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Structure, Diversity and Dynamics of Natural-Anthropogenic Landscapes of Phu Yen Province

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Abstract: The natural-anthropogenic landscape is a form of a modern landscape, formed on the common foundation of the geocomplex in which human activities become a basic factor participating in the creation and development of the landscape. Analysing the diversity and structure of the natural-antropogenic landscape is the basis for the planning of economic development, especially the agro-forestry economy as well as the conservation of biodiversity. Phu Yen Province is a coastal province of central Vietnam, but the western area is mostly hilly, bringing a high division of natural conditions, along with the typical economic development activities associated with indigenous culture. It is a territory with a high differentiation of natural and anthropogenic conditions, which is the foundation for the formation of a system of natural-anthropogenic landscapes with high diversity, including 1 system, 1 subsystem, 3 classes, 5 subclasses, 9 types and 132 kinds of landscape. Based on the results of the field survey and analyzed structure of the outstanding landscape types for Phu Yen Province, including natural forests, plantation forests, annual agricultural crops and perennial industrial plants. Biodiversity is expressed in the natural forest landscape type group distributed in the Hinh River - Deo Ca area, with the division of species according to different high belt structures. The annual agricultural crops are distributed largely across the river valleys and Tuy An plain, Tuy Hoa city. The group of plantation forests landscape is distributed on the form of low mountainous terrain, Son Hoa basalt plateau, Hinh River. The perennial industrial plants are distributed largely in western Phu Yen Province, with popular crops being *Litsea glutinosa, Hevea brasiliensis*, and *Piper nigrum*. Analysing the characteristics and structures of landscape types allows us to assess the suitability of the landscape for natural and socioeconomic conditions, which is the foundation for the orientation of agro-forestry economic development and biodiversity conservation for Phu Yen Province.

Keywords: Natural-anthropogenic landscape, Diversity, Seasonal dynamics, Components, Phu Yen

From the realistic requirements, the consequences of human impacts must be fully and vividly reflected in natural territorial complexes, which have promoted landscape science to a new development step, which is the birth of anthropogenic landscape science. The object of anthropogenic landscape science is anthropogenic landscape (AL) or natural-anthropogenic landscape (NAL). AL is a modern landscape formed on the common ground of geocomplex in which human activities become the basic factors participating in the creation, transformation and succession of landscape (Nguyen Dang Hoi 2007). AL are natural landscapes that have been affected, altered or built by technical methods (Nguyen Dang Hoi, Ngo Trung Dung et al 2021). Structure, diversity and dynamics are basic attributes of landscape in general and AL in particular (Dang Hung Cuong et al 2020). Research and analysis of landscape structural features allow the determination of the characteristics and roles of each landscape component, each landscape unit, and the interaction of its constituent components, especially anthropogenic components and factors (Nguyen Dang Hoi and Tishkov 2021). Based on the structure and dynamics of the landscape, it is possible to forecast changes, self-adjusting or under external factors of the landscape in the future (Nguyen Dang Hoi 2007). Humans are an important component of NAL when there are always direct and indirect impacts on landscapes, changing their composition and structure (Chase 2016, Nguyen Dang Hoi et al 2016, Nguyen Dang Hoi and Ngo Trung Dung 2017, Nguyen Dang Hoi et al 2019). To date, research on AL or NAL has been applied in many countries of the world, from Russia to Europe, America, Asia, and in the fields of conservation, territorial planning and economic development (Schreg 2019, Vasconcelos, Ventincinque 2021). The application of GIS in the establishment of thematic forms of maps has a long history of development, which is mentioned in many studies in many different countries (Kumar et al 2021, Sreedevi and Karthikeyan 2021, Venkatachalapathy, Kumar et al 2021). For the establishment of NAL mapping, GIS software with algorithms helps to increase accuracy, shorten time, and ensure data information for the resulting map.

In Vietnam, research on AL and NAL has been carried out for more than 20 years. However, the first phase of the research mainly focuses on discussing viewpoints, researching objects or some theoretical classification systems of AL (Nguyen Dang Hoi et al. 2019). From the establishment of principles and the classification system of NAL, the study focuses on clarifying the characteristics and roles of natural and anthropogenic components and factors forming NAL, their diversity and dynamics in Phu Yen Province, a coastal province in the south-central coastal region of Vietnam.

MATERIAL AND METHODS

Studied area: Phu Yen is located in the East of Truong Son range, in the geographical coordinate frame from 12°42'36" to 13°41'28" north latitude; from 108°40'40" to 109°27'47" east longitude. The province has 9 administrative units at the district level and 110 administrative units at the commune level (People's Committee of Phu Yen province 2019). The natural area of the province is 5,060 km^2. It borders Binh Dinh Province to the north, Khanh Hoa Province to the south, Gia Lai and Dak Lak Provinces to the west, and the East Sea to the east with a coastline of 189 km (Fig. 1). The territory of Phu Yen Province is located in the fault between the Kon Tum uplift and the Lam Dong zone. The geological structure includes formation factors such as sedimentary, metamorphic and eruptive sediments that range in age from Proterozoic to Kanozoic. Phu Yen's topography is quite complex, including hills, mountains, plains, plateaus, and valleys interspersed with each other. The mountainous terrain forms extending from the Cu Mong pass in the north to the Ca pass in the south of the province. Phu Yen Province has a tropical monsoon climate influenced by the oceanic climate. The year is divided into 2 distinct seasons: the dry season lasts from January to August, and the rainy season lasts from September to December. The annual average temperature ranges from 23-27°C, and the weather is warm and stable. The average annual rainfall ranges from 1,600-2,100 mm, increasing gradually from valleys and coastal plains to high mountain. The hydrological network is thick and relatively evenly distributed throughout the province. There are four main river systems: the Ba River, Ban Thach River, Ky Lo River and Tam Giang River.

Fig. 1. Location of Phu Yen Province

Phu Yen Province has 11 soil groups (sand soil, saline soil, alkaline soil, alluvial soil, gray and infertile soil, black soil, red–yellow soil, yellow humus in mountains, valley soil, inert eroded soil with gravel rocks and other soils) with 27 soil types. The most common and large area is red–yellow soil on shale and metamorphic rock (37.9%) and gley-formed alluvial soil (23%). The vegetation cover of the Phu Yen is characterised by three main forest types: evergreen broad-leaved closed forest, sparse deciduous forest and plantation forest. Within Phu Yen Province, typical human activities are resource exploitation for the development of agriculture - forestry - fishery, industry, urban areas and tourism.

Component mapping system: The article is made on the basis of the database made by the authors themselves within the framework of the project E.1.2, task 2: "Features and changes of NAL of Phu Yen Province" implemented by the Institute of Tropical Ecology, Vietnam-Rusian Tropical Center, including proportional component maps at scale of 1/100000: Geological map, geomorphological map, soil map, vegetation map of Phu Yen Province (Fig. 2).

NAL map: To create the landscape map of Phu Yen Province, the component maps were superimposed using Arcgis software based on the Intersect algorithm (Fig. 2). The Intersect tool calculates the geometric intersection of any number of feature layers. The feature, or part of the features, for all input information is written to the output feature class. When multiple classes or feature classes are specified in the list of input features, the order of the entries in the list does not affect the output feature type, but the spatial reference of the entry on the same dialog box tool (first entry in the script) of the list used during processing and set to output (ESRI 2021).

Taxonomic systems and classification criteria for the landscape: In accordance with the type viewpoint, a taxonomic system comprising six ranges (Nguyen Dang Hoi et al. 2016), namely, System → Subsystem → Class → Subclass → Type → Kind, was chosen. Using these six ranges, a landscape map of the studied area was built at a scale of 1/100.000 (Table 1).

Differentiation and diversity of the NAL: The differentiation in nature and human activities has formed the system of NAL in Phu Yen Province with a high diversity of low-level taxa. Accordingly, the territory includes 1 system, 1 subsystem, 3 classes, 5 subclasses, 9 types and 132 kinds of landscapes (Fig. 3).

Field survey: To assess the current status and check the areas as well as assess the dynamics of NAL in Phu Yen Province, field surveys were conducted in April 2020 and January 2021 (Fig. 4), identifying and visually describing the current status of component-forming typical landscape kinds. Conducted interviews with local people and rangers to determine the timelines of changes in afro-forestry landscapes in the area, determining the dynamics of each specific landscape kind in the studied area on the basis of spatial differentiation and landscape diversity.

In April 2020, the survey focused on the Song High protected forest area, assessing the current status, defining the contours of tropical forest landscapes and interviewing local rangers about the timelines for structural change and local forestry policies. In January 2021, a survey along the main national and provincial roads of the Phu Yen was conducted to check the borders of landscapes, determine the crop structure, and interview local people and forest rangers to collect information on structural changes in agricultural crops and forestry. This is an important source of data to determines the dynamics of NAL in Phu Yen Province. The identification of plant and vine species was carried out on the basis of synthetic studies of morphological signs and documents of Pham Hong Ho (1999), Takhtajan A. L. (1987).

Diversity and dynamics of NAL: On the basis of the resulting map, the research team analysed the differentiation

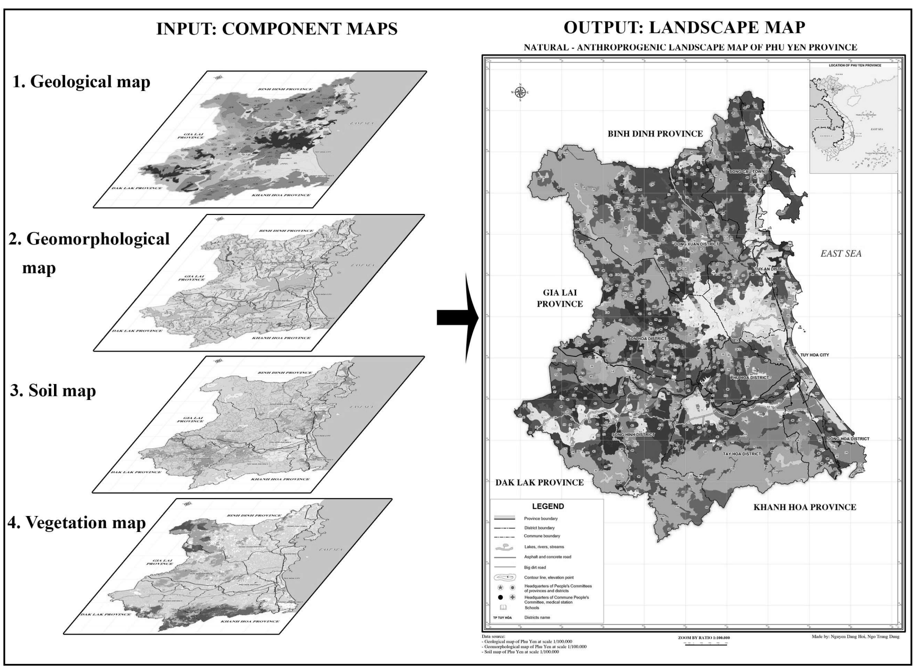

Fig. 2. The process of overlaying component maps to create a landscape map

Table 1. Taxonomic ranges and classification criteria for the landscape of Phu Yen Province

Taxonomic range	Classification criteria
System of landscape	Decisive role of the atmospheric circulation regime in the process of climate formation in the belt area.
Subsystem of landscape	Decisive role of the regime of atmospheric circulation in the process of climate formation and ecological region of the flora
Class of landscape	Relief configuration, which determines the homogeneity of two large processes, namely, erosion and concentration, in the real cycle
Subclass of landscape	Peculiarity of the formation of large relief forms manifests the property of nonzone on the basis of the combination of relief and typical geomorphological processes
Type of landscape	Quantitative peculiarity of bio climate and anthropogenic activities, which determines the formation of vegetation groups (except for the type of reservoir)
Kind of landscape	Differentiation of vegetations on different types of soils (except for the kind of reservoir)

Due to the homogeneity of landscape systemand subsystem levels, the legend of the landscape map does not show these two taxonomic ranges

LANDSCAPE SYSTEM	LANSCAPE SUB-SYSTEM	LANDSCAPE TYPE / LANSCAPE KIND		- Tropical monsoon climate on Highland, average annual air temperature 22oC; - Total annual rainfall fluctuate 1500 — 2800 mm, slightly lacking in humidity; - The rainy season lasts 8-9 months, from January to August, the dry is short 3-4 months, from September to December								
				Natural-anthropogenic Vegetation			*Anthropogenic Vegetation*					*Inland waters*
				Evergreen tropical closed forest	Bamboo forest	Grassland, shrub	Plantation forest	Perennial industrial plants	Annual agricultural crops	Wet Rice	Vegetation in resident	
Mountain	**Medium Mountain**	*Abrasion planar surface on basalt*	X-cr-h (*)	1		2	3	4	5	6		
			Fd-c-hu						7			
			X-h			8	9		10			
			X-hu-nh				11		12			
			X-tm-hu					13	14			
			Pc-a						15			
			C						16			
			E				17		18			
			All of Soils								19	
	Low mountain and hill	*Abrasion planar surface on different bedrock*	X-cr-h				20					
			Fd-c-hu				21					
			X-h	22		23	24		25			
			X-hu-nh			26			27			
			X-tm-hu				28					
			A	29								
		Gravititional slope on different bedrock	X-cr-h	30		31	32		33	34		
			Fd-c-hu				35		36	37		
			X-h	38		39	40	41	42	43		
			X-hu-nh	44		45	46		47			
			X-tm-hu	48		49	50	51	52	53		
			Pc-a				54		55	56		
			C				57					
			E				58		59			
			A	60			61					
			M							62		
			All of Soils								63	
Plain - Valley	**Plain**	*River floodplain*	X-cr-h						64			
			Fd-c-hu						65	66		
			X-h			67	68		69	70		
			X-hu-nh						71			
			X-tm-hu	72			73		74	75		
			Pc-a				76		77	78		
			C						79			
			E						80			
			M			81			82	83		
			All of Soils								84	
		River/marine/marsh deposition surface	Fd-c-hu						85			
			Pc-a							86		
			C				87		88			
			M						89	90		
			All of Soils								91	
		Mixed plain with relict hill and rock	X-cr-h						92			
			Fd-c-hu						93			
			X-h			94	95					
			C						96			
			E			97	98					
		Aeolian sand ,and dune	X-cr-h						99			
			C			100	101			102		
			M					103	104	105		
			All of Soils								106	
	Valley	*Deposition-erosion valley bed*	X-cr-h						107			
			Fd-c-hu						108	109		
			X-h	110		111	112		113	114		
			X-hu-nh				115		116			
			X-tm-hu	117		118	119		120	121		
			Pc-a				122		123	124		
			C						125	126		
			E	127					128			
			M						129	130		
			All of Soils								131	
Inland waters												132

(*): X-cr-h: Hapli-Chromi Acrisols; Fd-c-hu: Humi-Acric Ferralsols; X-h: Haplic Acrisols; X-hu-nh: Alumi-Humic Acrisols; X-tm-hu: Humi-EndoLeptic Acrisols; Pc-a: Dystri-Arenic Fluvisols; C:Arenosols; E: Dystri-Lithic Leptosols; A: Chromi-Humic Alisols; M: Molli salic fluvisols

Fig. 3. Legend of the NAL map of Phu Yen Province at a scale of 1/100000

and diversity of NAL in Phu Yen Province according to the classification and followed the principle from high to low levels and paid attention to the differentiation of kinds or group of landscape kind in each landscape class and subclass. The dynamics of NAL were determined based on the seasonal dynamics of the components and factors that formed NAL. Analysis of landscape seasonality was based on natural fluctuations and human activities, especially related to types of territorial use.

RESULTS AND DISCUSSION

Diversity and dynamics of NAL: The territory of Phu Yen Province has 3 landscape classes, including mountains, plains and valleys, and inland water (Table 2).

Mountain landscape class: Consists of 2 subclasses: medium mountains, low mountains and hills. In the subclass of medium mountains, there are 2 types: K1 (natural - anthropogenic vegetation on the leveled surface, eroded and developed on basalt) and K2 (planted vegetation on the leveled surface, eroded and developed on basalt). The subclass of low mountains and hills has 2 types: K3 (natural - anthropogenic vegetation growing on the leveled and eroded surface and gravity slopes) and K4 (planted vegetation on the leveled surface and gravity slopes).

Landscape type K1 includes 3 landscape kinds, numbered 1, 2 and 8. The total area was 1,006.27 ha, with vegetation cover mainly natural forest, grassland and shrub growing on soil types X-cr-h and Fd-c-hu. Landscape type K2 includes 16 landscape kinds, numbered 3÷7 and 9÷19, with a total area of 42,460.39 ha. The dominant group is the group of landscape kinds of annual agricultural crops growing on different soil types, with 8 landscape kinds. Landscape type K3 includes 13 landscape kinds, with a total area of 161,176.55 ha, in which there are similarities in the number of landscape kinds in the natural forest and shrub-grassland groups (numbered 7 and 8, respectively). Landscape type K4 includes 31 landscape kinds, with a total area of 165,826.95 ha. Landscape kinds of plantation forests and annual agricultural crops dominate, number 13 and 9, respectively.

Landscape class of plain and valley: Consists of 2 landscape subclasses: plain and valley. In the plain landscape subclass, there are 2 types: K5 (natural - anthropogenic vegetation on the plain surface accumulating river, river - sea and swamps, formed by sea winds and coastal plains interspersed with the rocks shore and hills); K6 (planted vegetation on the surface of the plain accumulates river, river - sea and swamps, formed by sea winds and coastal plains interspersed with the rock shore and hills). The valley landscape subclass has 2 landscape types: K7 (natural - anthropogenic vegetation growing on the bottom of valley with erosion and accumulation) and K8 (planted vegetation growing on the bottom of the valley with erosion and accumulation).

Landscape type K5 includes 6 kinds with a total area of 1,385.16 ha. Vegetation cover is mainly grassland and shrub growing on soils C and E. Landscape type K6 includes 57 kinds and has the highest level of landscape diversity, with a total area of 74,650.26 ha. The group of annual agricultural crops grew on different soil types, with 17 kinds. Next is the group of wet rice with 9 kinds and the group of plantation forests with 7 kinds. Landscape type K7 includes 5 kinds with a total area of 4,428.85 ha, in which there are similarities in the number of kinds of natural forest and shrub-grassland (numbered 2 and 3, respectively). Landscape type K8 includes 25 kinds, with a total area of 29,040.39 ha. The group of annual agricultural crops dominates has 9 kinds, and the group of wet rice has 6 kinds.

Landscape class of inland water: This is a special landscape class, not according to classification criteria such as terrestrial landscapes. This class is not differentiated but only has 1 subclass, 1 type (K9) and 1 kind number 132, with an area of 25,989.17 ha. This class includes inland water bodies such as natural lakes, man-made lakes, and lagoons. Landscape class plays an important role in supplying water

Table 2. Differentiation and diversity of NAL of Phu Yen Province

NAL class	NAL subclass	NAL type	NAL kind	Area (ha)
Mountain	Medium mountains	K1	1, 2, 8	1,006.27
		K2	3÷7, 9÷19	42,460.39
	Low mountains and hill	K3	22, 23, 26, 29, 30, 31, 38, 39, 44, 45, 48, 49, 60	161,176.55
		K4	20, 21, 24, 25, 27, 28, 32÷37, 40÷43, 46, 47, 61÷63	165,862.95
Plain and valley	Plain	K5	67, 72, 81, 94, 97, 100	1,385.16
		K6	64÷66, 68÷71, 73÷80, 82÷93, 95, 96, 98, 99, 101÷106	74,650.26
	Valley	K7	110, 111, 117, 118, 127	4,428.85
		K8	107÷109, 112÷116, 119÷126, 128÷131	29,040.39
Inland water	Inland water	K9	132	25,989.17

for agricultural landscapes, forestry, and the development of coastal aquaculture. Some reservoir landscapes can be mentioned: Song Hinh hydropower reservoir, reservoir capacity 357 million m^3; Ba Ha River hydropower reservoir, capacity 395 million m^3; Dong Tron irrigation reservoir (Tuy An District) with a capacity of 19.5 million m^3; Phu Xuan irrigation reservoir (Dong Xuan District) with a capacity of 11 million m^3.

Structural characteristics of typical landscape groups in Phu Yen Province: Phu Yen Province has a high level of differentiation, creating a diversity of taxa, especially types and kinds of landscape. In the landscape groups of the Phu Yen, natural forests, plantation forests, annual agricultural crops and perennial industrial plants are typical.

Landscape group of natural forests on low mountains and hills: Currently, in Phu Yen Province, natural forest landscapes are concentrated in the southern and western areas of the province, such as the Deo Ca - Hon Vong Phu mountain range, including Song Hinh protected forest (Fig. 4a, c, d, e), Deo Ca protected forest (Figure 4b) and Nature Reserve Krong Trai. Research results at Song Hinh protection forest show that the natural forest landscape is composed of shale, granite and basalt.

Vegetation consists of 4 floors. The first floor is 30-40 m high, formed by the following species: *Dipterocarpus turbinatus*, *D. hasseltii*, *D. retusus*, *Hopea odorata*, *Shorea cochinchinensis*, *Tarrietia javaniva*, *Pometia pinnata*, *Canarium album*, *Garcinia ferrea*, and *Endospermum sinensis*. The second floor is 18-24 m high. Species that form forest vegetation include *Balacata baccata*, *Cinnamomum iners*, *Schima wallichii*, *Swintonia floribunda*, *Dillenia blanchardii*, and *Lagerstroemia macrocarpa.* The 3rd floor is 8-12 m high. The main species recorded are as follows: *Arenga pinnata*, *Flacourtia jangomas*, *Stenomurus perobtusus*, *Helicia longeteticate,* and *Livistona saribus*. The 4th floor is 1.5-5 m high and is formed from species of the families Euphorbiaceae, Palmae, Apocynaceae, Rubiaceae, Melastomataceae, and Simaroubaceae. The herbaceous floor is quite developed but unevenly distributed in the landscape, depending on the microterrain structure. Species of the families Araceae, Primulaceae, Pandanaceae, Melastomataceae, and Poaceae have been recorded.

