102705	95002	87299	79596
Siwan	106954	98932	90910	82889
Supaul	71141	65806	60470	55134
Vaishali	177612	164291	150970	137649
West Champaran	319676	295701	271725	247749
Total	8256394	7637162	7017936	6398708

Table 4. Energy ('000' kWh) requirement for pumping of groundwater under efficient water management options of Case- 3(a) to Case-3(c) (laser land levelling)

Districts	Prevailing energy requirement	Case -1 (a)	Case -1 (b)	Case -1 (c)
Araria	93407	92473	91539	90605
Arwal	79286	78494	77701	76908
Aurangabad	190405	188500	186596	184692
Banka	44397	43953	43509	43065
Begusarai	266925	264256	261587	258918
Bhagalpur	74501	73756	73011	72266
Bhojpur	464755	460107	455459	450812
Buxar	52743	52215	51688	51160
Darbhanga	543512	538077	532641	527206
E.champaran	299943	296944	293944	290945
Gaya	303358	300324	297290	294257
Gopalganj	200461	198456	196452	194447
Jamui	58474	57889	57304	56720
Jehanabad	171842	170124	168405	166687
Kaimur	393017	389086	385156	381226
Katihar	196359	194396	192432	190469
Khagaria	332823	329495	326167	322839
Kishanganj	76124	75362	74601	73840
Lakhisarai	132086	130765	129444	128123
Madhepura	151960	150440	148921	147401
Madhubani	181101	179290	177479	175668
Munger	118017	116837	115657	114477
Muzaffarpur	399866	395868	391869	387870
Nalanda	845720	837262	828805	820348
Nawada	220079	217878	215677	213476
Patna	375224	371472	367720	363967
Purnia	79909	79110	78311	77512
Rohtas	86136	85275	84414	83552
Saharsa	51631	51114	50598	50082
Samastipur	541268	535856	530443	525030
Saran	314639	311493	308346	305200
Shekhpura	86729	85862	84994	84127
Sheohar	51609	51093	50577	50061
Sitamarhi	102705	101678	100651	99624
Siwan	106954	105884	104814	103745
Supaul	71141	70430	69718	69007
Vaishali	177612	175836	174060	172284
West Champaran	319676	316480	313283	310086
Total	8256394	8173830	8091263	8008702

Table 5. Energy ('000' kWh) requirement for pumping of groundwater under efficient water management options of Case- 4(a) to Case-4(c) (Bed planting)

Districts	Prevailing energy requirement	Case -1 (a)	Case -1 (b)	Case -1 (c)
Araria	93407	89671	85934	82198
Arwal	79286	76115	72944	69772
Aurangabad	190405	182788	175172	167556
Banka	44397	42621	40845	39069
Begusarai	266925	256248	245571	234894
Bhagalpur	74501	71521	68541	65561
Bhojpur	464755	446164	427574	408984
Buxar	52743	50633	48523	46414
Darbhanga	543512	521771	500031	478290
E.champaran	299943	287946	275948	263950
Gaya	303358	291223	279089	266955
Gopalganj	200461	192442	184424	176405
Jamui	58474	56135	53796	51457
Jehanabad	171842	164969	158095	151221
Kaimur	393017	377296	361575	345855
Katihar	196359	188505	180651	172796
Khagaria	332823	319510	306197	292884
Kishanganj	76124	73079	70034	66989
Lakhisarai	132086	126803	121519	116236
Madhepura	151960	145881	139803	133725
Madhubani	181101	173857	166613	159369
Munger	118017	113297	108576	103855
Muzaffarpur	399866	383872	367877	351883
Nalanda	845720	811891	778062	744233
Nawada	220079	211276	202473	193669
Patna	375224	360215	345206	330197
Purnia	79909	76713	73517	70320
Rohtas	86136	82691	79245	75800
Saharsa	51631	49566	47500	45435
Samastipur	541268	519618	497967	476316
Saran	314639	302054	289468	276883
Shekhpura	86729	83260	79791	76321
Sheohar	51609	49545	47481	45416
Sitamarhi	102705	98597	94488	90380
Siwan	106954	102675	98397	94119
Supaul	71141	68296	65450	62604
Vaishali	177612	170508	163403	156299
West Champaran	319676	306889	294102	281315
Total	8256394	7926141	7595882	7265625

Table 6. Carbon emission (in tonnes) from pumping of groundwater under efficient water management options of Case- 1(a) to Case-2(c)

Districts	Prevailing carbon emission	Sprinkler irrigation			Drip irrigation		
		Case-1(a)	Case-1 (b)	Case-1 (c)	Case-2(a)	Case-2 (b)	Case-2 (c)
Araria	9145	17709	8230	7773	8459	7773	7087
Arwal	7762	7374	6986	6598	7180	6598	6016
Aurangabad	18641	17709	16777	15845	17243	15845	14446
Banka	4346	4129	3912	3694	4020	3694	3369
Begusarai	26132	24825	23519	22212	24172	22212	20252
Bhagalpur	7294	6929	6564	6200	6747	6200	5653
Bhojpur	45499	43224	40950	38675	42087	38675	35262
Buxar	5164	4905	4647	4389	4776	4389	4002
Darbhanga	53210	50549	47889	45228	49219	45228	41238
E.champaran	29364	27896	26428	24960	27162	24960	22757
Gaya	29699	28214	26729	25244	27471	25244	23017
Gopalganj	19625	18644	17663	16681	18153	16681	15209
Jamui	5725	5438	5152	4866	5295	4866	4437
Jehanabad	16823	15982	15141	14300	15562	14300	13038
Kaimur	38476	36553	34629	32705	35591	32705	29819
Katihar	19224	18262	17301	16340	17782	16340	14898
Khagaria	32583	30954	29325	27696	30140	27696	25252
Kishanganj	7453	7080	6707	6335	6894	6335	5776
Lakhisarai	12931	12285	11638	10992	11961	10992	10022
Madhepura	14877	14133	13389	12645	13761	12645	11530
Madhubani	17730	16843	15957	15070	16400	15070	13741
Munger	11554	10976	10399	9821	10687	9821	8954
Muzaffarpur	39147	37190	35232	33275	36211	33275	30339
Nalanda	82796	78656	74516	70377	76586	70377	64167
Nawada	21546	20468	19391	18314	19930	18314	16698
Patna	36734	34898	33061	31224	33979	31224	28469
Purnia	7823	7432	7041	6650	7236	6650	6063
Rohtas	8433	8011	7589	7168	7800	7168	6535
Saharsa	5055	4802	4549	4296	4676	4296	3917
Samastipur	52990	50341	47691	45042	49016	45042	41067
Saran	30803	29263	27723	26183	28493	26183	23872
Shekhpura	8491	8066	7642	7217	7854	7217	6580
Sheohar	5053	4800	4547	4295	4674	4295	3916
Sitamarhi	10055	9552	9049	8547	9301	8547	7792
Siwan	10471	9947	9424	8900	9685	8900	8115
Supaul	6965	6616	6268	5920	6442	5920	5398
Vaishali	17388	16519	15649	14780	16084	14780	13476
West Champaran	31296	29732	28167	26602	28949	26602	24255
Total	808303	776906	727471	687056	747678	687056	626433

Table 7. Carbon emission (in tonnes) from pumping of groundwater under efficient water management options of Case- 3(a) to Case-4(c)

Districts	Prevailing carbon emission	Sprinkler irrigation			Drip irrigation		
		Case-1(a)	Case-1 (b)	Case-1 (c)	Case-2(a)	Case-2 (b)	Case-2 (c)
Araria	9145	9053	8962	8870	8779	8413	8047
Arwal	7762	7685	7607	7529	7452	7141	6831
Aurangabad	18641	18454	18268	18081	17895	17149	16404
Banka	4346	4303	4260	4216	4173	3999	3825
Begusarai	26132	25871	25609	25348	25087	24041	22996
Bhagalpur	7294	7221	7148	7075	7002	6710	6418
Bhojpur	45499	45044	44589	44134	43680	41860	40040
Buxar	5164	5112	5060	5009	4957	4750	4544
Darbhanga	53210	52678	52146	51613	51081	48953	46825
E.champaran	29364	29071	28777	28484	28190	27015	25841
Gaya	29699	29402	29105	28808	28511	27323	26135
Gopalganj	19625	19429	19233	19036	18840	18055	17270
Jamui	5725	5667	5610	5553	5496	5267	5038
Jehanabad	16823	16655	16487	16319	16150	15477	14805
Kaimur	38476	38092	37707	37322	36937	35398	33859
Katihar	19224	19031	18839	18647	18455	17686	16917
Khagaria	32583	32258	31932	31606	31280	29977	28673
Kishanganj	7453	7378	7303	7229	7154	6856	6558
Lakhisarai	12931	12802	12673	12543	12414	11897	11379
Madhepura	14877	14728	14579	14431	14282	13687	13092
Madhubani	17730	17552	17375	17198	17021	16311	15602
Munger	11554	11438	11323	11207	11092	10630	10167
Muzaffarpur	39147	38755	38364	37973	37581	36015	34449
Nalanda	82796	81968	81140	80312	79484	76172	72860
Nawada	21546	21330	21115	20899	20684	19822	18960
Patna	36734	36367	36000	35632	35265	33796	32326
Purnia	7823	7745	7667	7588	7510	7197	6884
Rohtas	8433	8348	8264	8180	8095	7758	7421
Saharsa	5055	5004	4954	4903	4852	4650	4448
Samastipur	52990	52460	51930	51400	50871	48751	46631
Saran	30803	30495	30187	29879	29571	28339	27107
Shekhpura	8491	8406	8321	8236	8151	7811	7472
Sheohar	5053	5002	4952	4901	4850	4648	4446
Sitamarhi	10055	9954	9854	9753	9653	9250	8848
Siwan	10471	10366	10261	10157	10052	9633	9214
Supaul	6965	6895	6825	6756	6686	6408	6129
Vaishali	17388	17214	17040	16867	16693	15997	15302
West Champaran	31296	30983	30670	30357	30045	28793	27541
Total	808303	800218	792135	784052	775969	743637	711305

groundwater pumping was 845720 MWh for Nalanda district. The total carbon emission from pumping of groundwater for the whole state was 808301 tonne. Nalanda contributes highest to carbon emission followed by Darbhanga and Samastipur. The lowest carbon emission was for Banka district. The groundwater development in hard rock areas like Banka, Kishanganj and Jamui districts of Bihar are difficult and aquifer storage capacity is limited.

Impact of efficient water management options on energy requirement and carbon emission.

Case-1 If 10 , 20 and 30 % of tube well command area is converted from surface to sprinkler irrigation system: The reduction in the total energy requirement under sprinkler irrigation system water management options in case-1(a), case-1(b) and case-1(c) were 5, 10 and 14% respectively. The total carbon emission reduction under sprinkler irrigation system water management options in case-1(a), 1(b) and -1(c) were 4, 10 and 15%, respectively. Sprinkler Irrigation can be a useful irrigation method for crop like wheat, mustard, pulses. Sprinkler irrigation coupled with underground pipeline may further reduce energy requirement and carbon emission.

Case-2. If 10, 20 and 30 % of tube well command area is converted from surface to drip irrigation system: The reduction in the total energy requirement under drip irrigation system water management options in case 2(a), 2(b) and 2(c) were 8, 14.6 and 23.1%, respectively. The total carbon emission reduction under drip irrigation system water management options in case-2(a), 2(b) and (c) were 7.5, 15 and 22.5%, respectively. Drip irrigation can be useful irrigation method for vegetables, fruits, sugarcane, rabi maize etc. This study clearly indicates the% importance of micro irrigation in reducing ground water use, energy requirement and carbon emission.

Case-3. If 10, 20 and 30 % of tube well irrigated area is levelled with laser land leveller: This scenario includes three case 5% (3a), 20% (3b) and 30 % (3c) of tube well irrigated area is levelled with laser land leveller. The reduction in the total energy requirement under laser land levelling water management options in case 3(a), 3(b) and 3(c) were 1.25, 2.5 and 3%, respectively. The reduction in carbon emission under laser land levelling water management options in case 3(a), 3(b) and 3(c) were 1, 2and 2.5%, respectively.

Case-4. If 10, 20 and 30 % of tube well irrigated area is converted into bed planting cultivation system: In this system, the land is prepared conventionally and raised bed and furrows are prepared manually or using a raised bed planting machine. Crops are planted in rows on top of the raised beds and irrigation water is applied in the furrows between the beds.

If 10, 20 and 30 % of tubewell irrigated area is of converted into bed planting cultivation system reduction in the total energy requirement under bed planting system water management options in were 3.6. 8.5 and 12% , respectively. The total carbon emission reduction under bed planting system water management options in case 4(a), 4(b) and 4(c) were 3.7, 7.5 and 11%, respectively. Resource conservation technology are one of the important mechanism through which water use, energy use and carbon emission can be reduced for rice-wheat cropping system.

CONCLUSIONS

This study gives us insight for sustainable groundwater resource management along with energy management in agriculture. Among the different water management options, drip irrigation was most efficient for reduction in energy requirements and carbon emissions for groundwater pumping. The different water management options which included sprinkler, drip, laser land levelling and raised bed planting for rice-wheat cropping system can also be used as an alternative for a reduction in energy requirement and carbon emission for groundwater pumping. Climate resilient agriculture approach is required for efficient water, energy, and resource management. The combination of policies to optimize energy and water use in agriculture is required to respond to these hydro-climatic challenges.

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Heat Unit Requirement for Different Phenophases in Mango Varieties under South Gujarat Conditions

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***Abstract*:** The present investigation was conducted at Navsari Agricultural University, Navsari, Gujarat, India during the year 2019-20 and 2020-21. The, requirement of heat units *viz.,* growing degree days (GDD), photo thermal units (PTU) and heliothermal units (HTU) for each phonological event was assessed for seven mango varieties *viz*., Sonpari, Alphonso, Amrapali, Kesar, Dashehari, Totapuri and Rajapuri. Alphonso consumed minimum heat units *viz.,* GDD, PTU and HTU for attaining flower bud differentiation (1436.90, 17682.21 and 6805.82^{0}C, respectively) and grain stage fruits (38.68, 424.52 and 284.10^{0}C, respectively). For panicle initiation, Kesar required minimum GDD (40.73^{0}C) and PTU (452.23^{0}C). However, HTU requirement was minimum in Sonpari (275.61^{0}C). For initiation of flowering, Sonpari required minimum GDD (37.53^{0}C) and PTU (420.67^{0}C). The requirement for HTU was minimum (316.19^{0}C) in cultivar Dashehari. For attaining pea stage fruits, Alphonso required minimum GDD (48.70^{0}C) and PTU (542.44^{0}C). Whereas, HTU requirement was minimum (357.60^{0}C) in Kesar. Dashehari consumed minimum GDD (93.15^{0}C) followed by PTU and HTU for attaining marble stage fruits. However, minimum requirement of GDD (733.58^{0}C), PTU (9201.91^{0}C) and HTU (6717.69^{0}C) for maturity was observed in Sonpari.

***Keywords*:** Mango varieties, Heat units, Phenophase

Mango is undoubtedly the most important fruit crops among the tropical and subtropical fruits, grown in more than 110 countries of the world. The climate change has been perceived as a major threats and has maximum impact on mango production in India and particularly in coastal area of South Gujarat (Anon., 2015). This geographic location is continuously becoming increasingly vulnerable to thunder storms during late summer, this results in heavy pre-mature fruit drop which ultimately results in huge economic losses (Parmar et al 2012). In this context, it is necessary to escape crop produce from these losses by manipulating phenological crop cycle through different management practices *viz*., bahar treatment measures, application of paclobetrozol, choice of cultivar *etc*. In order to predict fruit maturity, requirement of heat units is most accurate among different options. Hence, it is necessary to know the varietal thermal requirements for each phenological event. Computational method using heat unit accumulation during the fruit growth and development has been used as an easy and feasible criterion for determining the fruit maturity (Halepotara 2018). However, information available on this aspect is rare and scanty. Hence, an effort was made to determine the requirement of growing degree days, (GDD) photothermal units (PTU) and heliothermal units (HTU) of different mango cultivars and hybrids for their different phenological events.

MATERIAL AND METHODS

The present investigation was carried out at Navsari Agricultural University, Navsari during two consecutive seasons 2019-20 and 2020-21**.** Uniform fifteen year old trees of seven mango varieties *viz*., V_1– Sonpari, V_2 – Alphonso, V_3 – Amrapali, V_4– Kesar, V_5– Dashehari, V_6– Totapuri, and V7 – Rajapuri were selected for this study. The dates of different phenological events were recorded and its respective durations were computed. Accordingly, mean heat units *viz*., growing degree days (^{0}C), photothermal units (^{0}C) and heliothermal units (^{0}C) were computed as described below.

Growing degree days (GDD): The degree days for completion of each phenophase were calculated.

$$Growing\,degree\,days\,(GDD) = \sum_{i=1}^{n} \frac{T\max. + T\min.}{2} - Tc$$

Maximum and Minimum temperatures of the day

Tc - Minimum threshold temperature of the crop called as base temperature or minimum threshold temperature. The base temperature of mango crop is 17.9 ^{0}C Oppenheimer (1947).

Representation

Day-1: A day with a maximum temperature of 35°C and a minimum temperature of 14°C would contribute 6.60 (GDD).
Narration: 35 + 14/ 2 - 17.9 = 6.60

Day-2: A day with a maximum temperature of 22°C and a

minimum temperature of 11°C would contribute – 1.40. So accumulation of GDD will be 0.00, because negative growth is not possible. Narration: 22 + 11/ 2 - 17.9 = –1.40 ≈ 0.00

Photo thermal units (°C): The photo thermal unit for a given day represents the product of GDD and the maximum possible sunshine hours and PTU was calculated.

$$\text{Photo thermal units (PTU)} = \sum_{i=1}^{n} \text{GDDXN}$$

Where, GDD - Growing degree days and N - Maximum possible sunshine hours. These maximum possible sunshine hours were taken in accounts from the findings obtained by Sahu (2003). In his report at latitude of Navsari (20^0 latitude) possible sunshine hours for different months *viz.,* Jan. (11.10), Feb. (11.50), March (12.00), April (12.60), May (13.10), June (13.30), July (13.20), Aug. (12.80), Sept. (12.30), Oct. (11.70), Oct. (11.20), Nov. (11.20) and Dec. (10.90) were observed.

Heliothermal units (^{0}C): The heliothermal units for a given day represent the product of GDD and the actual bright sunshine hours and HTU was calculated.

$$\text{Heliothermal units (HTU)} = \sum_{i=1}^{n} \text{GDDXN}$$

Where, GDD is the growing degree days and n is the actual bright sunshine hours as recorded by the Campbell-stokes sunshine recorder.

RESULTS AND DISCUSSIONS

Flower bud differentiation: For induction of flower bud differentiation, Alphonso (V_2) consumed minimum GDD (1436.90^{0}C), PTU (17682.21 ^{0}C) and HTU (6805.82^{0}C) in 153.67 days from 1st July. However, among all cultivars, Amrapali (V_3) consumed maximum GDD (1709.03^{0}C), PTU (20696.71^{0}C) and HTU (8759.62^{0}C) in 215.16 days from 1st July (Table 1).

Mango varieties *viz.,* Alphonso, Kesar and Rajapuri detected early FBD and required minimum GDD and HTU, which might be due to varietal character and their interaction with climatic parameters. Kanzariya et al (2015) reported requirements of GDD for FBD of Kesar (1549.42^{0}C in 170.71 days), Alphonso (1559.77^{0}C in 172.63 days) and Rajapuri (1572.81^{0}C in 177.17 days). These findings for thermal indices are in line with Cesaraccio et al (2001) and Halepotara (2018).

Panicle initiation: In panicle initiation, Kesar required minimum GDD (40.73^{0}C) and PTU (452.23^{0}C) accumulated during 9.33 days after FBD. Sonpari required minimum HTU (275.61^{0}C) which were accumulated in 10.83 days after FBD. For panicle initiation, Amrapali required maximum GDD (55.83^{0}C), PTU (639.23^{0}C) and HTU (462.69^{0}C) consumed during 10.33 days after FBD (Table 1). Cultivar Amrapali required more heat units than other cultivars under study, this was might be due to its hunger for heat units for the sake of panicle initiation. These results are in close confirmation with results documented by Burondkar et al (2000), Shinde et al (2001), Kanzaria et al (2015) and Souza et al (2015).

Flowering initiation: In flowering initiation, cultivar Sonpari required minimum GDD (37.53^{0}C) and PTU (420.67^{0}C) which were harnessed in 11.17 days from panicle initiation. However requirement for HTU was minimum (316.19^{0}C with 10.67 days) in cultivar Dashehari. However, cultivar Alphonso required maximum GDD (75.75^{0}C), PTU (841.63^{0}C) and HTU (557.83^{0}C) which were consumed during 14.16 days from panicle initiation.

The overall performance for thermal indices indicted that, Sonpari consumed minimum GDD, PTU and HTU, whereas, in case of Alphonso and other early cultivars these parameters were higher. These results show the adaptability of Sonpari to climatic conditions of South Gujarat. These results are in close conformity with the findings of Shinde et al (2001), Kanzaria et al (2015) and Souza et al (2015).

Table 1. Requirement of days and heat units for attaining FBD, panicle initiation and flowering in mango varieties (Two years mean)

Treatments	Flower bud differentiation				Panicle initiation				Flowering initiation			
	Days	GDD (^{0}C)	PTU (^{0}C)	HTUs (^{0}C)	Days	GDD (^{0}C)	PTU (^{0}C)	HTUs (^{0}C)	Days	GDD (^{0}C)	PTU (^{0}C)	HTUs (^{0}C)
Sonpari	193.17	1620.90	19715.23	8153.24	10.83	49.33	547.01	275.61	11.17	37.53	420.67	320.76
Alphonso	153.67	1436.90	17682.21	6805.82	9.67	47.73	530.94	369.64	14.16	75.75	841.63	557.83
Amrapali	215.16	1709.03	20696.71	8759.62	10.33	55.83	639.23	462.69	9.33	51.53	592.54	436.84
Kesar	161.17	1488.00	18253.46	7192.20	9.33	40.73	452.23	289.26	14.00	65.78	721.05	471.23
Dashehari	193.00	1626.95	19781.89	8120.18	12.50	50.23	557.50	378.01	10.67	37.98	425.00	316.19
Totapuri	199.83	1652.90	20069.93	8292.84	11.17	45.68	506.99	374.62	10.50	67.25	770.62	561.23
Rajapuri	158.16	1472.65	18081.54	7098.88	12.00	50.63	564.16	331.06	13.67	63.38	695.67	482.95
C.D. at 5%	30.50				NS				1.26			
C.V. (%)	3.52				14.32				8.97			

Grain stage: Alphonso consumed minimum GDD (38.68^{0}C), PTU (424.52^{0}C) and HTU (284.10^{0}C) during 9.67 days from initiation of flowering. However, Amrapali consumed maximum GDD (76.78^{0}C), PTU (890.32^{0}C) and HTU (712.03^{0}C) accumulated during 10.83 days from initiation of flowering. These variations in requirement of heat indices might be due to respective varietal characters. These results are in agreement with Burondkar et al (2000), Shinde et al (2001), Kanzaria et al (2015) and Souza et al (2015) (Table 2).

Pea stage: Alphonso required minimum GDD (48.70^{0}C) and PTU (542.44 ^{0}C) which were harnessed in 13.66 days after grain stage (Table 2). However, HTU requirement was minimum (357.60^{0}C with 12.00 days) in Kesar. The requirements of GDD, PTU and HTU was maximum (69.30, 798.63 and 622.06^{0}C, respectively) in cultivar Totapuri which were accumulated in 9.83 days after grain stage. The overall performance for thermal indices shows that Alphonso consumed minimum GDD, PTU and HTU, whereas, in Totapuri these parameters were maximum. Totapuri consumed more heat units, hence highlighted its hunger for heat units for attaining pea stage fruits. These results are in agreement with findings obtained by *Kanzaria* et al (2015) and Souza et al (2015).

Marble stage: Dashehari required minimum GDD (93.15 ^{0}C), PTU (1094.20^{0}C) and HTU (848.17 ^{0}C) which were consumed in 14.00 days after attaining pea stage fruits (Table 2). For attaining marble stage fruits, maximum GDD (132.35^{0}C) and PTU (1495.19^{0}C) were required by cultivar Alphonso (V_2) with 23.17 days after attaining pea stage fruits. However, consumption of HTU (1058.00^{0}C) was highest in Sonpari with 19.50 days after pea stage. The cultivar Alphonso required maximum GDD and Dashehari and Totapuri required minimum GDD. This highlighting requirement of specific variety for particular heat units. Similar results were reported by Shinde et al (2001), Kanzaria et al (2015) and Souza et al (2015).

Maturity: The thermal requirement for attaining fruit maturity predominately indicated that Sonpari required minimum GDD (733.58^{0}C), PTU (9201.91^{0}C) and HTU (6717.69^{0}C) which were absorbed in 65.67 days after attaining marble stage (Table 2). However, cultivar Totapuri consumed maximum GDD (961.73^{0}C) and PTU (12246.22^{0}C) which was harnessed in 82.83 days after marble stage. Requirement for HTU was highest (8658.33^{0}C) in cultivar Rajapuri with 95.00 days after attaining marble stage. The cultivar Sonpari consumed comparatively much less heat units than other cultivars under study. This was might be due to attaining maturity in relatively short time. This underlines the early maturing nature of this cultivar. On the contrary,

Table 2. Requirement of days and heat units for attaining grain, pea, marble and maturity in mango varieties (Two years mean)

Treatments	Grain Stage				Pea Stage				Marble Stage				Maturity			
	Days	GDD (^{0}C)	PTU (^{0}C)	HTUs (^{0}C)	Days	GDD (^{0}C)	PTU (^{0}C)	HTUs (^{0}C)	Days	GDD (^{0}C)	PTU (^{0}C)	HTUs (^{0}C)	Days	GDD (^{0}C)	PTU (^{0}C)	HTUs (^{0}C)
Sonpari	8.33	40.55	462.71	329.76	9.00	60.88	700.06	543.13	19.50	121.33	1421.31	1058.00	65.67	733.58	9201.91	6717.69
Alphonso	9.67	38.68	424.52	284.10	13.66	48.70	542.44	359.28	23.17	132.35	1495.19	1000.52	85.50	792.30	9760.23	7217.25
Amrapali	10.83	76.78	890.32	712.03	9.67	60.25	723.00	525.83	12.67	116.53	1398.30	932.42	77.50	924.13	11872.63	8144.26
Kesar	13.33	49.25	542.10	381.79	12.00	55.78	632.02	357.60	18.33	101.03	1145.18	862.21	92.16	927.13	11546.12	8496.70
Dashehari	11.17	54.83	630.49	479.39	9.50	61.18	703.51	492.83	14.00	93.15	1094.20	848.17	67.67	752.88	9447.92	6904.89
Totapuri	11.17	67.70	778.55	555.74	9.83	69.30	798.63	622.06	14.33	101.68	1214.73	909.54	82.83	961.73	12246.22	8576.02
Rajapuri	12.33	45.60	502.12	321.41	10.83	49.43	560.17	381.04	20.00	114.95	1306.15	904.83	95.00	941.98	11735.37	8658.33
C.D. at 5%	1.33				3.36				NS				15.55			

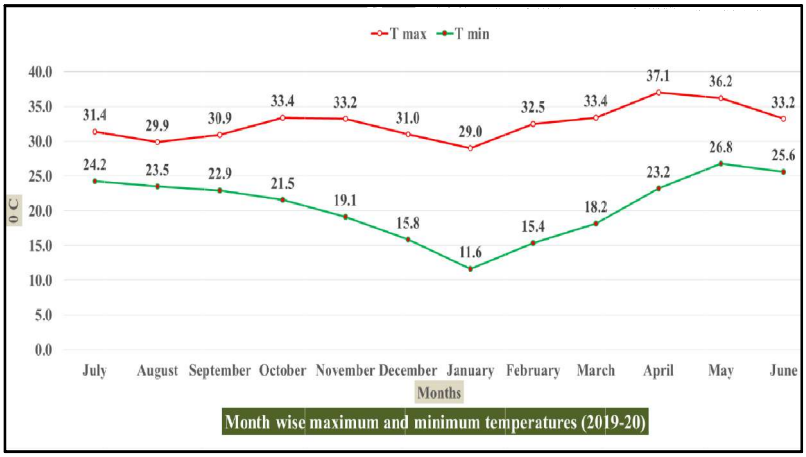

Fig. 1. Month wise maximum and minimum temperatures (2019-20)

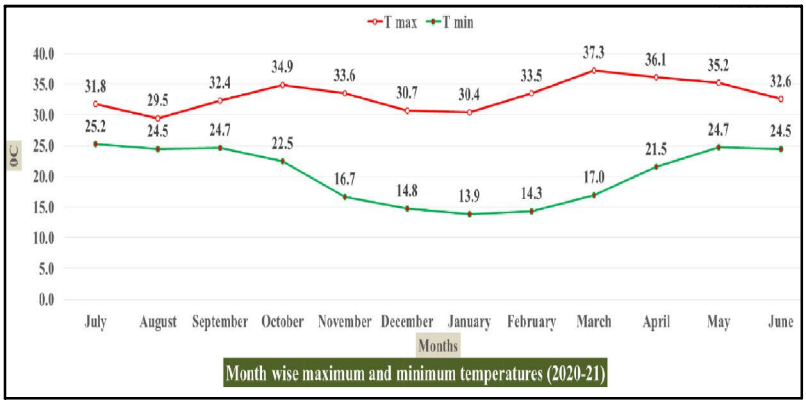

Fig. 2. Month wise maximum and minimum temperatures (2020-21)

Totapuri, Kesar and Rajapuri consumed higher heat units, because these cultivars required more days for maturity. These results are in close confirmation with findings of Kanzaria et al (2015) and Souza et al (2015).

CONCLUSIONS

The requirement of heat units for attaining different phenophase shown great varietal variations, which underlines the varietal specific heat requirement for each phenophases. Sonpari required minimum heat units for attaining maturity and thus ultimately required minimum days to attain the fruit maturity.

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Production Process of Urban Space: An Actor-Network Theory Analysis on Sociology-Translation of Ciliwung River Banks Public Space

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***Abstract*:** Urban spaces are not pre-existed and was enacted through social works. This research attempts to explain how urban spaces are enacted by social interaction, that is the work of building associations. Taking an empirical study at Komunitas Ciliwung Depok, this research utilizes Actor-Network-Theory as an approach to how every actant is involved in enacting their own spaces, later known as public open spaces around Ciliwung River. Drawing previous works of Michel Callon's moments of translation, public open spaces are perceived differently among actants, while carrying their actions in enacting space by enrolling other actants. This research finds that there are 3 stages where space is realized through translation, namely the interaction of 'environmental aspects' with 'key persons', the interaction of 'social aspects' that built and formed the collective, and the increased intensity of interaction between various actants and their mediators to carry their actions in enacting space.

***Keywords*:** Public spaces, Actor-Network-Theory, Ciliwung River, River bank

The existence of spaces in various areas is needed, both private and public spaces, from built-up spaces to open spaces. So that the realization of space must be able to reach all three dimensions simultaneously, namely land, sea, and air which are highlighted both horizontally and vertically (Jensen and Sandström 2020) with various aspects, namely economic, ecological, social and cultural as well as various interests in them such as community activities (working and living) (Allmendinger & Graham 2013). In the development of theories regarding the creation of existing space, it has spread widely with various understandings and meanings of each (Erdi-Lelandais 2014c, Kipferet al 2013, Merrifield 2013, Stanek 2011, Brenner and Elden 2009, Goonewardena, Kipfer 2008, Milgrom and Schmid 2008,Zieleniec 2007, Shields 2005 Elden2004, Lebas and Kofman 2003, Kipfer 2002, Elden 2001). Henri Lefebvre (1991: 375) in his book 'The Production of Space' introduces the idea that space is socially produced. The most frequent application of the Lefebvrean concept is in fields such as architecture and urban studies (Çınar 2014, Gegelioğlu and Aydınlı 2014, Karakaya 2010, Kaypak 2014a, 2014bY. Baş 2010,Turhanoglu 2010,, Koçak 2008,CS Wilson 2007) and social movements in urban areas (Erdi-Lelandais 2014a, 2014b,2013, Fırat 2011, Ergin 2006, Batuman 2003). Talking about social space, it is inseparable from the importance of creating a public open space whose needs are currently increasing along with the increasing number of private spaces that are built so that public open spaces are increasingly difficult to find. Then the problem arises when the need for space to live and work is not proportional to the rapid population growth and high land prices, especially in urban areas. It causes people in urban areas to have limited options in fulfilling the need for space to live (Aschwanden & Wallraff 2017). As a result, land conversion becomes the most logical consequence of increasing activity and population and other development processes. Land conversion at a certain stage is natural, but on the other hand, if it is not controlled, it will be more problematic because generally, the conversion of function occurs on land that is still productive(Arsyad and Rustiadi2008), such as carrying out development on a river bank which should be a public green open space area that has a function for the river ecological system can take place in a sustainable manner(Havinga 2020).In response to these problems, there is currently a new approach in space studies that can view a space no longer as a static physical container that waits to be used first but rather as the result of a manifestation of user interpretation that is constantly changing through the association of various actors occupying the space (Kim 2019). The Actor-Network Theory (ANT) approach is considered highly relevant in conceptualizing space as a phenomenon that can be explained through its network of relationships (Kim, 2019, Tornaghi 2014, Bricocoli and Savoldi 2013, Kärrholm 2007). Simply put, Actor-Network

Theory (ANT) can distinguish itself from other sociotechnical approaches by considering both human and non-human elements such as spatial material, natural conditions, to social relations as actors in a network (Webb 2011, Boelens 2010, Rydin 2010, Doak and Karadimitriou 2007)

This research intends to be able to contribute to realizing urban space by providing an overview of the interaction model of various parties (actors) by using Actor-Network-Theory (ANT) in the realization of public open space. Where ANT can easily know the interrelationship between actors involved in the process of manifesting a space**.** ANT focuses on tracking actants and their networks to understand their interactions within the organization (Guy and Moore 2005). According to opinion (Fox 2000), that ANT can be used to investigate how actors can come as a group to achieve something desired. Yuliar (2009), defined the actor as the initiator of an action. Actors also have roles in an agency or group called human actors and non-human actors have roles in a network. This research wants to investigate actors involved in a project to determine the optimal institutional application.

MATERIAL AND METHODS

This research utilizesthe qualitative method with the Callonian Actor-Network-Theory framework. That is a qualitative method with the sociology of association in mind in guiding its question or observation. Actor-Network-Theory was introduced by Bruno Latour (2005) in his works to follow scientists in action. Callonian version of ANT utilize moments of translation that divide the works of doing association into four phases, namely problematization, intersegment, enrollment, and mobilization. The qualitative method is used in this research to study the interaction of actors in the realization of green open space for the Ciliwung River bank and formulate a model of interaction patterns in the realization of sustainable public green open space in the Ciliwung River banks.The study began by describing this research data as a chronological narrative. Data for this research is drawn from the border of the Ciliwung River, Depok City, West Java, Indonesia. The Ciliwung River is one of the rivers that has the highest level of land criticality in Indonesia, reaching a very critical level from 2014 to the present and its size has continued to increase to reach 4,904 hectares or 11.65% of the total area of the Ciliwung River bank from the upstream to the downstream. (Ministry of Agrarian Affairs and Spatial Planning / National Land Agency 2018, p III-45).This research was conducted for 12 months from March 2019 to March 2020 using both primary and secondary data analysis. Data collection was delayed by the establishment of Large-Scale Social Restrictions (PSBB) in Jakarta due to the Covid-19 outbreak, which took effect on April 10, 2020. Primary research data included the results of direct deep interviews and the implementation of focus group discussions (FGD) involving approximately 60 informants/actors consisting of the Central Government, Depok City Government, area developers, communities, and communities around the Ciliwung River Depok. Meanwhile, secondary research data is obtained through the results of indirect data collection, such as through the internet, literature, and internationally accredited journals.Data was gathered and analyzed by imposing Callon's Moments of Translation on the respected actants and constructing their translation phase. After that, we extract particular characteristics of the actants involved and describe how they built an association with other actants. These characteristics are then juxtaposed with the facts and findings in the field obtained through the translational actor-network theory, so that they can produce targeted planning, and can create an active and environmentally friendly public space near the Ciliwung River in the future.

Depok city is one part of the Ciliwung River whose banks are also experiencing land criticism. This is a concern of many parties so environmental organizations specifically have a role in preserving and maintaining the existence of the Ciliwung river. one of them is the Organization of Depok Ciliwung Community, which this community becomes one of the communities that have a role in maintaining the sustainability of the Ciliwung River while utilizing the river bank as an active public open space to be the location of positive activities that are non-destructive to the ecological functions of the environment.Starting in 2009, a developer from Anyelir Park Estate was doing land restoration activities. These activities were criticized forharmingthe environment by lowering the land area to build parking area, potentially causing the flood to the downstream part of the river. Attempts were made to cleanse the river or restore the river and got the attention of the Depok City Government.Finally in Komunitas Ciliwung Depok (Depok Ciliwung Community/KCD) was formed in 2010. KCD has begun to be more active in voicing its programs to the community, especially students from the University of Indonesia who have been invited to work together before to participate in various KCD programs, one of which is the Run4River activity which aims to introduce the Ciliwung River to children, youth and rubber boat training activities which are a collaborative program between BLH Depok City, MAPALA UI, and KCD.Various cooperation programs between institutions, companies, and universities through KKN and Community were organized. The service together with the University of Indonesia are considered successful and have

resulted in a new open space located under the Grand Depok City Bridge, and until now it has been used as the base camp of the Depok Ciliwung Community itself. Seeing the success of the collaboration between the University of Indonesia and KCD and various other stakeholders entered into the organizational structure of the Depok Ciliwung Community.

The open space of the Ciliwung River School **(**Sekolah Sungai Ciliwung/ SSC) is geographically the same as the KCD public space, which is located on the banks of the Ciliwung river and close to the main road.Boundary-wise,SSC is locatedin the administrative area ofSouth Jakarta. In addition, the social characteristics are also similar, namely being in the middle of an urban residential area.Starting in 2006, several residents living around the Ciliwung River realized that the space on the riverbank was getting full of garbage day by day. This waste mainly piles up in empty spaces where there are no buildings and should be used as a place for plants along the river as a catchment area. Because these spaces seem they have no property and are responsible for protecting them, people can throw garbage and do anything to destroy the physical quality of the space. Seeing the condition; The citizen as well as people who often do activities on the riverbank then invites the community and other space users to clean the space on the riverbank. The movement that began in 2006 continues to involve various parties. One of those who are encouraged to contribute to the preservation of the environment on the riverbank is PLN. With this support at the same time in 2012 formed community of Masyarakat Peduli Ciliwung (Mat Peci) (The Community Cares for Ciliwung) to accommodate support provided by the Government, PLN, and other private sectors. At first Mat Peci had a basecamp on the road MT Haryono Jakarta, but because the area is not wide enough to accommodate activities then there is the idea for basecamp to be moved to Lenteng Agung (the current location) in 2016 .So that the moment of problematization and interesment that occurred from 2006 to 2016 occurred not on the banks of the Ciliwung River that are now used as the place of SSC. After the basecamp was moved to Lenteng Agung in 2016, it can accommodate various parties who wish to contribute to various programs initiated by SSC. There, various facilities and buildings were built to accommodate various community and community activities related to river conservation, for example, there are shelters, jetty, toilets, prayer rooms (musholla), camping ground, and others. Since then, SSC has been increasingly recognized, and many parties have contributed, and this includes receiving various awards from the Government. In addition to parties from outside the region who are interested in contributing to environmental care activities in the SSC area, various activities also involve people living around the SSC. People who initially do not pay attention to the river as an important element of the environment then begin to assume that the river has an important role in environmental sustainability, namely providing ecological, social, and economic benefits with the activities carried out.

The mobilization process mainly occurred when activities

Case Study A: Public Open Space in Komunitas Ciliwung Depok

Source: Private Documentation, 2019

Fig. 1. Orthophoto of Ciliwung Depok Community Public Space

Source: Private Documentation, 2019

Fig. 2. Orthophoto in the School of Ciliwung River (SSC) Public Space

Source: Private Documentation, 2018

Fig. 3. Various activities in the School of Ciliwung River (SSC) Public Space

Table 1. Sociology-translation analysis of case study

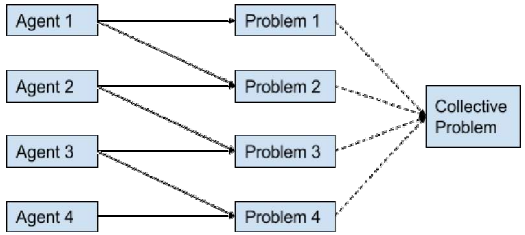
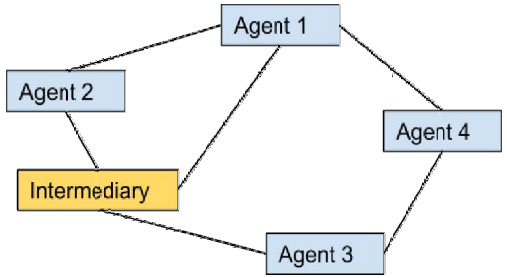
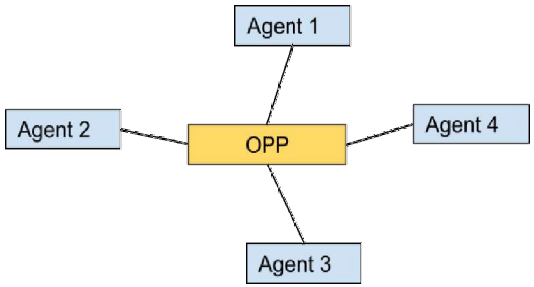
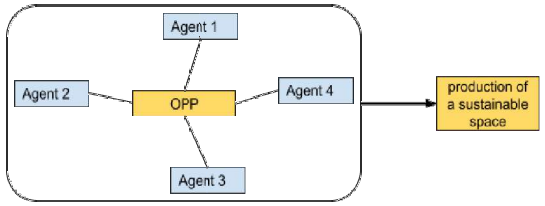
Translation moment	Interaction between actor	Synthesis	Diagram
Problematization	KCD Public Space There has been a change in the function of the riverbank area to become a residential area and does not pay attention to the sustainability of the function of the river which has resulted in the reduction of green open space on the riverbank as a water catchment area in the Ciliwung Drainage Basin (DAS). SSC Public Space The accumulation of garbage in the space on the banks of the Ciliwung River has caused slum conditions, silting, and narrowing of the river width, and it is feared that it will become a source of disease for the surrounding community.	In each location, there is a condition that is perceived as a problem by actors who are interested in the sustainability of the Ciliwung River and the surrounding environment. Even though the social and economic conditions are different from each actor, the environmental conditions that occur are both considered wrong conditions so that they become common problems (collective problems). (diagram 1)	 Diagram 1
Interessement	KCD Public Space Mr. Taufik as a resident who lives around the river quite actively interacts with other actors in discussing and planning with actors involved in the problematization phase in solving common problems. With a background as a journalist, strong interactions occur with the environmental community and local governments to voice problems and find solutions. SSC Public Space The community concerned about the environment of the Ciliwung River (Mat Peci), which was declared earlier in another area, interacted with Mr. Usman a resident of Lenteng Agung who became one of the residents. Mr. Usman then established strong interactions with residents who felt the same environmental and social problems regarding the condition of waste and interacted with PLN (State Electricity Company) to offer programs for their CSR (Corporate Social Responsibility) activities.	With the capital of equality of problems (collective problems), there will be contestations between actors who are moved to be more active than other actors to solve these common problems. The more interactions that are done, the more active actors will be able to influence/attract other actors to follow the opinion of the active actor. This is referred to as an intermediary in the process of translation interessment. (diagram 2)	 Diagram 2

Table 1. Sociology-translation analysis of case study

Translation moment	Interaction between actor	Synthesis	Diagram
Enrollment	KCD Public Space Along with the interaction process between actors that continues to be established, Mr. Taufik as an intermediary, and other actors including Ms. Ismala who has a background in law lecturers in UI formed Depok Ciliwung Community (KCD) SSC Public Space People who live on the banks of the Ciliwung river in the great temple together with Mr. Usman as intermediary at the previous moment, interacted with Mat Peci, PLN, and formed the Ciliwung River School (SSC) located on the banks of the Ciliwung river in the area of the great temple and the planned activities of the SSC.	Institutional form becomes the Obligatory Passage Point (OPP) of actors involved in previous interactions. The institution binds actors in common interests and circulates various media (legal documents, technical implementation proposals, funding proposals, technical training, technical manual documents, etc.). Through the institutional formation, the actors began to delegate each other's roles to each other by exploring each other's various competencies and at the same time becoming a knot for other actors who want to contribute to solving the common problems revealed earlier. (Diagram 3)	 Diagram 3
Mobilisation	KCD Public Space In line with the growing actors and the stronger interaction between the actors involved, KCD is more stable and sustainable from the environmental and organizational side. This is evidenced by the activities that are widely carried out on the edge of the Ciliwung so that an actor interaction space is formed. These activities are done routinely or there are incidental. SSC Public Space SSC and other actors who interact, various community activities and community activities began to be actively carried out both by the SSC management itself and initiated by outside parties, such as the parade Getek Ciliwung River. But generally, the activities carried out are still very dependent on the initiator of actors from outside the region.	Stable and sustainable interactions between actors are intertwined. The interaction of the actor is in line with realizing the same goal which is to solve the problem together is expressed at the time the moment of the problem. In this case, space manifests in line with the interactions that occur between actors to realize the goal. So that the space formed in line with the moment of mobilization. (Diagram 4)	 Diagram 4

were held in the SSC area, either by the SSC management itself or initiated by external parties. Routine activities held by SSC administrators include the Ciliwung River *Getek* parade as part of the socialization of the Ciliwung Clean program. However, most of the activities in the SSC space came from the initiation of outsiders living in that space. So the "space" that occurs is quite dependent on the initiation of actors from outside the area.

RESULTS AND DISCUSSION

Based on both case studies it can be known that each spacehas its moment in the process of its existence. The space in the Depok Ciliwung Community was formed because of community concerns about the sustainability of the Ciliwung River therefore Depok Ciliwung Community space was formed through various complex stages to fight for the sustainability of the Ciliwung Riverand the surrounding environment. The space in the Ciliwung River School is the result of Corporate Social Responsibility (CSR) programs in maintaining and preserving the Ciliwung River through the establishment of public spaces that can be used for various socialization and learning activities about the Ciliwung River.The two spaces have the same goal of protecting and preserving the Ciliwung River, but they are formed through different approaches. Physically, the space created in the Ciliwung River School is much better and permanent. It is different from the space created in the Depok Ciliwung Community which seems simple. This is because the sources of funding obtained by the two spaces are different. Ciliwung River School gets better funding because it is supported by private donors, namely Limited Liability Company ofPerusahaan Listrik Negara (PLN), while The Ciliwung Community of Depok relies on self-help assistance from local communities and other communities in helping it to fight for space on the Ciliwung river.The conditions for bothspaces are different. The space created by the Depok Ciliwung Community tends to be more active and lively when compared to the space created by the Ciliwung River School. This is because the Depok Ciliwung Community has become a shared space since the beginning of its planning.The Depok Ciliwung Community has involved many actors in cooperation, especially since these actors are not only temporarily involved, but sustainable by contributing to protecting the community, and contributing materially. towards the creation of space in the Depok Ciliwung Community. This causes the space in the Depok Ciliwung Community to have a greater sense of belonging when compared to the Ciliwung River School which only involves fewer community contributions in the realization of its space.

This comparative case study shows that the concept of 'space' was abstracted from associations between actants that are involved in the works of establishing a relationship. In line with Kim's (2019) and Tornaghi's (2014) arguments, to describe how 'space' is enacted, we should pay attention to how various networks were made and sustained.However, this research also finds that the 'space' to live (Aschwanden & Wallraff 2017) is not the cause of the loss of living options for people. Instead, 'the space' itself has its trajectory that can be changed, evolving, according to the actant involved and completed translations. More actants involved imply a more diverse and heterogenous 'space' to live. While more actant completes the translation, the more 'space' can accommodate collective life. In other words: the more sustainable it is. On the account of sustainability, this research also finds that the concept of 'sustainability' in developing riverbanks as Hanvinga (2020) wrote, can't be taken at face value as an ethical proposition. Should the 'space' be understood as an evolutionary, ever-changing construct, the 'sustainability' also should consider the flexibility and malleability of the actants involved in the collective, rather than be taken as a fixed ethical proposition for an account.

CONCLUSIONS

This research finds that the conception of 'space' should be taken as a means, rather than an end. While the term open space or public space is usually taken as a fixed concept with certain characteristics embedded to explain the development of an area, this term prevents us to see that the associations between actants are ever-changing, some succeeded while some aren't. The realization of the public green open space has required a series of activities included in the four-moment of translation. Where these four stages can create a pattern of interconnected interaction in the realization of space. The created space can be said to be successful if the space can live and have continuity in it. The space created on the border of the Ciliwung River by the Depok Ciliwung Community can survive and be active every day because it has a very big sense of belonging from many actantsin the process of its realization, it is not such a space created unilaterally by the Ciliwung River School. The “sense of belonging” refers to how various actants do numerous trials and enact their own 'space' as a means for living together.

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Spatial and Temporal Variation of Air Quality in Himachal Pradesh

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Abstract: Study was conducted to investigate the air quality status during national highway expansion by analyzing the relationship of air quality pollutants (PM_{10}, NO_2 and SO_2) with meteorological parameters at different locations. The elevated concentration of PM_{10}, NO_2 and SO_2 was observed around the road construction activities, which indicated vehicular emissions, dust generation and biomass burning for energy purpose to be the main sources of these pollutants. Since combustion of fuel generates these pollutants and are directly released in the atmosphere. PM_{10} have higher values (74.56 to 116.77 µg m^{-3}) and was above the permissible limit (100 µg m^{-3} of National Ambient Air Quality Standards (NAAQS). NO_2 ranged from 16.96 to 26.33 µg m^{-3} and SO_2 from 3.72 to 7.94 µg m^{-3}, respectively and were below the prescribed permissible limits. Seasons played significant role in catalyzing the air pollutants, especially higher values of PM_{10}, NO_2 and SO_2 in post monsoon season i.e. in winter months. Air Quality Index of study region was satisfactory to moderate.

Keywords: Highway expansion, Ambient air quality, PM_{10}, SO_2. NO_2, Impact assessment

Air pollution is a major challenge and concern in the present world causing serious health issues, loss of life and adversely affecting the economic productivity of a country like India say $5 trillion by 2024 (Pandey et al 2021). Air quality degradation has caused around 4.2 million deaths per year due to heart disease, bronchitis, lung cancer and respiratory diseases, since 9 out of 10 people breathe impure air that exceeds the permissible limits of air quality standards (WHO 2022). There have been numerous research studies, which significantly demonstrate that India is experiencing high rates of development and urbanization to strengthen the economic growth but on the verge of air quality and its adverse effects on different ecosystem services (Diaz et al 2020). The degradation of air quality subsequently is due to increased burden of anthropogenic activities like transportation, power generation, industries, agriculture and biomass burning since these activities have great potential to disturb the homeostasis of atmospheric system (Ganguly and Thapa 2016, Sharma et al 2020a). These human induced undertaking in context to road construction activities involving transportation material, equipment and other vehicular movements act as acts as source of gaseous pollutants such as carbon monoxide (CO), sulphur dioxide (SO_2), oxides of nitrogen (NO, NO_2, NO_x), ozone (O_3), particulate matter in different range (PM_{10}, $PM_{2.5}$) and heavy metals, catalyze the degradation of air quality (Sharma et al 2019, Sharma et al 2020, Sharma et al 2020b). According to Environment Protection Agency (EPA) there are six criteria air pollutants such as particulate matter (PM), oxides of nitrogen (NOx), carbon monoxide (CO), sulphur dioxide (SO_2), ozone (O_3) and lead (Pb); which are generally used to measure air quality status, based on the permissible limits of National Ambient Air Quality Standards (NAAQS) (Pattinson et al 2015). The air quality degradation is significantly affected by the meteorological conditions such as solar radiation, temperature, cloud cover, rainfall, wind speed, humidity and mixing height and further hampering the dispersion, accumulation and chemical transformation processes of air pollutants to extreme levels (Liu et al 2020). Increased vehicular activities and linked emissions has been contributing significant deterioration of air quality. Henceforth, there is need of assessment of ambient air quality of a particular region, by an index named Air Quality Index (AQI) based on eight pollutants *viz*., PM_{10}, $PM_{2.5}$, NO_2, SO_2, NH_3, CO, O_3 and Pb, which further compromises six categories, i.e., good, moderate, unhealthy for sensitive group, unhealthy, very unhealthy and hazardous (Pant et al 2020). Additionally, elaborates information on how much air quality has been degraded or polluted with a value ranging from 0 to 500. In this study we have investigated that how road expansion project in Himachal Pradesh have impacted ambient air quality.

MATERIAL AND METHODS

The present study deals with ambient air quality status in and around national highway expansion of NH-154 (Kiratpur-Nerchowk Expressway) which is being upgraded from its present two lane layout, to a four lane divided carriageway.

The present stretch of national highway falls in Himachal Pradesh at Garamoura in Bilaspur to Nerchowk in Mandi districts in the altitude ranging from 650-940 m amsl. Bilaspur district is located between 31°19'48" North latitude and 76°45'0" East longitude and Mandi district is located between 31°42'25" North latitude and 76°55'54" East longitude.

Ambient air quality was assessed at four locations around road expansion activities during the years 2016 and 2017. The parameters considered for this study were $PM_{10,}$ NO_2 andSO_2. The monitoring of these pollutants was carried out on a yearly basis at four locations namely Garamoura & Kainchi Mod in Bilaspur district and Jarol & Chaumukha in Mandi district (Fig. 1); during pre-monsoon (May-June) and post monsoon (October-November) seasons considered as treatments and at three periodic assessments (1^{st}, 15^{th} and 30^{th} day) of the selected month considered as replications. In total there were eight treatment combinations (4 x 2) which were replicated three times under randomized block design.

Respirable dust sampler (RDS) was used to assess PM_{10}based on the principle of gravimetric, which is operated at an average flow rate of 1.0-1.5 m^3 min^{-1}.The sampling was carried out for 8 hours and the sampler was installed at a height of 1.5 m above the ground level and flow rate was noticed after 5 minutes of starting of sampling. For this, Whatman filter paper (20.3×25.4 cm) was used for the collection of particulate matter.The gaseous pollutants such as NO_2 and SO_2 were monitored simultaneously in glass impinger attached internally with the RDS for 8 hours. Then the collected samples were brought to the laboratory and analyzed by following the Jacobs and Hochheiser (1958) method for NO_2 pollutant and West and Gaeke (1956) method for SO_2concentration, respectively. Ambient NO_2 was collected by bubbling air through a solution of sodium hydroxide. The concentration of nitrite ion produced during sampling was determined by reacting the nitrite ion with phosphoric acid and sulphanilamide and N-(1-napthyl)-ethylenediamine di-hydrochloride (NEDA). The absorbance of the solution was recorded on Spectrophotometer (Model-Spectronic-20) at 540 nm.