Forest landscapes in the plain valley: This group is widely distributed in Phu Yen Province and has been strongly affected by humans. In areas that still retain the natural structure, such as in the Krong Trai Natural Reserve, Song Sinh protection forest, landscapes develop on many different types of soil, but mainly sandy soil mixed with light flesh derived from river, basalt soil, and soil on schist. The vegetation has a structure of 3-4 floors. The first floor is 30-35

Fig. 4. Natural forestland scape of Phu Yen Province: Landscape of medium mountain forest (a, b); tree structure (c); bark root (d); profile of forest soil (e)

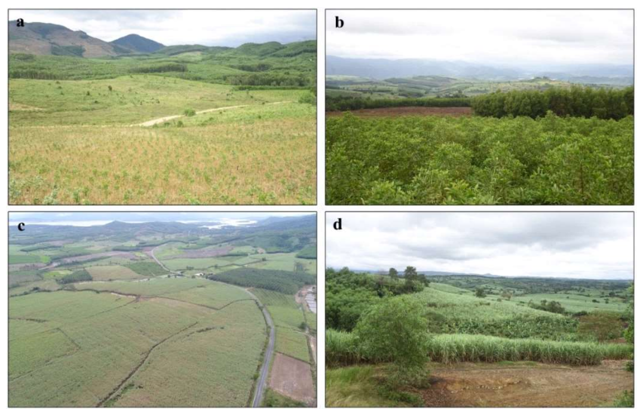

Fig. 5. Plantation forest and agricultural landscapes of Phu Yen Province: plantation forest landscape (*Acacia auriculiformis*) on plain and valley (a, b); sugarcane landscapes (c, d)

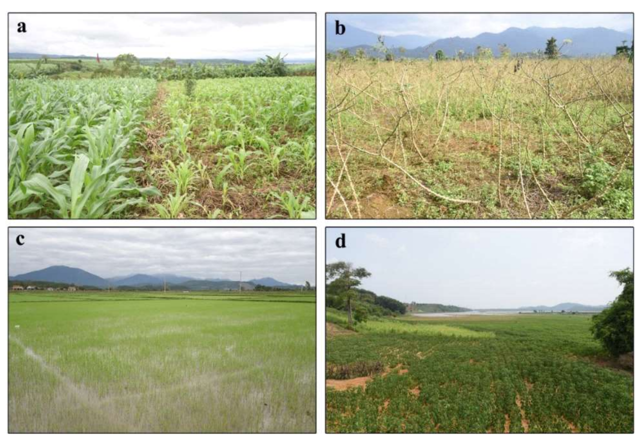

Fig. 6. Agricultural landscapes: corn and cassava (a, b, d); wet rice (c)

m high, with representatives such as *Dipterocarpus turbinatus*, *Shorea cochinchinensis*, *Herritiera javanica, and Endospermum sinensis*. The second floor is 15-20 m high, with representatives such as *Mangifera dongnaiense*, *Litsea monopetala*, *Cinnamomum* sp., *Cratoxylum cochinchinensis*, *Parkia sumatrana*, *Pometia pinnata, Diospyros buxifolia*, and *Metadina trichotoma*. The 3rd floor is 6-10 m high and has a diverse structure. Representative species belong to the families Rosaceae, Euphorbiaceae, Phyllanthaceae, Rubiaceae, Lamiaceae, Palmae, and Melastomataceae. The 4th floor is 1-4 m high and has a diverse structure, with representatives of the families Rubiaceae, Apocynaceae, Violaceae, Palmae, Melastomataceae, Rutaceea, and Zinginberaceae.

Landscape group of plantation forests: Commonly distributed, especially in the landscape subclass of low mountain - hill. The main plantation forests are *Acacia auriculaeformis* (Earleaf acacia), *Hopea ordorata*, *Dipterocarpus alatus*, and *Michelia mediocris*. The area of the Earleaf acacia forest landscape dominates absolutely in all subclasses in Phu Yen. Depending on the age of the plant, the height of the cover varies but usually does not exceed 15 m. The landscape of plantations with Earleaf acacia usually has a density of 2400 - 2500 trees/ha. Single-species plantation forest (Figure 5a,b). The tree has good vitality, increasing biomass evenly over the years from the first year to harvest (6th or 7th year).

Landscape group of annual agricultural crops: Widely distributed, from plains, valleys to medium mountains. Landscape develops on almost all types of soil at slopes below 25°, common below 15°. The most popular agricultural crops are wet rice (2 crops/year), cassava, corn (Fig. 6a), sweet potato, peanuts, beans, and sugarcane. In addition to wet rice (Fig. 6c), cassava and sugarcane are the dominant crops (Fig. 5c,d and Figure 6b,d). The composition of species in each landscape unit is quite simple and is usually planted with pure species. Sugarcane is planted in rows 1 m apart. Productivity and product quality depend on the farming conditions of the people. In mountainous areas, yields are often low due to a lack of people's care.

Landscape group of perennial industrial plants (including fruit trees): Widely distributed, but the size of each unit is usually small. The main plants grown in the landscape are *Litsea glutinosa, Hevea brasiliensis*, and *Piper nigrum*. In addition to landscpes of industrial plants, there are landscpes of fruit trees. These are landscapes with high economic value, but the area is small and undeveloped in Phu Yen Province.

Dynamics and transformation of NAL of Phu Yen Province: The dynamics of the landscape are reversible changes that do not lead to a radical structural shift. State changes are reversible, provided that changes in the parameters of the external environment do not exceed some critical value. The seasonal changes in the elements constituting the landscape must include the parameters of climate, hydrology, phenology of vegetation and the seasonal change of agricultural landscapes. The seasonality of the landscape of the study area is reflected in the air temperature regime, rainfall and processes occurring in the landscape. Seasonal rhythms are represented by the dry season lasting from January to August and the rainy season from September to December. The temperature range between the two seasons is 6-7°C. Rainfall is mainly concentrated in the rainy season, with 85% of the total annual rainfall. The flow of water in the landscape of water bodies also varies with the seasons, and the flood season is approximately 1 month slower than the rainy season. In the dry season, many landscapes of water bodies are dry, even exposing the bottom. At that time, the aquatic fauna and flora in the landscape no longer exist but instead are vegetation with the predominance of herbaceous species. Some landscapes of large water bodies do not run out of water, but in shallow zones, they are also "bottomed out" at the end of the dry season, losing the properties of water mass in the landscape, instead of annual agricultural crops such as the shallow water zone of Song Hinh hydropower reservoir, Ba Ha River hydropower reservoir, Dong Tron irrigation reservoir and Phu Xuan irrigation reservoir. In the rainy season, the landscape returns to the maintained state of the aquatic landscape with the characteristics of water mass and aquatic fauna and flora.

Landscape groups of agricultural crops are widely distributed throughout the province but are mostly concentrated in the lowlands of river basins. Vegetation changes seasonally. For example, with the landscape of sugarcane or cassava in districts of Song Hinh and Son Hoa, in the rainy season, the vegetation thrives, but in the dry season, the crops are harvested to create a bare land surface, changing the climate and soil parameters, especially illumination, temperature of the air and soil in the landscapes.

The landscapes of wet rice are distributed in the coastal areas of Tuy An, Dong Hoa, Tay Hoa dictricts and Tuy Hoa city. These are mainly two-crop rice fields, creating seasonality in the structure of the surface covers, unlike the landscapes of sugarcane or cassava. In these landscapes, the rhythm of the vegetation within 3-5 months creates 2 cycles each year. Meanwhile, in the rice landscapes in the western mountains, many areas are single-crop rice, and after fallow, create a dynamic cycle of vegetation in a year, consistent with other landscape factors. Thus, in agricultural landscapes of wet rice or crops with rotation of 2 or more crops in a year, the rhythm of the plant cover is out of phase with the climatic and hydrological conditions, creating the complexity of the NAL of Phu Yen Province. For forest landscapes, seasonal dynamics clearly show the harmony of forming components and factors. There, the elements of the climate and hydrological components determine the seasonal rhythms of vegetation and forest soil. The phenomenon of budding, young leaves, flowering, fruiting, deciduous and decomposing of falling plant layers on the ground, climatic and hydrological conditions of forestland comply with the seasonal laws of the local climate. When studying the seasonal dynamics of forest landscapes in Song Hinh protected forest, the results show that the deciduous period of trees is concentrated in January and February. After the period of old leaves fall, new leaves develop in 2-3 weeks. During the months of March and April, the trees under the canopy are partially deciduous, and some species are completely deciduous. On the surface of the soil, dry leaves and twigs of different sizes were observed. The falling plant layer only has traces of bones of some leaves from the previous year, which shows that the ability to decompose fallen leaves of plants in the landscape subclass of low mountains and hills completely takes place according to the annual cycle under the influence of seasons of soil animals and forest fungi.

CONCLUSIONS

Phu Yen Province is a territory with a high differentiation of components and elements that make up the NAL. On the basis of taxonomic range and classification criteria, a landscape map of Phu Yen Province was established at the scale of 1/100000. Accordingly, the landscape of the Phu Yen includes 1 system, 1 subsystem, 3 classes, 5 subclasses, 9 types and 132 kinds of landscapes. The highest diversity belongs to the landscape class of plain and valley with 2 subclasses, 4 types and 68 kinds. The mountain landscape class has a high degree of differentiation of the low mountain and hill subclasses, with 2 types and 44 kinds, respectively. The subclass of medium mountains has low diversity, with 2 types and 19 kinds. Landscape groups of plantation forests and annual agricultural crops dominate in terms of area and high diversity, consistent with the differentiation of natural and anthropogenic factors in the territory. The landscape of Phu Yen Province has a complex structure, especially its internal structure. In addition to the natural forest landscapes whose structure is quite consistent with the formation rules of natural components, in many groups of NLA, especially agricultural landscapes, plantation forest landscapes have many elements in their structure that are built by human activity. The seasonal dynamics of Phu Yen's NAL are clearly reflected in the seasonal rhythms of the natural elements of the climate, hydrological and forest vegetation components and the seasonality of anthropogenic activities, especially the seasonality of agricultural landscapes, in which the rhythm of the plant cover is out of phase with the climatic and hydrological conditions, creating the complexity of the NAL of Phu Yen Province. For natural forest landscapes, seasonal dynamics represent the suitability of landscape components and elements occurring in seasons as well as the transition period from winter to summer or from summer to winter. Meanwhile, agricultural landscapes, especially landscapes of annual crops, and landscape dynamics are strongly influenced and dominated by people in mountainous areas, plateaus or plains and valleys. This is a feature that should be noted in the process of landscape design and selection of plants suitable to the technical conditions of cultivation of each area and each district in the whole Phu Yen Province.

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Flora in Truong Sa Islands, Khanh Hoa Province, Viet Nam

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Abstract: The flora of Truong Sa islands has a number of features of tropical island flora. The research results had recorded 265 species, belonging to 200 genera and 74 families of vascular plants, in which 146 new species were added to the flora of Truong Sa islands. Representatives of the dominant families such as Fabaceae, Poaceae, Malvaceae, Cyperaceae or Asteraceae had the most species-rich genus- Cyperus (8 species). The flora of Truong Sa islands had a quite even proportion of lifeforms, Phanerophytes occupied the highest proportion with only 33.58%, while Hemicryptophytes madeup 23.40%, and Therophytes accounted for 21.51%. Species were distributed mainly in biotopes of island (H1) (88 species), 57 species that were divided into 2 biotopes were widely distributed. The study had counted 157 natural and naturalized species, accounting for 59.25% of the total species investigated on the islands. The flora of Truong Sa islands had typically tropical characteristics with 57.74% of the species belonging to the tropical element group. The flora had a relatively high conservation value with 39.24% of species and 66.21% of plant families listed on the IUCN Red List. There were 3 species were vulnerable (VU) in IUCN Red List 2021, 1 species was endangered (EN) and 1 species was vulnerable (VU) in Viet Nam Red Data Book 2007.

Keywords: Conservation, Diversity, Flora, Truong Sa islands

There were about 684,865 floating islands in the world which were located mostly in 50 countries and territories and account for about 5% of the global land area (www.worlddata.info 2020). Although the island's biodiversity was assessed to be poorer than that of the neighboring mainland due to many different natural conditions (isolation, shape, size, topography, climate, geology, etc.), but species on islands and peninsulas had a higher degree of endemism. It was estimated that more than 20% of the world's endemic vascular plant species were found on islands (Whittaker & Ferna´ndez Palacios 2007, Holger et al 2008, www.biodiversity. europa.eu). The populations of species on the island were also the most vulnerable to climate change, sea level rise, and the invasion of alien species, in which the species belonging to terrestrial ecosystems were being destroyed seriously affected. Even so, the biological world on the island still had a lot of new things for scientists to discover (Pysˇek & Richardson 2006, Buckley and Jetz 2007). Truong Sa islands (Spratly Islands) belongs to Truong Sa district, Khanh Hoa province is a part of Viet Nam's territory, associated with the development of Viet Nam for thousands of years. The rresearch results showed that Truong Sa islands had the highest level of biodiversity in the Asia-Pacific region (Thanh 2001, Thung et al 2014). Although research on biodiversity in Truong Sa islands started in the 20th century, the database is still limited so far. The first relatively systematic and complete study on flora of Truong Sa islands in 1997 mainly at 4 islands, Truong Sa island (Spratly island), Nam Yet island (Namyit island), Song Tu Tay island (Southwest Cay island) and Son Ca island (Sand Cay island) had recorded 119 species of vascular plants (Khoi and Phuong 2001). Over the past two decades, there had been natural and human impacts on terrestrial ecosystems, so a comprehensive study of terrestrial flora characteristics was conducted to find solutions for conserving species and terrestrial ecosystems in the region in the context of climate change, sea level rise for 3-year from 2020 to 2022.

MATERIAL AND METHODS

Study area: Truong Sa islands is located from 6°30' to 12°00 North latitude and 111°20'-117°20' East longitude. The islands had about 130 islands, shoals and sandbars, scattered in an area of about 170,000 square kilometers, with a length of about 800 kilometers from east to west and 600 kilometers from north to south (Cuong et al 2020). The area had the following meteorological and tidal characteristics: the average annual temperature was about 27.7°C, the average annual humidity was about 83%. The average wind speed was about 6 m/s, the strongest was about 34 m/s (in thunderstorms and storms), the wind usually gets strong from November to February the next year. Prevailing winds were from the southwest in summer and from the northeast in winter.

Thunderstorms and tornadoes can happen at any time of year. The average annual rainfall was about 176.0 mm, the highest rainfall was about 104.7 mm (November) and the lowest was about 0.1 mm (February and March). The islands in Truong Sa islands were strongly affected by waves. The average monthly wave height in Song Tu Tay island and Truong Sa island was 2.1 m and 1.9 m respectively. In March, April and May there are usually small waves while in January, August, and December there are usually big waves. The salinity of seawater in Truong Sa islands area was almost uniform, its variation was low. Surface seawater salinity in summer was 33.5‰, and in winter 33 - 33.5‰ (Luong et al 2016). The study was carried out on 9 floating islands of Truong Sa islands, including: Truong Sa Dong island (Central Reef), An Bang island (Amboyna Cay), Truong Sa island (Spratly island), Sinh Ton Dong island (Grierson Reef), Sinh Ton island (Sin Cowe island), Phan Vinh island (Pearson Reef), Son Ca island, Nam Yet island and Song Tu Tay island (Fig. 1).

MATERIAL AND METHODS

Material: Research subjects are vascular plants.

Methods

Field investigation method: 5 main survey routes with 10m wide were designed across each island: Route 1 surrounds the outermost edge of the island, the remaining 4 routes follow 4 directions East, West, South and North, counting from the position of the island's centre until it meets Route 1. Depending on the shape and area of each island, herringbone-shaped auxiliary routes were additionally designed so that the investigated area covers all the biotopes and habitats of the species on the islands. Collecting specimens, information, and taking photos of the plant species under the study according to the research methods of Thin (2007) and Chung (2009).

Method of data processing: Handling and preserving the collected plant specimens according to the guidelines of Thin (2007). Identifying, assessing samples and looking up the scientific names of species by morphological comparison and expert methods on the basis of documents of Ho (2003), Ban (2003, 2005). Preserving specimens after being processed and classified at the Viet Nam - Russia Tropical Center, Ha Noi, Viet Nam. Assessing conservation value according to the IUCN Red List in 2021. Determining lifeform characteristics and geographical elements of species based on the documents of Raunkiaer (1934), Chan (1999) and Thin (2004).

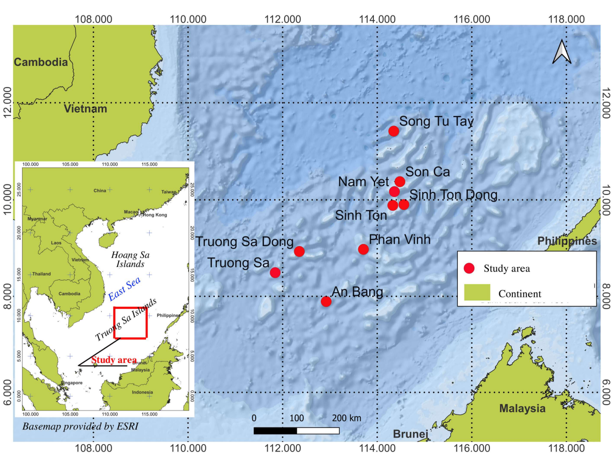

Fig. 1. Study area

RESULTS AND DISCUSSION

Diversity of Plant Taxon

Floristic composition: The survey results recorded 265 species of vascular plants belonging to 200 genera, 74 families, 36 orders, 5 classes and 3 phyla, in which 146 new species of higher plants were added to Truong Sa islands's flora according to the most recently published study of Khoi and Phuong (2001). Polypodiophyta, Gymospermae and Angiospermae are the representatives of the three phyla, in which Polypodiophyta had only 1 species, accounting for 0.38% of the total species, Gymospermae possessed 3 species (1.13%). Angiospermae dominated with 261 species (98.49%), 196 genera (98%), 70 families (94.59%), 33 orders (91.67%) from the total species, genera, families and orders recorded, respectively (Table 1). Most of the taxa of Truong

Table 1. Diversity of plant taxon in Truong Sa islands

Division	Class	Order		Family		Genus		Species	
	N	N	%	N	%	N	%	N	%
Polypodiophyta	1	1	2.78	1	1.35	1	0.50	1	0.38
Gymospermae	2	2	5.56	3	4.05	3	1.50	3	1.13
Angiospermae	2	33	91.67	70	94.59	196	98.00	261	98.49
Dicotyledones		25	69.44	55	74.32	152	76.00	199	75.09
Monocotyledones		8	22.22	15	20.27	44	22.00	62	23.40
Dicotyledones/Monocotyledones		3.13		3.67		3.45		3.21	
Total	5	36	100	74	100	200	100	265	100

Sa islands's flora were distributed in Angiospermae, in which the taxonomic ranks of Dicotyledones were 3.13 to 3.67 times higher than those of Monocotyledones (Table 1). This ratio is similar to that of the inland flora of Viet Nam (3.2 to 3.8) (Chan 1999, Ho 2003). On the other hand, when assessing taxonomic diversity by the ratio between the number of species, families and genera, the results showed that the Truong Sa islands flora had a family diversity index of 265 species/74 families in proportion to the ratio of 3.58/1, or each family had 3.58 species. The genera diversity index was 265 species/200 genera, corresponding to the ratio of 1.33/1, or each genus had 1.33 species. As for the flora of Con Co island, the ratio was 2.86/1 and 1.26/1, respectively (Cam 2008). Thus, the flora in Truong Sa islands had a higher diversity index in terms of species composition than that in Con Co island.

Diversity of plant families and genera: The 10 most species-rich families of Truong Sa islands flora only accounted for 13.5% of the total families, they had 126 species, belonging to 86 genera, corresponding to 47.55% of the total species and 43% of the total genera recorded. Fabaceae dominated with 21 species (7.92%), Poaceae with 18 species (6.79%), Malvaceae and Cyperaceae with 15 species (5.66%) and Asteracea with 13 species (4.91%). Some families such as Rubiaceae, Euphorbiaceae, Moraceae, Lamiaceae, Convolvulaceae had from 8 to 10 species. The 10 most species-rich genera owned 46 species, accounting for 17.36% of the total species, of which *Cyperus* had the most species with 8 species (3.02%) (Table 2). Most of the recorded families were considered to be the most species-rich ones of the flora in Viet Nam. Besides, some of the species-rich families such as Cyperaceae, Malvaceae, Convolvulaceae, and of the genera such as *Cyperus*, *Fimbristylis*, *Ipomoea* have reflected the characteristics of the flora of Truong Sa islands and the obvious influence of geographical and geological conditions, and marine climate on the flora species composition of the islands.

Diversity of plant life forms: Phanerophytes (Ph) accounted for the highest percentage at 33.58% of the total species, in which the number of species are mainly Microphanerophytes (Mi) with 17.36% and Mesaphanerophytes (Me) with 9.06% (Table 3). However, the proportion of Ph group of Truong Sa islands flora was much lower than that of typical tropical flora, such as Kon Tum province's flora (65.35%) (Cuong et al 2020), Xuan Nha nature resereve (78.85%) (Hoa and Sam 2016). Instead, species belonging to the group

Table 3. Statistical results of plant lifeforms in Truong Sa islands

Life form	Code	No. of species	Percent
Phanerophytes	Ph	89	33.58
Megaphanerophytes	*Mg*		
Mesaphanerophytes	*Me*	24	9.06
Microphanerophytes	*Mi*	46	17.36
Nanophanerophytes	*Na*	9	3.40
Epiphytes	*Ep*	3	1.13
Parasitephanerophytes	*Pp*		
Lianophanerophytes	*Lp*	5	1.89
Herbacephanerophytes	*Hp*	1	0.38
Succelentphanerophytes	*Sp*	1	0.38
Chamaephytes	Ch	29	10.94
Hemicryptophytes	Hm	62	23.40
Cryptophytes	Cr	28	10.57
Therophytes	Th	57	21.51
Total		265	100

Table 2. Most diverse families and genera in the flora of Truong Sa islands

Family	Genus		Species		Genus	Species	
	N	%	N	%		N	%
Fabaceae	18	9,00	21	7,92	*Cyperus*	8	3.02
Poaceae	15	7,50	18	6,79	*Fimbristylis*	6	2.26
Malvaceae	11	5,50	15	5,66	*Ficus*	6	2.26
Cyperaceae	3	1,50	15	5,66	*Euphorbia*	5	1.89
Asteraceae	12	6,00	13	4,91	*Ipomoea*	5	1.89
Rubiaceae	9	4,50	10	3,77	*Citrus*	4	1.51
Euphorbiaceae	4	2,00	9	3,40	*Plumeria*	3	1.13
Moraceae	3	1,50	9	3,40	*Cleome*	3	1.13
Lamiaceae	7	3,50	8	3,02	*Sida*	3	1.13
Convolvulaceae	4	2,00	8	3,02	*Syzygium*	3	1.13
Total	86	43	126	47.55	Total	46	17.36

Hemicryptophytes (Hm), Cryptophytes (Cr), Therophytes (Th) accounted for a relatively high proportion, from 10.57%-23.40%. The lifeform spectrum formula of the flora in Truong Sa islands was as follows:

SB = 33.58 Ph + 10.94 Ch + 23.40 Hm + 10.57 Cr + 21.51 Th.

The research results have shown the high adaptation of flora to the hot weather, hoarfrost and dry land of the Truong Sa islands.