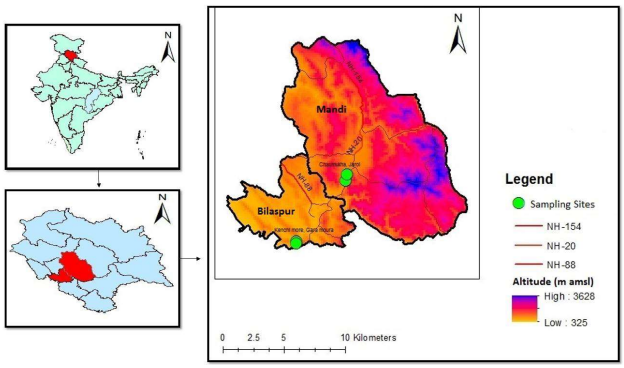

Fig. 1. Study area

$$\mu g \frac{NO_2}{ml} = \frac{\mu g / NO_2 \times Vs(ml)}{Vt(ml) \times Va\,(m^3) \times 0.82}$$

Where

µg per NO_2 = NO_2 concentration from calibration curve,

Vs, Vt, Va = Volume of sample, aliquot taken for analysis and air sampled

Overall sampling efficiency = 0.82

Sulphur dioxide from air is absorbed in a solution of potassium tetrachloromercurate (TCM) and dichlorosulphitomercurate complex is formed. This complex is made to react with pararosaniline and formaldehyde to form the intensely coloured pararosaniline methyl sulphonic acid. The absorbance of the solution was recorded on Spectrophotometer (Model-Spectronic-20) at 560 nm.

$$SO_2\left(\frac{\mu g}{m^3}\right) = \frac{\frac{\mu g}{SO_2} \times Vs(ml)}{Vt(ml) \times Va\,(m^3)}$$

Where

µg per SO_2= SO_2concentration from calibration curve, Vs, Vt, Va = Volume of sample, aliquot taken for analysis and air sampled

Furthermore, the results obtained for PM_{10} concentration and gaseous pollutants, were compared with the air quality permissible limits given by National Ambient Air Quality Standards, to know whether the pollutant is above or below the permissible limit (Table 2).

Climate and weather condition: The climate of the study region is sub-tropical type and there is a considerable variation in the seasonal temperature. In general, May and June are the hottest months and December and January, are the coldest ones in the region. The average maximum and minimum temperature varies from 22.50 to 38.77°C and 2.40 to 20.40°C. The average annual rainfall in the region is 1200 mm, the bulk of which is received during monsoon months (June-September) with few pre monsoon showers during early June period (Fig. 2 and 3).

Air quality index (AQI): The quality of air in the study region has been estimated with help of air quality index. AQI had been calculated from open access internet source provided by Central Pollution Control Board (http://app.cpcbccr.com/AQI_India/).

To convert concentration to AQI, the equation used is:

$$I = \frac{I_{high} - I_{low}}{C_{high} - C_{low}}(C - C_{low}) + I_{low}$$

Where;

I: The air quality index

C: The pollutant concentration

$C_{low,} C_{high}$: The concentration breakpoint that is $\leq C$ *and* $\geq C$

I_{low}, I_{high}: The index breakpoint corresponding to C_{low} *and* C_{high}

RESULTS AND DISCUSSION

PM_{10} concentration: There was a slight change in PM_{10} during road expansion/construction activities in the study region. The highest concentration of PM_{10}(114.56 µg m^{-3}) was at Chaumukha, while lowest of 80.39 µg m^{-3} was in Garamoura during the highway expansion activities (Table 4). This indicated that PM_{10} during the observation was slight above the permissible limit of 100 µg m^{-3} given by National Ambient Air Quality Standards (Table 2). During post monsoon, the significantly higher concentration of PM_{10} was observed (97.85 µg m^{-3}) whereas lowest of 92.85 µg m^{-3} was in pre monsoon season. The interaction between locations and seasons was significant. Highest PM_{10} concentration of 116.46 µg m^{-3} was at Chaumukha in post monsoon season mainly in October and November while lowest of 74.77 µg m^{-3} was at Kainchi Mod during pre-monsoon (summer) season especially in May and June. Higher PM_{10} concentration recorded during winter season may be ascribed to low temperature, cool and dry period, strong atmospheric stability and less rainfalls which reduces vertical mixing of pollutants in the atmosphere leading to less dilution and further dispersion of particulate matter (Bodor et al 2020).

Table 1. Location, latitude, longitude and elevation of the study area

Location	Latitude	Longitude	Elevation (m asl)
Garamoura	31°12'52.6"N	76°39'08.4"E	663
Kainchi Mod	31°13'58.2"N	76°39'48.5"E	698
Jarol	31°27'35.2"N	76°51'40.2"E	705
Chaumukha	31°30'54.8"N	76°52'36.9"E	927

The local sources can be vehicular emissions and forest fires in the study region which comes under high risk zones of forest fires in Himachal Pradesh mainly caused by both man-made and natural sources (Kumar et al 2019).

Gaseous Pollutants

Nitrogen dioxide (NO_2): Highest NO_2 concentration of 26.33 µg m^{-3} was at Chaumukha while lowest of 16.96 µg m^{-3} was at Garamoura (Table 4). Elevated NO_2 levels in the atmosphere are usually ascribed to combustion of fuel (petrol and diesel), emissions from road traffic, construction activities and catalyzed by weather parameters as reported by Niepsch et al (2021).The effect of different seasons was significant. The highest NO_2 concentration of 23.90 µg m^{-3} was during post monsoon months whereas lowest of 19.56 µg m^{-3} was in the summer season. Higher NO_2 concentration during post monsoon may be attributed to combination of anthropogenic emissions and weather parameters. This include winter temperature inversion (i.e. low temperature conditions) and increased energy needs by biomass burning for domestic heating (Rowell et al 2021). The interaction between different locations and seasons was significant. Highest NO_2 concentration of 30.79 µg m^{-3} was at Jarol during winter season (i.e. October and November), while lowest of 16.58 µg m^{-3} was at Garamoura in the summer season (May and June), which was at par with Jarol and Garamoura in post monsoon at Kainchi Modduring pre-monsoon (summer months). The nitrogen dioxide is well within permissible limits prescribed by National Ambient Air Quality Standards (Table 2).

Sulphur dioxide (SO_2): Significant highest SO_2 concentration of 7.94 µg m^{-3} was at Chaumukha whereas lowest of 3.72 µg m^{-3} was at Kainchi Mod (Table 4). The main considered source of SO_2 may be due to emissions released from transportation system i.e. vehicular movement and burning of fuel (Sudalma et al 2015).Seasonal effect on SO_2 was significant. Highest SO_2 concentration of 6.09 µg m^{-3} was

Table 2. Permissible limit standards of ambient air quality parameters

Pollutant	Time weighted average	Concentration in ambient air		Methods of measurement
		Industrial area residential, rural & other areas	Ecologically sensitive area (Notified by Central Govt.)	
Particulate matter (size less than 10µm) or PM_{10}, µg/m^3	Annual*	60	60	-Gravimetric -TOEM -Beta attenuation
	24 hours**	100	100	
Sulphur dioxide (SO_2), µg/m^3	24 hours**	100	100	-Improved West and Gaeke method -Ultraviolet fluorescence
	24 hours**	80	80	
Nitrogen dioxide (NO_2), µg/m^3	Annual*	40	30	-Modified Jacob and Hochheiser -Chemiluminescence
	24 hours**	80	80	

Source: NAAQS (2020)

*Annual Arithmetic mean of minimum 104 measurements in a year at a particular site taken twice a week 24 hourly at uniform interval.

**24 hourly or 8 hourly or 1 hourly monitored values, as applicable shall be complied with 98 % of the time in a year. 2% of the time, they may exceed the limits but not on two consecutive days of monitoring.

during post monsoon (winter season) while lowest of 5.63 µg m^{-3} was in the pre monsoon months. Higher SO_2 concentration in winter season can be attributed low temperature, lesser precipitation and dry conditions that limits the dispersion of SO_2 (Kuttippurath et al 2022). The interaction between locations and seasons was have significant effect on SO_2 concentration. The highest SO_2 concentration of 8.43 µg m^{-3} was at Chaumukha during post monsoon season while lowest of 3.37 µg m^{-3} was noticed at Kainchi Mod in the summer months. The Sulphur dioxidewas well within permissible limits prescribed by National Ambient Air Quality Standards (Table 2).

The higher values of NO_2, SO_2 and PM_{10} were estimated during post monsoon season i.e. (October and November months) in comparison to pre monsoon months (May and June) (Table 5). The year wise assessment showed slight differences in the pollutants. Moreover, the analysis identified higher PM_{10} in comparison to gaseous pollutants $NO_2$and SO_2.

Air quality index (AQI): AQI during pre-monsoon and post monsoon seasons was moderate (101-150) at two locations i.e. Jarol and Chaumukha, whereas at Garamoura and Kainchi Mod it was satisfactory (51-100) (Table 6). The AQI is one of the most important tools for knowing overall air quality condition. Based on the overall conditions, ambient air quality of the study region was satisfactory to moderate (Table 3).

Local meteorology: The maximum monthly average temperature at Mandi and Bilaspur districts was observed to be 34.9ºC and 32.2ºC in 2016 which was followed by 35.1ºC and 32.3ºC in 2017 during observation period, minimum temperature for Mandi district was minus 0.6ºC in December 2016 and 0.3ºC in January, 2017. In Bilaspur, minimum temperature was minus 0.9ºC in January 2016 and minus 1.8ºC in January 2017. The monthly highest rainfall at Bilaspur district was 241.6 mm in June 2016 followed by 521.0 mm in August 2017. The monthly maximum rainfall at Mandi was 388.0 mm in August 2017

Table 3. Air quality index-category and range

AQI	Remark
0-50	Good
51-100	Satisfactory
101-150	Moderate
151-200	Poor
201-300	Very poor
301-500	Severe

Source: Pant et al (2020)

Table 4. Spatial and seasonal variations in ambient air pollutantconcentration (µg m^{-3}) around national highway

Locations	PM_{10} (µg/m3)			NO_2 (µg/m3)			SO_2 (µg/m3)		
	Pre-monsoon	Post-monsoon	Mean	Pre-monsoon	Post-monsoon	Mean	Pre-monsoon	Post-monsoon	Mean
Garamoura	78.35	82.42	80.39	16.58	17.35	16.96	3.64	4.14	3.89
Kainchi Mod	74.77	84.27	79.52	18.48	20.95	19.71	3.37	4.06	3.72
Jarol	105.61	108.26	106.94	17.00	30.79	23.90	8.06	7.73	7.89
Chaumukha	112.67	116.46	114.56	26.17	26.50	26.33	7.45	8.43	7.94
Mean	92.85	97.85		19.56	23.90		5.63	6.09	
C.D. (p=0.05)									
Seasons		0.46			1.70			0.17	
Locations		0.32			1.20			0.12	
Seasons x Locations		0.65			2.41			0.25	

Table 5. Concentration of PM_{10}, NO_2 and SO_2 (2016 and 2017)

Parameters	NO_2 (µg/m3)		SO_2 (µg/m3)		PM_{10} (µg/m3)	
	2016	2017	2016	2017	2016	2017
Pre monsoon	19.30	19.81	92.57	93.13	5.56	5.70
Post monsoon	23.61	24.18	97.52	98.19	5.97	6.20
Maximum	26.16	26.50	114.23	114.89	7.81	8.06
Minimum	16.86	17.07	79.18	79.85	3.67	3.76
SD*	5.09	5.41	16.94	17.04	2.18	2.28
SE*	2.54	2.71	8.47	8.52	1.09	1.14

Table 6. AQI in the selected sites during study period

Locations	Air quality index	
	Pre monsoon	Post monsoon
Garamoura	78	82
Kainchi Mod	75	84
Jarol	104	105
Chaumukha	109	111

followed by 317.2 mm in July 2017. These meteorological conditions play their role dominantly in affecting the PM_{10} and gaseous pollutant concentrations at experimental sites. The weather data was collected from India Meteorological Department, Meteorological Centre Shimla, Himachal Pradesh (Table 7).

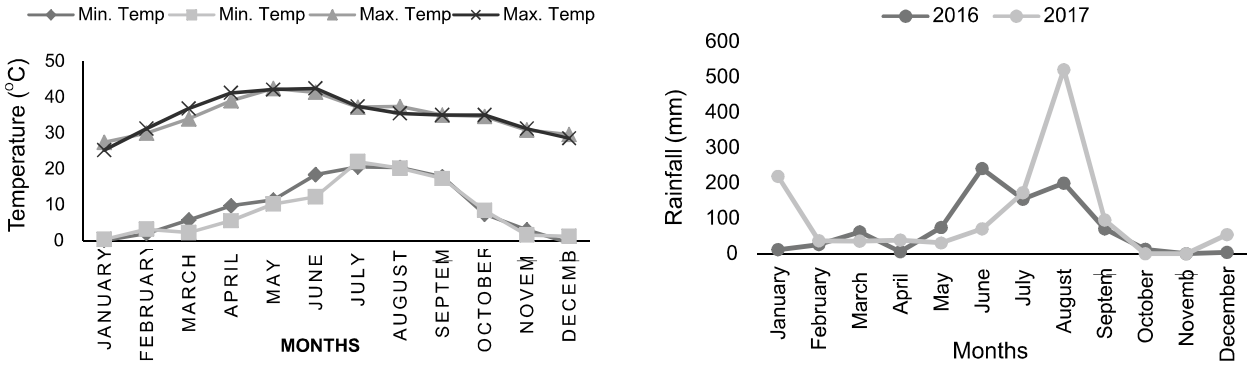

Fig. 2. Maximum and minimum temperature (°C) and rainfall (mm) conditions at Barthein, Bilaspur (2016 and 2017)

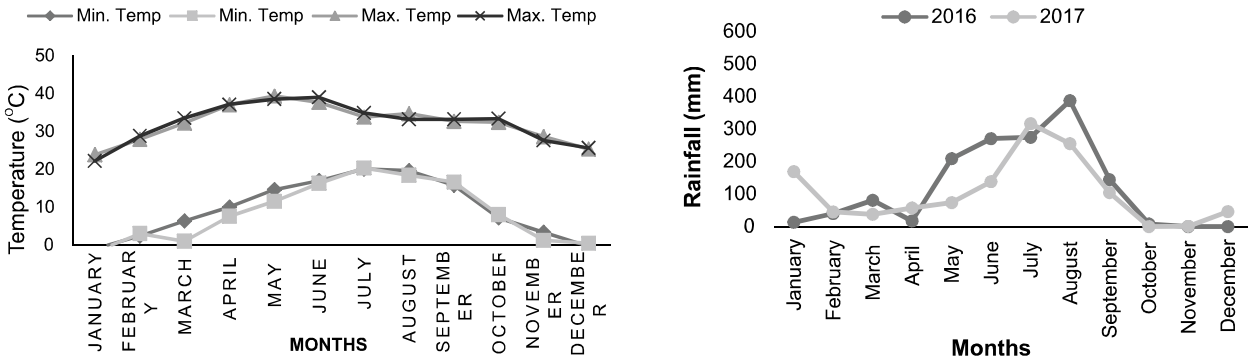

Fig. 3. Maximum and minimum temperature (°C) and rainfall (mm) conditions at Sundarnagar, Mandi (2016 and 2017)

Table 7. Average maximum and minimum temperature (°C) and monthly rainfall (mm) of the study sites during study period

Months	Bilaspur						Mandi					
	Minimum Temp		Maximum Temp		Rainfall (mm)		Minimum Temp		Maximum Temp		Rainfall (mm)	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
January	0.1	0.3	27.4	25.2	11.6	219.2	-0.9	-1.8	23.9	22.2	13.8	169.1
February	2.0	3.2	30.0	31.2	26.4	36.8	2.4	3.0	27.9	28.8	40.2	45.5
March	5.8	2.2	34.0	36.8	61.6	36.0	6.4	1.0	32.2	33.6	81.1	37.8
April	9.8	5.6	39.0	41.2	6.0	38.4	10.1	7.6	37.1	37.2	17.6	57.4
May	11.4	10.2	42.4	42.2	74.0	30.4	14.6	11.6	39.4	38.6	209.4	74.4
June	18.4	12.2	41.4	42.4	241.6	70.2	17.0	16.3	37.7	39.0	270.8	138.8
July	20.6	22.0	37.2	37.4	154.9	172.3	20.1	20.4	33.8	34.9	274.7	317.2
August	20.4	20.2	37.4	35.5	200.4	521.0	19.7	18.4	34.7	33.2	388.0	255.1
September	17.8	17.4	35.0	35.0	70.8	95.8	15.8	16.7	32.7	33.2	144.9	105.0
October	7.4	8.4	34.6	35.0	12.8	0.0	7.2	8.0	32.4	33.4	8.3	0.0
November	3.0	1.6	30.8	31.2	0.0	0.0	3.4	1.3	28.6	27.7	0.0	1.7
December	-0.6	1.2	29.6	28.6	4.0	54.0	-0.3	0.5	25.4	25.6	0.4	46.2

Source: India Meteorological Department, Meteorological Centre Shimla, Himachal Pradesh

CONCLUSIONS

The concentrations of gaseous pollutants i.e. NO_2 and SO_2 in the air are within the National Ambient Air Quality Standards at all the sites in Bilaspur and Mandi district. However, the concentration of PM_{10} was slight higher in study sites than the prescribed limit i.e. 100 µg m^{-3}. Thus, particulate matter has been observed to be the major pollutant identified in the study sites. Moreover, meteorological parameters have played a significant role in catalyzing the air pollutants in the atmosphere, since higher values of the pollutants was observed post monsoon season i.e. winter season in comparison to pre monsoon months i.e. summer season both the years. Concluding values of Air Quality Index showed that the air quality index during post monsoon and pre monsoon seasons was satisfactory to moderate.

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Stock Structure Analysis of Channidae Family from River Sutlej in Punjab by using Truss Networking System

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***Abstract*:** The present study was carried out to distinguish the phenotypic stocks of fish species under Channidae family from selected stretches of river Sutlej in Punjab based on morphometric variations using truss network system. Sampling was conducted at monthly intervals from three different sampling sites of Sutlej river i.e. Rupnagar, Ludhiana and Harike pattan from November 2020 to October 2021. A total of 120 specimen representing different age groups of three fish species under Channidae family (*Channa striata, C. marulius* and *C. punctata*) were analysed. Truss network was constructed by interconnecting 12 homogenous landmarks form 26 truss distances. *tpsUtil32, tpsdig2* software packages were used to extract truss distances from digital photos of specimens. Under principal component analysis (PCA); PC1, PC2 and PC3 accounted 92.9, 3.5 and 1.2% variance in *C. striata*, whereas 86.3, 7.2 and 6.5% variance was observed in *C. marulius. C. punctata* accounted 44.8, 35.4 and 17.7% variance in PC1, PC2 and PC3, depicting highest and lowest variability in *C. striata* and in *C. marulius*, respectively. The study revealed no significant morphometric differences within species, indicating the presence of same phenotypic stock of *Channa* species in River Sutlej. The outcome of the study can be a scientific base for future selective breeding and river ranching programme.

***Keywords*:** River Sutlej, Channidae**,** Phenotypic characters, Truss networking system, Principle component analysis

River Sutlej is one of most important tributaries of Indus river system. It is the longest tributaries with total length of 1450 km, out of which 740 km lies in India. It originates from trans-Himalayas at an elevation of 4630 msl at south-west of Tibetian lakes, Rakshastal. Shipki pass of Great Himalayan range (Himachal Pradesh) is a place where river Sutlej enters into India. It enters the plains near district Rupnagar in Punjab and flows up to Fazilka via industrial city of Ludhiana after confluence with river Beas at Harike Pattan. In the realm of ecosystem-based fisheries management, fish stock characterization is considered as crucial because stocks with a variety of life cycle characteristics, such as growth, mortality and reproductive potential, are thought to be vital for fisheries management. Different body shapes within a species result from difference in environmental factors, genetic makeup are frequently observed (Cadrin 2000). Differences in growth and maturation rates are the indications of patterns of morphometric variation in fish which, in turn, are the result of ontogeny. One of the most common and cost-effective approaches for stock identification is biometric features. To overcome the inherent flaws of standard morphometric methods, the 'Truss network system,' a system of morphometric measurements that effectively discriminates phenotypic stock structure and population features, has become increasingly popular for stock identification (Cadrin 2005). It is more accurate and modern technique of classification of individuals than traditional morphometric methods since it helps to overcome size dependent variance and takes shape-related characteristics into account (Strauss and Bookstein 1982). Dean et al (2001) observed that truss analysis distinguishes physically identical species. Zhang et al (2016) mentioned that the truss network could be attributable to spatial variation in the environmental factors of different locations. Many researchers employed truss morphometry to distinguish between fish stocks of Indian waters such as *Catla*, *Labeo rohita, Rastrelliger kanagurta* and *Megalaspis cordyla* (Jayasankar et al 2004, Sajina et al 2011, Ujjania and Kohli 2011) and also worked on *Channa* species like *C. punctatus*, *C. striata* and *C. gachua* (Khan et al 2013, Kashyap et al 2016, Jearranaiprepame et al 2017, Khan et al 2019).

There are three genera (*Aenigmachanna, Channa* and *Parachanna*) and 50 species available in the Channidae family (Actinopteri, Anabantiformes) (Britz et al 2019). The extant three species of *Parachanna* are African, despite *Aenigmachanna and Channa* that two genera are purely Asian. Britz et al (2019) also reported a new species from Malappuram, Kerala i.e. *Aenigmachanna gollum*. Due to the appearance of massive scales on the skulls of most species, Channids are popularly referred to as “Snakeheads”. The fish

species under Channidae family have huge demand in domestic and international markets because of their taste, absence of intramuscular spine, flavour and medicinal values. In Indian waters phylogenetic study using truss networking system was conducted on *C. punctata* (Khan et al 2013) and *C. striata* (Kashyap et al 2016) but no related study has been conducted on *C. marulius*. Therefore, the current study was designed to use truss network system, a modern morphometric method, to separate the phenotypic stocks of Channidae species collected from selected stretches of river Sutlej in Punjab to determine morphometric variations.

MATERIAL AND METHODS

Sample collection: The study was conducted at the College of Fisheries, GADVASU, Ludhiana, Punjab for a duration of November 2020 to October 2021. Sampling was conducted at monthly intervals from three different sampling sites of Sutlej river i.e.Rupnagar Head works (30°59′ 52.9404″N, 76°32′ 00.636″E), Rail/road Bridge at Phillaur, Ludhiana (30°59′ 35.2608″N, 75°47′ 28.2516″E) and Harike Pattan (31°08′32.334″N, 74°56′55.0032″E) with a distance of about 90 km from one station to another. A total of 120 specimens out of which *Channa striata* (n=40)*, C. marulius* (n=40) and *C. punctata* (n=40) of different age groups were collected from selected sites.

Sample preparation and measurement: Measurements of fish samples were taken by piercing the paper with a needle at relevant anatomical landmarks. Fish samples were placed on a water-resistant laminated graph sheet. To identify each specimen in the graph, it was labelled with a unique code. Freshly caught samples were washed properly under running water, drained and wiped dry before taking images. The digital image of each individual was obtained using a Sony (Japan) camera at a fixed distance (Zhang et al 2016). Digital images of fish specimens implied a complete body shape also allow making repeated measurements (Cadrin and Friedland 1999). Images were captured from the same height as well as angle to minimize the human error as per procedure described by Mir et al (2013).

Statistical analysis: The truss network was constructed by interconnecting the 12 homogenous landmarks form 26 truss distances (Table 1). The network extended across the whole body of the fish to depict the full dimension of the body (Moore and Bronte 2001 and Mir et al 2013).

Measurement of truss distances: *tpsUtil32, tpsdig2* (Rohlf 2006) and the PAST software package (Hammer et al 2001) were used to extract truss distance from digital photos of specimens. The *tpsUtil32* software converted the 'JPEG/JPG' format to 'TPS' file as *tpsdig2* software works only in TPS file. The *tpsdig2* software set the landmarks and measured the truss distances of each captured image (Fig. 1). All the calculated measurements were transferred to Paleontological Statistics (PAST) software package in a spread sheet file (Hammer et al 2001) and the coordinate data (X-Y) was transformed into linear distances using the Pythagorean Theorem by the software for subsequent analyses.

Normalization of the data: For the analysis of morphometric data, an important stage in the data preparation was to eliminate the size effect while comparing the different sizes of the fish. Considering allometric growth, before statistical analyses all the measurements were log transferred to remove the size effect by applying an allometric approach. The significant correlation exists between the body length and the morphometric variables and hence, the variation in

Table 1. Particulars of truss distances between landmarks used in fish species under Channidae family

Landmark numbers	Truss distance
1-2	Tip of snout- Termination point of snout
1-12	Tip of snout- Termination of mouth
2-12	Termination point of snout - Termination of mouth
2-3	Termination point of snout – Forehead
11-12	Most posterior point of maxillary- Termination of mouth
2-11	Termination point of snout - Most posterior point of maxillary
3-12	Forehead - Termination of mouth
3-11	Forehead - Most posterior point of maxillary
3-4	Forehead - Origin of dorsal fin
9-10	Origin of Anal fin- Point of Pectoral fin insertion
10-11	Point of Pectoral fin insertion- Most posterior point of maxillary
3-9	Forehead- Origin of Anal fin
3-10	Forehead- Point of Pectoral fin insertion
4-11	Origin of Dorsal fin- Most posterior point of maxillary
4-10	Origin of Dorsal fin- Point of Pectoral fin insertion
4-5	Origin of Dorsal fin- Termination of dorsal fin
8-9	Termination of Anal fin- Origin of Anal fin
4-8	Origin of dorsal fin- Termination of Anal fin
5-9	Termination of Dorsal fin- Origin of Anal fin
4-9	Origin of dorsal fin- Origin of Anal fin
5-6	Termination of dorsal fin- Dorsal side of Caudal fin
7-8	Ventral side of Caudal fin- Termination of Anal fin
5-7	Termination of Dorsal fin- Ventral side of Caudal fin
6-8	Dorsal side of Caudal fin- Termination of Anal fin
5-8	Termination of Dorsal fin -Termination of Anal fin
6-7	Dorsal side of Caudal fin- Ventral side of Caudal fin

the whole data may discriminate the populations based on the size of fish. Therefore, each distance was corrected as per Elliott et al (1995).

$$M_{adj} = M (L_s / L_0)^{b}$$

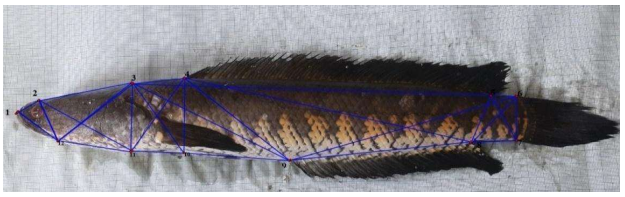
Where, M_{adj} = Transformed the Truss measurement, M = Original Truss measurement

Ls = Overall mean standard length, L_o = Standard length of Fish

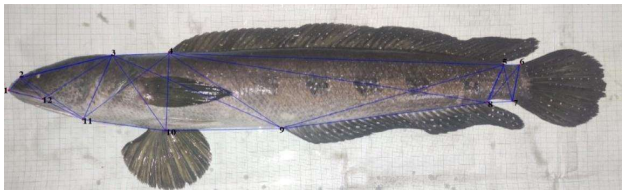
'b' is the within group slop regression for plot of M and L_s on logarithmic scale

Since variation should be immutable to body shape thus it should not be related to the relative size of fish (Reist 1985). The significance of the connection between transformed variables and standard length was tested on the standardized data (Turan 1999).