Immigration pattern and habitats: The immigration pattern of plant species recorded in Truong Sa islands can be divided into 2 main groups: (1) Group N - Natural species (from ocean currents, from wind, from birds, from humans, etc.) or Naturalized; (2) Group C - Cultivated species. As a result of the investigation, there were 157 species of group N, accounting for 59.25% and 108 species of group C accounting for 40.75% of the total 265 species of vascular plants recorded in Truong Sa islands (Table 4 and 5). The species of group N dispersed by ocean currents, wind, and

Table 4. Immigration pattern and habitats of vascular plants in Truong Sa islands

Division	Immigration pattern		Habitats			
	N (Natural and naturalized species)	C (Cultivated species)	H1	H2	H3	≥ 2 Habitats
Polypodiophyta	1					1
Gymospermae		3	3			
Angiospermae	156	105	85	59	61	56
Dicotyledones	121	78	71	46	45	38
Monocotyledones	35	27	14	13	16	18
Total	157	108	88	59	61	57

H1: Natural, naturalized and cultivated species - on open place near/beside/between constructions or in the campus;
H2: Cultivated species - on the incremental garden
H3: Natural, naturalized species - on the island's edge, on open places, beach or hanging on coastal trees/shrubs

Table 5. The distribution patterns of species of vascular plants in Truong Sa islands

Distribution pattern	Immigration type				Total (%)
	N (Natural and naturalized species)		C (Cultivated species)		
	No. of species	Percent	No. of species	Percent	
Endemic element	3	1.13	5	1.89	3.02
Bac Bo endemic	2	0.75	1	0.38	1.13
Trung Bo endemic		0.00		0.00	0.00
Nam Bo endemic	1	0.38	1	0.38	0.75
Viet Nam endemic		0.00	3	1.13	1.13
Tropical element	103	38.87	50	18.87	57.74
Indochina endemic	10	3.77	9	3.40	7.17
Southern China endemic	0	0.00	3	1.13	1.13
Haiman, Taiwan, Phillippines element	3	1.13	2	0.75	1.89
Hymalaya element		0.00		0.00	0.00
India element	14	5.28	7	2.64	7.92
Malaysia element		0.00		0.00	0.00
Indonesia Malaysia element		0.00	1	0.38	0.38
Indonesia Malaysia Ocean Australia element	3	1.13		0.00	1.13
Tropical Asia element	35	13.21	22	8.30	21.51
Palaeotropical element	24	9.06	3	1.13	10.19
Newtropical and circum tropical element	14	5.28	3	1.13	6.42
Temperate element	15	5.66	4	1.51	7.17
East Asia element	2	0.75	1	0.38	1.13
Asia element	13	4.91	3	1.13	6.04
Northern temperate element		0.00		0.00	0.00
Other elements	36	13.58	49	18.49	32.08
Wide disposing element	9	3.40	8	3.02	6.42
Midern imported and migrant element	27	10.19	41	15.47	25.66
Total	157	59.25	108	40.75	100.00

birds had characteristics including fruit with light and sticky seeds such as *Triumfetta repens, Sida rhombifolia, Bidens pilosa, Chromolaena odorata, Xenostegia tridentata,* etc. The species, which are naturalized for the purpose of landscape and protection consist of *Casuarina equisetifolia, Calophyllum inophyllum, Heliotropium arboreum, Coccoloba uvifera, Cocos nucifera, etc.* Group C was relatively diverse with species with different values which were used for ornament (*Bougainvillea spectabilis*, *Combretum indicum*), and serve as food (*Carica papaya, Benincasa hispida, Zingiber officinale,* etc.). There were 88 species of plants only found in open places, constructions or campus on the islands. Up to 59 species of plants were grown in incremental gardens, 61 species distributed on the island's edge were mainly herbaceous, shrubs, vines found in bare ground, coral sand, coastal trees or shrubs, and 57 species were widely distributed throughout the island (Table 4) .

Diversity of geographic elements: The results of classification of geographical elements according to the classification framework of Chan (1999) have indicated that the flora of Truong Sa islands had the characteristics of tropical flora with 57.74% of the recorded species belonging the tropical element group, in which the group of natural and naturalized species accounted for 38.87%, most concentrated in tropical Asia (13.21%). The endemic element group in Truong Sa islands accounted for 3.02%, of which endemic species with natural distribution occupied 1.13% (Table 5). In comparison with the endemic rate of the flora of Viet Nam in the mainland (11.49%) (Chan 1999), the flora of Truong Sa islands had a very low endemic rate. However, due to the different conditions in the islands, the flora was less diverse than that in the mainland, so the endemic species such as *Ruellia tuberosa, Acalypha lanceolata, Portulaca pilosa* need special attention for research and conservation in Truong Sa islands.

Diversity of conservation value: Although the flora of Truong Sa islands had a low species diversity, 104 species belonging to 49 families have been counted, accounting for 39.24% of the total species and 66.21% of the total families listed on the IUCN Red List (iucnredlist.org 2021-3). In which, there were 3 species at vulnerable level (VU) of IUCN 2021, 1 species at endangered level (EN) and 1 species at vulnerable level (VU) in Viet Nam Red Data Book 2007 were in urgent need of conservation actions, especially natural species on the islands, such as: *Tribulus terrestris, Barringtonia asiatica* and *Euphorbia atoto.*

CONCLUSIONS

Although the flora of Truong Sa islands was not very diverse, it had the characteristics of tropical island flora. The study recorded 265 species, belonging to 200 genera, 74 families and 3 phyla of vascular plants (ferns, gymnosperms and angiosperms). As a result, 146 species were added to the flora in the Truong Sa islands. The proportion of lifeform groups Hemicryptophytes (23.40%), Cryptophytes (10.57%), Therophytes (21.51%) was relatively high, which reflects the dry, hot character through the flora of Truong Sa islands. The flora of Truong Sa islands was relatively diverse in terms of geographical elements, the tropical element group accounted for the highest proportion with 57.74% of the total species. Two main immigration types of species were identified (N - natural and naturalized and C - cultivated). In which, the natural and naturalized species were dominance, with accounting for 59.25% of the total species. Recorded species were distributed in many different biotopes including 3 main groups: island's edges; incremental gardens: shelves or pots; and islands or constructions. There were 57 species widely distributed from the 2 groups of biotopes. The total species were listed on the IUCN Red List up to 39.24%, some of which were endangered and need to be preserved. The flora of Truong Sa islands still had many unknowns that need to be further investigated, researched and monitored for changes in the coming time, which contributes to the conservation of flora in particular, as well as the biodiversity of terrestrial ecosystem in Truong Sa islands in general.

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Floristic Diversity, Nativity and Endemism of High Altitude Forested Landscape of Kedarnath Wildlife Sanctuary

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Abstract: The high altitude zones of the Himalaya are the rich pockets of native and endemic species, and contributes vast range of flora and fauna in the Himalayan biological diversity. In the present study conducted in the high altitude forested zone of Kedarnath Wildlife Sanctuary (KWS) following extensive survey. The total of 474 species belonging from 276 genera and 78 families were recorded. Of this 321 (67.7%) were herbs, 11.6% shrubs,9.3% trees, 8.4% grasses, 3% sedges and 1.3% climbers. Out of total recorded families and genera, 27 families and 192 genera were monotypic, indicating poor genetic base or recent speciation with this Himalayan region. A good proportion of endemic (29.54%) and native species (68.57 %) were recorded within the region, highlighting the significant contribution of this region (KWS) in the regional (Himalaya) flora.

Keywords: Western Himalaya, Kedarnath Wildlife Sanctuary (KWS), Floristic diversity, Himalayan endemic, Native species

The Indian Himalayan Region (IHR) forms a significant part of Himalayan Biodiversity Hotspot (HBH) and with an area of about 5 lakh km^2 comprises about 16% of country's total geographical area. The IHR is well known for its representative, unique, natural, and socio-economically important flora and fauna. The wide range of physiographic and phytoclimatic conditions within the region support diverse and luxuriant vegetation and are species rich particularly in native and endemic species (Singh 2018). IHR supports nearly 50% of the total flowering plants in India of which 30% flora are endemic. The flora of the IHR includes about 8000 species including angiosperm, gymnosperm, pteridophytes bryophytes, lichen and fungi (Palni and Rawal 2013). At present, the IHR has 28 National Parks, 98 Wildlife Sanctuaries, and 06 Biosphere Reserves, covering a total geographical area of 51,899.239 Km^2 excluding North East India (Samant et al 2003). Out of these 98 Wildlife Sanctuaries of IHR the Kedarnath Wildlife Sanctuary (KWS) is recognized well for its wide range of altitude (1100-7068 m asl) and support vast range of floral and faunal diversity as well and considered as one of the largest protected area in the western Himalaya (Rai et al 2017). There is great significance in floristic and ecological studies due to the availability of vast range of forests types along the altitudinal gradient from temperate (closed canopy) to subalpine/timberline, tree line to the alpine meadows (covers the vegetation of various high altitude ecotone zones). The planning, monitoring and management of the forests ecosystem the studies on species diversity, structure and composition are crucially important (Bilyaminu et al 2021). The region was explored by various workers from late 1970s in several disciplines and the available published information (nearly 300 research articles and other publications) which suggests that maximum research in this region were done in the vegetation and ecosystem related studies followed by the faunal studies. Knowing importance of the region the aim was to document the floristic diversity of the region from above 2000 m and to provide the valuable information (habit, habitat, nativity, endemism and worldwide distribution) of the individual plant species.

MATERIAL AND METHODS

Study site: The study was conducted in Chopta-Tungnath area (30°30'03.0" to 30°29'23.8" N and 79°09'52.8" to 79°12'42.3" E) of Kedarnath Wildlife Sanctuary (KWS), Uttarakhand, Western Himalaya. The extensive survey was conducted between the altitude range 2000-3500 m asl in the high altitude forested zone of KWS. Forests of the area fall in temperate to sub-alpine zone, the latter giving way to alpine meadows beyond the tree line ecotone (Gairola et al 2008, Rai et al 2012a).

Methods

Floristic inventorization: The baseline of floristic inventory includes qualitative sampling, an extensive survey was conducted in the representative site during August 2016 to September 2020, covering all seasons in three transects along the altitudinal gradient from 2000-3300 m asl. The plant specimens collected in field were brought to the GBP-NIHE for herbarium preparation and further examination and identification. The herbarium specimens were prepared following standard methods of herbarium preparation (Jain and Rao 1977). The specimens were identified with the help of recently published literature of that region and Northern regional centre of Botanical Survey of India (Dehradun). The checklist of study site flora was prepared and voucher specimens were deposited at herbarium of G.B. Pant National Institute of Himalayan Environment (GBP-NIHE). Further, for the nomenclature of the species i.e. updated botanical names and other details, online websites were consulted i.e. Tropicos, The Plant List, eFlora of Pakistan, eFlora of China and Plants of the World. Information on habit, altitude zones etc. were taken and list of species was updated. The information was also used to assess patterns of species to genera and species to family ratio for different life forms.

Nativity and endemism: The prepared list of species was further categorized in four categories: (i) endemic; (ii) near endemic (both as Himalayan endemics); (iii) native and (iv) non-native species. The species special range restriction to the Himalaya (i.e. Indian Himalayan Region, Nepal, Bhutan, Pakistan Himalaya) were considered as endemic and species having range extension beyond the Himalaya were referred as near-endemic (Samant 2015). The native and non-native species were identified based on; the species having their origin in Himalayan region and distribution in the region and neighboring countries/states were considered as natives (Samant 2015).

RESULTS AND DISCUSSION

Floristic diversity: A total of 474 species of vascular plants (angiosperms and gymnosperms) belonging to 276 genera and 78 families were recorded within the investigated altitude zone (2000-3300 m asl) in Chopta-Tungnath region of Kedarnnath Wildlife Sanctuary, Uttarakhand. This accounts for 32.22% species, 54.55% families and 37.76% genera of the reported floristic diversity pool within the forested high altitude zone (2000-3500 m) of Uttarakhand (Rawal et al 2018). The representative site covers 4.51% species from total reported species richness for the Himalaya (Rana and Rawat 2017). Of the total recorded species (474), 67.7% were herbs, 11.6% shrub, 9.3% trees, 8.4% grasses, 3% sedges and 1.3% climbers (Table 1 and 2) and this covers 9.48% species (8.18% trees, 6.11% shrubs and 8.03% herbs) from the reported species numbers (from sub-tropical to alpine zone) for the Uttarakhand state (Samant 2015). Bhatt et al (2020) observed richness of tree species in high altitude forest zone (2000-3500 m) as 10.4% for IHR. This indicates the representative site and altitude zone have a significant proportion of the total floristic diversity of Uttarakhand and the Himalaya as well. The comparative account of floristic diversity of Himalaya with the present study is given in (Table 3).

Species to genera (S/G) and species to family ratio (S/F): The species to genera ratio (S/G) for entire altitude zone was 1.71 and species to family ratio (S/F) was 6.08 (Table 1). In the present study the species to genera ratio (S/G) was recorded greater in sedges (3.55) followed by shrubs (1.72), while the species to family ratio (S/F) again in sedges (14) followed by herbs (6.69).This indicates that the representation of all genera and families among the species was greater in these life forms. The S/G and S/F ratio is high

Table 1. Distribution of numbers and percent of family, genus, species, S/F (Species/Family) and S/G (Species/Genera) ratios among different life forms

Life forms	Family	Genus	Species	S/F	S/G
Trees	22 (28.2%)	35 (12.7%)	44 (9.3%)	2	1.26
Shrubs	17 (21.8%)	32 (11.6%)	55 (11.6%)	3.23	1.72
Herbs	48 (61.5%)	195 (70.7%)	321 (67.7%)	6.69	1.65
Climbers	3 (3.8%)	4 (1.4%)	6 (1.3%)	2	1.5
Grasses	2 (2.6%)	26 (9.4%)	40 (8.4%)	20	1.54
Sedges	1 (1.3%)	4 (1.4%)	14 (3%)	14	3.5
Total	78	276	474	6.08	1.71

when environmental condition is inflammable and habitat suitability is high. In such conditions number of individuals is high.

Dominant and Monotypic families and genera : Among higher taxa (angiosperms and gymnosperms) Compositae dominated with 30 genera and 49 species, followed by Poaceae with 25 genera, 39 species and Rosaceae with 16 genera and 39 species (Fig. 1). Similar observation was made for the overall Western Himalaya in an earlier study by Samant (2015). The family Lauraceae (5 genera, 7 species) was dominant followed by Betulaceae and Rosaceae in tree taxa (Fig. 2). Bhatt et al (2020) have also reported family Lauraceae as the most dominant family among trees in IHR. In shrubs, family Rosaceae (7 genera, 17 species) was the dominant family, followed by Berberidaceae and Poaceae (Fig. 3). Mehta et al (2019) reported family Rosaceae as a second dominant family in shrubs for the entire Western Himalayan states. In herbaceous vegetation, family Compositae (30 genera, 49 species) was the dominant family followed by Poaceae and Orchidaceae (Fig. 4).

Among the genera, *Carex* (10 spp.) and *Potentilla* (10 spp.) were the species rich genera followed by *Anaphalis*, *Impatiens*, *Persicaria*, *Berberis*, *Rubus* in the entire high altitude forested zone (2000-3300 m). Analyzing dominant genera for different life forms, genus *Quercus* (4 spp.) in trees; *Berberis* (7 spp.) and *Rubus* (7 spp.) in shrub; *Potentilla* (10 spp.), *Anaphalis*, *Impatiens* and *Persicaria* (8 spp. each) in herbs; *Poa* and *Stipa* (6 spp. each) in grasses, *Carex* (10 spp.) in sedges and *Clematis* (2 spp.) in climbers were recorded dominant genera (Table 4). *Carex* and *Potentilla* were the dominant genera from the study site for Uttarakhand (*Carax*-74 and *Potentilla*-46) and Western Himalaya (*Carax*-99 and *Potentilla*-71) by Samant (2015).In the region there were 27 families which were represented by only one genus and species (termed as monotypic families) and 192 genera represented by single species (termed as monotypic genera (Table 5). Such families and genera are likely to have either a poor genetic base or were recently introduced to the area. The four families which were recorded monotypic in the present study were Daphniphyllaceae, Myricaceae, Paeoniaceae and Saururaceae are also reported monotypic families for the entire Western Himalaya (Samant 2015). This indicates a poor genetic base and recent speciation of these four families in this Himalayan zone.

Distribution of native and endemic species (Endemicity and Nativity): The present study showed a high nativity and

Table 2. Representation of floristic diversity across different plant groups and life forms.

Plant groups	Sub-divisions	Number of life forms recorded in extensive survey																				
		Total numbers			Woody vegetation						Herbaceous vegetation											
					Trees			Shrubs			Herbs			Climbers			Grasses			Sedges		
		F	G	S	F	G	S	F	G	S	F	G	S	F	G	S	F	G	S	F	G	S
A	Dicots	66	207	365	20	32	40	14	26	49	40	161	265	3	4	6	-	-	-	-	-	-
	Monocots	12	69	109	-	-	-	2	5	5	8	34	50	-	-	-	2	26	40	1	4	14
G		3	4	5	2	3	4	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-
Total		78	276	474	22	35	44	17	32	55	48	195	315	3	4	6	2	26	40	1	4	14

A-Angiosperms, G-Gymnosperms, F-family, G-genus, S-species

Table 3. Comparative account of floristic diversity within the Himalayan for

Study site	Altitude zone (m asl)	Numbers of species				Reference
		Total	Trees	Shrubs	Herbs	
Entire Himalayan Timberline zone	>3000	58	58	-	-	Singh et al 2020
Himalaya	>100	10503	-	-	-	Rana and Rawat 2017
Indian Himalayan region	>200	1466	1466	-	-	Bhatt et al 2020
West Himalaya	>200	582	-	582	-	Mehta et al 2019
Western Himalaya (sub-tropical to alpine zone)	>200	>5000	538	900	>4000	Samant 2015
High altitude forests (2000-3500 m) of Uttarakhand	2000-3500	1471	106	233	1133	Rawal et al 2018
Sub alpine region of Tungnath (KWS)	1100-7068	433	13	42	349	Rai et al 2012
Tungnath (KWS)	2000-3300	474	44	57	373	Present study

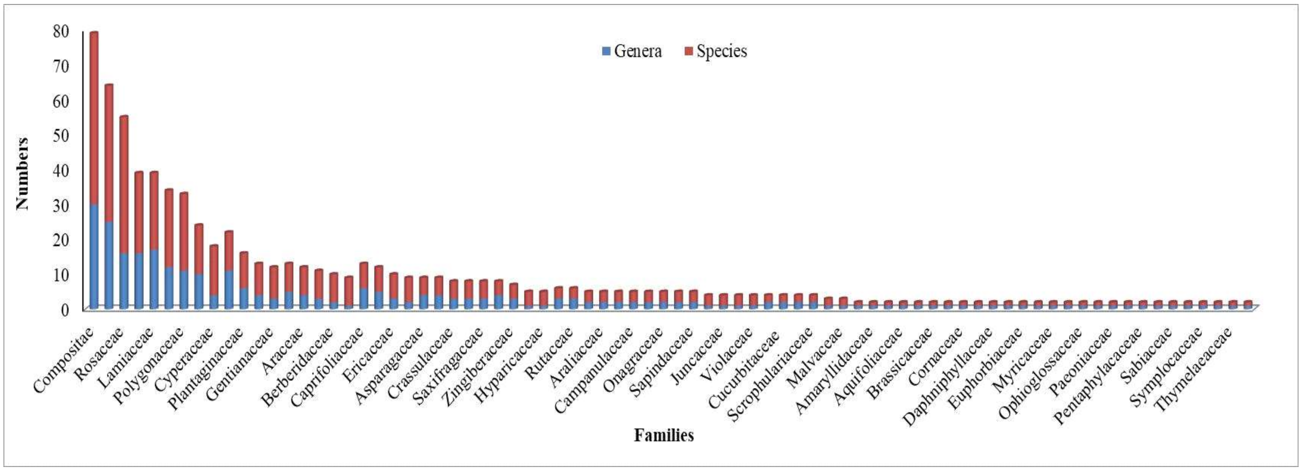

Fig. 1. Number of species and genera distribution among recorded families in the entire high altitude forested zone (2000-3500 m asl)

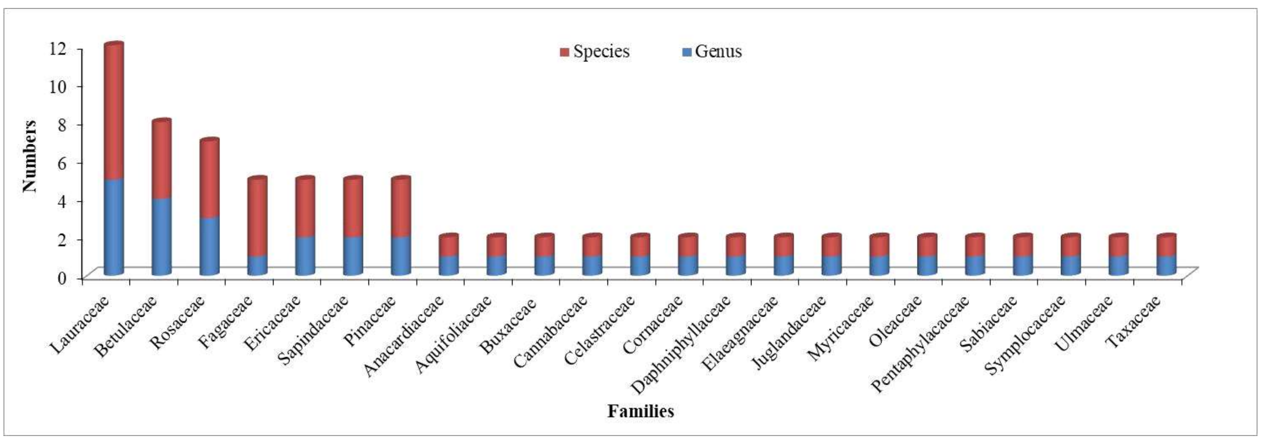

Fig. 2. Number of species and genera distribution among families in tree taxa in the high altitude forested zone (2000-3500 m asl.)

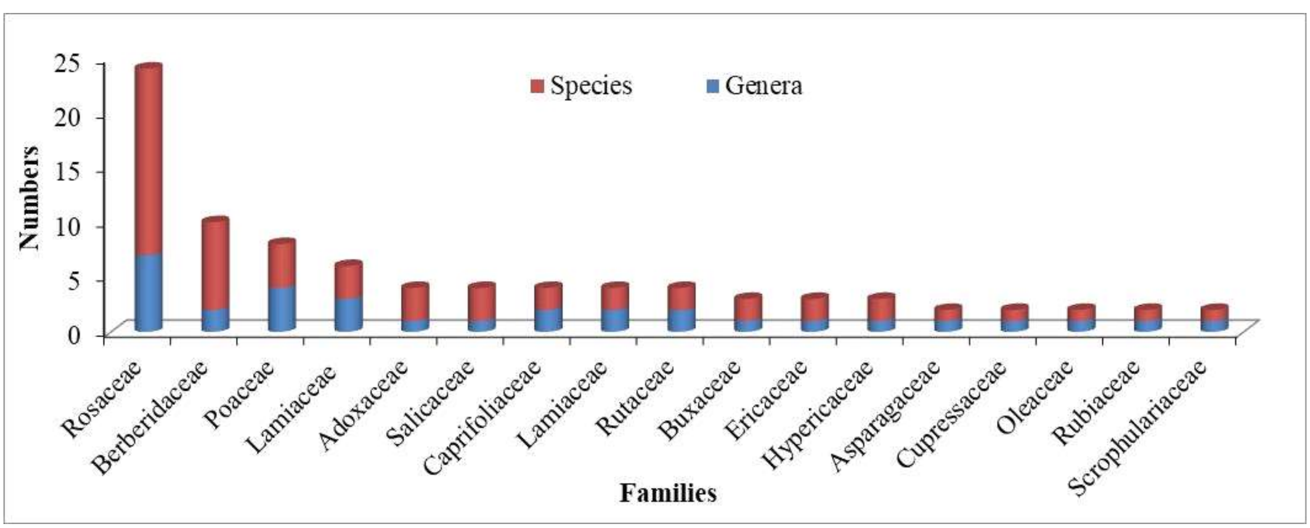

Fig. 3. Number of species and genera distribution among families in shrubs in the high altitude forested zone (2000-3500 m asl.)

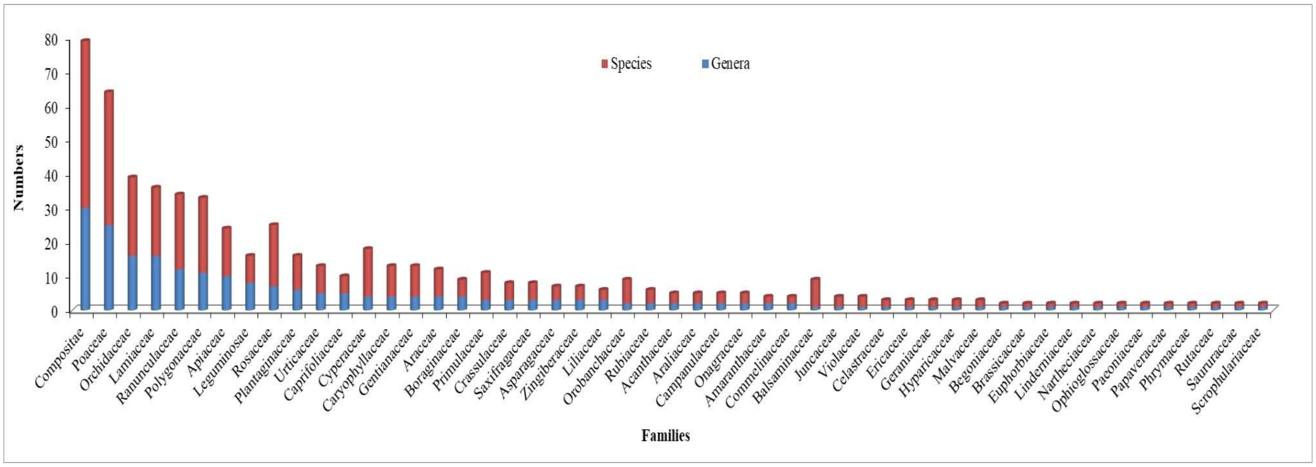

Fig. 4. Number of species and genera distribution among families in herbaceous its life from in the high altitude forested zone (2000-3500 m asl.)

Table 4. Species distribution among dominant genera of high altitude forested zone

Dominant genera	Species numbers	Dominant genera	Species numbers	Dominant genera	Species numbers
Carex	10	*Elsholtzia*	3	*Chaerophyllum*	2
Potentilla	10	*Erigeron*	3	*Clematis*	2
Anaphalis	8	*Galium*	3	*Conyza*	2
Impatiens	8	*Gentiana*	3	*Cynoglossum*	2
Persicaria	8	*Habenaria*	3	*Epilobium*	2
Berberis	7	*Juncus*	3	*Fragaria*	2
Rubus	7	*Neolitsea*	3	*Gaultheria*	2
Pedicularis	6	*Pilea*	3	*Geranium*	2
Poa	6	*Platanthera*	3	*Geum*	2
Stipa	6	*Rhodiola*	3	*Goodyera*	2
Thalictrum	6	Rosa	3	*Hedera*	2
Arisaema	5	*Salix*	3	*Leycesteria*	2
Plantago	5	*Saussurea*	3	*Ligularia*	2
Polygonum	5	*Saxifraga*	3	*Liparis*	2
Primula	5	*Senecio*	3	*Lysimachia*	2
Swertia	5	*Sibbaldia*	3	*Malva*	2
Anemone	4	*Silene*	3	*Myriactis*	2
Hypericum	4	*Viburnum*	3	*Parnassia*	2
Nepeta	4	*Viola*	3	*Polygonatum*	2
Oplismenus	4	*Abies*	2	*Prunus*	2
Quercus	4	*Acer*	2	*Roscoea*	2
Ranunculus	4	*Aconitum*	2	*Sarcococca*	2
Rhododendron	4	*Agrostis*	2	*Scutellaria*	2
Stellaria	4	*Ainsliaea*	2	*Selinum*	2
Bistorta	3	*Angelica*	2	*Strobilanthes*	2
Bupleurum	3	*Artemisia*	2	*Synotis*	2
Calanthe	3	*Bulbostylis*	2	*Taraxacum*	2
Cotoneaster	3	*Campanula*	2	*Urtica*	2

Table 5. Monotypic families and genera recorded in the study site

Monotypic Families

Amaranthaceae, Amaryllidaceae, Anacardiaceae, Aquifoliaceae, Begoniaceae, Brassicaceae, Cannabaceae, Cornaceae, Cupressaceae, Daphniphyllaceae, Elaeagnaceae, Euphorbiaceae, Juglandaceae, Myricaceae, Nartheciaceae, Ophioglossaceae, Oxalidaceae, Paeoniaceae, Papaveraceae, Pentaphylacaceae, Phrymaceae, Sabiaceae, Saururaceae, Symplocaceae, Taxaceae, Thymelaeaceae and Ulmaceae.

Monotypic Genera

Achillea, Achyranthes, Acomastylis, Aconogonum, Acronema, Actaea, Adenocaulon, Aesculus, Ageratina, Agrimonia, Aletris, Allium, Alnus, Androcorys, Andropogon, Androsace, Aquilegia, Ariopsis, Arthraxon, Aruncus, Asparagus, Aster, Astragalus, Barleria, Begonia, Bergenia, Betula, Boenninghausenia, Botrychium, Buddleja, Buxus, Calamagrostis, Caltha, Caragana, Cardamine, Carpesium, Carpinus, Cautleya, Cedrus, Celtis, Cephalanthera, Cerastium, Chrysopogon, Chrysosplenium, Circaea, Cirsium, Clinopodium, Commelina, Cornus, Corydalis, Corylus, Cremanthodium, Cyananthus, Dactylorhiza, Daphne, Daphniphyllum, Delphinium, Desmodium, Digitaria, Doronicum, Drepanostachyum, Elaeagnus, Elatostema, Elymus, Epipactis, Eragrostis, Euonymus, Euphorbia, Euphrasia, Eurya, Fagopyrum, Fallopia, Festuca, Fraxinus, Gagea, Galinsoga, Gerbera, Gnaphalium, Gypsophila, Hackelia, Halenia, Hedychium, Helictotrichon, Hemiphragma, Herminium, Himalayacalamus, Houttuynia, Hydrocotyle, Hylodesmum, Ilex, Indegofera, Isodon, Jasminum, Juglans, Juniperus, Jurinea, Kashmiria, Kobresia, Lactuca, Lamium, Lecanthus, Leptodermis, Lespedeza, Lindenbergia, Lindera, Lindernia, Litsea, Lonicera, Lyonia, Machilus, Maharanga, Mahonia, Maianthemum, Malaxis, Mazus, Meliosma, Mentha, Micromeria, Microstegium, Momordica, Morina, Muhlenbergia, Murdannia, Myrica, Nardostachys, Neobrachyactis, Ohwia, Ophiopogon, Oreorchis, Origanum, Oxalis, Oxyria, Paeonia, Parochetus, Pennisetum, Perilla, Persea, Phlomis, Pimpinella, Piptanthus, Piptatherum, Pleione, Pouzolzia, Prinsepia, Prunella, Pseudognaphalium, Pseudomertensia, Pyracantha, Pyrus, Reinwardtia, Remusatia, Rheum, Rhus, Rhynchospora, Rosularia, Roylea, Rubia, Rumex, Salvia, Sanicula, Satyrium, Sauromatum, Sedum, Setaria, Shuteria, Skimmia, Solidago, Sorbus, Spiranthes, Sporobolus, Stachys, Streptopus, Symplocos, Tanacetum, Taxus, Tenaxia, Thamnocalamus, Thymus, Trachydium, Tragopogon, Trifolium, Triplostegia, Tripogon, Ulmus, Valerian, Verbascum, Veronica, Vicatia, Wulfeniopsis, Yushania, Zanthoxylum and *Zehneria*

Table 6. Representation of nativity and endemism across different life forms

Life forms		Himalayan endemic		Native	Non-Native
		Endemic	Near- endemic		
Woody vegetation	Trees	2 (4.55 %)	13 (29.55 %)	33 (75 %)	8 (18.18 %)
	Shrubs	2 (3.64 %)	16 (10.91 %)	45 (80 %)	11 (20 %)
Herbaceous vegetation	Herbs	15 (4.67 %)	85 (26.50 %)	223 (69.47 %)	95 (29.60 %)
	Climbers	0	0	4 (1.23 %)	2 (1.47 %)
	Grasses	1 (2.5 %)	2 (5 %)	13 (32.5 %)	14 (35 %)
	Sedges	1 (7.14 %)	3 (21.43 %)	7 (50 %)	6 (42.86 %)
	Total	21	119	325	136

endemicity in the representative high altitude forested zone. Of the total recorded species, 325 (68.57%) were native (herbs- 223, 69.47%, shrubs- 45, 80% and trees- 33, 75%) and the remaining species were non-native representing various biogeographic provinces of the globe. Out of total recorded species 140 (29.54%) were Himalayan endemic (includes endemic species- 21, 4.43% and near endemic species- 119, 25.11%), when the endemism was seen among different life forms in herbs-100, 31.15%, shrubs-17, 30.91% and trees-15, 45.45% were recorded endemic to the Himalayan region, representing a good number and percent of Himalayan endemic species. This higher endemism of species might be governed by difficult terrain present in the region (Vetaas and Grytnes 2002). The occurrence of high number of endemic and native species in the present study site indicates a good health of ecosystems in the high altitude forested zone and they have high conservation values (Samant 2015). However, there is tendency of increase in non-native species because of anthropogenic activities, which is a disturbed sign. Among the various life forms, maximum percent of endemic species were recorded in herbs 4.67% (15 spp.), near-endemic in trees 29.55% (13 spp.), native in shrubs 80% (45 spp.) and non-native in sedges 42.86% (6 spp.; Table 6)

CONCLUSION

Among the various forest types of the Himalaya the high altitude forested zone have their own unique identity as an exchequer of multifarious evergreen and deciduous species along with the various species association which have high ecosystem values. In the present study the representative high altitude forested zone covers 32.22% of vascular plant species out of reported species diversity for the high altitude forested zone (2000-3500 m asl) of Uttarakhand and has a good proportion of native (68.57%) and endemic (29.54%) species of the Himalaya, highlighting the significant contribution of this forested zone in the regional flora. Many of the recorded species are important for medicinal, fodder, fuel etc. purposes and the local inhabitants are fully or partially dependent in these forests from centuries. Furthermore, having such number of plant species diversity within this forested zone, it is critically important for the ecotone studies (timberline, treeline etc.) as well, as it is sensitive to the climate change and recently the habitat degradation, heavy anthropogenic activities, tourism has created a big challenge to the biological diversity, ecology and conservation biology within the region. This investigation suggests the conservation and management of this high altitude forested zone for having rich pocket of Himalayan plant species.

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Endangered Liana of the Western Ghats, *Coscinium fenestratum* (Gaertn.) Colebr: An Overview

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Abstract: *Coscinium fenestratum* (Gaertn.) Colebr. is a rare and endangered liana present in the Western Ghats, which is recently gaining greater importance in the pharmaceutical sector due to its medicinal properties. Globally, this species is distributed in India, Sri Lanka, Taiwan, Cambodia, Vietnam, Peninsular Malaysia, Western Java, and Borneo. Medicinal plants are important components of the biodiversity of the Western Ghats. The high anthropogenic pressures and associated fragmentation of natural forests have resulted in the loss of habitat and species. Increasing public interest in phytochemical-based medicine along with rapid expansion in pharmaceutical industries has resulted in the increase in demand for medicinal plants like *Coscinium fenestratum* resulting in a major threat due to over-exploitation from natural habitats in the absence of cultivation. This review article discusses the background of this endangered species of Western Ghats to create awareness of the conservation and research of such an important natural resource in the country.

Keywords: Rare, Endangered, Fragmentation, Anthropogenic

Since time immemorial, different classical medicinal systems such as Ayurveda, Siddha and Unani are being practiced in the country along with innumerable local folk medicinal traditions. The rich medicinal plants used in such systems are mainly distributed in two hot spots of diversity - Northeastern region and the Western Ghats. The Western Ghats cover a mere 5 per cent of the country's total land area in the country, it is believed to be more than 27 percent of the country's plant species which is a remarkable high level of endemism ranging from 25 to 60 per cent of recorded species. *Coscinium fenestratum* (Gaertn.) Colebr is a woody climber which grows wild in the natural rainforest reserves in Sri Lanka and India. This species is listed as endangered in the IUCN Red Data Book and in the Convention on International Trade in Endangered species of wild Fauna and Flora (CITES) listing because of its large-scale harvesting for medicinal use. *Coscinium fenestratum* is a slow growing liana which takes 15 years to reach its reproductive stage. But due to its huge demand for industrial consumption, it gets chopped down before it is fit to regenerate, and also the traders directly engage tribes and other collectors for the supply of the raw drug. The combination of rampant destruction of the forests along with over-exploitation of the species for the raw drug market and very slow rate of regeneration has seriously depleted its population (Tushar and Udayan, 2005).This belongs to the order Ranunculales, and family Menispermaceae.

Geographical distribution and status of *Coscinium fenestrstum*: *C. fenestratum* is a dioecious and a large woody climber of the family Menispermaceae, indigenous to the Indo-Malayan region. In India, it is restricted to the Western Ghats, mostly in the high rainfall wet evergreen forests, moist evergreen, semi-evergreen and semi-deciduous forests at 500-750 m altitude (Mohanan and Sivadasan 2002). In India, *C. fenestratum* is found in Kerala, Tamil Nadu, and some regions of Karnataka. In Kerala, natural populations have been identified in Thiruvananthapuram, Thrisuur, Malappuram, Palakkad, Kollam, Idukki, Waynad Kannur and Kozhikodi districts (Udayan et al 2004). Only a tiny band of high rainfall hilly slopes of the central Western Ghats in Karnataka was predicted to be highly suitable for the species, suggesting a high habitat-specificity and restricted distribution of *Coscinium fenestratum*. A total of 163 adult individuals and 975 regenerating individuals were enumerated in all the eight natural populations studied in three populations both adult and regeneration structures were healthy; large deficiencies of higher size class of adults and of individuals in class I (< 40 cm height) regeneration were observed in other five populations (Thriveni et al 2015). Due to a lack of information on the population size, trend, and threats in Cambodia, Vietnam, and West Malaysia, as well as information on whether this species is wild or introduced in these countries, this species is categorized as Data Deficient. Further research is needed on the native distribution of this species and on the population size, trends, and threats in Cambodia, Viet Nam and West Malaysia (Ved et al 2015). The threat status of this species has been assessed as Critically Endangered for Karnataka, Kerala and Tamil Nadu in India, due to a more than 80% decline in the wild populations over the last 30 years (Ravikumar and Ved 2000).

Morphology and anatomy of liana: Malamanonmani and Mehalingam (2019) studied the transverse section of the leaf of *Coscinium fenestratum* and reported the presence of a circular hyaline hypodermal layer with three-layered parenchymatous cells. The presence of thick-walled parenchyma and small isolated calcium oxalate crystals were also observed in the cortical region. The anatomy of the stem revealed the occurrence of 8 to 10 parenchymatous layers in the cortical zone and thick-walled sclerenchymatous zone with lignification.

Germination and dormancy of seeds: Senerath (1991) provided best method of fruit collection and seed germination in *C. fenestratum* in populations of Sri Lanka and ripe fruits must be depulped and exposed to direct sunlight for six hours to increase germination. Ramasubbu et al (2011) conducted an experiment to analyze the biological characters and germination rate of seeds of *C. fenestratum* in the natural as well as in the laboratory condition. The fresh seeds showed 65% viability and 28% germinability. However, the seeds pretreated with GA3 (1000-4000 ppm) showed 55 to 70% germinability and 79% of the seed germination was observed after 6 months. The temperatures above 30°C and dark conditions facilitate germination of the mature seeds after split opening the hard seed coats by exposing them to direct sunlight for 6 hours followed by dipping the seeds in 2250mg/L GA_3 solution for 24 hours to reduce the time taken for germination by removing inhibitory chemicals, facilitating embryo growth and reducing inherent ABA/GA_3 ratio (Warakagoda et al 2014). Bhat (2015) investigated the seed germination of five medicinal plants of Western Ghats. In case of *C.fenestratum*, a total of 91.25% germination was recorded with the highest of 42.5% in the 4th week. Anilkumar et al (2010) observed that the germination of seeds of the liana was enhanced to 93% as the initial seed moisture content was reduced to 10% upon exposure to open laboratory conditions. When fresh seeds were pre-treated with 2 to 10% KNO_3 or GA_3 3000 ppm, the germination was enhanced to 95% respectively. Seeds kept exposed for two months at laboratory conditions lost their viability, while the seeds stored for four to six months inside the polycarbonate bottles expressed 90% germination within a month of sowing.

Medicinal uses of the liana: The stem of *C.fenestratum* has long been used in South India and Sri Lanka as a yellow dye and bitter tonic and in Europe under the name False Calumba or Tree Turmeric. The roots and stem contain alkaloids berlambine, dihydroberlambine, noroxyhydrastine, berberine, etc. The medicinally active compound of *C. fenestratum* is berberine, an isoquinoline alkaloid with numerous bioactivities (Birdsall and Kelly1997). Ashalatha and Gopinath (2019) analysed the leaf and stem samples of *C. fenestratum* by High-performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) and the compounds identified were berberine, jatrorrhizine, palmatine, tetrahydropalmatine, tetrahydroberberine, magnoflorine, isocorydine, glaucine and ecdysterone which is a plant sterol compound. Phytochemical investigation on *C.fenestratum* (Gaertn.) Collebr, revealed the presence of significant amounts of ecdysterone in the stem (0.22%) and leaves (0.12%), in addition to berberine. Ecdysterone was characterized using High-Performance Liquid Chromatography (HPLC), Infrared Spectroscopy (FT-IR), and Liquid Chromatography-Mass Spectroscopy (LC-MS). Isolation of this multi- functional bioactive compound will throw light on the chemical basis for the various pharmacological effects of *Coscinium* plant extract. Boberok

Source: Tushar et al (2008)

Fig. 1. *Coscinium fenestratum*

et al (2010) mentioned that Berberine had a weak activity against Gram-negative bacteria and is more potent against Gram-positive bacteria, including *Mycobacterium tuberculosis* and MRSA (Methicillin-Resistant *Staphylococcus aureus*) by the MDR pump NorA inhibition. Wang et al (2019) observed the impact of berberine on human hepatocarcinoma cell survival, demonstrating that its anti-tumor properties could be mediated both by apoptosis and autophagy. Pongkittipha et al (2015) reported that berberine showed a better direct-antioxidant activity of the derivatives containing phenolic groups than berberine in a cell-free system. For cell-based system, berberine was able to exert better cytotoxic activity than its derivatives. Berberine derivatives containing a single and four phenolic groups showed improved up-regulation of SOD gene expression. Tran and Stefan (2001) reported that the old parts or roots of the medicinal *liana C. fenestratum* was crushed and boiled for drinking in case of stomachache in the buffer zone of Bach Ma National Park, Vietnam. Shirwaikar et al (2005) concluded that alcoholic stem extract of *C. fenestratum* effect carbohydrate metabolism and observed the antioxidant status in streptozotocin–nicotinamide induced type 2 diabetic rats. Oral administration of *C. fenestratum* stem extract in graded doses caused a significant increase in enzymatic antioxidants such as catalase, superoxide dismutase, glutathione synthetase, peroxidase, and glutathione peroxidase and in the nonenzymatic antioxidants ascorbic acid, ceruloplasmin and tocopherol. The medicinal plants used by Kaadar tribe in Thrissur, Kerala were studied by Udyan et al. (2005) which revealed that the mature stem cuttings of *C. fenestratum* a liana of the family Menispermeaceae were boiled in water and taken internally against jaundice and joint pain. Among the different extracts of *C.fenestratum*, methanolic stem extract showed moderate activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Betula subtilis* . Methanol leaf extract had maximum activity against *Staphylococcus aureus*) and lowest against *B. subtilis* respectively (Goveas et al 2013). Preliminary phytochemical screening of *C.fenestratum* fruit extracts revealed the presence of alkaloids phenols, flavonoids, tannins, steroids, and resins, which are responsible for biological properties. The combined aqueous and methanol extract resulted in significant anthelmintic and antioxidant properties in a dose-dependent manner. The anthelmintic activity test was carried out against *Pheretima posthuma* and *Taenia solium* with the extract at varying concentrations of 25, 50, 100 and 150 mg/mL and compared with standard albendazole (25 and 50 mg/mL) and saline (0.9%) as a control. All the extracts exhibited concentration-dependent paralytic effect and a significant activity was observed with the combined methanol and aqueous extract (Das et al 2018).

Variation in berberine content: Yields of berberine content in crude extracts of *C. fenestratu* between 9.87-16.38% dry weight while Berberine content in the dried powder and in the crude extract was in the ranges of 1.71-2.89% w/w and 11.84-18.45% dry weight, respectively in a study. (Rojsanga and Gritsnapan., 2005). The recovery of standard berberine was 97.58-98.71% (%RSD = 3.85), and the limit of detection and quantitation were 25 and 50 ng/ spot, respectively. Eighty percent ethanol gave a higher content of berberine than 50% ethanol. Berberine contents from maceration, percolation and Soxhlet extraction with 80% ethanol were 3.37, 3.08 and 2.67 % w/w, respectively (Rojsanga et al., 2006). Khan et al. (2008) reported for the first time the role of phytohormones on the production of berberine in *C. fenestratum* from in vitro calli cultures. Berberine with the retention time of 8.49 min and enhanced dry weight (1.788%) from the petiole explant has been reported for the first time in this study. The presence of berberine was first checked by preparative thin layer chromatography (TLC) and then confirmed by High-Pressure Liquid chromatography (HPLC) and mass spectrometry. The root and stem bark samples of *Berberis asiatica* from lower altitudes, having a larger size class contained more Berberine. The Berberine concentration was highest in summer and lowest in the rainy season. Low level of soil moisture and higher level of soil potassium produced higher Berberine concentrations (Harish et al 2011). Method of extraction and method of drying significantly influenced the Berberine yield in *C. fenestratum*. Methanol cold extraction is the best and efficient method for the highest product recovery. Highest yield of Berberine (4.06 % w/w) was obtained in the shade dried samples followed by the sundried samples (3.21% w/w) extracted with cold methanol (Babu et al.,., 2012). The average berberine content irrespective of age, sex and tissues ranged between 0.64 to 3.01 %. Out of 45 adult individuals, 18 individuals yielded more than 5 % of berberine in the root samples. Further, the herbivore attack resulted in a significant increase in the berberine content of leaves. (Thriveni et al 2017).

Improvement of Berberine through selection and clonal multiplication: Nair et al (1992) conducted an experiment to study Berberine synthesis in callus and cell suspension cultures of *Coscinium fenestratum* which were established from sterile petiole segments on MS medium, supplemented with 2,4-dichlorophenoxy acetic acid (2,4-D) and benzyl amino purine (BAP). The cells in the culture produced Berberine as the major compound. NAA stimulated the product synthesis over 2,4-D and the presence of light enhanced the Berberine synthesis. The root samples collected from Sarhan provenance had the highest Berberine content. Practically all the sources of *Berberis aristata* suppressed more than 50% fungal growth of *Rhizoctonia solani* and less than 50% in the cases of *Colletotrichum* and *Fusarium* (Rashmi et al 2009).

Genetic diversity: Random amplified polymorphic DNA (RAPD) markers were used to assess the genetic diversity of 14 individuals belonging to seven populations of *C. fenestratum* (Gaertn.) Colebr. (Menispermaceae). 18 decamer primers used for the analysis generated 99 scorable bands of which 79 were polymorphic. Coefficient of similarity ranged from 0.6604 to 0.9809. Variation within population was slightly higher than between populations. The similarity between individuals within and between populations was found (Narasimhan et al 2006). Thriveni et al (2013) observed that the species, *Coscinium fenestratum* exhibited a moderate to low level of intra population genetic diversity among seven populations of Central Western Ghats. The population had weak structure (K = 2) with one single widespread gene pool indicated that gene flow and inbreeding have been likely the major driving force in shaping the current population genetic structure of *C. fenestratum*.