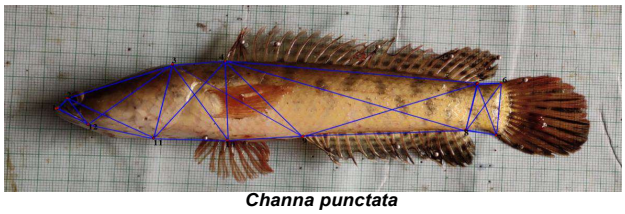
The Principal component analysis was performed to understand that which of the morphometric measurements could distinguish populations. The PCA was based on correlation or covariance matrix which considered all the data simultaneously rather than individually (Bookstein et al 1985). Eigen values were obtained using this procedure, which allowed for the major part of the variation of original variables to be explained with a small number of factors. The proximity in the space defined by the components is used for analyzing the relationship between populations (Khan et al 2012). PCA was performed using PAST software.



Channa striata



Channa marulius



Channa punctata

1. Tip of Snout 2. Termination point of Snout 3. Forehead 4.Origin of Dorsal fin 5. Termination of Dorsal Fin 6. Dorsal side of Caudal fin 7. Ventral side of Caudal fin 8. Termination of Anal fin 9. Origin of Anal fin 10. Point of Pectoral fin insertion 11. Most posterior point of Maxillary 12. Termination of Mouth

Fig 1. Consensus of Truss Morphometric Network (TMN) of *C. striata, C. marulius, C. striata* and *C. punctata*

RESULTS AND DISCUSSION

The three species under family Channidae were recorded i.e. *C. striata, C. marulius* and *C. punctata* from the selected stretches of River Sutlej in Punjab. Among these *C. marulius* was dominant in overall catch and available in all the three sites and seasons whereas, *C. striata* and *C. punctata* were at Site 2 (Ludhiana) and Site 3 (Harike) and it was absent at Site 1 (Rupnagar). *C. marulius* was dominant fish in catch throughout the study period (weight basis) under family Channidae (58%) followed by *C. striata* (37%) and *C. punctata* (5%) contributed significantly. In *C. striata* average weight (W), total length (TL) and standard length (SL) were recorded as 570.58 g, 41 cm, 34.97 cm, respectively. In *C. marulius* W, TL and SL were as 1482.39 g , 57.70 cm and 49.94 cm respectively. The W, TL and SL were recorded as 37.43 g , 16.88 cm, 14.10 cm respectively in *C. punctata*. Output data of the truss networking system was further analyzed through PCA to find out the interrelationship among intra and inter species variability. The first component (PC1) of morphometric is interpreted as the size axis (Bookstein et al 1985) and the second (PC2) and third components (PC3) as shape variables (Humphries et al 1981, Bookstein 1989, Sundberg 1989). In present study the main two factors which were responsible for the association among the truss measures were shape and size of the fish. The correlation coefficient between standard length and truss measurements were closed to one before the transformation for size correction. After the transformation, truss measurements did not show significant correlation with standard length of the fish species and were ready for further analysis. PC1, PC2 and PC3 of *C. striata* successively accounted 92.9, 3.5 and 1.2% variability, respectively. PC1, PC2 and PC3 contributed 86.3, 7.2 and 6.5%, of total variance in *C. marulius* whereas PC1, PC2 and PC3 contributed 44.8, 35.4 and 17.7% variability in *C. punctate* (Table 2 and Fig. 2-4).

In all of the Channidae species studied, the overall findings through PCA revealed that size is the most important contributor to variability, followed by shape. Khan et al (2013) studied the PCA of *C. punctata* (n=234) from three different River of India (i.e Ganga, Yamuna and Gomati) and find the different stock of *C. punctata*. Kashyap et al (2016) reported 83.25, 5.33 and 3.37% variability in PCI, PCII and PCIII, respectively in *C. punctata* from Northern and Eastern Regions of Gomati River in India. Jearranaiprepame (2017) calculated the PCA of population of one of the species *C. gachua* under same family Channidae; and observed that

first three components were contributing 49.98% variability due to body shape. Norainy et al (2018) analysed size and shape variation of four *Channa* species (*Channa striata, C. micropeltes, C. marulioides and C. lucius*) through truss networking system from Indonesian waters. First three components of PCA contributed 88.98% variability due to body size and 46.05 % variability due to body shape. Khan et al (2019) examined the PCA of *C. striata* population from the river Ganga as well as from its tributaries Yamuna and Gomti and found that the first three components were responsible for 40.2, 9.86 and 6.68% of variability due to body size.

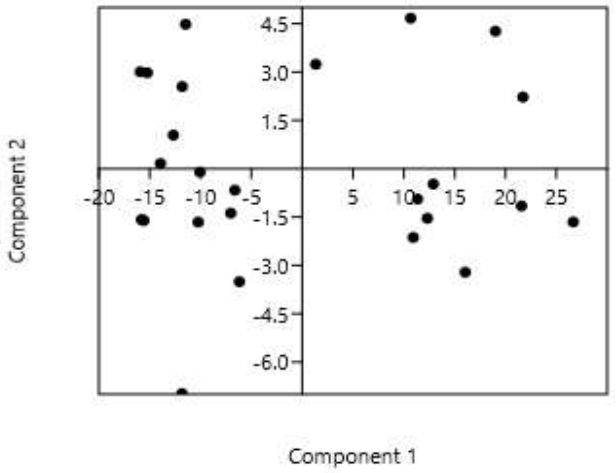


Fig. 2. Principal component analysis of *C. striata* (intra species) collected from river Sutlej

In present study, first five components, the eigenvalues of *C. striata* along river Sutlej was higher than one (209.5, 8.1, 2.7, 1.6 and 1.2, respectively). Higher eigenvalue factors contributed more to the variations in the variables, while lower eigenvalue factors have been dismissed as redundant with more relevant factors. The eigenvalue of the first six in *C. marulius* were also higher than one (98.8, 8.2, 7.5, 5.7, 4.5 and 1.8, respectively). In *C. punctata*, the eigenvalue were recorded more than one for the first five components (57.7, 45.6, 22.8, 2.5 and 1.0, respectively). Fig 2 - 4 depicted the intra species scatter plots of the *C. marulius, C. striata* and *C. punctata*. Based on the morphometric characterization

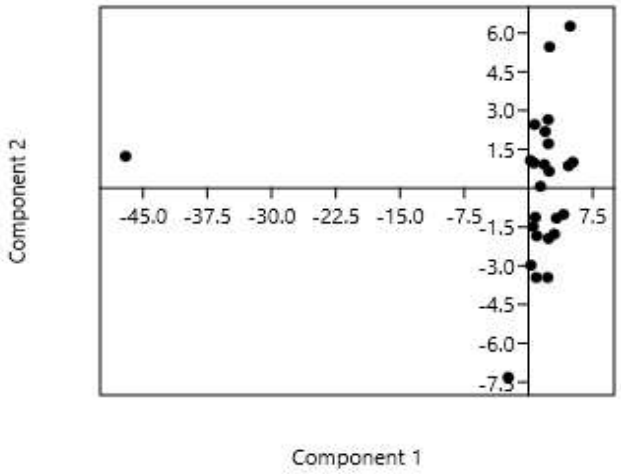


Fig. 3. Principal component analysis of *C. marulius* (intra species) collected from river Sutlej

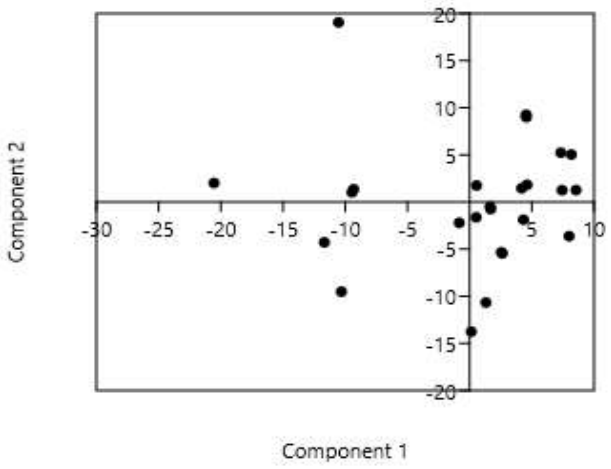


Fig. 4. Principal component analysis of *C. punctata* (intra species) collected from river Sutlej

Table 2. Percentage of variance accounted by variables having Eigen values more than 1 of within group PCA for *Channa striata, Channa marulius* and *Channa punctata* from Sutlej River

Principal component	*Channa striata*		*Channa marulius*		*Channa punctata*	
	Eigenvalue	% Variance	Eigenvalue	% Variance	Eigenvalue	% Variance
1	209.533	92.91	98.830	86.317	57.7525	44.812
2	8.113	3.597	8.2843	7.2354	45.6752	35.441
3	2.798	1.240	7.536	6.582	22.8739	17.748
4	1.699	0.753	5.718	4.994	2.517	1.953
5	1.212	0.537	4.5627	3.9851	1.097	0.851
6			1.818	1.588		

maximum variability within stock was observed in *C. striata* and it was minimal in *C. marulius*. No significant morphometric differences were recorded among three species. Khan et al (2013) studied the PCA of *C. punctata* (n=234) from three different River of India (i.e Ganga, Yamuna and Gomati) and find the different stock of *C. punctata*. Kashyap et al (2016) reported 83.25, 5.33 and 3.37% variability in PCI, PCII and PCIII, respectively in *C. punctata* from Northern and Eastern Regions of Gomati River in India. Jearranaiprepame (2017) calculated the PCA of population of one of the species *C. gachua* under same family Channidae; and observed that first three components were contributing 49.98% variability due to body shape. Norainy et al (2018) analysed size and shape variation of four *Channa* species (*Channa striata, C. micropeltes, C. marulioides and C. lucius*) through truss networking system from Indonesian waters. First three components of PCA contributed 88.98% variability due to body size and 46.05 % variability due to body shape. Khan et al (2019) examined the PCA of *C. striata* population from the river Ganga as well as from its tributaries Yamuna and Gomti and found that the first three components were responsible for 40.2, 9.86 and 6.68% of variability due to body size.

CONCLUSIONS

In Channidae family *C. marulius* was dominant fish in catch composition followed by *C. striata* and *C. punctata* contributed significantly. The truss analysis revealed no significant morphometric differences, indicating the presence of same phenotypic stock of *Channa* species in River Sullej. Among different species under Channidae family maximum variability was observed in *C. striata* and least in *C. marulius.*

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Food and Feeding Habit of *Wallago attu* from Bhadar Reservoir of Gujarat, India

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***Abstract*:** The present study is conducted at Bhadar reservoir landed of Rajkot in Gujarat, India. The Gastrosometic index (GaSI) of the male and female was higher in February and September and low in f August. Mean GaSI of male was higher than the female. The percentage of empty stomachs was the highest (26.67%) in July and in September was absent. The stomachs with one-half contents and three-fourth contents was high in October (34.22%) and in September (40%). The percentage of full stomachs was highest (50%) in February.

***Keywords*:** *Wallago attu*, GaSI, Stomachs, Bhadar reservoir

The study of the feeding habits of fish and other animals based upon analysis of stomach content has become a standard practice (Ajah and Udoh 2012). Stomach content analysis provides important insight into fish feeding patterns and quantitative assessment of food habits (Alam et al 2013). This is an important aspect of fisheries management provides important insight into fish feeding patterns (Zacharia and Abdurahiman 2004). Feeding of fish represents an integration of many important ecological components that includes behavior, condition, habitat use, energy intake and inter- and intra-specific interactions, etc (Demekeadmassu and Tadesse 2015). Accurate description of fish diets and feeding habits also provides the basis for understanding trophic interactions in aquatic food webs. Conceptually, trophic relations of fishes begin with food and feeding behaviour of individuals or species (Epko et al 2014). Diet composition analysis can be used to evaluate effects of ontogeny or the establishment of exotic species (Manko 2016). Catfish is an opportunistic and omnivorous feeder ingesting a wide variety of food items such as algae, macrophytes, zooplankton, insects, fish prey, detritus, amphibians and sand grains (Tesfahun 2018). Food and feeding habits of fishes have been a field of interest to fisheries researchers since very long (Manon and Hossain 2011, Nikolioudakis et al 2011, Ramesh and Kiran 2016). Feeding is the dominant activity of the entire life cycle of fish and food is the main source of energy which plays an important role in determining the population levels, rate of growth and condition of fishes (Mamun et al 2004, Gupta and Banerjee 2013, Tesfahun 2018). The present study was focus on the food and feeding habit of *Wallago attu* (fresh water shark) from the reservoirs of Bhadar, Gujarat.

MATERIAL AND METHODS

The present study is conducted at Bhadar reservoir landed of Rajkot district (Saurashtra region (22°30′N 70°78′33"E) in Gujarat, India. Bhadar reservoir (site) is located at 21°76′28"N 70°42′37" E near Bhukhi village Dhoraji, Taluka of Rajkot district during July 2018 to February 2019. Data collected from the sites at monthly interval. *W. attu* fishes were collected from selected site of reservoir. The fishermen are mainly using gill net for fishing. Fish samples were brought to College of Fisheries, Veraval and used 5% formalin solution in specimen jar according to the size of species. The samples were identified with the help of literature. The Index of relative importance (IRI) of various food items in the gut was calculated (Pinkas 1971).

$IRI = (\%N + \%V) \times \%F$

Where N = number, V = volume and F = frequency of occurrence

RESULT AND DISCUSSION

Gastro somatic index (GaSI): The higher peak in gastro-sometic index of both male and female during February and September 6.83 and 4.85 respectively (Fig. 1). The lower value of 2.91 was in August, because during spawning season female prefer less food than the male.

Feeding intensity: During the present investigation, a total of 225 stomach of *W. attu* ranged from 31.8 to 109.5 cm. The total length (TL) were examined the ontogenetic shift in the selection of food was observed as fishes with higher total length were with fishes and some prawns. Ontogenetic shift in diet is widespread among fish, which is a function of an increase in the sizes of body and mouth, permitting the individuals to capture preys of broader ranges of size and

types (Sahoo et al 2006). Most of the examined specimens were with full and three fourth stomach contents. Very less number of empty stomachs observed during study period. The percentage of empty stomachs was 32% in August, half contents (34.22%) in October, three-fourth contents (40%) in September, full stomachs was (50%) in February and absent in July, August. The percentage of empty stomach was high in female than the male. *Chanda nama* and digested fish, *M. rosenbrgii* and some other cat fishes were the favorite food of *W. attu* are highly carnivores species (Yousafzai et al 2010).

Feeding patterns: The stomach contents of *W. attu* composed mainly of prawns and other fresh water which *Chanda nama, Catla catla, Labeo rohita, Ompak spp., Laboe calbasu, Channa spp., Mytus spp.,* and *M. rosenbergii.* The *W. attu* species are highly carnivores in nature. Babare et al (2013) observed that gut of *W. attu* consists of a thick, muscular, roughly spherical, highly extensible stomach bag and narrow, medium and thick intestine. *W. attu* able to locate and consume different type of animals. Fishes consume 90% food items of animal origin of which 90% are locally available weed fish species. Ranjan et al (2009) reported *W. attu* as only carnivorous food and it was recorded in the gut of this fish. The 99.0 % included planktonic crustaceans, rotifers, insects and their larvae and small fishes. In addition, miscellaneous food particles (1.0%) were also present. Samina et al (2017) observed that in *W. attu* stomach content the adult crustaceans, insects and a number of small fishes.

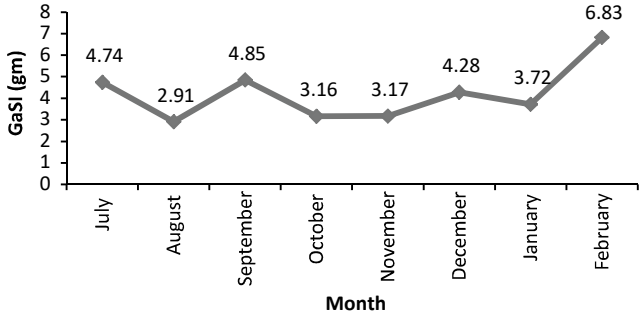

Fig. 1. Monthly variation in GaSI of *W. attu*

Frequency of occurrence of food items: The most frequent food item observed in the diet of *W. attu* was *Chanda nama and M. rosenbergii*., and digested fish, in all the months. It exhibited carnivores in nature more frequently in October, November, December, January and February month. As dietary components, *Chanda nama* and *M. rosenbergii* were more frequent in all months. *Ompaks spp., C. catla, L. rohita, Mystus sp., Channa sp.,* was a frequent food item during almost all the month except July and August.

Table 1. Month wise estimation of Gut condition in *W. attu*

Month	Percentage (distribution) of gut status in different				
	Empty	Quarter	Half	Three fourth	Full
July	26.67	60.00	13.34	-	-
August	32.00	36.00	32.00	-	-
September	-	33.34	20.00	40.00	6.67
October	15.79	5.27	34.22	34.22	10.53
November	23.53	20.59	20.59	29.42	5.89
December	16.22	8.11	13.52	18.92	43.25
January	13.80	10.35	6.90	20.69	48.28
February	4.55	4.55	18.19	22.73	50.00

Table 2. Monthly variation of IRI in the dietary component of *W. attu* (Percent)

Food prey item	July-18	Aug-18	Sep-18	Oct-18	Nov-18	Dec-18	Jan-19	Feb-19	Average
Digested material	33.42	29.72	13.99	14.60	12.20	11.13	11.37	10.71	17.14
Fishes	0	0	9.84	0	4.05	15.24	5.84	8.24	5.4
Chandanama	39.19	34.68	27.59	22.32	10.81	16.85	24.94	15.86	24.03
Ompak species	0	0	9.90	10.85	7.86	4.05	4.50	9.57	5.84
Prawns species	27.37	35.59	17.59	21.61	37.94	23.61	30.13	26.45	27.53
Catlas pecies	0	0	14.06	5.10	10.57	10.16	2.96	7.20	7.15
Rohus pecies	0	0	0	3.49	5.94	5.28	4.79	6.29	3.68
Calbasu species	0	0	0	4.25	0	0	6.75	4.63	2.6
Mystus species	0	0	6.63	9.39	5.12	7.29	4.80	4.78	6.33
Channa species	0	0	0	8.36	5.46	6.33	3.87	6.21	6.04

Table 3. Month wise percentage composition of food item of *W. attu*

Months	Digested material	Fishes	*Chanda nama*	*Ompak* spp.	*Prawns* spp.	*Catla* spp.	*Rohu* spp.	*Calbasu* spp.	*Mystus* spp.	*Channa* spp.
Jul-18	33.42	0	39.19	0	27.37	0	0	0	0	0
Aust-18	29.72	0	34.86	0	35.59	0	0	0	0	0
Sept-18	13.99	9.84	27.59	9.90	17.59	14.06	0	0	6.63	0
Oct-18	14.60	0	22.32	10.85	21.61	5.10	3.49	4.25	9.39	8.36
Nov-18	12.20	4.05	10.85	7.86	37.94	10.57	5.94	0	5.12	5.46
Dec-18	11.13	15.24	16.85	4.05	23.61	10.16	5.28	0	7.29	6.33
Jan-19	11.37	5.24	24.94	4.50	30.13	2.96	4.79	6.75	4.80	3.87
Feb-19	10.71	8.24	15.86	9.57	26.45	7.20	6.29	0	4.78	6.21

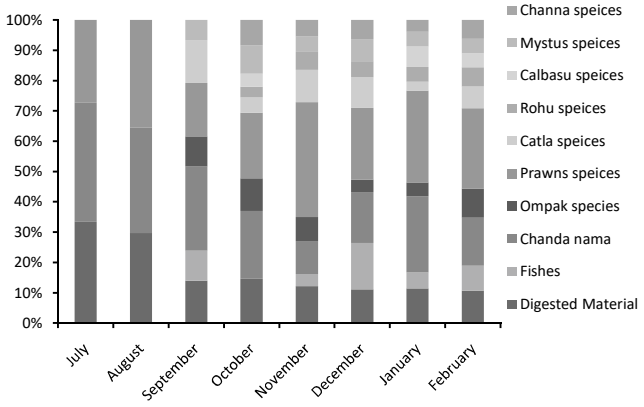

Fig. 2. Monthly variation of IRI in the dietary component of *W. attu*

L. calbasu was present in stomachs all the months except July, August, September, November and December.

Monthly index of relative importance: Variation in the index of relative importance (IRI) of food items ingested by *W. attu* during different months revealed that the percentage composition of different food items varied in different months according to their availability and preference. The food items of *M. rosenbergii* (27.53%), *Chanda nama* (24.03 %), *Ompak sp.* (5.84%), *Catla* (7.15%), *Labeo rohita* (3.68%), *Labeo calbasu* (2.6%), *Mystus sp.* (6.33%), *Channa sp*. (6.04%) and Digested material (17.14%) contributed during entire study period.

CONCLUSION

The gut content analysis of *W. attu* from the Bhadar reservoir in Gujarat showed that 90% of the food consumed by weed fish species in the area is of animal origin. In July and September the empty gut was observed and in February the gut was full. *W. attu* preferred a much smaller amount of diet at this time because of its larger in July and September due to breeding and spawning seasons. The Chanda nama (Glass fish) species was the single most common food item consumed each month.

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First Record of *Calanopia metu* Uysal & Shmeleva, 2004, A Mediterranean Calanoid Copepod from Indian Waters

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***Abstract*:** The present study deals with the first record of a calanoid copepod, *Calanopia metu* Uysal & Shmeleva, from coastal waters of Cochin region, which is situated in the south west coast of Arabian Sea, region of Indian Ocean. A total of 48 specimens ranged from 350 µm and 512 µm were recorded from the region. The mean abundance was 1±1.4 individuals per m^3. The species is native to the Mediterranean Sea and was originally described in 2004 from the Levantine basin. The occurrence of *Calanopia metu* in the study region is inferred as an introduction of non-native species through the ballast water of cargo hold ships. For the present study, the samples of *Calanopia metu* were collected from three stations of Cochin estuary during the Pre-monsoon period.

***Keywords*:** Zooplankton, Cochin estuary, Ballast water, Arabian Sea

Copepods are the most abundant and diverse group of small crustaceans distributed in almost all types of aquatic habitats. They have a promising role in the ecosystem, because they act as a connection link between the phytoplankton and the higher organisms. Order Calanoida, represents a major group of copepods with nearly 2266 valid species, belonging to 42 families (Boxshall and Halsey 2004, Razouls et al 2005-2016). Genus *Calanopia* under the family Pontellidae consist of a total of eighteen species (Unal and Shmeleva 2002, Al-Aidaroos et al 2016, El-Sherbiny and Al-Aidaroos 2017) of which fourteen species were reported from the Indo-Pacific waters and the remaining four species from the Mediterranean and Atlantic waters. Members of genus *Calanopia* are typically marine inhabitants but also reported from estuaries during high saline periods (Costello 2001, Vineetha et al 2015). Of the fourteen species reported from Indo-Pacific waters, the species such as *Calanopia elliptica* Dana, 1849, *Calanopia aurivilli* Cleve, 1901, and *Calanopia minor* Scott A, 1902 are the common species distributed in the marine and coastal waters of the Arabian Sea (Prusova et al 2011). *Calanopia metu* is reported first time from Mediterranean waters (Uysal and Shmeleva 2012). Cochin estuary is the largest estuarine system located in the south west coast of India and emptying into the south-eastern Arabian Sea of Western Indian Ocean. The estuary is called monsoonal estuary, because it is heavily influenced by south west monsoon (Vijith et al 2009). It permanently opens to the Arabian Sea at Cochin (width 450m) and at Azhikode (width250m) (Vineetha et al 2015, Vinita et al 2015). Estuaries becomes merely an extension of the adjoining Sea, with environmental features almost similar to those of the coastal waters (Sivaprasad et al 2013) and most of the coastal/marine species were encountered during the high saline pre-monsoon and post-monsoon season (Vineetha et al 2015). During the dry season, the Cochin estuary is also more productive and the diversity, richness and evenness of copepod species become high (Jyothibabu et al 2006). The present study deals with the new record of *Calanopia metu* from the coastal waters of India and this is the first report from the Indian Ocean.

MATERIAL AND METHODS

Samples were collected from three locations (Vallarpadam – 9.98°N, 76.25° E, Bolgatty-9.98°N, 76.26°E, Marine drive-9.97° N, 76.27°E) in bar mouth area of Cochin estuary (Fig. 1) during pre-monsoon period (February 2018). Copepod sampling performed in the morning between 6 am and 8 am during low tide by using the conical zooplankton net (mesh size- 200 µm; diameter -60cm) in the upper water column. A calibrated flow meter (Model: Hydro-Bios-438110) fitted with net used to estimate the volume of water filtered. The net was hauled horizontally for 10 minutes by maintaining a minimum speed of ~2 knots. Collected samples were properly washed and filtered with pre filtered

water and preserved with 4-5% formaldehyde solution and stored in sample containers for further analysis in the shore lab. Water samples were collected for analysing the physical parameters such as temperature and salinity. Temperature measured using 0-50^{0}C precision thermometer. Salinity using a calibrated hand refractometer (Model: ERMA accuracy ±0.001).

In the laboratory, copepods were sorted with help of a zoom stereo microscope (Model: Magnus MSZ-Tr) and the numerical abundance was recorded. Folsom plankton splitter was used to get the subsample in the case of large samples. The species identification was done by dissecting the fifth pleopods and mounted on a clean slide and observed under the microscope (Model: Leica DM500). Photographs and measurements were done by using Leica microscope Camera (Leica ICC50 HD) fitted with the microscope and image analysing software (LAS EZ) respectively. Taxonomic identification up to species level was done using the following manuals: Kasturirangan (1963), Uysal and Shmeleva (2004) and Conway (2006). Marine planktonic copepod databases were also used for identification (https://copepodes.obs-banyuls.fr/en/).

RESULTS AND DISCUSSION

During the study period, the salinity varied from 31 to 32 ppt and the surface temperature fluctuated between 28 and 30^{0}C. A total of 48 specimens of *Calanopia metu* were collected during the survey. All specimens were in the advanced copepodite IV and V stage of both males (43%) and females (56%). Mean abundance recorded during the period was 1±1.4 individuals per m^3 with a size range from 350 to 512 µm. The samples were deposited in the national zoological collection of crustacean division ZSI/FPS of Zoological Survey of India, Kolkata and the reference ID issued is C8930/2.

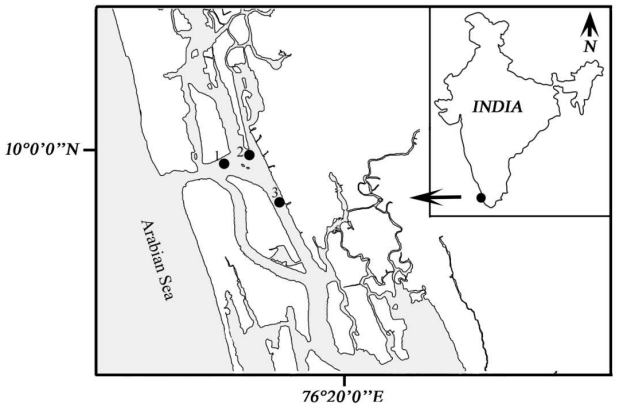

Fig. 1. Sampling locations of Cochin estuary-South-west of Arabian Sea

Classification

Phylum	: Arthropoda Von Siebold 1848
Subphylum	: Crustacea Brünnich 1772
Class	: Copepoda Milne Edwards 1840
Order	: Calanoida Sars GO 1903
Family	: Pontellidae Dana 1852
Genus	: *Calanopia* Dana 1852
Species	: *Calanopia metu* Usyal & Shmeleva 2004

Description of the species: Cephalosome lacks cephalic hook; rostral spines present. Specimens examined include 36 males and 46 females.