Recent advancement in sexual methods of reproduction: *In vitro* propagation study was undertaken to produce the multiple shoots in *Coscnium fenestratum.* Murashige and Skoog (MS) medium supplemented with 1.0 µM kinetin (Kin) and 0.25 µM 2,4-Dichlorophenoxy acetic acid (2,4-D) was used for the epicotyl explants. From each explant, maximum five shoots were obtained. It was reported that repeated subculture favoured the increase in shoot length and the number of shoots per explant in the media containing Kin and 2,4-D.The higher concentrations of either cytokinin used: butyric acid (BA) or Kin caused stunting of multiple shoots with small and narrow leaves (Senarath 2010). Warakagoda et al (2017) reported that in case of *Coscinium fenestratum*, mature double nodal cuttings resulted in the highest shoot proliferation rate (3.90 shoots/explant) when cultured on WPM medium supplemented with 2.0 mgL^{-1} 6-benzylaminopurine,1.0 mgL^{-1} thidiazuron and 0.4 mgL^{-1} 2,4-dichlorophenoxyacetic acid. Shoots were separated and transferred to WPM medium devoid of plant growth regulators for regeneration into plantlets and gave over 60 % survival rate. Karthika et al (2019) in a study *Coscinium fenestratum* reported that the nodal explants when inoculated on MS medium and supplemented with BAP and TDZ at 2.0 and 1.0mg/L respectively produced high frequency of shoots (91.01%) and the same shoots subcultured on half strength MS medium supplemented with IBA alone at 0.6mg/L produced high frequency of roots (97.42%). Renuka et al (2019) revealed that for direct regeneration of liana, the ideal explant was shoot tip with 20µM/l Kinetin + 0.25µM/l 2,4-D & 20µM/1 BAP + 0.25 µM/1 2,4-D and for indirect regeneration, petiole with 6µM//l 2,4-D+. Petiole and leaf explants of *Coscinium fenestratum* were induced to form callus when cultured on vermicompost extract media along with coelomic fluid. Vermicompost medium and vermicompost extract medium with coelomic fluid showed the maximum (100 per cent) callus induction. Light induced the darkening of the callus tissue. The suspension media turned deep yellow because of the release of alkaloid, which was extracted with 80 per cent ethanol and subjected to Dragendrof's reagent which showed fluorescent colour under UV which confirmed positive test for berberine (Kashyap et al 2016).

CONCLUSION

Herbal remedies have become more popular in the treatment of variety of ailments. Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today, face either extinction or loss of genetic diversity. Reserves of herbs and stocks of medicinal plants in developing countries are diminishing and in danger of extinction as a result of growing trade demands for new plant-based therapeutic markets. As the demand for medicinal plant is growing, some of them are increasingly being threatened in the natural habitat. *C. fenestratum* is one among the families having several medicinal values from root to fruit. Because of its medicinal uses in Ayurveda, Unani and Siddha systems of medicine, collection of wild plants from natural habitat has made the species endangered. Therefore, the present review attempts to create awareness for the protection of such genetically rich source in the country.

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Evaluation of the Phytomass of the *Pistacia lentiscus L*. for Rational Exploitation of Natural Resources in A Peri-Urban Forest of Algiers

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***Abstract*:** In Algeria, the *Pistacia lentiscus* L. is widely used in different industries. Its uncontrolled exploitation threatens its sustainability. In the peri-urban forest of Baïnem, the most important one in the capital Algiers and are witnessing degradation for several years by a strong exploitation for the benefit of small and medium-sized enterprises. This exploitation does not follow any sustainable management strategy. We measured the production of phytomass and essential oil in order to develop a rational management protocol for the exploitation of the lentisk pistachio tree. Ten productive facies are identified based on their floristic compositions, structures, and dynamic states: 4 matorrals and 6 types of reforestations. A significant variability of phytomass and essential oil production is noted between the different facies according to the density of the undergrowth. Exploitation classes are identified according to the facies and their respective productions. This protocol could be applied in the Mediterranean forests of North Africa.

Keywords: *Pistacia lentiscus* L., Aerial phytomass, Essential oil, Peri-urban forest, Baïnem, Algiers

Algiers, the capital of Algeria in continuous development, tends to be a large metropolis and natural spaces are reducing. The peri-urban forest of Baïnem remains the most important green space (Plate 1) and endowed with a much diversified biodiversity that conceals threatened species such as Onopordum Algerians (Munby) Pomel (Djelid and al 2020). The biodiversity it contains is an important component in studying the functioning of all natural and human made ecosystems (Pandiarajan and al 2019). This forest ecosystem contributes directly and indirectly to the well-being of the Algerian population. It is a forest that has always been historically exploited for its wood, its medicinal, and food species including the *Pistacia lentiscus* L. of family Anacardiaceae, and widespread in the Mediterranean basin, growing in the wild in scrub (maquis) and garrigues in all types of soils, although it prefers siliceous grounds (Bereksi Reguig and al 2021). Common in the thermo-Mediterranean zone (Benmehdi and al 2013). Furthermore, *Pistacia lentiscus* L. has been known for its medicinal properties since ancient times (Beldi and al 2021). Also called the mastic tree, its resin was used to make chewing gum for gums, for the relief of abdominal pain, stomach ache, dyspepsia, and peptic ulcer (Soulaidopoulos and al 2022), for the treatment of gastrointestinal disorders (Vasiliki and al 2020). Essential oil is one of the main components reported for different parts of the Pistacia species (Bozorgi and al 2013). The collection and the anarchic uprooting of the *Pistacia lentiscus* L. constitute a serious threat to the habitats which it forms and which welcome a diverse fauna. Its formations have suffered a strong degradation for the production and marketing of essential oil. The exploitation is not subject to any regulation. To our knowledge, no study is reported of the exploitation of the *Pistacia lentiscus* L. nor any measure to make it less anarchic. This has led us to undertake an estimate of production to have data that can develop a regulation and a specification of exploitation for the preservation of the resource. The study aims at estimating the potential quantity of the phytomass of the *Pistacia lentiscus* L. to be taken without affecting the biological potential of the shrub at the level of the various facies previously recognized. It also aims to facilitate the respect of its exploitation charges by the operators by defining production classes according to the facies.

MATERIAL AND METHODS

Study area: The study was conducted in the peri-urban forest of Baïnem, which has a public domain status and is subject to the forestry regime and managed by the General Directorate of Forests, under the Ministry of Agriculture. It covers an area of 504 ha, in the Bouzaréah massif (Fig. 1).

Located 15 kilometers west of Algiers, in a coastal position less than 1 kilometer from the Mediterranean Sea. It represents the largest natural green space in the city of Algiers. The bioclimate is subhumid with hot winter. The highest point of the forest reaches 320 meters. Geologically, the terrain is diverse with the presence of metamorphic rocks (schists, micaschists, gneiss) (ISL-BRGM 2006). The Baïnem massif presents various natural forest and pre-forest formations (matorrals) with different facies based on *Pinus halepensis* Mill. and *Quercus suber* L. There are also plantations occupying a large area mainly with *Eucalyptus camaldulensis* Dehnh., *Eucalyptus gomphocephala* DC. , *Eucalyptus cladocalyx* F. Muell., *Eucalyptus leucoxylon* F.Muell. and *Acacia decurrens* (J.C.Wendl.) Willd.

Plate 1. Overview of the peri-urban forest of Baïnem

Fig. 1. Baïnem forest location map

Study method: The evaluation of the biomass of *Pistacia lentiscus* L. was done in spring, a period coinciding with the maximum production. Two methods were used, one direct, called destructive, and the other indirect, called allometric. These two methods lead us to measure the phytomass of the considered species. To do this, we first carried out 14 phytoecological surveys (Fig. 2) with an area of 400 m^2 representative of each visually recognized facies. In a second step, we stretched, in each survey area, a 20 m line to estimate the global cover. The latter will be used in the application of the indirect method by determining the measured cover-phytomass relationship.

Direct method: Along the 20 m line, we chose 10 m^2 to make the cuts on 50% of the surveys. To minimize the destructive effects of the method, a previous experiment where several sampling rates were tested on this species, led to determining the rate of 75% as a potential sampling threshold to not harm the good development of the sampled subjects and collected 75% of the plant material (twigs and leaves without fruit) from all the *Pistacia lentiscus* L plants on a 10 m² area. The aerial phytomass represents the quantitative expression of the vegetation at a given time and place, in the 10 m² of each plot with cutting (Nedjraoui and Touffet, 1994). The cut vegetation is sorted, possibly by separating living material from dead material or by separating leafy branches from wood. Once sorted, weighed on-site to determine its

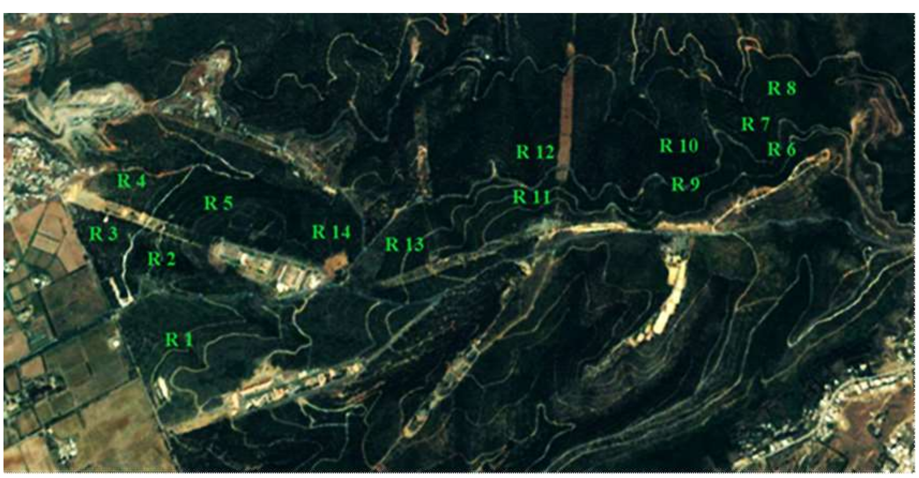

Fig. 2. Map of the location of the sampling plots

fresh weight. We then take a sample of known green weight, which will be placed in an oven at a temperature of 75-80°C for drying, for 48-72 hours (until constant weight) to obtain the dry matter (DM) weight following the formula (Honvou and al 2018) :

$$DM\ \% = \frac{\text{Dry weight of the sample}}{\text{Fresh weight of the sample}} \times 100$$

Indirect or allometric method: This technique is of very common use for the evaluation of phytomass in tree formations (Clifford and al 2013), allows the evaluation of phytomass using parameters that have a significant correlation with it. Based on the cover and the structure of the mastic bushes of the stations used for the direct method, we measured the cover of the species using the line method to estimate the biomass (Long and al 1970). The latter consists of setting up a device consisting of a graduated line represented by a 20 m long ribbon and taking readings along this line by pointing a needle at the elements of the soil surface and vegetation with a10 cm spacing between two readings. The frequency of a feature was recorded at 200 points which allowed for the measurement of vegetation cover and other soil surface features (Fig. 3).

Production of pistachio oil: The extraction of the essential oils was carried out by hydrodistillation of 100 g of samples of the leaves of the *Pistacia lentiscus* L during 2 h 30 mn using a system of "Clevenger" (Clevenger 1928). The distillation lasts three hours after the appearance of the first drop of distillate at the exit of the steam condensation tube. The essential oil content, expressed in ml of the distillate per 100 g of dry matter, is expressed by the following relationship.

THE= (V/DMX100) ± (ΔV/DM100)

THE: Content of essential oils, V: Volume of essential oils collected (ml), ΔV: Error on the reading, DM: Dry plant mass (g).

RESULTS AND DISCUSSION

The analysis of the phytoecological and linear surveys indicated 10 potentially productive facies. These facies are represented by 4 types of matorral and 6 types of plantation. These two physiognomic types cover 79 ha and represent nearly 16% of the total surface of the peri-urban forest of Baïnem (Table 1). The total production of phytomass exploitable area is estimated at 181485, 52 kg.dm with a production of essential oils of about 40 kg, the plantations produce 21, 52 kg while the natural matorrals produce 18, 64 kg. Phytomass and essential oil production per hectare at the matorral level are higher than at the plantation level (Table 2).

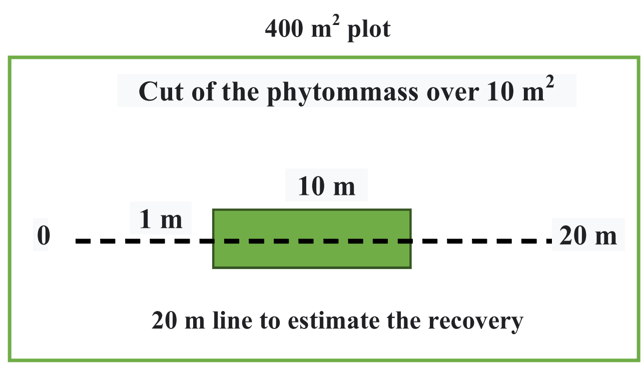

Fig. 3. Sample plot, linear survey, and cut surface

Table 1. Area of potentially productive and exploitable facies of *Pistacia lentiscus* L.in the Baïnem forest

Facies	Area (ha)
Facies 1 : Löw and moderately dense matorral with *Pistacia lentiscus* L., *Cistus monspelliensis*L. and *Olea europea subsp. europaea* L.	10,56
Facies 8 : Low and moderately dense matorral with *Pistacia lentiscus* L., Arbutus unedo L. and *Cistus monspelliensis* L.	5,22
Facies 5 : Low and dense matorral with *Arbutus unedo*,L.,*Pistacia lentiscus* L.,*Olea europea subsp. europaea* L., *Phillyrea angustifolia* L. and *Cistus monspelliensis*L.	3,08
Facies 9 : Matorral low and clear to *Quercus suber* L., *Arbutus unedo* L., *Pistacia lentiscus*L. and *Cistus monspelliensis* L.	9,12
Facies 4 : Plantation of *Eucalyptus camaldulensis*Dehnhwith dense undergrowth of *Pistacia lentiscus* L., *Calycotomespinosa* (L.)Link.and Cistusmonspellienis L.	9,04
Facies 3 : Plantation of*Eucalyptus bosistoana*F.Muell. a light undergrowth of *Oleaeuropea subsp. europaea* L., *Pistacia lentiscus*L.and *Cistusmonspeliensis*L.	4,2
Facies 10 : Plantation of *Eucalyptus cladocalyx*F.Muellwith moderately dense undergrowth of *Pinus halepensis*Mill. , *Pistacia lentiscus* L .,*Arbutus unedo*L. and *Myrtus communis* L.	1,72
Facies 6 : Plantation of *Pinus halepensis Mill* with a light undergrowth of*Olea europea subsp. europaea L., Arbutus unedoL.*and*Pistacia lentiscus L.*	5,84
Facies 2: Plantation of *Eucalyptus bosistoana*F.Muell.With moderately dense undergrowth of *Quercus suber* L.,*Olea europea subsp. europaea*L.,*Quercuscoccifera* L. and *Calycotome spinosa* (L.)Link.	4,32
Facies 7: Plantation with *Eucalyptus gomphocephala*DC.and*Pinus halepensis Mill.*with a light undergrowth of *Quercus coccifera*L.*, Arbutus unedo*L. and*Pistacia lentiscus*L*.*	26,04
Total exploitable area	79,14

Phytomass of *Pistacia lentiscus* L. in the matorrals: The total exploitable phytomass at the level of matorrals was estimated at 84292 kg of dry matter or 46.45% of the total production for an area of 27.98 ha. The quantity of phytomass produced per hectare varies from 4080 kgms/ha for the most productive facies 1 to 1060 kgms/ha for the least productive (facies 9). The phytomass produced by these matorrals can provide up to 18.64 kg of essential oil (Table 3).

Facies 1 covers 13,3% of the potentially productive area and is characterized by low, moderately dense matorral with *Pistacia lentiscus* L., *Olea europea subsp. europaea L.*, *Cistus monspelliensis* L., and *Myrtus communis* L.. This type of formation represents, in the Mediterranean region, regressive stages of forest ecosystems made up of sclerophyllous species that offer a heterogeneous physiognomic diversity (Thompson 2020). Together with facies 8 and 5, it forms formations composed of an undergrowth of species indicative of degradation, and where the *Pistacia lentiscus* L. is abundant ,which explains the high production of phytomass, which exceeds 3800 Kg.dm/ha (Fig. 4).

The matorral with *Quercus suber* L. (facies 9) is characterized by the lowest production compared to the other three and is just over 1000 Kg.dm/ha. This low production is essentially due to the low cover of *Pistacia lentiscus* L.. The presence of this species nevertheless characterizes degraded subterranean forests (Aime, 1976). Excessive logging may intensify the degradation of the undergrowth, the opening of the matorral, and promote therophytization (Barbéro and al 1990).

Phytomass of the *Pistacia lentiscus* L at the level of the plantations: The greatest quantity of the exploitable phytomass of the *Pistacia lentiscus* L. is at the level of the plantations with 97193, 52 Kg.dm distributed on a surface of 51,16 ha that is to say 64,64% of the exploitable surface and which can give more than 21kg of essential oil (Table 4).

Indeed, the plantations at the level of the forest of Baïnem are essentially constituted of Eucalyptus which occupy more than 50% of the total surface of the plant cover. These plantations do not favor the development of the undergrowth, hence a lower phytomass (facies 6, 2 and 7). However, the greatest production of exploitable phytomass per hectare is on facies 4, represented by the *Eucalyptus camaldulensis* Dehnh plantation with a dense undergrowth dominated by *Pistacia lentiscus* L.. This phytomass is evaluated at 5500 Kg.dm/ha or11,4% of the total production, which is explained by the abundance of *Pistacia lentiscus* L., which is in more than 60% of the total surface area of the facies with a coefficient of abundance dominance of 4 according to Braun Blanquet (1932), and where the production of essential oil exceeds 11 kg. These are old Eucalyptus plantations dating from 1956 that were thinned out in the 1990, which allowed the development of *Pistacia lentiscus* L. in abundance after the opening of the tree layer. The presence of *Calicotome*

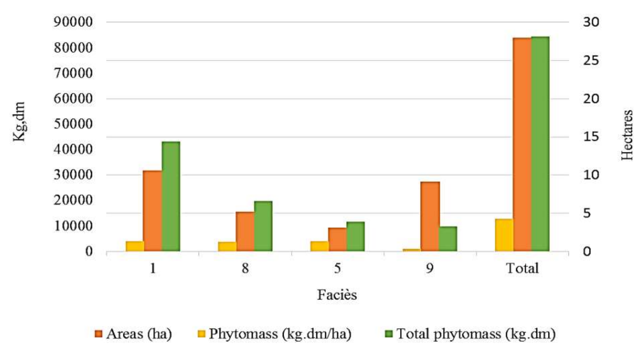

Fig. 4. Phytomass production by facies

Table 2. Usable phytomass and essential oil production of *Pistacia lentiscus* L.at the level of plantations and matorrals

Physiognomic types	Area (ha)	Average phytomass (kg.dm/ha)	Total phytomass (kg.dm)	Average essential oil production (kg / ha)	Total production of essential oils (kg)
Plantations	51,16	2 439,66	97 193,52	0,52	21,52
Matorrals	27,98	3210	84 292	0 ,69	18,64
Total	79,14	5649,66	181485,52	1,21	40,16

Table 3. Phytomass and production of essential oils of *Pistacia lentiscus* L. by facies on the matorral level

Facies	Area (ha)	Phytomass (kg.dm/ha)	Total phytomass (kg.dm)	Essential oil production (kg/ha)	Total production of essential oils (kg)
1	10,56	4080	43084,8	0.98	10.35
8	5,22	3800	19836	0.72	3.76
5	3,08	3900	11704	0.85	2.62
9	9,12	1060	9667.2	021	1.91
Total	27,98	12840	84 292	2.76	18.64

Table 4. Phytomass and production of essential oils of *Pistacia lentiscus* L by facies at the level of the plantations

Faciès	Area (ha)	Phytomass (kg.dm/ ha)	Total phytomass (kg.dm)	Essential oil production (kg / ha)	Total production of essential oils (kg)
4	9.04	5500	49720	1.27	11.48
3	4.2	3700	15540	0.67	2.81
10	1.72	2250	3870	0.51	0.87
6	5.84	1000	5840	0.23	1.34
2	4.32	1600	6912	0.32	1.38
7	26.04	588	15311.52	0.14	3.64
Total	51.16	14638	97193.52	3.14	21.52

Table 5. Phytomass production classes

Phytomass classes (Kg.dm/ha)	Facies	Production (Kg.dm/ha)
I : P> 5000	4	5500
II : 5000 > P > 4000	1	4080
III : 4000 > P> 3000	5	3900
	8	3800
	3	3700
IV : 3000 > P > 2000	10	2250
V : 2000 > P > 1000	2	1600
	6	1000
	9	1060
VI : P < 1000	7	588

spinosa (L.)Link.is the result of the degradation of the subterranean forests to the point of the disappearance of the cork oak and its usual cortège, and the installation of a flora belonging to other degraded groupings. The degradation of this grouping is followed by an opening of the tree and shrub stratum as evidenced by the presence of *Pistacia lentiscus* L . and which also reflects the high and regular frequency of fires.

The *Eucalyptus bosistoana* F.Muell plantation (facies 3) is also characterized by a fairly high phytomass production reaching 3700 Kg.dm/ha. Facies 10, on the other hand, covers the smallest exploitable area and is represented by the *Eucalyptus cladocalyx* F. Muell plantation, producing 2250 kg.dm/ha. It is characterized by a moderately dense undergrowth of *Pinus halepensis* Mill.,*Pistacia lentiscus L.*, *Arbutus unedo* L., and *Myrtus communis* L. Facies 6 has a total essential oil production of 1,34 kg or 0,23 kg/ha. These last facies with facies 2 produce a phytomass equal to or greater than 1000 kg.dm/ha which is much greater than that produced by the mixed plantation of *Eucalyptus gomphocephala* DC. and *Pinus halepensis* Mill.(facies 7). This latter plantation occupies the largest area with 26, 04 ha but with the lowest production, which is around 588 kg.dm/ha. The coverage of the tree stratum formed by Eucalyptus and Aleppo pine means that the development of the undergrowth is very low, hence the low production of the phytomass of the *Pistacia lentiscus* L. The low coverage of the latter is also due to the overexploitation of this species by the pickers (the removal exceeds 75%).

Proposal of exploitation classes: Six classes are defined by the interval of phytomass produced across the different facies (Table 5). This classification is proposed for use in the management of *Pistacia lentiscus* L. leaf collection. This proposal remains dependent on the dynamics of this peri-urban natural space. Although it has a recreational vocation, the Baïnem forest is subject to frequent fires. The rainfall regime, concentrated over two seasons, causes significant flooding that can lead to soil erosion when it occurs after the fire. The opening of the forest and pre-forest cover, the consequence of these elements, favors to a certain degree the installation and the extension of the *Pistacia lentiscus* L.

CONCLUSION

Algeria still lacks a lot of data on the current state of plant biodiversity of peri-urban forests and its exploitation. However, certainly, the absence of this kind of information does not facilitate the task of managers. This work is the first of its kind in this forest, so the results obtained are a source of information for managers. It poses a new problem for scientists and users. The implementation of a management plan would contribute to the safeguard of this shrub object of exploitation and the fauna and flora of which it constitutes the habitat.

This study shows the production capacity of the forest of Baïnem in terms of phytomass and therefore the profitability that *Pistacia lentiscus* L. offers by its oil which is one of the most expensive on the international market. These results will make it possible to set up specifications for the exploitation of lentisk in the Baïnem forest but also can be used elsewhere in the same thermo-Mediterranean facies. The preservation and development of this resource require the implementation of rational exploitation methods and a participatory management.

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Phenological Patterns of Selected Tree Species in Amrabad Tiger Reserve, Telangana, India

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Abstract: The present paper deals with phenology of selected tree species like *Phyllanthus emblica, Dalbergia paniculata, Hardwickia binata, Anogeissus latifolia, Albizia thompsonii, Chloroxylon swietenia, Diospyros melanoxylon, Givotia moluccana, Buchanania axillaris, Terminalia alata, Sterculia urens, Strychnos nux-vomica, Bombox ceiba, Butea monosperma, Madhuca indica, Eriolaena lushingtonii, Albizia odoratissima, Terminalia bellirica, Pterocarpus marsupium, Firmiana colorata, Careya arborea* in Amrabad Tiger Reserve, Telangana, India. The phenological observations include leaf flush, leaf mature, leaf fall, leaf less periods, flowering, fruiting, fruit fall, among the selected tree species. A total of ten individuals (≥50 cm gbh), for each of the selected 21 tree species were observed at fifteen days interval during 2018-2020 . It was observed that there were species specific phenophases relationship with deciduous period and initiation of seasonal rainfall and warm periods. In addition, intra species asynchrony in phenological activities was also recorded. Leaf flush activity was initiated in March and reached peak in the month of April and completed before the initiation of South-West monsoon. Leaf maturity started in the month of May and peak was recorded in June and completed in September. Leaf fall activity was initiated in the month of November and reached peak in January before the arrival of intense dry period. Deciduous period was recorded in December to April and the peak period was recorded in February. The reproductive phenophases like Flowering, Fruiting and Fruit fall have significantly varied across the different seasons among the observed tree species. Majority of tree species (43%) revealed synchronous flowering with Leaf flush activity. The results indicate that Leafing (48%) and flowering phenophases (70%) occur during the dry period before the onset of first rains and fruiting, fruit fall timing was in consequence to utilize the growing season. Thus, species specificity was recorded with respect to Phenophases were found to be in relation with the seasonal rainfall distribution and in turn soil moisture availability in the study area.

Keywords: Phenophases, Amrabad Tiger Reserve, Synchronous flowering

Among the plants, the variations in phenological activities such as leaf flesh, leaf fall, and flowering were directly related to deciduous period, seasonal distribution of rainfall, soil moisture and temperature (Moza and Bhatnagar 2005). Tropical dry deciduous forest consists of tree communities which grow in climates with marked pronounced dry and wet conditions in an annual period (Singh and Kushwaha 2006). Nanda et al (2014) observed that these forests constitute high variations in vegetative and reproductive phonological patterns at both large scale and small scales. The phenophases of tree species were mainly found to be based on the seasonal changing events such as availability of soil moisture, stem water status, photoperiod, changes in temperature and irradiance (Singh and Sahoo 2019) and biotic factors like pollinators attraction, competition for seed dispersers and avoidance of herbivore have been proposed to influence different phenological patterns in tropical dry forests (Singh and Kushwaha 2005). Thus pheonological events should be assessed by both abiotic factors and plant functional traits to achieve integrative understanding of tree community (Saha 2007). In seasonal tropical forests, plant phenological patterns were controlled by various interactions between biotic and climatic factors; especially seasonal variation in rainfall, dry periods which influence soil moisture, tree water status are considered as the principal factors influencing the timings of the periodic phenophases of growth and reproduction (Sakai 2001). In dry forests of southern Eastern Ghats the peak leaf flushing activity and flowering events occur during the dry period before the onset of first rains and fruit maturation period is high and fruit fall timing is in consequence to utilize the rains for germination. Thus, seasonal rains (soil moisture availability) and extent of deciduous period (photoperiod) influence the leafing and reproductive phenological events in dry deciduous forest (Mastan et al 2020). Few communities wide phenological studies in dry forests were carried out in dry forests of India, (Singh and Kushwaha, 2005, Nanda et al 2014, Mastan et al 2020). But no phenological studies were carried out in the dry deciduous forests of Telangana. Hence the present study was undertaken with an objective to examine the various phenophases for the selected tree species in relation to the prevailing climatic and to test the hypothesis that different phenophases among the tree species do not show seasonality and seasonal rainfall has no role in the sequence of phenophases in Amrabad Tiger Reserve, Telangana.

Study site (NL 15°.21'- 16° 30' NE 78° 30'-80°10'): The Amrabad Tiger Reserve (ATR) is a part of the Nallamalais of Southern Eastern Ghats and it harbors rich floral and faunal diversity. ATR is one of the largest Tiger Reserves of India in terms of geographical area (2611.4 km^2) and it is home for largest number of Tigers in Telangana State. It spreads in Nagarkurnool and Nalgonda districts of Telangana State with 3 forest divisions namely Achampet, Amrabad (Nagar Kurnool District) and Nagarjunasagar (Nalgonda District). The hilly terrain of this Tiger Reserve with deep valleys and gorges forms the catchment of the Krishna River. The reserve was notified as Amarabad Tiger Reserve in 2014. Amrabad plateau stands as extension of Eastern Ghats and is cut into many slopes and valleys running south-southeast directions. The elevation in ATR ranges from 120 to 900m MSL. The climate of ATR is warm from January to March with an average temperature varies from 24° to 30°C and in April and May the climate be too hot with an average temperature of 35°C-45°C. During the succeeding four months of rainy season, the wind blows from western side and the average rainfall for ATR is about 900-1000mm and is mostly due to south-west monsoon. At the end of September, the onset of north-east monsoon occurs and rainfall is scarce in November and December. From November to February the temperature falls as low as 8°C. The average minimum temperature 18°C and maximum temperature of 40°C. The ATR comprising geologically ancient rocks and two principal geological formations the Kurnool and Cuddapah. The major portion of the ATR consists of the oldest geological formation known as the Archeans and mainly three types of soils red, black and mixed varieties were observed in ATR.

MATERIAL AND METHODS

The total of 21 tree species with 10 individuals of each species having girth at breast highest (GBH) ≥50cm were selected in the study site for the phenological observations. Two branches (A & B) of 21 reproductively matured individuals of each species were marked and monitored for every 15 days intervals over a period of 3 years from January 2018 to December 2020. The phenophases like leaf flush, leaf mature, leaf fall, leaf less, flowering, fruiting and fruit fall were recorded. If at least five individuals in the species feature a phenophase it was considered as the initiation of that particular activity and the extension of that activity was noticed till at least eight individuals have promulgated the phenophase activity. The average of the three years data was taken into consideration for the study (Table 1). Spearman rank correlations were carried to obtain coefficients for the number of tree species in each phenophase in a month by the current and one to three month lag period rainfall data.

RESULTS AND DISCUSSION

The leaf initiation was first recorded during March in two species *Phllanthus emblica* and *Strychnos nux-vomica* (9.5%) and reached peak during the months of April and May (38%) followed by June (14%). In all the 21 tree species, leaf flushing activity has conspicuously started on the total leafless twigs. Further, majority of tree species (85.7%) have promulgated their first leaves in summer period before the onset of rains, while three species *Terminalia bellirica, Firmonia colorata* and *Eriolaena lushingtonii* have produced new leaves after the first rains concurring with the onset of monsoon period. Leaf flushing activity revealed non-significant negative correlation with current month rainfall and a significant negative relation with three-month lag rainfall amounts (Fig. 1, Table 2). The leaf initiation period was in the range of 31 to 77 days with a minimum period in *Chloroxylon swietenia* and *Albizia thompsonii* and maximum period was among *Firmonia colorata* and *Eriolanea lushingtonii* individuals. Leaf expansion period has started in May among four species (19%) with a peak in June (52%) and extended during July (14%), August (9.5%) and up to September by *Eriolaena lushingtonii*. High duration of leaf maturation (>200 days) was among species *Phyllanthus emblica*, *Caraya arborea*, *Buchananaia axillaris* and *Strychnos nux-vomica* and at least four months of growing season was recorded in the rest of 17 species. Leaf fall was recorded just after the end of monsoon period and well before the onset of dry period in November among six species (28.5%) followed by four species (19%) in December and reached peak condition in January (9; 43%) and two species namely *Careya arborea* and *Diospyros melanoxylon* have shed their leaves in February. Species under leaf fall activity and rainfall had significant negative relationship with current rainfall and the negative relationship was highly correlated with one month lag rainfall (Fig. 2, Table 2). Distinct leaf less period (Deciduous period) was as low as 14, 16 and 17 days in *Strychnos nux-vomica, Pterocarpus marsupium, Caraya arborea* respectively and as high as 106 days in *Sterculia urens* and 105 days in *Anogeissus latifolia.*

Flowering and fruiting phenophases : Flower bud break was first recorded in *Butea monosperma* during January, followed by *Phyllanthus emblica* in February and got pronounced in March (47%) extended during April (10.5%), May (15.8%) and *Dalbergia paniculata* and *Buchanania axillaris* have produced flowers in July. There was no flowering activity for *Hardwickia binata* and *Eriolaena lushingtonii* in the study period and all the tree species have revealed unimodal type (annual) of flowering except *Strychnos nux-vomica* in which bi-modal flowering in the months of March and July. Flower bud break and rainfall indicated negative relationship with current rainfall; but showed significant positive relationship with three-month lag rainfall (Fig. 3, Table 2). Flowering was recorded on completely leaf less twigs in 10 species in the summer season, four species have registered synchronous leaf initiation and flower initiation and another four species have produced flowers after the leaf initiation period was

Table 1. Vegetative and reproductive phenology data with initiating, ending days and duration period of the selected tree species

	Deciduous period	Leaf Initiation	Leaf maturation	Leaf Fall	Flowering	Fruiting	Fruit dispersal
Phyllanthus emblica	Jan16-Feb 15 (30)	Feb 01-Mar 30 (58)	Apr 01-Dec 30 (274)	Jan 01-15 (15)	Feb 16-Mar 30 (45)	Aug 01-Nov 15 (46)	Nov16-Dec 30 (45)
Dalbergia paniculata	Feb 01-Apr 30 (89)	Apr 15-June30 (76)	June 16-Oct 30 (138)	Oct16-Jan 30 (106)	May 01-July 30 (92)	Aug 01 -Oct 30 (62)	Nov 01-Dec 30 (60)
Hardwickia binata	May 01-30 (30)	Apr 15-June 15 (61)	June 16-Jan 30 (229)	Feb 01-Apr 30 (89)	ABSENT	ABSENT	ABSENT
Anogeissus latifolia	Jan01-Apr 15 (105)	Apr 16-June 15 (61)	June 16-Oct 30 (138)	Nov01-Dec 30 (60)	Sep 01 -Oct 15 (45)	Oct 16-Dec 15 (75)	Dec 16-Jan 15 (30)
Albizia thompsonii	Feb 01-Mar 15 (43)	Apr 15-May 15 (31)	May 16-Nov 15 (184)	Nov 16-Jan 30 (76)	Mar 16- Apr 30 (46)	May 01-Nov 30 (214)	Dec 01-Jan 01 (31)
Chloroxylon swietenia	Feb01-Mar30 (59)	Apr 01-May 15 (45)	May 16-Oct 31 (169)	Nov 01-Jan 30 (91)	March 01-31 (31)	April 01- 30 (30)	May 01-30 (30)
Diospyros melanoxylon	March 16 - Apr 30 (46)	May01-June 30 (61)	July 01-Jan 30 (214)	Feb 01-Mar 15 (43)	Apr 01-May 30 (61)	June 01-July 30 (61)	July 16-Aug 15 (30)
Givotia moluccana	Feb 16-Apr 30 (74)	May 01-July 15 (76)	July 16-Dec 30 (169)	Jan 01-Feb 15 (45)	May 01- 30 (30)	July 01-Oct 30 (122)	Nov 01-30 (30)
Buchanania axillaris	April 01-30 (30)	Apr 01-June 15 (76)	June 16-Jan 15 (215)	Jan 16 –Ma r30 (45)	June 01-July 30 (61)	Aug 01-Dec 30 (152)	Jan 01-Feb 28 (58)
Terminalia alata	Mar 01-Apr 30 (60)	Ap r01-May 30 (61)	June 01-Oct 30 (152)	Nov 01-Feb 28 (119)	May 01-June 30 (61)	July 01-Oct 30 (122)	Nov 01-Jan 31 (91)
Sterculia urens	Dec30-Apr15 (106)	Apr 30-May 30 (31)	June 15-Oct 30 (138)	Nov 15-Dec 15 (30)	Mar 30-Apr 30 (30)	May 15-Aug 15 (92)	Aug 30-Oct 15 (46)
Strychnos nux-vomica	Feb15-Feb28 (14)	Mar 15-Apr 15 (31)	Apr 30-Dec 30 (245)	Jan 15-Jan 30 (15)	Mar 15-Mar 30 & July 30-Aug 15 (32)	Apr 15-June 15 & Aug 30-Oct 30, (62) &(60)	June 30-July 15 & Nov 15-Feb 28 (16) & (73)
Bombox ceiba	Feb 28-Apr 30 (62)	May 15-June 15 (32)	June 30-Dec 30 (184)	Jan 15-Feb 15 (31)	Mar 30-Apr 15 (16)	Apr 30-May 30 (30)	June15-July15 (30)
Butea monosperma	Jan15-Mar 30 (74)	Apr 15-May 30 (45)	Jun 15-Oct 30 (137)	Nov 15-Dec 30 (45)	Jan 15-Feb 15 (31)	Feb 28-Mar 30 (31)	Apr 15-May 15 (30)
Madhuca indica	Feb 15-Apr 15 (59)	Ap r30-May 30 (31)	Jun 15-Dec 15 (184)	Dec 30-Jan 30 (31)	Mar 30 -Apr 30 (30)	May 15-Aug 30 (107)	Sep 15-Oct 30 (45)
Eriolaena lushingtonii	Mar 15-June 15 (92)	June 30 -Aug 30 (62)	Sep15-Dec 30 (106)	Jan 15-Feb 28 (44)	ABSENT	ABSENT	ABSENT
Albizia odoratissima	Feb 15-Ma r30 (43)	Apr 15-May 15 (30)	May 30-Nov 15 (169)	Nov 30-Jan 30 (91)	Mar 15 -Apr 15 (30)	Apr 30-Aug 15 (107)	Aug 30-Sep 30 (31)
Terminalia bellirica	Mar 30-May 30 (60)	June15-July 30 (46)	Aug 15-Nov 15 (91)	Nov 30-Mar 15 (134)	May 15- June 15 (30)	June 30-Oct 15 (122)	Oct 30-Nov 30 (30)
Pterocarpus marsupium	Feb 28-Mar 15 (16)	Mar 30-Apr 30 (31)	May 15-Dec 15 (211)	Dec 30-Feb 15 (46)	Apr 15 - June 15 (62)	June 30-Oct 15 (122)	Oct 30-Dec 15 (46)
Firmiana colorata	Feb 28-May 15 (89)	May 30-July 30 (62)	Aug 15-Oct 30 (76)	Nov 15-Feb 15 (92)	Mar 15 -Apr 15 (30)	Apr 30-Sep 15 (139)	Sep 30-Oct 30 (31)
Careya arborea	Mar 30-Apr 15 (17)	Apr 30-May 30 (31)	June 15-Jan 30 (230)	Feb 15-Mar 15 (31)	Mar 30-Apr 15 (16)	Apr 30-Oct 15 (169)	Oct 30-Nov 30 (30)

Figure in parentheses indicate number of days completed. The total of nine tree species (47%) have registered lesser flowering period (≤ 30 days) and the rest ten species have > 30 days flowering period with a maximum period of 61 days among *Dalbergia paniculata, Terminalia bellirica* and *Buchanania axillaris*. The fruit bud break was observed distinctly among *Butea monosperma* in February and *Anogeissus latifolia* has produced flowers lately in the whole forest during October. The peak fruiting activity was registered in April (31.5%), extended during May (15.8%), June (15.8%) and another lower peak was recorded in August (26.3%). Fruit maturation period was in the range of 30 – 214 days with a remarkably high period for *Albizia thompsonii* and species such as *Careya arborea* (183 days) and *Buchanania axillaris* (153 days) have registered high fruit maturation periods. In the same order of first tree to show flowering, fruiting buds break up, *Butea monosperma* has scored the first position for the fruit/seed dispersal also in April (Fig. 4). The peak condition for fruit/seed dispersal was registered in November and the range was 30-90 days with a minimum period shown by *Anogeissus latifolia* and higher period for *Terminalia bellirica* and *Buchanania axillaris* in which dispersal occurred during January of the next calendar year. Fruiting phenophase activity among the species and rainfall had positive relationship and with one-month lag rainfall the relationship was significant (Fig. 5, Table 2).

Tree species of Amrabad Tiger Reserve showed concentration (85.7%) of leaf initiation activity during the dry season and negative relation with a time lag of three months rainfall was noticed as in dry deciduous forests of Bhadra wildlife sanctuary (Nanda et al 2014). The triggering events for new set of leaves may be influenced by the photoperiod (increasing day length) as the timings of high frequency leaf initiation events corresponds with the initiation of spring equinox period where the day length period starts increasing. The leaf initiation timings in the dry period suggests that trees make sure of better utilizing the short growing season by

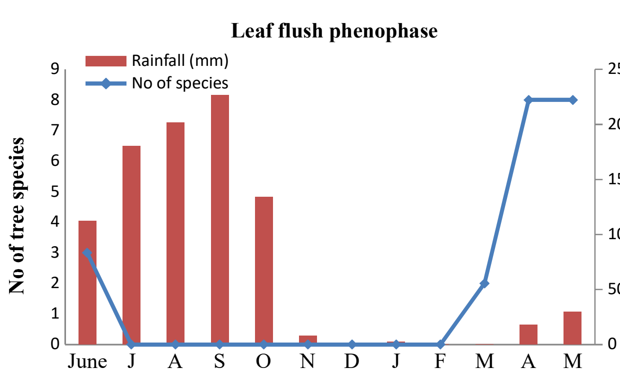

Fig. 1. Leaf flush phenophase

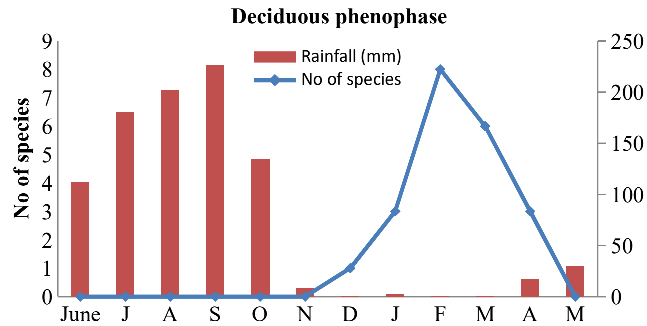

Fig. 2. Deciduous phenophase

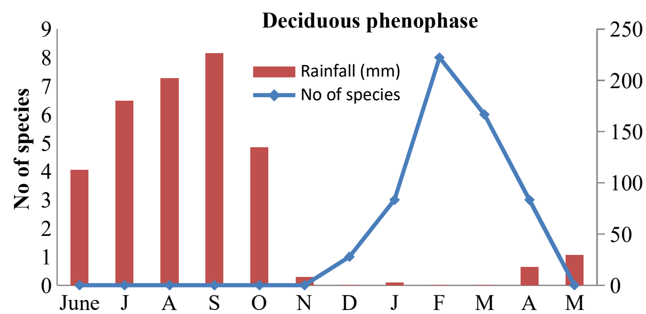

Fig. 3. Flowering phenophase

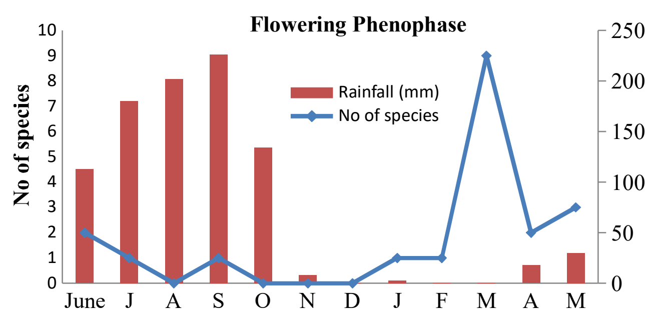

Fig. 4. Fruit bud break phenophase

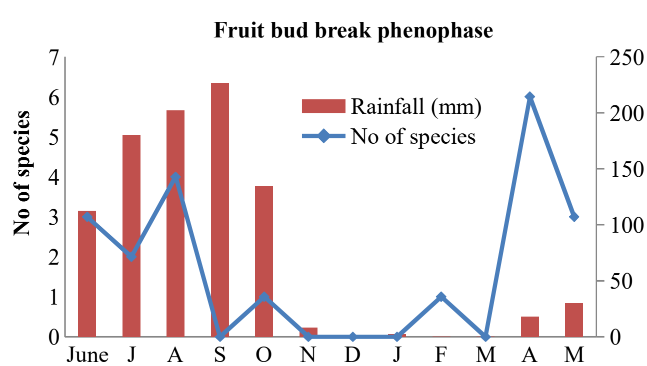

Fig. 5. Fruit/seed dispersal Phenophase

Table 2. Spearman rank correlation values between four different phenophase activities and current rainfall amounts and one to three months lag rainfall amounts

Phenophase activity	Correlation type	Correlation with variable	Significance NS= Not Significant S*= Significant at 0.05 level
Leaf flush	Negative	With current monthly rainfall amount	r = -0.138 P>0.05 (NS)
	Negative	With three-month lag rainfall amount	r = -0.77 P<0.05 (S*)
Leaf fall	Negative	With current rainfall	r = -0.57 P<0.05 (S*)
	Negative	With one month lag rainfall amount	r = -0.78 P<0.05 (S*)
Flower bud break	Negative	With current monthly rainfall amount	r = -0.38 P>0.05 (NS)
Flower bud break	Negative	With three month lag rainfall amount	r = -0.86 P<0.05 (S*)
Fruit bud break	Positive	With current monthly rainfall amount	r = 0.41 P>0.05 (NS)
Fruit bud break	Positive	With one month lag rainfall amount	r = 0.58 P<0.05 (S*)

projecting well-formed leaves well before the advent of monsoon periods as also observed in Vindhyan dry forests (Singh and Kushwaha, 2005). Majority of tree species like *Chloroxylon swietenia, Strychnos nux-vomica, Albizia thompsonii* have initiated the leaf senescence 3-4 months before the initiation of dry season. Such high frequency of leaf senescence events at the advent of low temperature dry period helps the trees to maintain turgidity in the shoots which helps in leaf bud and flower bud break up in the dry period itself strengthening the water stress hypothesis (Elliot et al 2006). Species have showed varied deciduous period (30-136 days) which indicates that certain species can tolerate, and few species avoid the water stress conditions in the hot dry season. In the study, six species featured nearly 30 days leafless period showing their tendency towards high tolerance for water stress conditions; while another six species had 31-70 days deciduous period and nine species projected greater than 70 days deciduous period indicating ability to avoid water stress conditions. Majority of species produced flowers during the dry season as well as on leaf less twigs. These observations are in line with reports that water deficit conditions in branch tips will induce the flower bud development and break ups in dry forests and the presence of flowers on the leafless twigs provide better opportunities for pollinators visit (Singh and Kushwaha, 2005). More over synchrony between flower and leaf initiation was noticed during dry season in species like *Pterocarpus marsupium, Phyllanthus emblica, Givotia molluccana*as also recorded in Mudumalai dry forests and dry forests of Rajasthan (Yadav and Yadav, 2008) which seems to related to moisture and day length. It indicates that water storage ability in tree trunks make few species to maintain high stem water potential and as well produce flowers in the dry period (Schongart et al 2002). Except *Butea monosperma* all other tree species in the present study are insect pollinated. *Butea monosperma* pollinated by squirrels and sun birds.