Male: Total length of the body varied from 350µm to 487µm. Cephalosomes are similar to females. Antennule 14 segmented, reaching up to the first urosomal segment (Fig. 2E). Urosome four segmented (Fig. 2F). Fifth leg asymmetrical left one slightly longer than the right one; exopod of the left leg 3 times wider than the right with 4 small spines on ventro-lateral margin. Right leg exopod bearing 3 small spines (Fig. 2G). In males, all the characters were identified the same as in the original, male described as copepodite V stage in the original description.

Female: Body robust without cephalic hooks. (Fig. 2A). Total length of the body varied from 350µm to 512µm. Antennule 16 segmented, reaching up to first urosomal segment

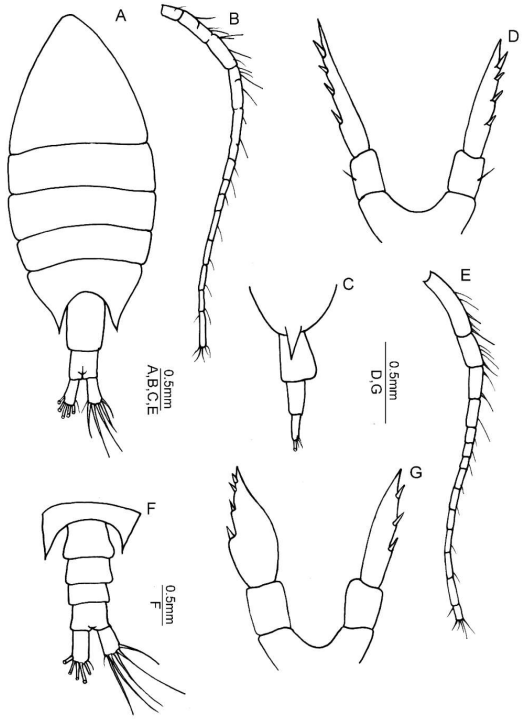

Fig. 2. External morphology of Calanopia metu A) Calanopia metu female dorsal view; B) Female antennule; C) Female urosome; D) Female P5; E) Male antennule; F) Male urosome; G) Male P5

(Fig.2B); Postero lateral segment of the 5th pedigerous somite produced symmetrical acuminate spines. Urosome two-segmented; genital somite bulged Postero ventrally (Fig. 2C). Fifth leg three segmented, asymmetrical; right leg slightly shorter than left. Basis bearing short setae, exopod having four short, sharp spines (Fig. 2D).

The detailed examination of the specimen showed urosomal structure and fifth pedigerous somite are similar to *Calanopia metu* and were noticeably varied when compared to other species of the genus commonly in the region such as *Calanopia elliptica* and *Calanopia aurivilli* (Table 1) (Fig. 3). Even though all the specimens collected were late copepodite stages, the species identification characteristics were prominent and according to Czaika (1982) copepodites can also be used for the species identification. *Calanopia metu is a truly* marine species, first described in 2004 by Uysal & Shmeleva from the Levantine basin of Eastern Mediterranean Sea. According to Uysal and Shmeleva (2012), this is an abundant species in the Levantine basin of the Eastern Mediterranean Sea and they emphasized the suitability of environmental condition of the basin to support all developmental stages of *Calanopia metu*. Temperature and salinity of the Mediterranean Sea varied from 16 to 29°C and 38.5 to 39.4 ppt, respectively which is conducive for the successful survival of this species. The observation on the occurrence of *Calanopia metu* in the Cochin estuary i.e., the present study forms the first report of this species from the entire Indian Ocean region (Al-Aidaroos et al 2016).The species *Calanopia metu* was observed in Cochin estuary during the high saline pre-monsoon season. The

Table 1. Comparison of *Calanopia metu* with *Calanopia aurivilli* and *Calanopia elliptica*

Calanopia metu	*Calanopia aurivilli*	*Calanopia elliptica*
Female	**Female**	**Female**
Cephalosome without lateral hook	Cephalosome without lateral hook	Cephalosome without lateral hook
Prosome in dorsal view, slightly less than twice as long as wide. The 5th segment with symmetrical acuminate spines, inclined a little outward.	Prosome produced in to posteriorly directed acute spines	Thoracic segment reduced posteriorly in to strong process reaching middle genital segment
Urosome 2-segmented, symmetrical and unarmed	Urosome 2-segmented	Urosome 2- segmented
Genital somite lacking ventral spine, distinctly evolved laterally, slightly protuberant ventrally and genital operculum rosette shaped	Genital segment shorter than anal segment	Genital segment same in length as urosomite
P5 slightly asymmetrical, last segment having 4 shorts spines resembling well developed finger like projections	P5 symmetrical and uniramous; exopod 1-segmented apex terminates in 3 spines, inner being distinctly longer and plumose at its distal margin	P5 asymmetrical left leg longer than right. Exopodal segment 1with 1 median and distolateral sine; exopodal segment 2ending in an acute spine with 2 outer marginal spine
Male	**Male**	**Male**
Description of Copepodite stage V Body similar to female Metasome 2.2 times longer than urosome 4-segmented with symmetrical caudal rami	Body similar to female. Cephalosome without hook.	Body similar to female except the right thoracic process is longer than left and pointed inward
A1 extending to first urosomite	Right A1 geniculate	Right A1 geniculate, segments 13-16 swollen while segments 19-21 denticulate. A1 extended to the genital segment
Urosomite 1 1.6 times longer than urosomite 2. Ventral spines of urosomite 2 absent	Urosome 5-segmented	Urosome 5-segmented, urosomite 2 with 1 spiniform process on distal right margin
Copepodite V leg 5 is asymmetrical with length of the third segment of right leg twice longer than the second segment and with smaller spines. Length of third segment of left leg 3 times as long, with 4 small spines on ventro-lateral margin.	P5 asymmetrical and chelate; right leg 4-segmented, proximal inner margin of basis swollen, exopodal segment 1 with well-developed thumb, claw spoon shaped, slightly swollen at tip with 1 outer marginal seta, 1 terminal and 2 inner marginal; left leg basis swollen and gibbose, exopod segment 1 with distolateral seta, terminal segment with 2 unequal apical spines	P5 asymmetrical; right leg exopodal segment 2 with 3 process along the inner margin; left leg, exopodal segment 1with 2 outer marginal spines, 1 at mid –outer margin and another distolaterally; exopodal segment 2 with 2 outer marginal spines and terminates in a strong acute spine with setules on the outer margin

The characters were taken from the original description as well as from the copepod database (https://copepodes.obs-banyuls.fr/en/) for the comparative purpose

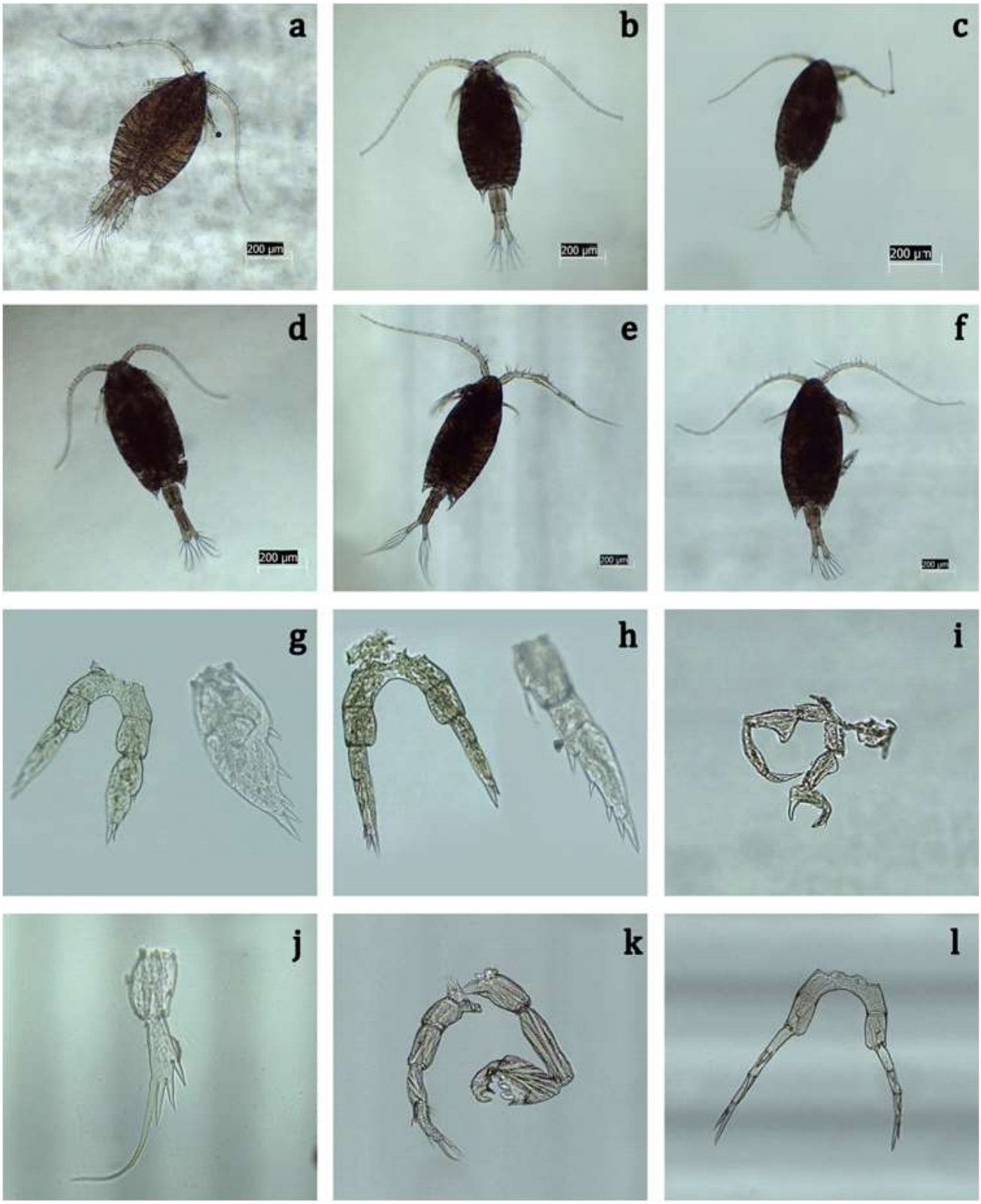

Fig. 3. External morphology of *Calanopia metu, Calanopia aurivillii* and *Calanopia elliptica* a) *C. metu* male dorsal view; b) *C. metu* female dorsal view c) *C. aurivillii* male dorsal view; d) *C. aurivillii* female dorsal view; e) *C. elliptica* male dorsal view; f) *C. elliptica f*emale dorsal view; g) *C. metu* male leg 5 and right exopod; h) *C. metu* female leg 5 and left exopod ; i) *C. aurivilli* male leg 5; j) *C. aurivilli* female right leg 5; k) *C. elliptica* male leg 5; l) *C. elliptica* femaleleg 5

environmental condition persisted during the study period may be conducive for the survival of this Mediterranean species in the estuaries which point out the tolerance of this species to wide range of salinity and temperature

The presence of a non-native species in the Arabian Sea from its native place to a far distance new environment by two possibilities transport of species through oceanic circulation or water mass and through human interaction such as marine traffic/ ballast water. The first possibility can easily be ruled out from the present study because of the rare chance of anti erythraean or anti lessepsian migration (Zakaria 2015). The second possibility strongly supports the occurrence of this species in the Cochin estuary i.e., introduction through the ballast water of commercial cargo ships. Cochin bar mouth is the major site for portal activities and it is one of the largest port cities in India. The country's first Trans - shipment hub-Kochi International Container Trans-shipment Terminal (ICTT) and the Cochin Shipyard were located in Cochin Estuary. Being an active/busy maritime destination, the chance of introduction of foreign species to the Cochin estuary through ballast water is quite high. Due to the vicinity of the International Container Transhipment Terminal, Cochin estuary is exposed to non-native species (Jayachandran et al 2019a). Considering these facts, can evince the second possibility of introduction of the species through the ballast water into the new environment.

CONCLUSION

Calanopia metu is introduced into the Cochin areas of Indian waters through the ballast waters. Occurrence of this species in the region suggests that the environmental conditions prevailed in the region is favourable for the survival of this species. The low relative abundance recorded may be due to the competition pressure on resources such as food and space with the native species. There may be a possibility of distributional extension of this species to the Arabian Sea. In order to ensure this, a comprehensive studies is required.

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Bird Diversity of Acharya Narendra Deva University of Agriculture & Technology Campus, Ayodhya

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***Abstract*:** A bird survey was conducted using point count method in the Acharya Narendra Deva University of Agriculture and Technology campus from October 2020 to September 2021. A total of 124 bird species representing 101 genera, 61 families and 16 orders were recorded from the campus. Passeriformes was recorded as the most dominant order with 62 species. Muscicapidae was the most dominant family with 10 species and the highest RDi value (8.06). Six groups were formed according to their feeding habits: omnivore birds (37%) were the most dominant, followed by insectivores (26%), carnivores (25%), granivores (6%), frugivores (5%) and nectarivores (1%). Out of 124 bird species, 78% were resident, 19% were winter visitors and 3% were summer visitors. Of the species recorded, one species is classified as Endangered, one species as Vulnerable and four species as Near Threatened as per the IUCN Red List of Threatened Species. This campus also supported birds having a decreasing population trend globally (21%). This study is the first attempt to prepare a checklist of birds at the ANDUAT campus, which will provide a baseline for future research and monitoring of birds in the campus.

Keywords: Avifauna, Conservation, Feeding guild, Threatened, Uttar Pradesh

The avian diversity is not just restricted to the protected areas of the country but also can be found in urban spaces, public parks, backyards of houses and university campuses. The university campuses are suitable habitats for birds as they are heterogeneous in nature and have continuous human disturbances. As majority of the universities are very old in their establishment their campuses have different habitats such as remnant vegetation, gardens, plantations, buildings, and housing colonies with kitchen gardens. The agricultural universities also have agricultural fields, agroforestry plots, wetlands, ponds etc. meant for experiment purpose. All these diverse habitats of the university provide suitable micro and macro habitats which help in harboring a wide range of bird species. The exotic and indigenous trees in the campuses attract not only the residential but also the migratory birds.

Uttar Pradesh is home to 358 bird species (Hegde and Venkatraman 2014). Out of the 52 threatened species recorded in India, 14 species occur in Uttar Pradesh (BirdLife International 2001). In Uttar Pradesh, checklists of avifauna have been published from Lucknow University (Kanaujia et al 2012), Chaudhary Charan Singh University, Meerut (Rana et al 2013), Gorakhpur University (Singh et al 2018a), Banda University of Agriculture and Technology (Singh et al 2018b) and Integral University, Lucknow (Singh and Khalid 2020). But there has been no documentation of birds from the campus of Acharya Narendra Deva University Agriculture and Technology, Ayodhya. Taking this into consideration, a study was taken up to understand the diversity and distribution of birds in the campus of Acharya Narendra Deva University of Agriculture and Technology, Ayodhya. This study will be helpful in preparing a baseline data on the bird diversity available in the campus.

MATERIAL AND METHODS

Study site: The Acharya Narendra Deva University of Agriculture and Technology (ANDUAT) main campus is located at 26.543322 ^{0}N and 81.835374 ^{0}E, Kumarganj, Ayodhya district, Uttar Pradesh. The campus has parks, plantations of teak and Eucalyptus, orchards of mango, guava, jackfruit, amla, agricultural fields, homgardens, ponds, grasslands etc. It is situated 93 m above MSL (Mean Sea Level). ANDUAT enjoys a tropical climate. The average temperature varies from 32 ^{0}C in summers to 16 ^{0}C in winters and the average annual rainfall is 1067 mm (Anonymous 2021). There are three distinct seasons – summer (March to June), rainy (July to October) and winter (November to February).

Methodology: The study was carried out from October 2020 to September 2021. Point count method (Bibby et al 2000) was used to record bird diversity in the university campus. Point counts were laid in diverse habitats of the campus such as agricultural fields, homegardens, plantations, parks, remnant vegetation patches, wasteland, grasslands and

ponds. The observations were made between 1100 to 1200 hrs in winter season and between 0600 to 0700 hrs during the rest of the seasons. During the entire study period each point count was surveyed 24 times. At each point count, bird species were observed with the help of binoculars (Nikon 7x35) for 15 mins. Grimmett et al (2011) was referred for identification of birds and assigning its residential status. The taxonomic positions and names were assigned referring to Praveen et al (2020). Ali and Ripley (1987) were followed for classifying birds into feeding guilds. The conservation and global population status were assigned following (IWPA 1972; CITES 2012; IUCN 2021). The relative diversity of bird families was calculated using Torre-Cuadros et al (2007). The formula is given below:

$$RDi = \frac{\text{Number of bird species in a family}}{\text{Total number of species}} \times 100$$

RESULTS AND DISCUSSION

A total of 124 bird species belonging to 101 genera, distributed among 61 families and 16 orders were recorded from the campus of Acharya Narendra Deva University of Agriculture and Technology (Table 1). The bird species recorded in the current study is higher than the bird species observed at Jawaharlal Nehru University, New Delhi (Singh et al 2017), Calicut University, Malappuram (111 species) (Jose and Zacharias 2003), OUAT, Bhubaneswar (95 species) (Mallik et al 2015) and BUAT, Banda (61 species) (Singh et al 2018b), but lower than the bird species recorded at Sabaragamuwa University, SriLanka (145 species) (Surasinghe and de Alwis 2010). Passeriformes had the highest diversity with 62 species and 26 families, followed by Pelecaniformes with 9 species and 5 families (Fig. 1). This result is similar to the findings of Mallik et al (2015) and Singh et al (2018b). Muscicapidae was the most dominant family with 10 species and the highest RDi value (8.06). In India, Passeriformes are known to be the most dominant order (Praveen et al 2016) and Muscicapidae is known to be the most diverse family (Manakadan and Pittie 2001). This was followed by Columbidae with 7 species (Table 2). Aegithinidae, Anatidae, Anhingidae, Bucerotidae, Coraciidae, Dicruidae, Falconidae, Gruidae, Jacanidae, Monarchidae, Nectariniidae, Paridae, Phalacrocoracidae, Ploceidae, Rallidae, Sittidae, Stenostiridae, Threskiornithidae, Turdidae, Upupidae, Vangidae and Zosteropidae were represented by just a single genus and were the least represented. The mosaic of habitats such as grasslands, forest patches, plantations, orchards, homegardens, wetlands and parks in the university campus might have met the requirements of different bird species leading to the occurrence of high bird diversity. According to the seasonal status of the birds, 78% were resident, 19% were winter visitors and 3% were summer visitors. Similar results were reported by Jose and Zacharias (2003) at Calicut University. The occurrence of higher number of winter migrants must be due to the fact that this study area is a part of the Central Asian Flyway and therefore may act as a wintering and stop over site for the winter migrants that breed in the Palearctic zone (Kumar et al 2016).

The foraging habit of the bird community indicated six major feeding guilds. The omnivore guild was the most dominant (37%), followed by insectivore (26%), carnivore (25%) (Fig. 2). The higher representation of omnivores and insectivores suggests the presence of diverse food resources in the campus. Due to their specialized feeding structure, the nectarivores were the least represented. The diverse habitats and microhabitats of the campus provide conditions preferable for many invertebrates, thereby meeting the diverse food requirements of different feeding guilds of birds. Apart from food, the campus provides shelter, breeding and nesting sites to the birds. The birds such as

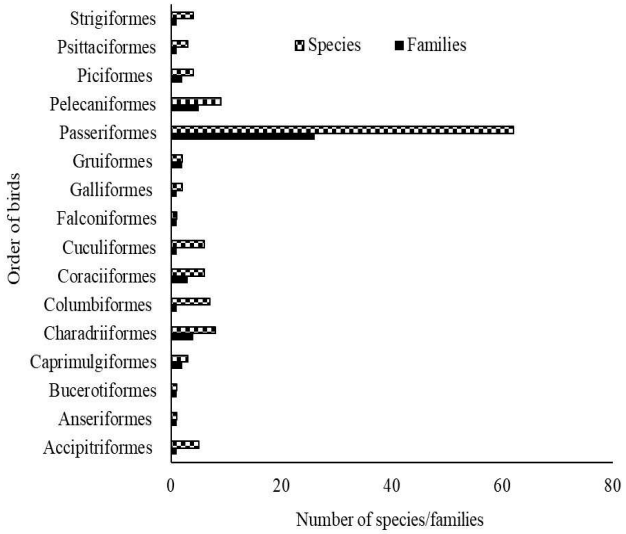

Fig. 1. Order wise bird community composition in ANDUAT campus, Ayodhya.

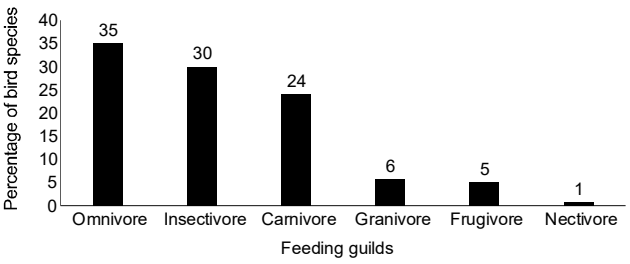

Fig. 2. Foraging guild-based classification of bird species in ANDUAT campus, Ayodhya

Table 1. Checklist and status of avifauna recorded in Acharya Narendra Deva University of Agriculture and Technology, Ayodhya

Order/Family/Common name	Scientific name	Residential status	Feeding status	Conservation status				Habitat
				IUCN (2021)	CITES (2012)	IWPA (1972)	Global status	
Accipitriformes Accipitridae (5)								
Black Kite	*Milvus migrans* (Boddaert 1783)	R	C	LC	II	I	→	AG,PL
Black-winged Kite	*Elanus caeruleus* (Desfontaines 1789)	R	C	LC	II	I	→	AG
Egyptian Vulture	*Neophron percnopterus* (Linnaeus 1758)	R	C	EN	-	I	↓	GL
Shikra	*Accipiter badius* (Gmelin 1788)	R	C	LC	II	I	→	HG, PK, PL, RV
White-eyed Buzzard	*Butastur teesa* (Franklin 1831)	R	C	LC	II	I	→	GL, AG
Anseriformes Anatidae (1)								
Lesser Whistling Duck	*Dendrocygna javanica* (Horsfield 1821)	R	O	LC	-	IV	↓	PD
Bucerotiformes Bucerotidae (1)								
Indian Grey Hornbill	*Ocyceros birostris* (Scopoli 1786)	R	O	LC	-	IV	→	PL, RV
Caprimulgiformes Apodidae (2)								
Asian Palm Swift	*Cypsiurus balasiensis* (Gray 1829)	R	I	LC	-	IV	→	AG, GL
Indian House Swift	*Apus affinis* (Gray 1830)	R	I	LC	-	IV	↑	AG, GL
Upupidae (1)								
Common Hoopoe	*Upupa epops* (Linnaeus 1758)	R	O	LC	-	IV	↓	GL, AG, PK, WL
Charadriiformes Burhinidae (2)								
Eurasian Thick-knee	*Burhinus ioedicnemus* (Linnaeus 1758)	R	O	LC	-	IV	↓	GL, AG, PK, WL
Great Thick-knee	*Esacus recurvirostris* (Cuvier 1829)	R	C	NT	-	IV	↓	GL, AG, PK, WL
Charadriidae (2)								
Red-wattled Lapwing	*Vanellus indicus* (Boddaert 1783)	R	O	LC	-	IV	?	PD, GL, WL, PK,AG
Yellow-wattled Lapwing	*Vanellus malabaricus* (Boddaert 1783)	R	C	LC	-	IV	→	AG, GL, WL, PD
Jacanidae (1)								
Bronze-winged Jacana	*Metopidius indicus* (Latham 1790)	R	O	LC	-	IV	?	PD
Scolopacidae (3)								
Common Sandpiper	*Actitis hypoleucos* (Linnaeus 1758)	WV	C	LC	-	IV	↓	PD
Temminck's Stint	*Calidris temminckii* (Leisler 1812)	WV	O	LC	-	IV	?	PD
Wood Sandpiper	*Tringa glareola* (Linnaeus 1758)	WV	O	LC	-	IV	→	PD
Columbiformes Columbidae (7)								
Eurasian Collared Dove	*Streptopelia decaocto* (Frivaldszky 1838)	R	G	LC	-	IV	↑	AG, GL, WL, PK
Laughing Dove	*Streptopelia senegalensis* (Linnaeus 1766)	R	G	LC	-	IV	→	AG, GL, WL, PK

Cont...

Table 1. Checklist and status of avifauna recorded in Acharya Narendra Deva University of Agriculture and Technology, Ayodhya

Order/Family/Common name	Scientific name	Residential status	Feeding status	Conservation status				Habitat
				IUCN (2021)	CITES (2012)	IWPA (1972)	Global status	
Oriental Turtle Dove	*Streptopelia orientalis* (Latham 1790)	WV	G	LC	-	IV	→	AG, GL, WL, PK
Red Collared Dove	*Streptopelia tranquebarica* (Hermann 1804)	R	G	LC	-	IV	↓	AG, GL, WL, PK
Rock Pigeon	*Columba livia* (Gmelin 1789)	R	G	LC	-	IV	↓	AG, GL, WL, PK
Spotted Dove	*Streptopelia chinensis* (Scopoli 1786)	R	G	LC	-	IV	↑	AG, GL, WL, PK
Yellow-footed Green Pigeon	*Treron phoenicopterus* (Latham 1790)	R	F	LC	-	IV	↑	AG, PL, PK
Coraciiformes Alcedinidae (3)								
Common Kingfisher	*Alcedo atthis* (Linnaeus 1758)	R	C	LC	-	IV	?	PD, AG
Pied Kingfisher	*Ceryle rudis* (Linnaeus 1758)	R	C	LC	-	IV	?	PD, AG
White-throated Kingfisher	*Halcyon smyrnensis* (Linnaeus 1758)	R	C	LC	-	IV	↑	PD, AG, GL
Coraciidae (1)								
Indian Roller	*Coracias benghalensis* (Linnaeus 1758)	R	C	LC	-	IV	↑	AG, GL, WL, PD
Meropidae (2)								
Blue-tailed Bee-eater	*Merops philippinus* (Linnaeus 1767)	SV	I	LC	-	IV	→	AG, GL
Green Bee-eater	*M. orientalis* (Latham 1801)	R	I	LC	-	IV	↑	AG, GL
Cuculiformes Cuculidae (6)								
Asian Koel	*Eudynamys scolopaceus* (Linnaeus 1758)	R	O	LC	-	IV	→	PL, RV
Common Hawk Cuckoo	*Hierococcyx varius* (Vahl 1797)	R	O	LC	-	IV	→	PL, RV
Greater Coucal	*Centropus sinensis* (Stephens 1815)	R	O	LC	-	IV	→	AG, HG, PK, PL, RV
Indian Cuckoo	*Cuculus micropterus* (Gould 1838)	SV	O	LC	-	IV	↓	PL, RV
Pied Cuckoo	*Clamator jacobinus* (Boddaert 1783)	SV	O	LC	-	IV	→	PL, RV
Sirkeer Malkoha	*Taccocua leschenaultii* (Lesson 1830)	R	O	LC	-	IV	→	PL, RV
Falconiformes Falconidae (1)								
Common Kestrel	*Falco tinnunculus* (Linnaeus 1758)	WV	C	LC	II	IV	↓	AG, GL
Galliformes Phasianidae (2)								
Grey Francolin	*Francolinus pondicerianus* (Gmelin 1789)	R	O	LC	-	IV	→	AG, GL, WL
Indian Peafowl	*Pavo cristatus* (Linnaeus 1758)	R	O	LC	III	I	→	AG, HG, PK, PL, RV
Gruiformes Gruidae (1)								
Sarus Crane	*Antigone* (Linnaeus 1758)	R	O	VU	-	IV	↓	AG, PD
Rallidae (1)								
White-breasted Waterhen	*Amaurornis phoenicurus* (Pennant 1769)	R	O	LC	-	IV	?	PD, AG, GL

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Table 1. Checklist and status of avifauna recorded in Acharya Narendra Deva University of Agriculture and Technology, Ayodhya

Order/Family/Common name	Scientific name	Residential status	Feeding status	Conservation status				Habitat
				IUCN (2021)	CITES (2012)	IWPA (1972)	Global status	
Passeriformes Acrocephalidae (2)								
Blyth's Reed Warbler	*Acrocephalus dumetorum* (Blyth 1849)	WV	O	LC	-	IV	↑	PD, AG, GL
Booted Warbler	*Iduna caligata* (Lichtenstein 1823)	WV	I	LC	-	IV	↑	AG, GL
Aegithinidae (1)								
Common Iora	*Aegithina tiphia* (Linnaeus 1758)	R	O	LC	-	IV	?	PL, RV
Alaudidae (4)								
Ashy-crowned Sparrow-Lark	*Eremopterix griseus* (Scopoli 1786)	R	O	LC	-	IV	→	AG, GL, WL, PK
Bengal Bushlark	*Mirafra assamica* (Horsfield 1840)	R	O	LC	-	IV	→	AG, GL, WL
Crested Lark	*Galerida cristata* (Linnaeus 1758)	R	O	LC	-	IV	↓	AG, GL, WL
Sand Lark	*Alaudala raytal* (Blyth 1845)	R	O	LC	-	IV	→	AG, GL, WL
Cisticolidae (4)								
Ashy Prinia	*Prinia socialis* (Sykes 1832)	R	I	LC	-	IV	→	AG, HG, PK, PL, GL
Common Tailorbird	*Orthotomus sutorius* (Pennant 1769)	R	I	LC	-	IV	→	AG, HG, PK, PL, GL
Plain Prinia	*Prinia inornata* (Sykes 1832)	R	I	LC	-	IV	→	AG, HG, PK, PL, GL
Zitting Cisticola	*Cisticola juncidis* (Rafinesque 1810)	R	I	LC	-	IV	↑	AG, HG, PK, PL
Corvidae (3)								
House Crow	*Corvus splendens* (Vieillot 1817)	R	O	LC	-	V	→	HG, AG, PK, RV, WL, PL
Large-billed Crow	*C. macrorhynchos* (Wagler 1827)	R	O	LC	-	IV	→	HG, AG, RV, WL,PL
Rufous Treepie	*Dendrocitta vagabunda* (Latham 1790)	R	O	LC	-	IV	↓	HG, AG, PK, RV, PL
Dicruridae (1)								
Black Drongo	*Dicrurus macrocercus* (Vieillot 1817)	R	C	LC	-	IV	?	AG, PK, PL, RV, GL
Estrildidae (3)								
Indian Silverbill	*Euodice malabarica* (Linnaeus 1758)	R	G	LC	-	IV	→	GL, AG, PK, WL
Red Munia	*Amandava* (Linnaeus 1758)	R	O	LC	-	IV	→	GL, AG, PK, WL
Scaly-breasted Munia	*Lonchura punctulata* (Linnaeus 1758)	R	O	LC	-	IV	→	GL, AG, PK, WL
Hirundinidae (3)								
Barn Swallow	*Hirundo rustica* (Linnaeus 1758)	WV	I	LC	-	IV	↓	AG, PD, GL
Plain Martin	*Riparia paludicola* (Vieillot 1817)	R	I	LC	-	IV	↓	AG, PD, GL
Wire-tailed Swallow	*Hirundo smithii* (Leach 1818)	R	I	LC	-	IV	↑	AG, PD, GL

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Table 1. Checklist and status of avifauna recorded in Acharya Narendra Deva University of Agriculture and Technology, Ayodhya

Order/Family/Common name	Scientific name	Residential status	Feeding status	Conservation status				Habitat
				IUCN (2021)	CITES (2012)	IWPA (1972)	Global status	
Laniidae (2)								
Bay-backed Shrike	*Lanius vittatus* (Valenciennes 1826)	R	C	LC	-	IV	→	AG, GL, PL, RV
Long-tailed Shrike	*Lanius schach* (Linnaeus 1758)	R	C	LC	-	IV	?	AG, GL, PL, RV
Leiothrichidae (2)								
Common Babbler	*Argya caudata* (Dumont 1823)	R	O	LC	-	IV	→	AG, HG, PK, PL, RV, GL, WL
Jungle Babbler	*Argya striata* (Dumont 1823)	R	O	LC	-	IV	→	AG, HG, PK, PL, RV, GL, WL
Monarchidae (1)								
Indian Paradise-flycatcher	*Terpsiphone paradisi* (Linnaeus 1758)	SV	I	LC	-	IV	→	PL, RV
Motacillidae (4)								
Citrine Wagtail	*Motacilla citreola* .(Pallas 1776)	WV	I	LC	-	IV	↑	PD, AG
Grey Wagtail	*M. cinerea* (Tunstall 1771)	WV	I	LC	-	IV	→	PD, AG
Paddyfield Pipit	*Anthus rufulus* .(Vieillot 1818)	R	C	LC	-	IV	→	AG, GL,WL
White-browed Wagtail	*Motacilla maderaspatensis* (Gmelin 1789)	R	I	LC	-	IV	→	PD, AG
Muscicapidae (10)								
Black Redstart	*Phoenicurus ochruros* (Gmelin 1774)	WV	I	LC	-	IV	↑	AG, HG, PK,PL,RV
Bluethroat	*Luscinia svecica* (Linnaeus 1758)	WV	I	LC	-	IV	→	PD
Brown Rockchat	*Oenanthe fusca* (Blyth 1851)	R	I	LC	-	IV	→	AG, GL, PK, HG, PL, RV
Indian Robin	*Copsychus fulicatus* (Linnaeus 1766)	R	C	LC	-	IV	→	AG, GL, PK, HG, PL, RV
Oriental Magpie Robin	*C. saularis* (Linnaeus 1758)	R	C	LC	-	IV	→	AG, GL, PK, HG, PL, RV
Pied Bushchat	*Saxicola caprata* (Linnaeus 1766)	R	I	LC	-	IV	→	AG, GL, PK, HG, PL, RV
Red-breasted Flycatcher	*Ficedula parva* (Bechstein 1792)	WV	I	LC	-	IV	↑	PL, RV
Siberian Stonechat	*Saxicola maurus* (Pallas 1773)	WV	I	LC	-	IV	→	AG, GL, PK, HG, PL, RV
Taiga Flycatcher	*Ficedula albicilla* (Pallas 1811)	WV	I	LC	-	IV	→	AG, GL, PK, HG, PL, RV
Tickell's Blue Flycatcher	*Cyornis tickelliae* (Blyth 1843)	R	I	LC	-	IV	→	AG, GL, PK, HG, PL, RV
Nectariniidae (1)								
Purple Sunbird	*Cinnyris asiaticus* (Latham 1790)	R	N	LC	-	IV	→	AG, GL, PK, HG, PL, RV
Oriolidae (2)								
Black-hooded Oriole	*Oriolus xanthornus* (Linnaeus 1758)	R	O	LC	-	IV	→	PK, HG, PL, RV
Indian Golden Oriole	*Oriolus kundoo* (Sykes 1832)	R	O	LC	-	IV	?	PK, HG, PL, RV

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Order/Family/Common name	Scientific name	Residential status	Feeding status	Conservation status				Habitat
				IUCN (2021)	CITES (2012)	IWPA (1972)	Global status	
Paridae (1)								
Cinereous Tit	*Parus cinereus* (Vieillot 1758)	R	I	LC	-	IV	↑	PK, HG, PL, RV
Passeridae (2)								
House Sparrow	*Passer domesticus* (Linnaeus 1758)	R	O	LC	-	IV	↓	AG, GL, PK, HG
Yellow-throated Sparrow	*Gymnoris xanthocollis* (Burton 1838)	R	O	LC	-	IV	→	AG, GL, PK, HG
Phylloscopidae (3)								
Blyth's Leaf Warbler	*Seicercus reguloides* (Blyth 1842)	WV	I	LC	-	IV	→	AG, GL, PK, HG, PL, RV
Common Chiffchaff	*Phylloscopus collybita* (Vieillot 1817)	WV	I	LC	-	IV	↑	AG, GL, PK, HG, PL, RV
Greenish Leaf Warbler	*Seicercus trochiloides* (Sundevall 1837)	WV	I	LC	-	IV	↑	AG, GL, PK, HG, PL, RV
Ploceidae (1)								
Baya Weaver	*Ploceus philippinus* (Linnaeus 1766)	R	O	LC	-	IV	→	AG, GL
Pycnonotidae (2)								
Red-vented Bulbul	*Pycnonotus cafer* (Linnaeus 1766)	R	O	LC	-	IV	↑	AG, GL, PK, HG, PL, RV
Red-whiskered Bulbul	*Pycnonotus jocosus* (Linnaeus 1758)	R	O	LC	-	IV	↓	AG, GL, PK, HG, PL, RV
Sittidae (1)								
Chestnut-bellied Nuthatch	*Sitta castanea* (Lesson 1830)	R	O	LC	-	IV	?	PL, RV
Stenostiridae (1)								
Grey-headed Canary Flycatcher	*Culicicapa ceylonensis* (Swainson 1820)	WV	I	LC	-	IV	→	PL, RV
Sturnidae (5)								
Asian Pied Starling	*Gracupica contra* (Linnaeus 1758)	R	O	LC	-	IV	↑	AG, GL, PK, RV
Bank Myna	*Acridotheres ginginianus* (Latham 1790)	R	O	LC	-	IV	↑	AG, GL, PK, PL
Brahminy Starling	*Sturnia pagodarum* (Gmelin 1789)	R	O	LC	-	IV	?	AG, GL, PK, HG
Common Myna	*Acridotheres tristis* (Linnaeus 1766)	R	O	LC	-	IV	↑	AG, GL, PK, HG, PL, RV
Common Starling	*Sturnus vulgaris* (Linnaeus 1758)	WV	O	LC	-	IV	↓	AG, GL, PL, RV
Turdidae (1)								
Black-throated Thrush	*Turdus atrogularis* (Jarocki 1819)	WV	G	LC	-	IV	?	PL, RV
Vangidae (1)								
Common Woodshrike	*Tephrodornis pondicerianus* (Gmelin 1789)	R	I	LC	-	IV	→	AG, HG, PL, RV
Zosteropidae (1)								
Indian White-eye	*Zosterops palpebrosus* (Temminck 1824)	R	I	LC	-	IV	↓	HG, PL, RV

Cont...

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Order/Family/Common name	Scientific name	Residential status	Feeding status	Conservation status				Habitat
				IUCN (2021)	CITES (2012)	IWPA (1972)	Global status	
Pelecaniformes Anhingidae (1)								
Oriental Darter	*Anhinga melanogaster* (Pennant 1769)	WV	O	NT	-	IV	↓	PD
Ardeidae (4)								
Cattle Egret	*Bubulcus ibis* (Linnaeus 1758)	R	C	LC	-	IV	↑	AG, PD, GL
Indian Pond-heron	*Ardeola grayii* (Sykes 1832)	R	C	LC	-	IV	?	PD, AG
Intermediate Egret	*Ardea intermedia* (Wagler 1827)	R	C	LC	-	IV	↓	PD, AG, GL
Little Egret	*Egretta garzetta* (Linnaeus 1766)	R	C	LC	-	IV	↑	PD, AG, GL
Ciconiidae (2)								
Asian Openbill	*Anastomus oscitans* (Boddaert 1783)	R	C	LC	-	IV	?	PD, AG
Woolly-neck Stork	*Ciconia episcopus* (Boddaert 1783)	R	C	NT	-	IV	↓	PD, AG
Phalacrocoracidae (1)								
Little Cormorant	*Microcarbo niger* (Vieillot 1817)	R	C	LC	-	IV	?	PD
Threskiornithidae (1)								
Red-naped Ibis	*Pseudibis papillosa* (Temminck 1824)	WV	C	LC	-	IV	↓	PD, AG
Piciformes Picidae (2)								
Black-rumped Flameback	*Dinopium benghalense* (Linnaeus 1758)	R	O	LC	-	IV	→	PL, RV, PK
Brown-capped Pygmy Woodpecker	*Dendrocopos moluccensis* (Gmelin 1788)	R	I	LC	-	IV	↑	PL, RV, PK
Ramphastidae (2)								
Brown-headed Barbet	*Psilopogon zeylanicus* (Gmelin 1788)	R	F	LC	-	IV	→	PL, RV, PK
Coppersmith Barbet	*P. haemacephalus* (Muller 1776)	R	F	LC	-	IV	↑	PL, RV, PK
Psittaciformes Psittaculidae (3)								
Alexandrine Parakeet	*Psittacula eupatria* (Linnaeus 1766)	R	F	NT	II	IV	↓	PL, RV, PK
Plum-headed Parakeet	*P. cyanocephala* (Linnaeus 1766)	R	F	LC	II	IV	↓	PL, RV, PK
Rose-ringed Parakeet	*P. krameri* (Scopoli 1769)	R	F	LC	-	IV	↑	PL, RV, PK
Strigiformes Strigidae (4)								
Brown Fish Owl	*Ketupa zeylonensis* (Gmelin 1788)	R	C	LC	II	IV	↓	PD, AG
Jungle Owlet	*Glaucidium radiatum* (Tickell 1833)	R	C	LC	-	IV	→	PL, RV, PD
Mottled Wood Owl	*Strix ocellata* (Lesson 1839)	R	C	LC	II	IV	→	PL, RV, PD
Spotted Owlet	*Athene brama* (Temminck 1821)	R	C	LC	II	IV	→	PL, RV, PK, PD

IUCN: International Union for Conservation of Nature and Natural Resources; CITES: Convention on International Trade in Endangered Species of Wild Fauna and Flora; IPWA: Indian Wildlife Protection Act; R: Resident, WV: Winter Visitor, SV: Summer Visitor; C: Carnivorous; O: Omnivorous; I: Insectivorous; F: Frugivorous; G: Granivorous; N: Nectarivore; LC: Least Concern; EN: Endangered; VU: Vulnerable; NT: Near Threatened; CITES II: Schedule-II species of CITES are the ones that are not necessarily threatened now with extinction but may become so unless trade is closely controlled; III: Schedule-III species of CITES are those species which are already regulated for trade by the country and that needs the cooperation of other countries to prevent unsustainable and illegal exploitation; IWPA I: Schedule - I species of IWPA (high priority species); IV: Schedule - IV species of IWPA (relatively low priority species); V: Schedule - V species of IWPA (vermin - species which can be freely hunted) ?: Unknown; —: Stable; ↑: Increasing; ↓: Decreasing; AG: Agricultural field; HG: Homegarden, PL: Plantation; PK: Park; PD: Pond; GL: Grassland; RV: Remnant Vegetation; WL: Wasteland

Table 2. Number of species and relative diversity of various avian families in ANDUAT campus, Ayodhya

Avian family	Number of species (Rdi value)
Muscicapidae	10 (8.06)
Columbidae	7 (5.65)
Cuculidae	6 (4.84)
Accipitridae, Sturnidae	5 (4.03)
Alaudidae, Cisticolidae, Motacillidae, Ardeidae, Strigidae	4 (3.23)
Scolopacidae, Alcedinidae, Corvidae, Estrildidae, Hirundinidae, Phylloscopidae, Psittaculidae	3 (2.42)
Apodidae, Burhinidae, Charadriidae, Meropidae, Phasianidae, Acrocephalidae, Laniidae, Leiothrichidae, Oriolidae, Passeridae, Pycnonotidae, Ciconiidae, Picidae, Ramphastidae	2 (1.61)
Anatidae, Bucerotidae, Upupidae, Jacanidae, Coraciidae, Falconidae, Gruidae, Rallidae, Aegithinidae, Dicruridae, Monarchidae, Nectariniidae, Paridae, Ploceidae, Sittidae, Stenostiridae, Turdidae, Vangidae, Zosteropidae, Anhingidae, Phalacrocoracidae, Threskiornithidae	1 (0.81)

lapwings, thick-knees, pigeons, crows, red-naped ibis and purple sunbird have been observed nesting in the campus.

The campus supported one Endangered species viz., *Neophron percnopterus*, one vulnerable species viz., *Antigone* and four Near Threatened species viz., *Anhinga melanogaster, Ciconia episcopus, Esacus recurvirostris* and *Psittacula eupatria* as per the IUCN Red List (IUCN 2021) (Table 1). Moreover, ten species were included in the Appendix-II and one species was included in the Appendix-III of CITES (CITES 2012) (Table 1). Six species came under Schedule I of the Indian Wildlife (Protection) Act (1972). In addition to this, the university campus harbours bird species having stable (45%), increasing (21%), decreasing (21%) and unknown (13%) population trends globally. This result highlights the conservation importance of the university campus which is continuously changing due to development and construction of new buildings but yet has the potential to conserve globally threatened, population declining and migratory species. The campus with its varied habitats provides the birds with permanent and temporary refuge sites, roosting, foraging, nesting and breeding sites.

CONCLUSION

Acharya Narendra Deva University of Agriculture and Technology campus provides habitat for residential as well as summer and winter migratory birds. The diverse macro and micro-habitats meets the nutritional requirements of six different feeding guilds of bird. It also supports threatened bird species and bird species having stable, increasing and decreasing global population trends. This study has helped develop a database on avian diversity which will be useful for long-term studies on habitat and bird interaction. The result of this study can be used for better management and planning of habitats and conservation of rich bird diversity existing in the university campus.

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Estimating Occupancy and Abundance of Endangered Kashmir Musk Deer (*Moschus cupreus*) in Uttarkashi, Uttarakhand.

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***Abstract*:** The present study aimed to assess the occupancy and abundance of musk deer in Uttarkashi using camera traps from October 2018 to October 2019. Musk deer was detected in 9 out of 24 grids yielding a naïve occupancy estimate of 0.29. By using the null model, the estimates of the site occupancy was 0.39 and detection probability was 0.19. The overall abundance of musk deer was 28.40 in the study area, with an average density of 4.73/100 km^2. This study is the first attempt to estimate occupancy and abundance using camera traps, providing baseline information for future management and conservation strategies within the landscape.

***Keywords*:** Occupancy, Elusive, Camera trap, Habitat degradation, Threatened

Mammals which occur in low densities at high altitudes, are globally threatened due to habitat loss and anthropogenic disturbances (Woodroffe 2000, Sharief et al 2020). Monitoring the population of these mammals is pivotal for their long-term viability and is essential from both ecological and management perspectives. For effective conservation and management planning, especially for those species which are under increasing threat or on the verge of extinction. It is imperative to identify their habitats and estimate their abundance. Among these species, musk deer (*Moschus* spp.) is a globally threatened species and needs top priority conservation action (Singh et al 2020). Recent studies have highlighted that Kashmir musk deer (*Moschus cupreus*, hereafter KMD) is coverings parts of Afghanistan, Pakistan, India, and Nepal (Singh et al 2020). The species is distributed in continuous to fragmented patches of forested and alpine scrub habitats in the western Himalayas, which is experiencing a rapidly changing climate (Syed and Ilyas 2016, Singh et al 2020). Worldwide, the population of musk deer have dramatically dwindled to half of the original size in three generations (approximately 21 years) primarily because of poaching and habitat degradation (Green 1986, Homes 2004, Timmins & Duckworth 2015). Therefore, musk deer have been categorized as endangered (Timmins and Duckworth 2015) in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2015). In addition to poaching, the other prevailing threats to musk deer are habitat destruction and degradation (Yang et al 2003, Ilyas 2014). Habitats in the Himalayas are threatened by anthropogenic pressures such as intensive livestock grazing, fuelwood cutting, and fodder collection (Vinod & Sathyakumar 1999, Ilyas 2014, Sathyakumar 2015). The suitable habitat for musk deer is mainly confined to protected areas with fragmented habitats and therefore demands the urgent need for protection and conservation efforts. Information is documented on various aspects of the ecology of musk deer (Green 1986, Sathyakumar 1994, Vinod & Sathyakumar 1999, Syed 2014, Ilyas 2014, Sathyakumar & Rawat, 2015, Singh et al 2018, Wangdi et al 2019). However conservation and management planning need information on species' different life history traits, especially population estimation. Hence the present study assessed the occupancy and abundance of KMD in Uttarkashi using camera traps. It has been documented that camera trapping is particularly useful by allowing population densities of the species to be estimated when the identification of individuals is possible (Singh et al 2014b). In some mammals, including musk deer, individuals cannot be identified positively because of the lack of a distinct spot or stripe pattern; however, abundance estimates can be made with occupancy surveys that rely on a species being detected, or not, at a particular site (MacKenzie et al 2002). Occupancy yields unbiased maximum likelihood estimates of numerous variables relevant to a wide variety of conservation and management applications research. (Mackenzie et al 2006, Singh et al 2015). The objective was to estimate the site occupancy and abundance of KMD in Uttarkashi district using remotely triggered cameras and to provide information

for managers to formulate effective conservation and habitat management strategies for the concerned species.

MATERIAL AND METHODS

Study area: For the most part, the district Uttarkashi is known as a sacred town situated on the banks of Bhagirathi, and located at 30.73°N 78.45°E with an average elevation of 1165 m. Uttarkashi is rich in biological resources, biodiversity, and other religious, cultural, and eco-tourism places. Besides, several mammal species occur in this prestine valley, such as Asiatic black bear, Brown bear, Musk deer, Common leopard, Snow leopard, Blue sheep, Himalayan tahr, and Serow. Moreover, the district also harbours the population of the Himalayan monal and many other important bird species. Whereas the floral resource of the district contains western Himalayan broadleaf woods at its most reduced heights, changing to western Himalayan subalpine conifer backwoods and western Himalayan alpine shrub and meadows at its most noteworthy rises. Trees exhibited in the lower parts are pine, deodar cedar, oak, and deciduous species.

Methodology: The study area was divided into 5×5 Km grids to maximize our effort so that all logistically accessible grids could be covered. The field surveys were conducted from 2018 to 2019 in all the ranges of Uttarkashi. A team of researchers visited the selected grids to detect/non-detection of musk deer. A total of 55 camera traps were deployed in selected grids. Camera traps were placed at knee height from the ground on animal trails or a few meters away from animal trails. The camera-trap was designed to evaluate the site occupancy and abundance of KMD in Uttarkashi. During the study, ultra-compact SPYPOINT FORCE-11D trail cameras (SPYPOINT, GG Telecom, Canada, QC) were used. In addition, we conducted seven continuous camera-trapping replicate surveys between October 2018 to October 2019.

Occupancy estimation: The single season occupancy analysis for estimating the site occupancy probability (ψ) and detection probability (ρ) of musk deer using a likelihood-based method was adopted(MacKenzie et al 2002) . Analysis was carried out using the PRESENCE v.2.12.25 software package (Proteus Wildlife Research Consultants, New Zealand; http://w. proteus.co.nz;Hines 2006). The detection/non-detection of musk deer was recorded over 12 months, from October 2018 to October 2019. Those surveys were divided into seven sampling occasions of around 15 days each. The data from all the camera trap locations at the respective sites was pooled and constructed standard detection histories for each site (Mackenzie et al 2002). Detection histories of musk deer was constructed for each site over the seven sampling occasions. Modelled occupancy and detection probability, applying single-season occupancy model on the pooled data set, keeping occupancy and detection probability constant ψ (.) ρ (.).

Abundance and density estimation: Estimation approaches developed for occupancy surveys incorporate detection probability directly into the estimation process (MacKenzie et al 2002) and thus deal appropriately with this fundamental component of animal abundance estimation. This was assumed that the detection of individuals was independent, individuals were equally detectable across the whole sampling site, and the site-specific abundance of individuals followed a Poisson distribution. The Royle–Nichols model provides estimates of the (λ) and r, representing the average abundance per site and innate species detectability, respectively (Royale and Nicholas 2003). The overall density will depend on the number of grid cells used by individuals was considered (Thorn et al 2011). This approach and divided abundance (λ) by the area of the sampling unit (n=24; area=600 km^2) to estimate the average KMD density of the sites in the study area was adopted. Royle–Nichols heterogeneity constant model λ(.),r(.) to estimate the abundance and associated parameters KMD was used.

RESULTS AND DISCUSSION

Sampling effort of 2819 camera trap nights yielded 10 independent detections of musk deer in 24 sites which were above >2500 m elevation. KMD was detected in 7 out of 24 sites (naïve occupancy=0.29) during the study period. The estimated site occupancy was 0.39with a detection probability of 0.19 (i.e., probability of detection of KMD on each survey) using the null model ψ (.) ρ (.) in which occupancy and detection probability are kept constant. Using Royle- Nicholas heterogeneity null model λ(.), r(.) keeping abundance and detection probability constant, evaluated the abundance of KMD in Uttarkashi. The abundance was λ=0.51KMD/site with a detection probability of r=0.12 (Fig.2). The overall abundance was 28.40KMD in the study area. The estimated density was 4.73/100 km^2 (Table 1).