Fruiting was recorded in both wet and dry seasons registering two peaks in these dry forests. This indicates that both advent of rains and concentrated of rainfall events trigger the fruit bud break ups and similar trend was observed in Lankamalleswara forests (Mastan et al 2020). Among 16 species fruit maturation was completed before the onset of pre monsoon period with relatively lengthy period of fruit retention and 12 species had rapid maturation period. Thus Fruit/ seed dispersal events coincide with the onset of monsoon period for better germination success rates. Fruit dispersal in *Phyllanthus emblica, Diospyros melanoxylon, Madhuca indica Terminalia bellirica, Careya arborea* are zoochory in wet season and the remaining species were dispersed by the wind in dry season.

CONCLUSIONS

Occurrence of leaf flush events before the advent of dry period, flowering activity in the peak dry period, synchrony between leaf initiation and flowering in the late dry period, leaf expansion in the wet monsoon period and fruit fall in the post monsoon period and before rains indicates that seasonality prevails in the occurrence phenophases. Photoperiod seems to majorly influence the phenological patterns of the majority of tree species. Phenophases relate with dry period and rainfall period either in the particular month or in the previous months of their occurrence making them prominent factors that influence the phenophases that occur in the dry forests.

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Characterization of Wild *Begonia* Species of Sikkim Himalaya: A Study for Morphological and Antioxidants Analysis

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***Abstract*:** Sikkim Himalaya harbors many flora and fauna diversity and is rich in wild ornamental and medicinal plants. To know its valuable resources, present paper emphasizes on three wild *Begonia* species found in Sikkim Himalaya. Survey and collection of samples was done with randomized sampling methods and collected specimens were evaluated at Sikkim University for its morphological variation and antioxidant potential. Three species viz. *Begonia picta*, *Begonia josephi* and *Begonia hatacoa* were selected for current experiment. All three species showed distinct phenotypic variation between and within the species level. All of them were found in two variations: red (dark) and green phenotypic variant. Dark variants showed superiority in phytochemical screening having higher anthocyanin, flavonoid, phenolic and antioxidant content as compared to green phenotypic variants. The study validates the folklore of these *Begonia* species having medicinal value and are edible in nature.

Keywords: Wild Begonia, *Begonia picta, Begonia hatacoa, Begonia josephi,* Antioxidant

Sikkim Himalayas falls in the eastern Himalayan region of India (27°4'46" - 28°7'48" N and 88°58"- 88°55'25" E), covers 7096 km^2 area, which harbor number of wild medicinally important plants which are edible as well as of ornamental importance. Sikkim is rich in flora and fauna due to its diverse agro-ecological variations. From this diverse region, *Begonia* under Begoniaceae is a widely distributed and morphologically diverse genus. Known for its showy foliage with different patterns and colour, *Begonia* flowers in diverse variations of white, yellow, pink to scarlet. *Begonia* is the sixth largest angiosperm genera of flowering plants (Hughes 2008, Moonlight et al 2018), with more than 1800 recorded species around the world (Averyanov et al 2019). *Begonia*s are native to moist subtropical to tropical climates, popular ornamental plant which is commonly grown ornamental houseplants in cold climates. Some of *Begonia* species are also known for their medicinal and edible properties. More than 10,000 registered artificial hybrids of *Begonia* are used for commercial purpose, many wild species are too taken into cultivation as parents for new hybrids development. *Begonia* traditionally used for medicine, vitamin C and as a source of food. Leaves of *Begonia* are eaten fried or in soups or salads and used to treat sore throats, also used for the treatment of diarrhea, skin diseases, blood cancer, respiratory tract infections, anti-HIV activity and anti-tumor activity (Girmansyah 2009, Rajbhandary 2013). Apart from the ornamental importance of *Begonia* species, the aim of present study is to validate the traditional knowledge known from literature cited by various researchers for its medicinal properties. Meanwhile, literature revealed that some species of *Begonia* were recognized as medicinal plants and may possess some amounts of phytochemical constituents in it (Karima et al 2017, Shrestha et al 2018). Bhattarai and Rana (2020) also study the wild *Begonia* of Sikkim Himalaya and reported varied morphological characters having potent antioxidant properties and anthocyanin in it. The phytochemical constituents present in the plant species such as flavonoids, alkaloids, phenols, anthocyanin and other compounds are responsible for antioxidant property and antimicrobial properties (Saffidine et al 2015, Awasthy et al 2016). The presence of these phytochemicals in the plants are responsible for their medicinal properties, as these phytochemicals involve in some biological activity in man and animals (Bagchi et al 2021). The present study investigated the morphological diversity, preliminary phytochemical analysis of *Begonia* plant extract in three different solvent *viz.* methanol, hexane and ethyl acetate for total phenolic, flavonoid and anthocyanin content along with DPPH test.

MATERIAL AND METHODS

Whole plant of *B. picta, B. josephi* and *B. hatacoa* was collected from the wild and maintained in the experimental field of Department of Horticulture, Sikkim University. Detailed study of morphological characters was evaluated from the fresh plant including: plant height, plant form, no of leaves/plant, no of shoots/plant, nodes and internodes, shoot length, root length, stem diameter, length of petioles, leaf diameter, length of peduncles. Observation for various taxonomic parameters was taken during the flowering stage of the plant as per the procedure of Dorrenbos et al (1998). For biochemical analysis extract prepared from fresh plant parts, which were allowed to shade dry, powdered and successively extracted with hexane, ethyl acetate and methanol. The solvents were removed completely under reduced pressure and a semi solid mass was obtained. The extracts were qualitatively analyzed (preliminary phytochemical screening) to find out the presence of alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, carbohydrates, glycosides and amino acid following standard methods (Harbone 1976, Kandelwal 2001, Kokatae 2002, Edeoga et al 2005, Gopinath et al 2012 and Yadav et al 2014).

Total phenolic content was determined by using Folin–Ciocalteu assay (Meda et al 2005). The absorbance of the reaction mixtures was measured at 760 nm by using a spectrophotometer. Gallic acid was used as a standard and TPC of *Begonia* extracts was expressed in milligram gallic acid equivalents (mg GAE g^{-1} extract). Total flavonoid content was determined by the aluminum chloride colorimetric method (Lamaison and Carnet 1990), with some modifications. The absorbance of the solution was measured at 435 nm by using a spectrophotometer. The flavonoid content was expressed as milligram quercetin equivalent (mg QAE g^{-1}) extract and anthocyanin content (mg L^{-1}) was determined by following Sutharut and Sudarat (2012) methods. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) test is widely used to determine antioxidant activity in plant extracts. Test was performed according to methodology described by (Shen et al 2010). 0.1mM solution of DPPH in methanol was prepared and 1ml of this solution was added to 3ml of all the extracts in methanol at different concentrations (50, 100, 200 and 400 µg ml^{-1}). The mixture was vortex and kept in the dark for 30 minutes. The change in colour from dark blue to yellow was determined by measuring the absorbance at 517 nm using the UV-Vis Spectrophotometer. Ascorbic acid was used as standard and control was prepared using DPPH and methanol but without sample extract, whereas baseline correction was done using methanol. A lower absorbance value indicates the high radical scavenging activity. The IC_{50} (the microgram of extract to scavenge 50% of the radicals) value was calculated using linear regression analysis. Lower IC_{50} value indicates greater antioxidant activity.

RESULTS AND DISCUSSION

Morphological characters: The genus *Begonia* has distinct morphological variation among the species and within the species level. Morphological comparison of three *Begonia* species *viz. Begonia picta* Sm., *Begonia josephi* A.D.C and *Begonia hatacoa* Buch.-Ham. Ex D. Don was done during the flowering time. Morphological characterization was done by comparing the data with Flora of China (Gu Cuizh et al 2007), Flora of Sikkim (Hajra and Verma 1996), Flora of British India (Hooker 1854, Clarke 1879). Distinct character for each species was presented in Figure 1-3, which shows diverse species within the genus *Begonia* L.

***Begonia josephi* A.D.C.:** *B. josephi* is tuberous plant without stem, petioles directly arise from the tuber itself, monoecious herb having 32.60 to 37.54 cm high and 18.20 to 26.10 cm plant spread. Leaves variations showed distinct in each green and red phenotype, green phenotypes showed green colour of leaves in both surfaces (upper and lower) whereas, red phenotypic variation showed maroon red colour in underside of the leaves and dark green in upper side of the leaves. Leaf pubescent was present in both variants. Both variations having lamina oblong ovate to broadly ovate, peltate and rounded base, leaf length having (23.00 to 25.28 cm), leaf width (17.60 to 17.90 cm) and length of midrib (18.84 to 20.84 cm), leaf apex acute to acuminated. Number of leaves per plant were recorded 1.60 in both phenotypic variations. Petiole's length varies from 22.40 to 27.62 cm with petioles hair in both variations. Inflorescence cymose, terminal, peduncle length from 24.14 to 25.20 cm, number of female and male flowers/plant was found maximum in green phenotype (8.49 and 14.24, respectively), flowers white to pinkish in colour, male flowers; 4 tepals and female flowers; 5 tepals, capsule oblong to ellipsoid, one long triangular wing and two smaller wings. Species was distributed mostly on moist and shaded areas, altitudinal ranges from 1500 to 2500 amsl, peak flowering season June to September.

***Begonia picta* Sm.:** *B. picta* belongs to tuberous rootstock and plant height was recorded between 18.66 to 19.18 cm, and plant spread was recorded about 12.67 to 16.14 cm, leaf base was unequally cordate to equally cordate, margin dentate to denticulate, leaf apex was acute to acuminate and leaf lamina was ovate to orbicular in both the phenotypic variants, length of leaf lamina was found 13.34 to 14.80 cm in both variants. Leaf length (15.02 to 16.94 cm), leaf width (11.32 to 14.12 cm), midrib length (12.70 to 14.80 cm) and densely leaf pubescent was recorded in both the variants. In the dark phenotypic variant, the upper surface of leaves was found dark green to maroon red leaf surface and reddish purple and green blotches in lower surface, whereas, green phenotypic variant showed greenish colour in upper side and light green in lower surface of the leaves. The number of leaves/plants was varied from 2.60 to 3.20 in both the variants. Length of petioles was around 4.94 to 6.20 cm and densely puberulous in both the variants. Inflorescence cymose, terminal, peduncles length was ranges from 16.02

to 16.28 cm), pink to white flower colour, capsule oblong, ellipsoid, one longest and two short wings, the number of female flowers per plant were ranges from 5.45 to 6.40 and male flowers per plant ranges from 6.46 to 8.20 in both variants, distributed on shady and moist places along with the low growing areas having fertile soil. Plants are mostly found around altitude-800-1500 amsl, with peak flowering season during July to September.

***Begonia hatacoa* Buch.-Ham. Ex D. Don:** *B. hatacoa* is rhizomatous, shade loving, monoecious herbs, upto a height of 31.04 to 35.02 cm and plant spread was around 18.54 to 22.34 cm, varied in their phenotypic appearances, dark or red, green and variegated phenotypic variant. Other characters of the plant remain the same, leaf lamina is ovate to lanceolate with cuneate leaf base and sub entire to denticulate leaf margin, apex acute to acuminate, leaf length varies from 12.82 to 15.08 cm, leaf width 5.52 to 5.70 cm, midrib length 10.94 to 12.24 cm in both dark and green

Table 1. Description of habit and leaf characteristics of wild *Begonia* species of Sikkim Himalaya

Species	Plant habit	Leaf lamina	Leaf margin	Leaf base	Leaf apex	Leaf surface upper side	Leaf surface lower side
B. josephi (Red)	Tuberous	Oblong ovate to broadly ovate, peltate	Acute lobes with denticulate	Rounded	Acute to acuminate	Dark green	Maroon red
B. josephi (Green)	Tuberous	Oblong ovate to broadly ovate, peltate	Acute lobes with denticulate	Rounded	Acute to acuminate	Green	Light Green
B. picta (Red)	Tuberous	Ovate to orbicular	Dentate to denticulate	Cordate	Acute to acuminate	Dark green to maroon red	Reddish purple and green blotches
B. picta (Green)	Tuberous	Ovate to orbicular	Dentate to denticulate	Cordate	Acute to acuminate	Green	Light Green
B. hatacoa (Red)	Rhizomatous	Ovate to lanceolate	Subentire to denticulate	Cuneate	Acuminate	Dark green	Maroon red
B. hatacoa (Green)	Rhizomatous	Ovate to lanceolate	Subentire to denticulate	Cuneate	Acuminate	Green	Light Green

Table 2. Description morphological characters of wild *Begonia* species of Sikkim Himalaya

Species	Leaf Pubescent	Stipules' colour	Petioles colour	Petioles hair	Flower colour	Flower pubescent	Capsule colour	Capsule hair	Peduncle colour	Peduncle hair
B. josephi (Red)	Present	Brown	Greenish brown	Rarely present	Pinkish white	Present	Dark brown	Absent	Red	Absent
B. josephi (Green)	Present	Brown	Green	Rarely present	Pinkish white	Present	Green	Absent	Reddish Green	Absent
B. picta (Red)	Dense	Green	Reddish	Dense	Pink	Present	Green	Present	Green	Present
B. picta (Green)	Dense	Green	Green	Dense	Pink	Present	Green	Present	Green	Present
B. hatacoa (Red)	Rare	Brownish green	Greenish brown	Rarely present	Pinkish white	Absent	Reddish green	Absent	Reddish green	Absent
B. hatacoa (Green)	Rare	Brownish green	Greenish brown to green	Rarely present	Pinkish white	Absent	Greenish brown	Absent	Greenish brown	Absent

Table 3. Morphological characters of wild *Begonia* species of Sikkim Himalaya

Species	PH (cm)	PS (cm)	LM (cm)	LL (cm)	LW (cm)	PTL (cm)	PL (cm)	NL/P	NFF	NMF
B. josephi (Red)	37.54±2.83	26.10±2.79	20.84±1.78	25.28±2.08	17.90±2.14	27.62±2.00	25.20±0.76	1.60±0.24	5.80±1.69	12.60±0.60
B. josephi (Green)	32.60±3.50	18.20±1.11	18.84±2.62	23.00±2.57	17.60±2.25	22.40±2.25	24.14±0.87	1.60±0.24	8.49±2.21	14.24±0.78
B. picta (Red)	18.66±0.79	12.67±0.65	13.34±0.97	15.02±1.02	11.32±0.88	4.94±0.38	16.28±0.41	2.60±0.51	6.40±0.75	8.20±0.66
B. picta (Green)	19.18±1.06	16.14±0.65	14.80±2.53	16.94±2.53	14.12±2.03	6.20±0.72	16.02±0.98	3.20±0.37	5.45±1.18	6.46±0.72
B. hatacoa(Red)	35.02±1.93	18.54±2.26	12.24±1.13	15.08±1.70	5.70±0.39	6.06±0.62	13.60±0.73	10.40±1.17	12.40±1.17	14.60±0.81
B. hatacoa (Green)	31.04±2.70	22.34±1.37	10.94±0.99	12.82±0.74	5.52±0.29	5.72±0.37	11.02±1.00	9.00±1.73	9.76±1.31	12.64±0.94

PH- Plant height, PS- plant spread, LM- length of midrib, LL- Leaf length, LW- Leaf width, PTL- Petiole length, PL- Peduncle length, NL/P- Number of leaves per plant, NFF- Number of female flowers per plant, NMF- Number of male flowers per plant

variants. Upper surface of the leaves of dark phenotypic variants showed dark green coloration and maroon red in the lower surface of leaves whereas, the green phenotypic variant showed green colour in the upper side and light green in the lower surface of the leaves. Petiole length ranged from 5.72 to 6.06 cm and sparsely pubescent in both variants. Peduncle length were ranges from 11.02 to 13.60 cm, inflorescence cymose, terminal, male flowers; (2+2) outer tepals, female flowers (2+3) 2 outer and 3 inner tepals, colour pinkish white, having pinkish streaked in outer tepals, capsule ellipsoid, glabrous, one long triangular and two short wings, number of female flowers per plant were ranges from 9.76 to 12.40 and male flowers per plant were ranges from 12.64 to 14.60 in both variants. Peak flowering season was recorded during June-September, and found mostly between altitude 500-1500 m.

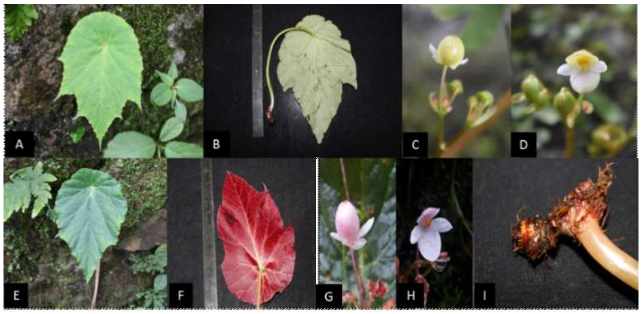

Fig. 1. *Begonia josephi* A.D.C: (A-D) Plant habit, Abaxial leaf surface, back side of flower petals and front view of flower petals of *B. josephi* Green Phenotype, (E-H) Plant habit, Abaxial leaf surface, back side of flower petals and front view of flower petals of *B. josephi* Red Phenotype and (I) tuber

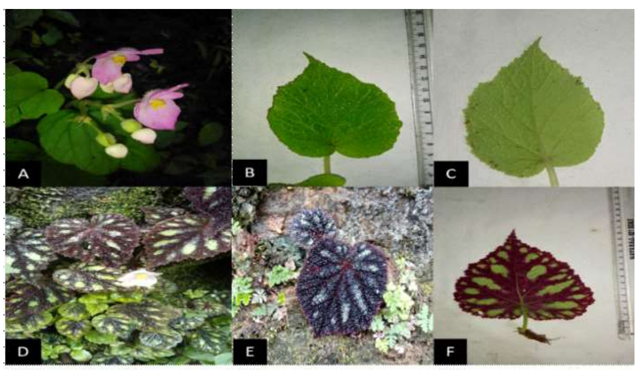

Fig. 2. *Begonia picta* Sm.:(A-C) Plant habit, Adaxial and Abaxial leaf surface of *B. picta* Green phenotype, (D-F) Plant habit, Adaxial and Abaxial leaf surface of *B. picta* Red phenotype

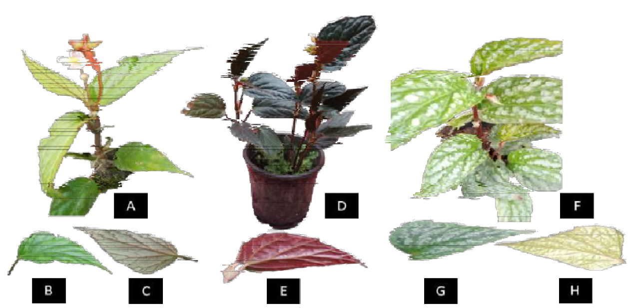

Fig. 3. *Begonia hatacoa* Buch.-Ham. Ex D.Don: (A-C) Plant habit, Adaxial and Abaxial leaf surface of *B. hatacoa* Green Phenotype, (D-E) Whole plant and Abaxial leaf surface of *B. hatacoa* Red Phenotype and (F-H) Plant habit, Adaxial and Abaxial leaf surface of *B. hatacoa* variegated Phenotype

Phytochemical screening of *Begonia* species: Phytochemical screening of different phenotypes does not vary within the species but great variation was found between the species level. The significant number of phytochemicals were present in each species level (Table 4).

Total phenolic, flavonoid and anthocyanin content: The total phenolic content and total flavonoid content was maximum in methanolic extract of *B. picta* (red) 55.03 QAE g^{-1} and 35.19 QAE g^{-1}, respectively while lowest amount was in hexane extract of *B. hatacoa* (green) 2.73 GAE g^{-1} and 0.92 QAE g^{-1} respectively. The result clearly indicates that phytochemical concentration varies from one species to another in different tested solvents, as well as between and within the species level. The red colour leaf variants showed highest phenolic and flavonoid content as compared to green leaves in each species tested. Anthocyanin content of tested *Begonia* species was in range from 31.83 to 185.33 mg L^{-1}, red phenotypes of *B. picta* showed the highest anthocyanin content. Similar kind of variation was also noted by Wang et al (2016) in *B. fimristupula* and Jose et al., (2016) in *B. trichocarpa*. Awasthy et al (2016) and Bhattarai and Rana (2020) found anthocyanin was one of the important phytochemicals present in *Begonia* species and is a potential source of natural antioxidant. Phytochemical constituents present in the plant species such as flavonoids, alkaloids, phenols, and other compounds are also responsible for antioxidant property and antimicrobial properties (Saffidine et al 2015, Bemmansor et al 2021). Phenolic compounds are the important class of antioxidants which have the ability to donate hydrogen atoms to free radical scavenging activity properties.

Difference in morphological and physiological traits of different plants of the same species or in a single plant exhibits phenotypic or ecotypic differentiation may be induced by environmental change (Deng et al 2012, Kurepin et al 2012). Alterations in phenotypic characters of same species allow the plant to modify its physiological characteristics and pigment content when habitat conditions change. It may also be due to the gene expression or hormonal regulation when the plant receives appropriate environmental signals (Schlichting and Smith 2002). Anthocyanins were commonly produced in the abaxial surface of leaves of understory plants and are responsible for red colour of the leaf blade (Lee and Collins, 2001). Previous studies on anthocyanins demonstrated that anthocyanin can significantly affect plant responses to environmental stress, protect organs and substances involved in photosynthesis processes, and relieve photo-oxidation damage to leaves. Therefore, anthocyanins can greatly improve viability and resistance of plants (Pourcel et al 2013).

DPPH (2, 2-diphenyl-1-picrylhydrazyl) test: Methanolic extract of *B. picta, B. josephi, B. hatacoa* and Ascorbic acid showed antioxidant activity in dose dependent manner in the rage of 50-400 μg ml^{-1} and produced maximum scavenging activity at a dose of 400 ug ml^{-1}. The mean IC_{50} values of three *Begonia* species and ascorbic acid were in the range of 376.50 to 17.89 μg ml^{-1}. Among all the tested samples of *Begonia* species, methanolic extract of *B. picta* (66.72 μg ml^{-1}) showed the high radical scavenging activity. The antioxidant potential of *Begonia* species between the various publications may vary both due to the methods used, as well as the method of bioactive substances extraction. The determination of antioxidant activity of plant extracts is an unresolved problem. The results from different antioxidant assays are even difficult to compare because of the difference in substrates, probes, reaction conditions and quantification methods. Loizzo et al (2016) observed that the different edible flowers which are largely consumed in Italy serve as natural source of antioxidants. Perez et al (2007) found that the methanolic extract of *Rosmarinus officinalis* L. had higher antioxidant properties than its ethanol and aqueous extract. Also, the methanolic extract of *R. officinalis* L. shows antibacterial properties (Bemmansor et al 2021). Wang et al (2016) studied antioxidant properties of 12

Table 4. Phytochemical screening of wild *Begonia* species of Sikkim Himalaya.