KMD is one of the least studied mammal in India, and less attention has been given to this dwindled species since the last few years despite its endangered status. This study provides insights into the occupancy and abundance of musk deer and the feasibility of using detection/non-detection surveys to assess the musk deer's population status in Uttarkashi. The species occupies distinct habitats and has fragmented distribution in Uttarkashi, with an estimated density equal to 4.73/100 sq km. The null model indicates the estimated site occupancy of KMD is greater than naïve

occupancy. The occupancy estimates suggest that the KMD is rare in the study area, and the low detection probability further indicates that it is not easily detected. Low estimates of detection probability across the sites suggested that KMD is difficult to detect in the study sites even though the site is occupied by the species. Density estimates (number/sq km) for musk deer in the subalpine forests of Shokharakh in Kedarnath Wildlife Sanctuary were 3.2/sq km in 1979-81 3.7 in 1989-91 (Sathyakumar 1994). The musk deer abundance in pellet group density was 58.8 9 pellet groups/ha (Ilyas

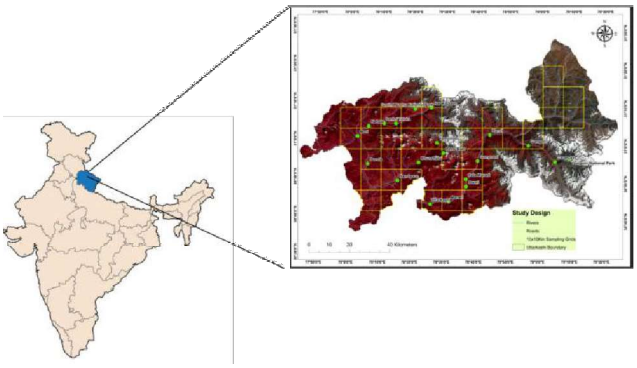

Fig. 1. Study area with camera trap locations in Uttarkashi

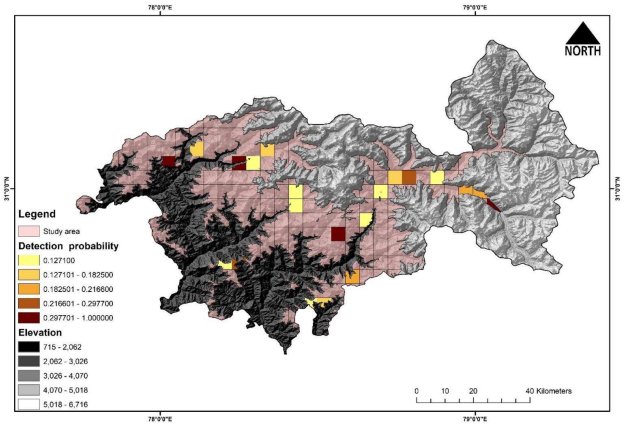

Fig. 2. Detection probability of Kashmir musk deer in Uttarkashi, Uttarakhand

Table 1. Occupancy and abundance estimation of Kashmir musk deer in Uttarkashi (above 2500 m)

Parameters	Estimation
Sampled area	600 km^2
Overall abundance	28.40±12.58
Naïve site occupancy	0.29
Site occupancy predicted/estimated	0.39±0.11
Detection probability of Musk deer	0.19±0.06
Density	4.73/100 km^2

2014). The current study indicate that KMD was not always detected at a site because the detection probability was low (0.19). The presence of KMD was captured in the alpine scrub and subalpine oak fir habitat at an elevation above 2500 m which is corroborated with the previous findings of Sathyakumar 1994, Green 1987, Ilyas 2014. However, despite the adoption of some conservation measures, the poaching of KMD continues virtually unchecked, and trading still persists on a large scale. Sathyamkumar (1994) documented that degradation and loss of musk deer habitat is due to the removal of understorey vegetation by extensive livestock grazing which has led to decreasing musk deer density in the Western Himalayas. Conservation of this rare animal is of utmost importance today, as it is fast heading towards total extinction. More effort to detect this species, the finding of this paper can be used as baseline information for making future management and conservation strategies for the species in Uttarkashi. The long term monitoring to assess KMD occupancy, population estimation and habitat utilization pattern within potential areas in Uttarkashi using different sampling methods is recommended.

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Seasonal Activity Pattern of Wild Boar (*Sus Scrofa*) and Temporal Overlap with Humans in the Uttarkashi Landscape of Western Himalaya, Uttarakhand

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Abstract: Wild boar is an invasive omnivore and an opportunistic feeder declared as a pest species in many countries. Despite of their invasive nature they play an essential role as ecological engineer in the ecosystem. Currently, very scanty information is available on wild boar ecology, behaviour and crop raiding pattern especially in Indian landscape. The, present study was aimed to understand daily activity pattern of wild boar and their activity overlap with human in Uttarkashi district. The 134 cameras consisted of 6220 trap nights during 2018 to 2020 before and after pre-monsoon season were deployed. The study indicated that wild boar are primarily nocturnal, remain most active during the midnight, and only occasionally interact with humans during afternoon. When compared activity patterns across the seasons, showed nocturnal activity pattern in the summer, spring, and diurnal during the winter. In order to understand their ecology and activity, present study can help to comprehend their activity pattern to avoid conflicts with human and effective population management of wild boar in the studied landscape.

Keywords: Wild boar, Environment, Camera trap, Invasive species, Western Himalaya

Wild boar (*Sus scrofa*) is a widely distributed mammal worldwide and native range extends from Western Europe to Southeast Asia (Massei and Genov 2004, Defra 2005). However, it is a generalist species and adapted for a wide range of habitats such as semi-desert to the tropical rain forest, temperate woodlands, and grassland (Barwal et al 2013). It is an omnivore species of the family Suidae and listed as Schedule V species under the Wildlife (Protection) Act, 1972 of India. Global populations of wild boar are rapidly increasing (Baubet et al 2004) and regroup-living animals, consisting of 4-14 individuals in a group (Allwin et al 2016) and when males join the group, can be larger than 20 individuals (Rosell et al 2004, Focardi et al 2015). Wild boars are highly active animal, opportunistic feeder, feed on variety of plants and animal matter and act as scavenger in the forest ecosystem. In addition to that wild boar also serves as an important prey base for large carnivores such as tiger, leopard and other large carnivores (Barwal et al 2013). Studying the activity pattern of species is essential to understand habitat use, behaviour and ecology (Tobler et al 2009). As internal biochemical processes regulate the species' activity rhythms following to the daily light–dark cycle and used for species classification as diurnal and nocturnal (Mistlberger and Antle 2011). There are many factors associated that changes the mammalian circadian rhythm, such as availability of food resources, light, competition, material behaviour, predation and human disturbances (Mistlberger and Skene 2004, Martin and Reále 2008 and Norris et al 2010).

Globally, there are several studies conducted on activity pattern of wild boar based on radio telemetry (Keuling et al 2015), acoustical detection (Cahill et al 2009), and camera trapping method (Ohashi et al 2013, Stolle et al 2015). On the other hands very few have been tested in India (Srivastava and Khan 2009, Barwal et al 2013). Camera trapping become popular tool in species monitoring, understanding activity pattern, human-wildlife conflict and habitat ecology analysis of elusive species (Bietti et al 2006, Vine et al 2009 and Ohashi et al 2013). Since the wild boar are one key species leading extensive conflict with human and results in huge agricultural loss (Scarcelli et al 2004) and designated as pest species in several parts of world (Meng et al 2009). Knowing the invasive species' activity patterns and their habitat use is essential for making effective conflict mitigation strategies and understand their ecological requirement (Guo and Quan 2017). Therefore, the goal of present study was to comprehend wild boar seasonal activity patterns and temporal overlap with humans, which can help to develop conflict mitigation strategies and population management of invasive species in Himalayan landscape.

MATERIAL AND METHODS

Study area: Uttarkashi is the largest district of Uttarakhand with a total area of 8016 km^2 lies between 38°28′–31°28′N latitude and 77°49′–79°25′E longitude. Two major rivers of India originated from this district, namely Bhagirathi (subsequently know as Ganga from Devparyag) from Gomukh and the Yamuna from the Bandarpuch glacier in Yamunotri. The elevation of district varies from 1158 to 6323 m. The terrain of the landscape is exceedingly mountainous, with tall snow-capped high peaks, small undulating bolder, steep mountains, and high ridges (Fig. 1). A varying range of climate and topography raises a wide range of vegetation and agriculture production. The faunal and floral diversity of the study landscape is diverse and is home for some globally endangered species and elusive species like snow leopard, musk deer, black bear, Himalayan tahr, common leopard etc. This district also has many threatened medicinal and economic flora such as *Taxus wallichiana*. *Myrica esculenta, Bergenia ligulata etc.*

Data collection: The study was conducted from 2018 to 2020 in this landscape. Study area was divided into a 10 km × 10 km and a reconnaissance survey were conducted after that we selected 26 logistically assessable grids of 10 X 10 km for the systematic survey and further divided in 5 km × 5km for intensive sampling.

Camera trapping: The total of 134 camera traps in various habitat types identified through reconnaissance survey and installed camera traps near meadows, natural trails, near water sources, grassland and subtropical and subalpine forest habitat in Uttarkashi district. The camera trap is placed at an average height of 30-45 cm from the ground based on terrain complexity and slop (Sathyakumar et al *2011 and Bashir* et al 2013). Ultra-compact SPYPOINT FORCE-11D trail camera (SPYPOINT, GG Telecom, Canada, QC) and Browning Trail Camera (Defender 850, 20 MP, Prometheus Group, LLC Birmingham, Alabama, <https://browningtrailcameras.com>) camera traps were kept operational for 20-30 days in the field.

Data analysis: The images of Wild boar were sorted and each independent image were considered in the interval of 1 hours (Tobler et al 2008). The species were identified based on expert opinion and images those were of poor quality and difficult to identify were excluded from the analysis. Further, time of each independent capture of human and wild boar from the camera traps were also recorded for the activity pattern analysis. All the captured of human activity were also recorded (Pei 1998). Daily activity pattern of wild boar and human were analysed for the temporal overlap and investigated using overlap package in R environment (Meredith and Ridour 2019). The daily activity index (DAI) was used to examine the daily activity pattern. The overlap coefficient (dhat) represented in scale of 0 to 1, where 0 indicates *'no overlap'* between the species, and 1 indicates '*complete overlap'* within the species.

RESULTS AND DISCUSSION

A total of 134 camera traps were remain operational for 6220 trap nights. In study used 273 individual captures of wild boar and 138 captures of human. Human capture were observed in 43 cameras. Based on activity pattern analysis, most of wild boars were active after the sunset to late at night. The peak time of activity of wild boar was 18:00 to 22:00 hrs. The highest peak showed during the 18:00 hrs, and a shorter peak observed at midnight 12:00 hrs (Fig. 2). The result also suggested that the wild boar was predominantly active during night hours with peak activity from 20:00 to 21:00 hrs (Fig. 2).

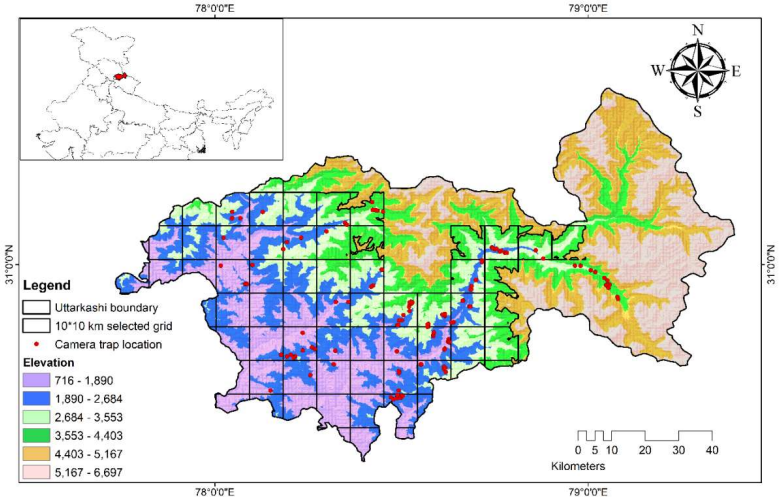

Fig. 1. Study area map showing the placement of camera traps

The activity overlap between wild boar and humans during all season was very low (dhat = Δ0.3; Fig. 3). Therefore, the results depict temporal overlap between wild boar and humans was only 30% in the study landscape. The camera trap data for three seasons: spring, summer, and winter were analysed and observed that the wild boar shows the nocturnal activity pattern, but also exhibit diurnal activity pattern during winter season. In the winter, wild boar is active during the afternoon at 12.00 hrs and increases with peak activity during 18.00 to 19.00 hrs. Wild boar shows high activity overlap with human during the winter (dhat = Δ0.76): Fig. 2). Further, during the spring, wild boar shows complete nocturnal activity pattern with highest activity peak during 18:00 to 19:00 hrs, with limited overlap with humans (dhat = Δ0.14). Similarly, in summer, the nocturnal activity pattern showed a peak between the 4:00 to 5:00 hrs in the early morning before sunrise and the second peak at 20.00 hrs (Fig. 2). Temporal overlap of wild boar with humans in the summer and spring season was avoided (dhat= Δ0.05).

The wild boar showed nocturnal activity pattern with peak activity during 18:00 to 22:00 hrs, which corroborated with previous studies of earlier researchers (Caruso et al, 2018, Oliver et al 2012). While, during the winter, wild boar exhibit daytime activity, especially in highly dense forests, which also observed in earlier studies (Keuling et al 2008, Ohashi et al 2013, Caruso et al 2018). However, the wild boar is a pest species as it destroys much of the agriculture land and leads to high economic losses for farmers (Apollonio et al 2010 and Ficetola et al 2014). Therefore, this species become one of major challenges for conflict mitigations throughout the globe. Present study indicates that wild boar show the less temporal activity overlap with humans and nocturnal activity gave it advantage to invade the agriculture lands during the night hours. However, in last few decades many species of carnivore, omnivore and ungulates became nocturnal due to high anthropogenic activity (George et al 2006, Ensing et al 2014). While in case of wild boar, this species adapted to human dominated areas and may adapted to nocturnal for changing crop raiding pattern or its thermoregulatory behaviour (Apollonio et al 2010). The high level of nocturnal activity is will be observed in wild boar during the summer, possibly due to the behavioural thermoregulation. When compare activity overlap according to the season, observed highest temporal activity overlap with humans during winters and limited during the summer and spring. The wild boar showed a high temporal activity overlap with humans during winter because of lack of availability of food and increased anthropogenic activity in forested areas for wood collection

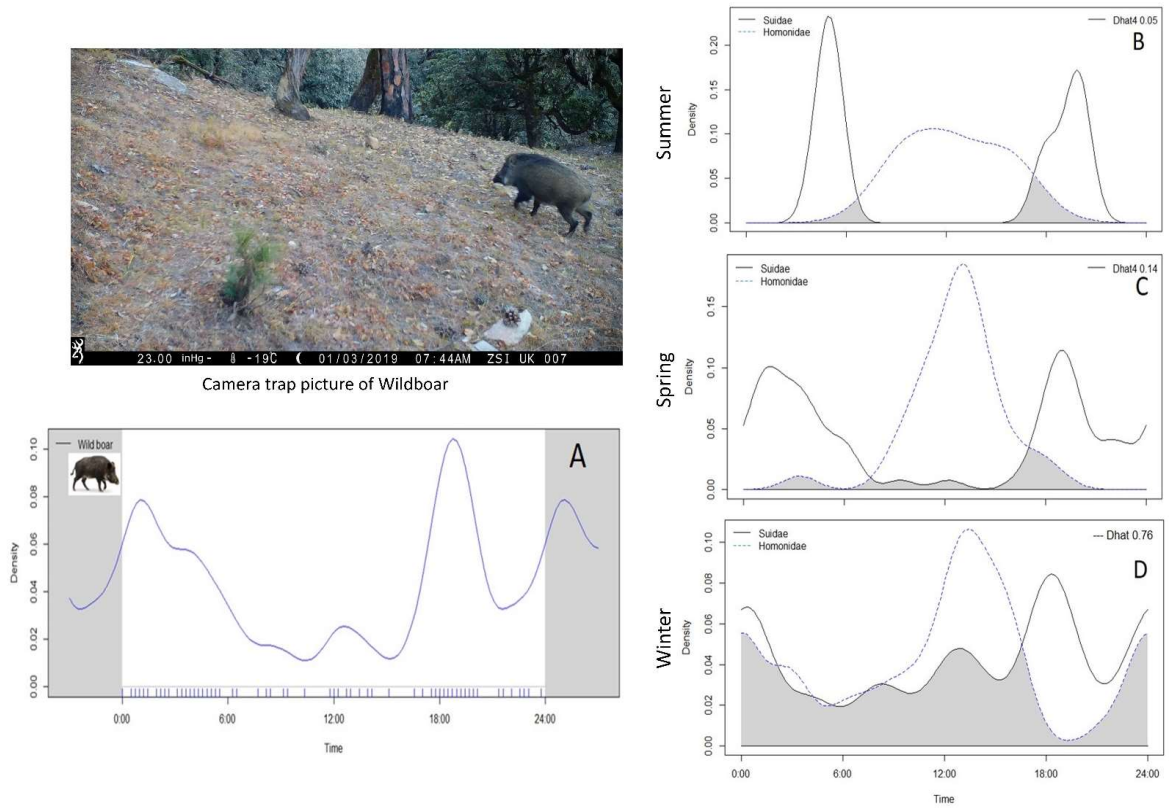

Fig. 2. Camera trap picture and activity pattern of Wild boar. (A) Wild boar (B) Summer (C) Spring (D) Winter

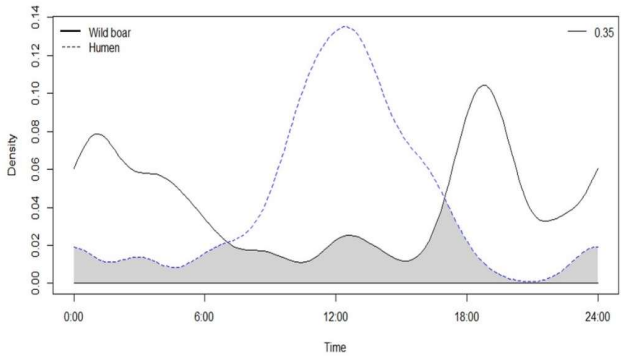

Fig. 3. Overall overlap of wild boar with human in all season

and livestock grazing which also corroborated with the other studies (Johann et al 2020).

In addition, some studies suggested that the wild boar activity changes seasonally due to the area and temperature (Campbell et al 2010). The wild boar's activity patterns vary according to the season in the study area during the summer are most active between the hours of 4:00 to 5:00 hrs and the hours of 20:00 to 21:00 hrs and followed the fully nocturnal pattern. The results are similar to the previous studies on wild boar (Brivio et al 2017, Maloney et al 2017). During the winter, diurnal activity peak was during 12:00-13:00. These results corroborated with study conducted in South Carolina, which indicates the nocturnal activity pattern of wild boar during summer and diurnal activity in the winter.

CONCLUSION

The present study monitored and assessed the activity pattern of Wild boar using camera trapping in the Uttarkashi district of Uttarakhand. The human-wildlife conflict has increased worldwide especially in case of wild boar, and their increasing populations often a major challenge to mitigate human wildlife conflict in India. Despite facing the conflict in majority of areas with wild boar and declared as a pest species, this species also works as an ecological engineer in the forest ecosystem and also an important prey base for large carnivore. Thus, population management required an intensive government intervention both in term of reduce conflict and maintain the ecosystem balances. The study indicates the nocturnal activity pattern of wild boar, which may increase the opportunity to invade in agriculture fields for easy access of food or may be due behavioural thermoregulation, which forced them to be nocturnal. The present study will be helpful for the management of the wild boar population and to mitigate the conflict. The results offered fundamental knowledge on wild boar activity pattern, which can be utilized by wildlife managers to rotate the agriculture crops and use of night deterrents to avoid the wildlife boar in agriculture fields.

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Phytoecology of the Atlas Pistachio (*Pistacia Atlantica* sub sp. *Atlantica*) in the Area of Laghouat (Algeria)

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***Abstract*:** The present work aims to study the floristic and ecological formations of *Pistacia atlantica* in the dayas of the wilaya of Laghouat phytoecological approach. One of the essential characteristics of the vegetation of this area is great plant diversity. The pistachio tree of the Atlas (*Pistacia atlantica*) is a very hardy xerophilous tree with great amplitude vis-à-vis the climatic factors not affected by the long periods of drought. The floristic inventory carried out has allowed us to identify 45 taxa, divided into 24 families and 40 genera. Systematically, the Asteraceae and Poaceae are the best represented, 20% of the floristic richness. The biological analysis shows the importance and predominance of therophytes on the other forms, this predominance is characteristic of the vegetation of arid regions which are adapted to the Saharan and steppe environment. From the biogeographic analysis, it emerges the predominance of the Mediterranean element. The numerical analysis, by the use of the analysis of the relaxed correspondences (DCA) and the ascending hierarchical classification (CHA), allowed us to individualize two floristic sets as well as the ecological factors which govern their distribution.

***Keywords*:** Laghouat*, Pistacia atlantica* formation, Numerical analysis, Ecology, Phytodiversity

The pistachio tree of the Atlas presents many interests at the ecological level as a protector of the pastoral steppe, soil preserver and air conditioner. Due very powerful root system, it can participate in the fixation of soils. Leaves constitute a good fodder for the feeding of the livestock. These trees as have nutritional values that can be used as fodder for ruminants. For several decades the natural resources of the steppe space (soil, water, vegetation) have suffered severe degradation due to the combined effects of an increasing human and animal pressure and an aggravating drought on these ecosystems (Bouderbala 2012) . The study of the vegetation concerns the description of the groups and their stationary conditions. The day as of Laghouat are characterized by tree vegetation represented by the Pistachio of the Atlas, a shrub vegetation represented by the wild Jujubier and a herbaceous vegetation.

The climate through its various factors (temperature, rainfall, wind) plays a determining role and intervenes in a decisive way on the growth and distribution of plants (Dahmani 2011). The temporal variation of monthly rainfall shows that the total rainfall is very irregular from one month to another. In Laghouat, the driest month is represented by July (5.6 mm) and the rainiest month is September with 22.8 mm. Temperature is a limiting factor of primary importance, as it conditions the distribution of species and communities of living beings in the biosphere (Ramade 1984). In Laghouat the coldest months are December, January and February, with minimum temperatures below 4 °C. The hottest are June, July and August, with maximum temperatures ranging from 35.44 to 39.23°C. The average maximum temperature reaches 39.23°C in July, the hottest month. On the other hand, in January, the coldest month, the average minimum temperature is 2.15°C. In the region of Laghouat, Salemkour et al (2013) had collected 66 species in the stations of El Houaita, Sidi Bouzid and Gueltet Sidi Saad. These authors made a comparative study of the floristic characteristics between free rangelands. This work on *Pistacia atlantica* is part of the preservation and conservation of this tree and to present the state of the flora of this important pastoral region (Laghouat), in order to appreciate its phytodiversity, as a model of highland area.

MATERIAL AND METHODS

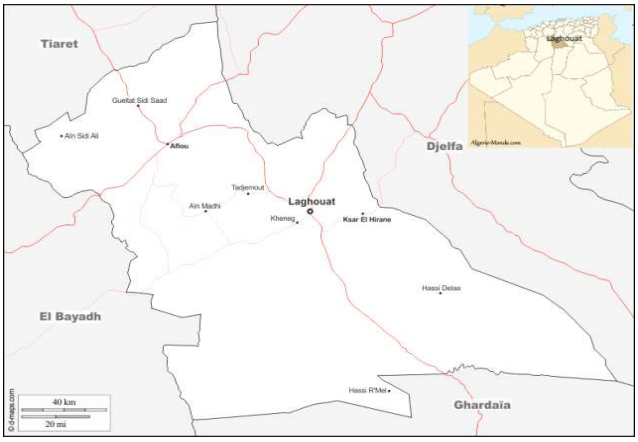
Study region: Due to its geographical position and climatic characteristics, the wilaya of Laghouat is part of the pastoral wilayas of the country, as well as the wilayas of the South (ANDI 2013).Laghouat, a former oasis, became chief town of wilaya in the administrative division of 1974(Benblidia et al 2006) .With an area of 25052 km², the wilaya of Laghouat is located 400 km as the crow flies in the south of Algeria of the Mediterranean coast (Aniref 2013). It is bounded to the north, by the wilaya of Tiaret, to the east, by the wilaya of Djelfa, to the south, by the wilaya of Ghardaïa, to the west, by the wilaya of El Bayadh (Fig. 1).

Prospecting methods: Several field missions were organized in the different zones of Laghouat. The study of

vegetation and natural environment are defined by several types of sampling, the one chosen for our study is the subjective sampling (Braun-Blanquetand De Bolos 1957). The surveys are carried out in physiognomically homogeneous areas. The floristic surveys were carried out on 100 m^2 plots.

The surface of 100 m^2 seems sufficiently representative of the minimum area in our study area (Benabadji and Bouazza 2002, Hasnaoui et al 2011). The sampling work was distributed in the field at the 52 sites. The coordinates of each site were noted using a GPS receiver. The 52 sites surveyed are located in the municipalities of Hassi Delaa, Ksar El Hiran, Kheneg, Ben Nacer Ben Chohra,Hassi R'mel,Ain El Madhi,Tadjrouna,Laghouat, Aflou and El Houita (Table 1, Fig. 2).

Data analysis: The numerical processing consists of factorial correspondence analysis (FCA) and detached correspondence analysis (DCA), followed by hierarchical ascending classification (HAC).



Source: d-maps.com

Fig. 1. Geographical location of the wilaya of Laghouat

Source: Photo taken on Google Earth

Fig. 2. Location of floristic surveys conducted in the Laghouat area

RESULTS AND DISCUSSION

The evaluation of the flora of Laghouat, was based on three main parameters: the biological, taxonomic and biogeographical diversity of the pre-Saharan and steppe ecosystems within the groups of *P. atlantica*. The flora encountered in the 52 dayas of Laghouat presents a diffuse spatial distribution, considering the surface, but very diversified: 45 species belonging to 40 genera and 24 families. Salemkour et al (2013) in the regions of El-Houaita, Sidi Bouzid and Gueltet Sidi Saad (wilaya of Laghouat) observed 66 species belonging to 21 families. Mallem et al (2017) collected in the region of Mokrane (wilaya of Laghouat) 30 species belonging to 29 genera and 14 families.The floristic richness is variable according to the different dayas, the highest is observed in the dayas of Tilghimt(Ayyat and Smahi). It is low in the dayas of Ajal, Saadia and Bouzidi with 03, 04 and 05 species. The flora of Laghouat reveals an unequal distribution of species between families: 06 families share alone more than 57% of species, while 18 families share the remaining 43%, where most are represented by only one taxon. Poaceae and Asteraceae contain 20% each and are the best represented. Malvaceae, Borraginaceae, Zygophyllaceae and Plantaginaceae contain 4.44% each. The other families Geraniaceae, Euphorbiaceae, Polygonaceae, Rutaceae, Lamiaceae, Brassicaceae, Capparidaceae, Convolvulaceae, Papaveraceae, Orobanchaceae, Cistaceae, Thymelaeaceae, Oxilidaceae, Caryophyllaceae and Apiaceae with 2.22% each (Fig. 3). This floristic diversity is related to the diversity of climates, geomorphology, nature of soils and anthropic action (Benaradj et al 2012). The Asteraceae family is the most dominant in the dayas with 09 species identified Poaceae are also well present on the wilaya's rangelands with a group of 8 species. Among the Fabaceae, the 2 species inventoried are *Astragalus armatus, Medicago polymorpha*, among the Malvaceae inventoried (*Malva parviflora, Malva aegyptica*) and Zygophyllaceae (*Fagonia glutinosa, Peganum harmala*) (Table 2).

The dayas of the communes of Hassi Rmel and Ksar El Hirane are the richest compared to other communes knowing that these dayas are plowed for a long time, which has favored the development of annual plants such as *Rumex vesicarius, Papaver rhoeas, Medicago polymorpha, Matricaria recutita* and *Avena sterilis* and perennials such as *Triticum repens, Malva parviflora, Echium humile, Ruta chalepensis* and *Reichardia tingitana*, namely the dayas of Tilghimt, Belil, Soltane, Boulehya, Zatacha, Baguira 1 and

Table 1. Location of the day as where the samples are taken

No. of the daya	Daya's name	Longitude	Latitude	Municipalitie
P4	Magrounat	33°28'54.30"N	3°30'57.36"E	Hassi Delaa
P5	Raysa	33°28'54.30"N	3°30'57.36"E	Hassi Delaa
P1	Kayed	33°32'15.17"N	3°33'46.87"E	Ksar El hiran
P6	Mansoura	33°37'12.48"N	3°10'5.80"E	Hassi Delaa
P2	khaled	33°32'15.04"N	3°21'36.13"E	Ksar El hiran
P7	Ben terbeh	33°37'4.40"N	3° 8'54.54"E	Hassi Delaa
P8	Saadi	33°31'20.77"N	3°33'19.54"E	Hassi Delaa
P34	Oum rzaime	(33°43'9.04"N	2°17'23.16"E	Kheneg
P27	Timzghit	33°31'6.99"N	2°56'23.93"E	Ben nacer ben chohra
P9	Bouezara	33°29'39.12"N	3°28'8.92"E	Hassi Delaa
P10	Magrounat 2	33°28'54.30"N	3°30'57.40"E	Hassi Delaa
P11	Marfouaa	33°29'55.70"N	3°32'2.82"E	Hassi Delaa
P12	Bouti	33°29'58.00"N	3°33'18.70"E	Hassi Delaa
P13	Abdelkader Bouchoucha	33°32'11.62"N	3°30'46.37"E	Hassi Delaa
P14	Hairech	33°29'59.36"N	3°32'39.59"E	Hassi Delaa
P15	Hneya	33°19'13.27"N	3°25'7.97"E	Hassi Delaa
P16	Hejaj	33°18'22.82"N	3°26'40.55"E	Hassi Delaa
P17	Bounoua	33°17'52.33"N	3°25'48.92"E	Hassi Delaa
P20	Tilghimt	33° 9'17.57"N	3°20'53.33"E	Hassi R'mel
P21	Belil	33°13'59.71"N	3°15'15.19"E	Hassi R'mel
P28	Smahi	33°30'37.07"N	2°59'32.01"E	Ben nacer ben chohra
P29	Ayyat	33°31'23.08"N	2°56'52.42"E	Ben nacer ben chohra
P38	Boumousi	33°30'41.19"N	2°14'35.57"E	Ain Madhi
P39	Legrar 1	33°28'31.47"N	2°13'42.39"E	Ain Madhi
P35	Lihoudi	33°38'59.19"N	2°33'33.40"E	Kheneg
P40	Gouffa	33°29'31.30"N	2°13'23.91"E	Ain Madhi
P41	Legrar 2	33°28'3.35"N	2°13'31.63"E	Ain Madhi
P45	Lelmaya 1	33°28'36.59"N	2° 2'16.23"E	Tadjrouna
P46	Lelmaya 2	33°27'1.88"N	2° 3'21.99"E	Tadjrouna
P22	Soltan	33°14'18.58"N	3°18'34.00"E	Hassi R'mel
P23	Boulehya	33°11'42.87"N	3°14'59.57"E	Hassi R'mel
P24	Zatacha	33°10'19.23"N	3°18'49.27"E	Hassi R'mel
P25	Baguira 1	33°13'14.83"N	3°14'49.37"E	Hassi R'mel
P26	Baguira 2	33°12'43.45"N	3°13'5.11"E	Hassi R'mel
P51	Gatte	33°43'22.43"N	2°53'43.49"E	Laghouat
P30	Talmzane	33°38'42.53"N	2°55'47.89"E	Ben nacer ben chohra
P31	Tinsafine	33°38'25.43"N	3° 2'46.46"E	Ben nacer ben chohra
P52	sidi bouzid	34°11'42.77"N	2°10'10.97"E	Aflou
P37	Rass mabzoug	33°30'7.74"N	2°24'27.27"E	El Houita
P47	Dakhla	33°21'34.87"N	2° 1'16.04"E	Tadjrouna
P3	Kebala	33°51'13.94"N	3°12'8.67"E	Ksar El hiran
p42	Saadia	33°45'0.09"N	2°17'11.43"E	Ain Madhi
P43	Ajal	33°51'4.58"N	3°14'47.60"E	Ain Madhi
P36	Bouzidi	33°41'23.93"N	2°39'44.71"E	Kheneg
P32	Latrech	33°36'19.91"N	3° 1'54.05"E	Ben nacer ben chohra
P33	Bsibisa	33°36'27.30"N	3° 4'55.44"E	Ben nacer ben chohra
P44	Lakhal	33°23'45.76"N	2°15'34.44"E	Ain Madhi
P48	Terkalal	33°34'11.39"N	2° 8'39.76"E	Tadjrouna
P49	Hotaiba	33°36'20.55"N	2° 8'5.60"E	Tadjrouna
P50	Boukhalkhal	33°31'53.53"N	2°12'32.33"E	Tadjrouna
P18	Terfas	33°27'47.31"N	3°40'24.63"E	Hassi Delaa
P19	Bouziane	33°27'47.31"N	3°40'24.63"E	Hassi Delaa

Baguira 2 for the municipality of Hassi Rmel and the dayas of Kayed, Khaled and Kebala for the municipality of Ksar El Hirane. Some dayas in the commune of Hassi Rmel and Ben Nacer Ben Chohra in Laghouat, have been cleared and cultivated with barley (*Hordeum vulgare*), such as Tilghimt, Smahi Zatacha while others in the same communes have been cultivated with soft wheat (*Triticum aestivum*) such as Ayyat, Belil and Boulehya. The problem of the cultivation of cereals in some dayas, which has an impact on the flora, ploughing and harvesting of cereals or note destruction of the natural vegetation in favor of agricultural spaces in the funds of dayas. Indeed, the extension of ploughing and the strong pressure with more and more systematic use of agricultural machines, within the dayas causes a clearing and a systematic eradication of the perennial species. Kaabèche (2003) observed that the use of the plough not only causes the disappearance of all plant cover but also destroys the soiland leads to the "physical" destruction of the rangeland. There are dayas that are well protected by the population and forestry services, such as that of Lelmaya (commune of Tadjrouna, wilaya of Laghouat) where the main plant cover is *Sonchus arvensis*. The jujube tree is present in all the dayas with the exception of some in the commune of Hassi Rmel such as Belil and soltane. According to the biological type, the species are divided into 21 therophytes, 14 hemicryptophytes, 07 chamephytes, 02 geophytes and 01 phanerophyte (Fig. 4). These eigenvalues quantify the amount of information contained in the data matrix and are therefore a first-order aid in determining the number of axes to be retained. For the present analysis, because of the gap between axis 2 and 3, only the first two axes will be retained for the analysis of the survey and species sets. The eigenvalues obtained from the AFC are close to those of the DCA (0.35 for the first axis and 0.32 for the second axis), but the individualization of the different ecological groups is

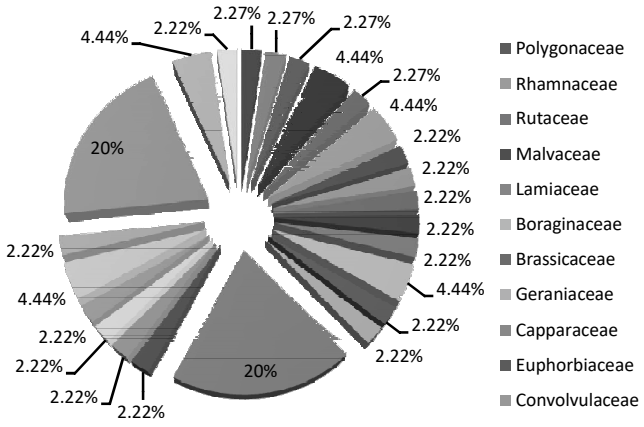


Fig. 3. Main families of the flora of the 52 day as of Laghouat

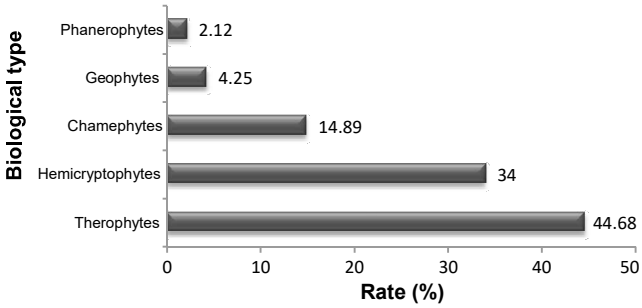


Fig. 4. Biological spectrum of the inventoried species

Table 2. Species identified in the study area

Family	Species
Asteraceae	*Matricaria recutita, Launaearesedifolia, Leontodon saxatilis, Onopordum macracanthum, Podospermum laciniatum, Reichardia tingitana, Silybum marianum, Santolina africana , Sonchus arvensis*
Poaceae	*Triticum repens, Triticum aestivum, Avena sterilis, Hordeum murinum, Cymbopogon schoenanthus, Stipa tenacissima, Cynodon dactylon, stipagrostis obtusa, Hordeum vulgare*
Fabaceae	*Astragalus armatus, Medicago polymorpha,*
Malvaceae	*Malva parviflora, Malva aegyptica*
Zygophyllaceae	*Fagonia glutinosa, Peganum harmala*
Boraginaceae	*Echium horridum, Echium humile*
Plantaginaceae	*Plantago ciliata , Plantago logopus*
Rutaceae	*Ruta chalepensis*
Lamiaceae	*Salvia verbenaca*
Brassicaceae	*Ammosperma cinereum*
Capparaceae	*Cleome arabica*
Convolvulaceae	*Convolvulus arvensis*
Papaveraceae	*Papaver rhoeas*
Orobanchaceae	*Orobanche cernua*
Rhamnaceae	*Ziziphus lotus*
Geraniaceae	*Erodium triangulare*
Euphorbiaceae	*Euphorbia calyptrata*
Cistaceae	*Hélianthemum lippii*
Oxilidaceae	*Oxalis corniculata*
Chenopodiaceae	*Arthrophytum scoparium*
Caryophyllaceae	*Paronychia argentea*
Apiaceae	*Pimpinella anisum*
Polygonaceae	*Rumex vescarius*

much clearer with the DCA. It is for this reason have opted for the latter to interpret results.

The climate has a dominant influence on the life of plants, both by rainfall and by temperature and light (Dahmani 2011). According to the rainfall gradient, Mediterranean and endemic elements decrease slightly with aridity, while the Saharan-Arabic and Mediteranean-Saharan-Arabic increase significantly. The water factor, associated with the thickness of the soil exploitable by plant roots, plays an essential role in the floristic composition and distribution of plants in the different habitats. Indeed, whatever the type of habitat, this factor acts by compensating the aridity of the climate and thus allows the maintenance of a relatively rich flora.

Three combined actions best explain the establishment of the flora, climatic changes, long-distance transport by wind and birds, and changes in geographic distribution. Finally, this study shows the importance of phytogeography, which allows us to study the phytodynamics. But also contributes to the knowledge of the impact of climatic and anthropic changes on ecosystems.

Detrended correspondence analysis and factor analysis of correspondence: The results of the DCA provide the eigenvalues for the first three axes (Table 3).

Ecological and floristic characterization of the identified groups: The characterization of the identified clusters is done by superimposing the factorial map of surveys (Fig. 6) and the species map (Fig. 7). In addition, the use of ecological and floristic information collected in the field allows us to describe the two sets of surveys obtained on the first

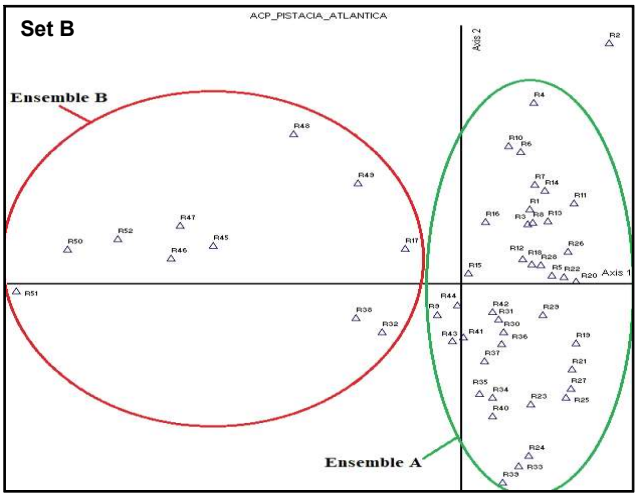


Fig. 6. Map of surveys on the ½ factorial plane of the DCA

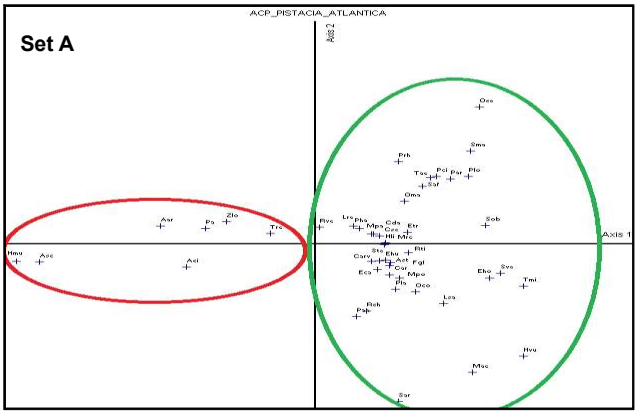


Fig. 7. Species on the factorial plane ½ of the DCA Ecological significance of the factorial axes

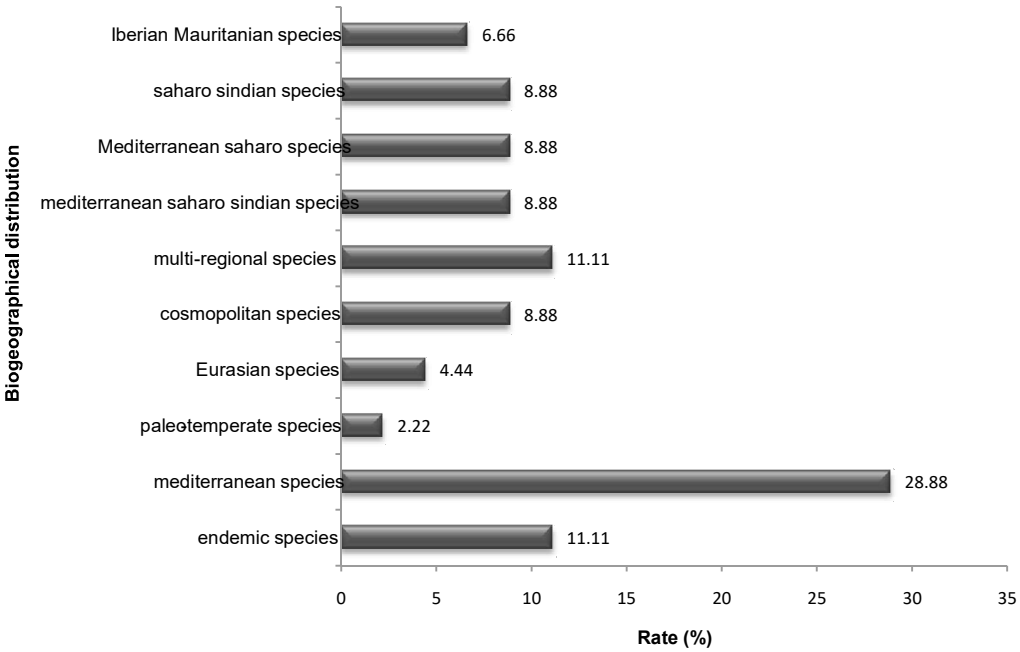


Fig. 5. Biogeographical distribution of collected taxa

factorial map. The individualization of the survey groups was carried out on the factorial map represented by axis 1 and 2.

Set A: Occupies the negative part of axis 1 represents the driest dayas. It contains 13 surveys: R9, R15, R17, R32, R36, R45, R46, R47, R48, R49, R50, R51 and R52 at an average elevation of 800 m on sandy-clay substrate characterized by a higher sand content compared to the surveys on the positive side. The overall vegetation cover varies from 5 to 10%. Physiognomically, this group is characterized by *Arthrophytum scoparium* and *Ammosperma cinereum*. There is also a good development of *Hammada scoparia* and *Ziziphus lotus*, which are well represented in this group. Although at low frequency, we note the presence of *Triticum repens.*

Set B: Occupies the positive part of axis 1. It contains 39 records: R1, R2, R3, R4, R5, R6, R7, R8, R10, R11, R12, R13, R14, R16, R18, R19, R20, R21, R22, R23, R24, R25, R26, R27, R28, R29, R30, R31, R33, R34, R35, R37, R38, R39, R40, R41, R42, R43 and R44. This set is represented by the surveys conducted under *Pistacia atlantica*. The 39 surveys carried out at an average altitude of 900 m on a clayey-sandy substrate. The global cover of the vegetation varies from 10 to 15 %. Physionomically an environment subject to anthropic action, including overgrazing, plowing and cultivation of cereals, which has allowed the development of ruderal species such as *Hordeum murinum, Hordeum vulgare* and *Triticum aestivum* as well as psamophilous species, elective of sandy sail such as Euphorbia calyptrate (Mallem et al 2017). The positive side includes grazing species that mostly grow on more or less encrusted surfaces, such as Erodium triangulare which is a grazing indicator therophyte, as well as *Malva eagyptiaca* and *Stipa parviflora* (Salemkour et al 2013) .

The ecological interpretation is often delicate because each axis can integrate several factors in different proportions. The highlighting of ecological factors acting on the distribution of the vegetation is based on the autoecology of the species, on the one hand, and on the stationary data collected in the surveys on the other hand. The species and the surveys taken into consideration are those whose relative contributions are the highest.

Axis 1 denotes the water stress and also the floristic richness which is important (positive part of axis 1) is reduced (negative part of axis 1). The presence of relatively water and fertility demanding species such as *Avena sterilis, Santolina africana* and *Schismus barbatus* was observed (Mallem et al 2017). Opposing the less water and soil fertility demanding species, well adapted to the arid conditions of these biotope types, such as: phanerophytes *Pistacia atlantica* and +3. accompanied by: *Plantago ciliata,Euphorbia calyptrata, Medicago polymorpha* and *Rumex vescarius* (Bouderbala 2012).

This group is marked by the dominance of species: *Pistacia atlantica, Ziziphus lotus* but the total number of species is reduced. These pistachio dayas have a high vegetation cover at 60-75%, an average litter rate hardly exceeding 20%.

The second axis is very clearly represented by a lithological gradient. It allows to classify the least degraded dayas (Tilghimt, Belil soltane, Boulehya, Zatacha, Baguira 1 and Baguira 2 for the municipality of Hassi Rmel and the dayas of Kayed, Khaled and Kebala for the municipality of Ksar El Hirane) in its positive part and those degraded (Daya lihoudi, saadia, ajal and bouzidi of the commune of Ain El Madhi) in the negative part, and confirms that a gradient of anthropic disturbance that allows to classify the dayas and corresponds to the determining factor in the distribution of grouping to *Pistacia atlantica*. The anthropic action is apparent through the presence of species of grazing, *Peganum harmala* testifies to the pressure of overgrazing and the postcultural character of these dayas, *Malva parviflora* known as ruderal and overgrazing. On the positive side, are grouped species for the most part therophytes that seem *Launaea resedifolia, Rumex vesicarius,* etc. and develop in arid bioclimate (Saharan). This richness in therophytes is linked to an occasional humidity.

On plan F1/F2, *Pistacia atlantica* contribution is high (7.35) and closer to the species *Ziziphus lotus* (4, 38). The 2 species are positioned closer in the same side, which explains that the regeneration of *Pistacia atlantica* occurs most often only in the shelter of the clump of *Z. lotus* (Monjauze 1968, Amara 2014, Benaradj 2010), so *P. atlantica* is sheltered in these clumps in order to protect the new plants from animals and strong winds. This association called *P. atlanticae* limited by two species: a tree layer (*P. atlantica*) and a shrub layer (*Z. lotus*). This regeneration is generally done within clumps of *Z. lotus* (Rhamnaceae) which protects the young shoots of the Atlas Pistachio from grazing, and promotes the germination of its seeds and the growth of its young shoots by enriching the soil in organic matter (Yaaqobi et al 2009). Kaabèche (2005) observed remarkable phenomenon called "facilitation" is to be pointed out about this species: this term "facilitation", gathers any

Table 3. Eigenvalues of the first three axes of the DCA

Axes	Eigenvalues
Axis 1	0,345
Axis 2	0,256
Axis 3	0,183

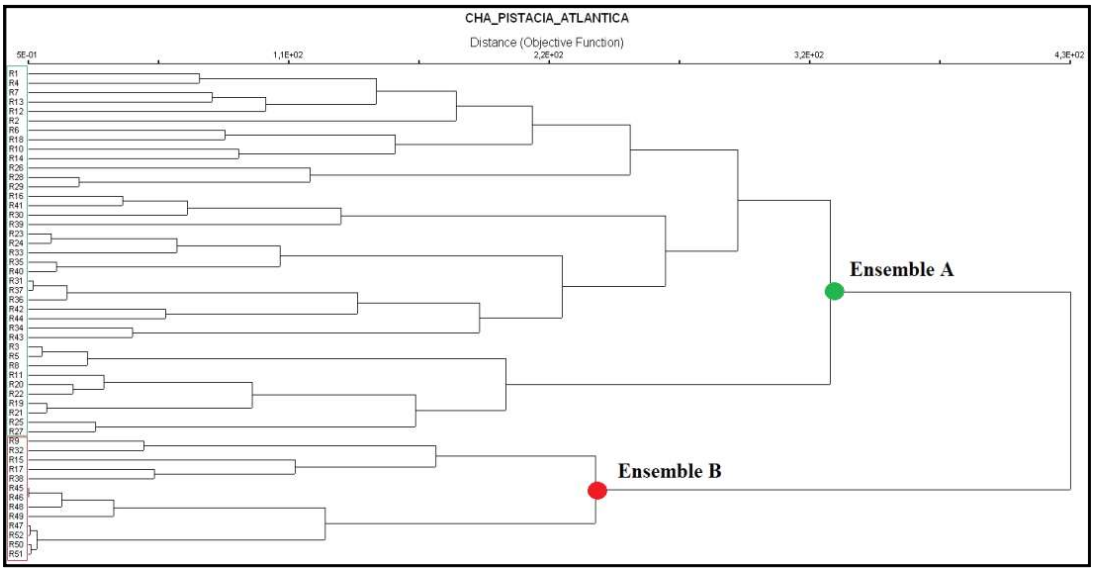


Fig. 8. Dendrogram of the hierarchical bottom-up classification of the total matrix

situation where a plant favors the establishment and the development of another plant thanks to privileged interspecific relations.

Hierarchical ascending classification: The CHA is the complement of a factorial analysis. It consists of grouping the individuals in a data set by similarity and thus allows for the confirmation of the results obtained previously with the DCA (Fig. 8). The 2 sets highlighted are thus delimited and confirm the results obtained by the DCA.

The CHA dendrogram shows two large clusters (A and B), which corroborates the results obtained during the numerical analysis, and confirms that geomorphology corresponds to the determining factor in the distribution of our *P. atlantica* ssp. atlantica grouping. Figure 9 represents the schematic distribution of the cores in the axes. The analysis has allowed a clear division of the floristic groups into floristically and ecologically homogeneous. Two groups (A and B) were highlighted and are thus delimited.

Group A: It is characterized by *Arthrophytum scoparium* and *Ammosperma cinereum*, we also note a good development of *Hammada scoparia*, and *Ziziphus lotus* well represented within this group.

Group B: represents the mosaic between the different biological types (phanerophytes, chaméphytes, Hemicryptophytes, Geophytes), with a remarkable wealth of therophytic species, among these species we distinguish: *Euphorbia calyptrata, Medicago polymorpha, Erodium triangulare, Reichardia tingitana, Pimpinella anisum*, etc.

CONCLUSION

The herbaceous vegetation and biomass of the selected *Pistacia atlantica* dayas varied significantly, which allowed us to appreciate the different adaptation strategies of the living organism. The predominance of therophytes is characteristic of the vegetation of arid regions that adapt to the Saharan and steppe environment. The predominance of the Mediterranean element confirms the Mediterranean affinity of the flora of the region. The extension of ploughing and the introduction of mechanization are parameters of degradation as important as overgrazing is retained as the main factor responsible for the floristic variations, it is imperative and urgent to create a strict protection zone specific to this species; otherwise, it risks to disappear in a very near future. To improve this form of protection, a balance between human activities and the natural dynamics of this species is necessary; in order to promote a continuity between the different strata: herbaceous, chamaephytes and phanerophytes. Also in the natural protection of Pistacia atlantica, by the creation of plant belts by species not consumed by livestock as *Ziziphus lotus* (L.) Desf. *Hammada scoparia*, and *Thymelaea microphylla*.

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Book Review

Book Title : Agroforestry Systems and Practices in Nepal (Revised Edition)
Authors : Swoyambhu Man Amatya, Edwin Cedamon and Ian Nuberg
Year : 2018

The Book "Agroforestry Systems And Practices in Nepal (Revised Edition)" has been written by Swoyambhu Man Amatya, Edwin Cedamon and Ian Nuberg is a revised version of "Agroforestry Systems and Practices in Nepal" authored by Swoyambhu Man Amatya in early 1994. This revisionincludes knowledge and experienced gained by the authors while collaborating on the project "Enhancing livelihoods and food security from agroforestry and community forestry in Nepal (EnLiFT)" funded by the Australian Centre for International Agriculture Research (ACIAR). The leading author, Swoyambhu Man Amatya, is an internationally renowned agroforestry scientist from Nepal and is also coordinator IUFRO 1.04.00 (Agroforestry). Edwin D. Cedamon andIan Nuberg are also internationally reputed scientists working in agriculture, environmental sciences, natural resource management and agroforestry. There are total ten chapters in the book.

Chapter 1 dwells on the physiographic, demographic, economic features and land resource assessment of Nepal.This chapter elaborates the basic concepts of agroforestry- definition, biological and socio-economic benefits, historical background of agroforestry in general and with special reference to Nepal.

Chapter 2 deals with methods of classification of agroforestry based on different criteria. The attributes of agroforestry has also been discussed.

Chapter 3 enlightens the readers about farming systems of Nepal, elements of analysis of land use systems, and agroforestry systems and practices of Nepal. Agroforestry systems of Nepal and their systems units have been delineated in Tabular form for the convenience of the readers.

Chapter 4 is quite comprehensive describing productive, protective and regulatory function of trees in agroforestry. Chapter also dwells on tree-crop interactions and factors affecting these interactions and management practices to neutralizenegative interactions with examples from Nepal and other countries. The role of tree roots in agroforestry, CO_2 sequestration and REDD+ initiatives in Nepal has been highlighted.

Chapter 5 on "Economic and Market Considerations of Agroforestry" is about principles of economic analysis and has lucidly elaborated methods and tools of economic assessment like discounted cash flow model, B:C ratio, IRR, annual equivalent value, valuation of inputs and outputs in agroforestry systems.

Chapter 6 on **"**Socio-Cultural Aspects of Agroforestry" highlights scope of agroforestry in Nepal, NTFPs, problems and potentials of domestication of NTFPs, women's role in agroforestry in Nepal, direct/indirect role of agroforestry in food security, reforms in legislative framework and their implications on agroforestry, specifically on the private forests in Nepal.

Chapter 7- "Policies and Regulations Relating to Agroforestry"discusses the constitution of Nepal, Forest Policy (2015) and Forestry Sector Strategy (2016) in relation to role of private forests, private-public-community partnership. Other acts, regulations of Nepal and amendments thereof with implications on private and community forests has been clearly and comprehensively dovetailed. Timber supply mechanism, policies related to herb development alongwith regulatory constraints in herb development has been highlighted.

Chapter 8 "Management Practices of Agroforestry" deals with different tending operations, pruning, pollarding, weeding, thinning and harvesting age, selection of tree species for agroforestry, pest and disease problems of trees.

Chapter 9 on "Agroforestry Research in Nepal" highlights the direction of agroforestry research in Nepal. It deals with common agroforestry trees and their uses, effect of trees on agricultural crops, estimates of fodder yield from different trees, lopping techniques and fodder quality. The chapter also delineates potential research areas and factors limiting agroforestry research and development in Nepal.

Chapter 10 "Tools and Techniques Used in Agroforestry Research" enlists the techniques used in diagnosis of agroforestry problems with special emphasis on D & D techniques, FSR and Agro-ecosystem research methods with reference to Nepal.

Each chapter of the book has its own reference list. The information provided in the book not only covers basic concepts of agroforestry but also describes all other sub-sectors of agroforestry with special reference to Nepal. It is great volume for students, teachers, researchers, planners and managers for augmenting their knowledge on basics and as well as advanced concepts of agroforestry, with specific reference to Nepal. Moreover the book succeeds in highlighting the direction of research in Nepal. All the authors of the book deserve compliments for brining interwoven elements of agroforestry in this book.

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