Species	*Begonia josephi*			*Begonia picta*			*Begonia hatacoa*		
Phytochemicals screened	Methanol	Ethyl acetate	Hexane	Methanol	Ethyl acetate	Hexane	Methanol	Ethyl acetate	Hexane
Flavonoid	+	+	+	+	+	+	+	+	+
Alkaloids	-	-	-	+	-	-	-	-	-
Tannins	+	-		+	+	-	+	-	-
Phenol	+	+	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+
Glycosides	+	-	-	+	-	-	+	-	-
Saponins	+	-	-	+	-	-	-	-	-
Amino acids	+	+	-	+	-	-	+	+	+
Terpenoids	+	-	-	+	+	-	+	-	-

Phytochemical present + ; Phytochemical absent -

Table 5. Total phenolic content, total flavonoid content and anthocyanin content of wild *Begonia* species of Sikkim Himalaya

Species/Solvent	*Begonia josephi*		*Begonia picta*		*Begonia hatacoa*	
	Red	Green	Red	Green	Red	Green
Total phenolic content (mg GAE g^{-1})						
Hexane	17.79±0.663	7.48±0.180	21.99±0.340	12.85±0.504	8.85±0.321	2.73±0.255
Ethyl acetate	20.79±0.387	8.48±0.351	36.34±0.326	16.22±0.509	13.61±0.866	5.44±0.403
Methanol	32.36±0.358	13.76±1.248	55.03±0.455	23.82±0.855	19.20±0.847	9.35±0.488
Total flavonoid content (mg QE g^{-1})						
Hexane	6.10±0.590	2.14±0.400	15.72±0.380	8.63±0.983	1.90±0.391	0.92±0.199
Ethyl acetate	5.82±0.258	4.18±0.543	17.33±0.225	12.91±1.098	4.12±0.538	2.17±0.973
Methanol	9.76±0.0.401	5.23±0.907	35.19±0.247	16.58±0.777	8.47±0.746	3.44±0.293
Anthocyanin content (mg L^{-1})						
	163.89±0.87	31.83±0.72	185.33±1.45	36.12±0.44	152.97±0.91	33.50±1.71

Chinese edible flowers, results show that the aqueous extract of *R. officinalis* L. and *Chrysanthemum morifolium* Ramat. possess good antioxidant *in vitro* and *in-vivo*. Previous researchers (Joshi et al 2015, Jose et al 2016) confirmed that *Begonia* species showed DPPH radical scavenging ability. In this experiment, the methanolic extract of *Begonia* species had significant scavenging effects on the DPPH which was increasing with the decreasing concentration of the sample from 50-400 µg ml^{-1}. Methanolic extract of *B. picta* showed best scavenging activity as compared to *B. josephi* and *B. hatacoa*. This might be due to the presence of flavonoid, phenol and anthocyanin content, the most required bio-compounds for scavenging activity in this extract.

CONCLUSION

This morphological and phytochemical study of *Begonia* gives an important insight into the diversity of the genus in the region. The study validated the medicinal potential of the three wild *Begonia* species of Sikkim Himalaya which are commonly used as folklore medicine as well as confirms its edibility by conducting phytochemical analysis. All three species show marked morphological and phytochemical variations. In all three species under study, *B. picta* red variant having higher antioxidant properties as compare to *B. josephi* and *B. hatacoa*. When comparing for various phytochemicals *viz.* total phenol, total flavonoid, anthocyanin and antioxidant activity of red and green variant of *Begonia*, red variant showed higher value against the green. Similarly red variant had more anthocyanin content as compared to green leaves, thus having better antioxidant properties. Ornamentally morphologically red variant species will be useful in breeding purposes for the development of new commercial varieties of Begonia.

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Effect of Altitude on Seed Dispersal and Seedling Establishment of *Shorea robusta* Roth. in Garhwal Himalaya

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Abstract: *Shorea robusta* is the most dominated species of tropical forest in India. The seed dispersal and regeneration status were studied during the period June 2019 to May 2021. Seed traps were placed for seed dispersal. Traps were round shape baskets with 60 cm open face diameter and place 1m above ground height. For the study of regeneration, 1*1 quadrates were laid down next to each trap. Total 30 traps and 30 quadrates were placed for each study site. The middle elevation (900m) shows better results in comparison to a lower elevation (600m) for seed dispersal and seed germination. The seed germination and seed survival were also compared as inside forest and outside *Shorea robusta* forest for both elevations. Seed germination is similar inside the forest for both elevation and outside the forest for elevation but there is a significant difference between inside forest and outside the forest for both elevations. The middle elevation shows better results for germination and survival of seeds of *Shorea robusta.*

Keywords: *Shorea robusta*, Seed dispersal, Seed trap, Seed germination, Inside forest, Outside forest

Indian Himalayan Region (IHR) sustains a variety of vegetation in the form of different forests. The part of IHR located within the state of Uttarakhand, known as Garhwal Himalaya, harbours forest types at different elevations i.e., extending from foothill to middle Himalaya. Among these one such type is the Sal forests dominated by the Sal tree (*Shorea robusta* Roth.) also, the major forest types in South Asia. Sal is a tropical tree species belonging to the family Dipterocarpaceae having well documented three sub-families, 17 genera and 511 species (Surabhi et al 2017) forming open to dense forests. The species is naturally found in Bhutan, Bangladesh, Nepal and India between 75º and 95º E longitude and 20º to 32º N latitude (Gautam and Devoe 2006, Sapkota, 2009). In India, Sal Forest is cosmopolitan in tropical regions covering 13.3% of the entire forest area within the country. The distribution extends up to the Assam valley (including Meghalaya and Tripura) in the east to the foothills of north-west Bengal, Uttar Pradesh, Uttarakhand, and Kangra region of Himachal Pradesh.

Seed dispersal is wind-driven. The recalcitrant seeds and short period of areal viability of *Shorea robusta* make the species of great interest to the forest researchers and plantation managers (Phartyal et al 2002 and Pattanaik et al 2015). Natural regeneration, the inherent ability of the species to breed itself is a direct indicator of the health of a forest ecosystem. Though, many factors reported to affect the process of natural regeneration (Doroski et al 2018) includes climate (humidity, temperature, light intensity, a span of light-receiving hours, precipitation and wind). soil (depth, aeration, moisture level, nutrients and erosion), seed (sensitivity, output and dispersal) and biotic conditions (wildlife, forest fire, and over-grazing), etc. seed viability losses leads by the long transportation period from collection site to nurseries (Dumroese et al 2016). The studies on the seed germination and seedling survival of Sal in a natural condition are well documented (Pattanaik and Dash 2015, Gautam and Devoe 2006). However, in the state of Uttarakhand prominent lack of such research for the study of forest covers where the Sal Forest cover especially in the Lansdowne Forest Division (LFD) region has been observed to be reduced. This forest division registered and accredited as Conservation Assured Tiger Standards (CATS) site in India dated 25 May 2017. CATS is a programme that satisfy a group of standards and criteria to promotes protected areas to assure effective and continuous tiger conservation, formed by conservation managers and international professionals. This region facilitates the movement of wild animals such as tiger and elephant between Corbett Tiger Reserve and Rajaji Tiger Reserve (Williams 2002, Harihar et al 2009). Thus, the region is ecologically important. The declining distribution of economically and ecologically important Sal forests underpins the importance of the present piece of work to investigate the seed production, seed dispersal and seed germination of this dominant species in natural conditions at different altitudes in the LFD.

MATERIAL AND METHODS

Study area: Lansdowne Forest Division located between Shivalik range and Lower Himalayan ecoregion and Terai Arc Landscape of India. It is situated between 29° 22' 12" - 30° 1' 12"N and 78° 11' 24" - 78° 25' 48"E in the district Pauri Garhwal. The study area includes Sal forests, grasslands, mixed forests (characterized by *Mallotus philippensis, Terminalia tomentosa, Anogeisus latifolia and Diospyros montana* etc), and pine forests and elevation range between 300-1300 m. There are five ranges (Kotdi, Dugadda, Lansdowne, Kotdwar, and Laldhang) in this division with 433 km^2 total geographical area. For the present study, the falling station was selected in different ranges according to elevation:

1. The sampling station first Aamsaur Forest Area falls under the Dugadda Forest Range. This station is located at elevation 600 masl and 29° 46' 52"N and 78° 34' 54"E. (Lower Elevation).
2. The second sampling station Fatehpur Forest Area also falls under Dugadda Forest Range. This station is situated at the altitude of 900 masl and 29° 49' 46"N and 78° 36' 56"E. (Middle Elevation).
3. The third sampling station Batkot Forest Area comes under the Lansdowne Forest Range. This station is situated at the altitude of 1200 masl and 29° 51' 45"N and 78° 38' 03"E. (Higher Elevation).

Methodology

Seed dispersal: Seed traps were placed to sample seed rain inside and outside the forest patches. The circular baskets with a 60 cm radius, made up of bamboo and lined with plastic sheets were used as seed traps. Three stakes (1.5-m high) were used to raise the seed traps above the ground to avoid predation by rodents. The traps height differs for undulate ground surface and steeply area. Traps were arranged inside the forest and within the matrix outside the forest. In the matrix outside the forest, the arrangement was along transects with side water bodies and drainage lines (total of 30 traps were set inside and outside the forest. The coordinates (x, y) of every trap and potential seed source tree were established. The traps were installed in June 2019 and

Table 1. GPS Location of laid quadrats in all three study sites

Quadrat N.	Aamsaur		Fatehpur		Batkot	
	Inside Forest	Outside Forest	Inside Forest	Outside Forest	Inside Forest	Outside Forest
1.	N 29° 46' 52.35" E 78° 34' 55.66"	N 29° 46' 54.82" E 78° 34' 56.66"	N 29° 49' 46.45" E 78° 36' 56.27"	N 29° 49' 49.46" E 78° 36' 56.80"	N 29° 51' 41.97" E 78° 38' 7.97"	N 29° 51' 40.26" E 78° 38' 7.82"
2.	N 29° 46' 52.21" E 78° 34' 55.21"	N 29° 46' 55.15" E 78° 34' 56.28"	N 29° 49' 46.38" E 78° 36' 55.86"	N 29° 49' 49.57" E 78° 36' 56.40"	N 29° 51' 41.99" E 78° 38' 8.62"	N 29° 51' 40.05" E 78° 38' 7.82"
3.	N 29° 46' 52.19" E 78° 34' 54.69"	N 29° 46' 55.38" E 78° 34' 55.82"	N 29° 49' 46.31" E 78° 36' 55.49"	N 29° 49' 49.62" E 78° 36' 56.09"	N 29° 51' 42.03" E 78° 38' 8.81"	N 29° 51' 39.82" E 78° 38' 7.89"
4.	N 29° 46' 52.11" E 78° 34' 54.34"	N 29° 46' 55.71" E 78° 34' 54.98"	N 29° 49' 46.28" E 78° 36' 55.14"	N 29° 49' 49.81" E 78° 36' 55.79"	N 29° 51' 42.01" E 78° 38' 8.32"	N 29° 51' 39.63" E 78° 38' 8.01"
5.	N 29° 46' 52.08" E 78° 34' 53.89"	N 29° 46' 55.58" E 78° 34' 54.60"	N 29° 49' 46.39" E 78° 36' 54.80"	N 29° 49' 50.07" E 78° 36' 55.59"	N 29° 51' 41.97" E 78° 38' 9.01"	N 29° 51' 39.51" E 78° 38' 7.82"
6.	N 29° 46' 52.66" E 78° 34' 55.91"	N 29° 46' 55.83" E 78° 34' 54.27"	N 29° 49' 46.83" E 78° 36' 56.33"	N 29° 49' 50.37" E 78° 36' 55.56"	N 29° 51' 41.79" E 78° 38' 7.96"	N 29° 51' 39.32" E 78° 38' 8.08"
7.	N 29° 46' 52.57" E 78° 34' 55.33"	N 29° 46' 56.20" E 78° 34' 54.01"	N 29° 49' 46.71" E 78° 36' 55.85"	N 29° 49' 50.61" E 78° 36' 55.48"	N 29° 51' 41.83" E 78° 38' 8.17"	N 29° 51' 39.08" E 78° 38' 7.92"
8.	N 29° 46' 52.52" E 78° 34' 54.84"	N 29° 46' 55.59" E 78° 34' 55.45"	N 29° 49' 46.59" E 78° 36' 55.62"	N 29° 49' 50.73" E 78° 36' 55.35"	N 29° 51' 41.86" E 78° 38' 8.35"	N 29° 51' 38.88" E 78° 38' 8.06"
9.	N 29° 46' 52.35" E 78° 34' 54.34"	N 29° 46' 56.44" E 78° 34' 53.83"	N 29° 49' 46.52" E 78° 36' 55.25"	N 29° 49' 50.88" E 78° 36' 55.47"	N 29° 51' 41.87" E 78° 38' 8.60"	N 29° 51' 38.77" E 78° 38' 8.24"
10.	N 29° 46' 52.30" E 78° 34' 53.82"	N 29° 46' 56.52" E 78° 34' 53.49"	N 29° 49' 46.61" E 78° 36' 54.94"	N 29° 49' 50.98" E 78° 36' 55.17"	N 29° 51' 41.79" E 78° 38' 8.81"	N 29° 51' 38.64" E 78° 38' 8.12"
11.	N 29° 46' 53.03" E 78° 34' 55.86"	N 29° 46' 56.71" E 78° 34' 53.19"	N 29° 49' 47.09" E 78° 36' 56.58"	N 29° 49' 51.18" E 78° 36' 55.02"	N 29° 51' 41.61" E 78° 38' 7.98"	N 29° 51' 38.49" E 78° 38' 8.23"
12.	N 29° 46' 52.90" E 78° 34' 55.26"	N 29° 46' 57.03" E 78° 34' 52.86"	N 29° 49' 47.01" E 78° 36' 56.13"	N 29° 49' 51.24" E 78° 36' 54.68"	N 29° 51' 41.71" E 78° 38' 8.25"	N 29° 51' 38.35" E 78° 38' 8.19"
13.	N 29° 46' 52.87 E 78° 34' 54.77"	N 29° 46' 57.21" E 78° 34' 52.52"	N 29° 49' 46.93" E 78° 36' 55.68"	N 29° 49' 51.20" E 78° 36' 54.36"	N 29° 51' 41.71" E 78° 38' 8.49"	N 29° 51' 38.26" E 78° 38' 8.11"
14.	N 29° 46' 52.91" E 78° 34' 54.29"	N 29° 46' 57.37" E 78° 34' 52.10"	N 29° 49' 46.87" E 78° 36' 55.26"	N 29° 49' 51.26" E 78° 36' 54.06"	N 29° 51' 41.64" E 78° 38' 8.69"	N 29° 51' 38.06" E 78° 38' 7.91"
15.	N 29° 46' 52.72" E 78° 34' 53.74"	N 29° 46' 57.60" E 78° 34' 51.76"	N 29° 49' 46.95" E 78° 36' 54.88"	N 29° 49' 51.23" E 78° 36' 53.72"	N 29° 51' 41.58" E 78° 38' 8.91"	N 29° 51' 37.95" E 78° 38' 7.82"

were monitored during the fruit setting period up to September 2019. Seeds were collected every week, counted at the spot. This routine was repeated until no more seeds were retrieved from the traps (Abiyu et al 2016).

Regeneration study: The next to every trap, 1 m^2 (1 m × 1 m) plots were prepared, where seeds were marked with toothpicks and monitored for emerging seedlings. The established plants and germinates are rare in the studied habitats, tree establishment was observed in areas with freshly exposed soil by human activity. To validate seed dispersal kernels with plant distribution, a further set of 15 plots (2 m × 2 m) were laid with additional transects running from seed sources into the open habitats (Abiyu et al 2016).

Statistical analysis: Descriptive statistics were used to visualize the number of seeds reaching different traps located in the various habitats at different altitudes. In the 1 m × 1m plots along the transects, an independent sample T-test with equal sample sizes was performed for the significant differences in the emergence of seeds between these habitats. The Minitab-18 statistical software were used to present the box plot.

RESULTS AND DISCUSSION

Seed dispersal: The middle elevation (Fatehpur Forest Area) shows the maximum number of seeds dispersed inside the forest as compared to the lower elevation (Aamsaur Forest Area). The average number of dispersed seeds outside the forest was 3.73 seeds in quadrate and 1.33 seeds in seed-traps for middle elevation (Fatehpur Forest Area) and 2.93 seeds in quadrate and 0.73 seeds in seed-traps for the lower elevation (Aamsaur Forest Area). The middle elevations (Fatehpur Forest Area) recorded an average number of dispersed seeds inside the forest being 27.4 seeds in quadrate and 11.46 seeds in seed-traps. The lower elevation (Aamsaur Forest Area) recorded 26.6 seeds in quadrate and 10.66 seeds in seed-traps. The dispersal of seeds was significantly different for the middle elevation and lower elevation and, into seeds in seed-traps and quadrate (Fig. 1). The variation in climatic conditions has a significant impact on seed production of Shorea robusta (Kumar and Chopra, 2018). Spring flushing, semi-evergreen *Shorea robusta* started out flowering with the onset of leaf fall in winter. Its flowering (January–April) coincided with the leaf transitional state (leaf fall, leaf initiation), and fruit formation and leaf flushing both were supported at the same time (Singh and Kushwaha 2006). In tropical forest, flowering is brought on by means of elements consisting of increased day period, solar intensity and temperature (Boulter et al 2006, Borah and Devi 2014). Based on the finding on phenology it may be stated that enough moisture content and temperature during favorable growth season are key determinants for growth and development in *S. robusta* (Kumar and Chopra 2018).

Distance of seed dispersal: The maximum distance (390m) was recorded for the middle elevation (Fatehpur Forest Area) outside the forest as compared to the (330m) lower elevation (Aamsaur Forest Area). The trend line shows the downward movement with the distance of the number of seeds dispersed. The seed dispersal distance is significantly different for the middle elevation and lower elevation (Fig. 2). The proximity of regeneration sites to the seed source has a significant impact on regeneration success (Holl 2008). For animal disseminated species Clark et al (2005) found up to 473 m dispersal distances. It has been found that the animal-dispersed tree species seeds have a longer dispersal distance than wind-dispersed tree species seeds (Montoya et al 2008, Nazareno et al 2021).

Germination of seeds: The germination percent of seeds of *Shorea robusta* was recorded outside the forest for the

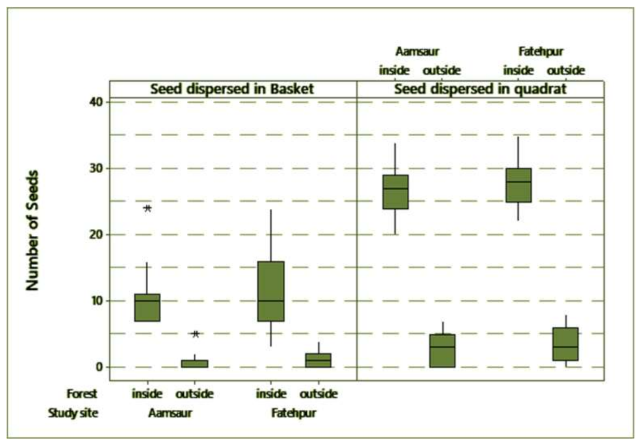

Fig. 1. Box plot for both study sites with comparative seed dispersal between inside and outside the forest and in basket and quadrates

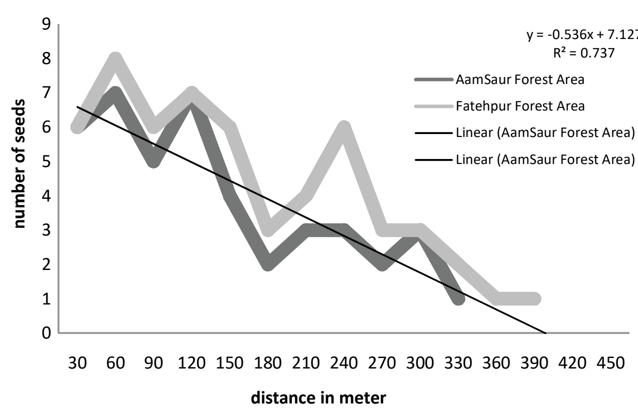

Fig. 2. Seed dispersal distance from the forest area for both forest sites

Aamsaur forest area was 40.41% and for the Fatehpur, Forest Area was 45.87%. The middle elevation (Fatehpur Forest Area) shows the highest percent of germination 73.09% and 71.8% in the lower elevation (Aamsaur Forest Area) inside the forest. The germination of *Shorea robusta* in both sites is found significantly different at 1%. The numbers of seeds produced varied between both elevations (Fig. 3). Seed viability of Sal last till a weak. The occurrence of rain within a week of seed fall take germination rate upto 90 %. Delay in rain can reduce the germination of seeds. The germination pattern of different plant communities is changed with the change in the elevation (Devi et al 2019). The range of seedlings was recorded from 38.89 to 67.35% (Mittal et.al 2020). Germination of seeds is also led by the maturity level of seeds (Phartyal et al 2002). Regeneration status was very poor in natural forest (Ganguli and Joshi, 2020). Sal has a tendency of germination in mass of seedlings in favourable conditions (light, soil, moisture with true drainage) in even aged vegetation, which can be highly natural (Saatkamp 2014). The 5000 seedlings per hectare and (Behera et al 2021) and 50000-100000 seedlings per hectare Sal forests beneath uniform Shelterwood system found after regeneration felling (Awasthi et al 2015, Gaire and Ghimire 2019).

Survival of seedling: The survival of seedlings outside the forest for the Aamsaur Forest Area was 6.0% and for the Fatehpur Forest Area 9.33%. The lower elevation (Aamsaur Forest Area) shows the percent of survival was 32.47% inside the forest and 39.14%in middle elevation (Fatehpur Forest Area). The survival of *Shorea robusta* in both sites was significantly different. The survival percent for the seedling of *Shorea robusta* is deficient outside the forest (Fig. 4) and Sal ranges between 3.01 to 7.59 (Mittal et al 2020). The survival of seedlings was about 2% in natural condition (Awasthi et al 2015). Survival of seedlings was good as high number of

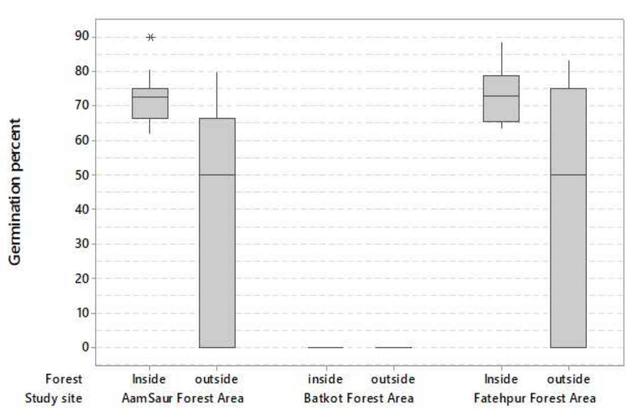

Fig. 3. Box plot for both study sites with comparative germination percent between inside and outside the forest

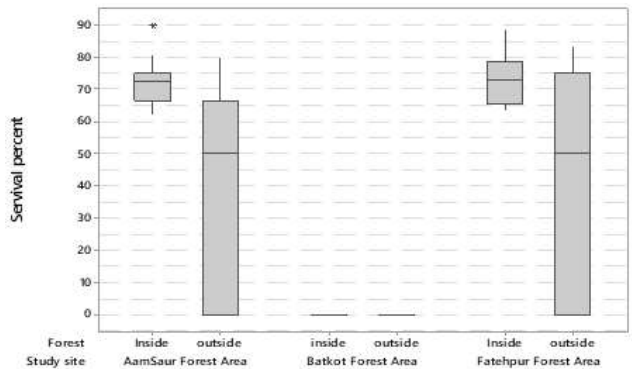

Fig. 4. Box plot for both study sites with comparative survival percent between inside and outside the forest

saplings was recorded under natural conditions (Ganguli and Joshi 2020). The dispersed seed shows a different variance in survival at both elevations. Depending on the number of shelter trees, the die-back rate varied from 4 to 10 percent but among tallest 2000 seedling per hectare observed no die-back (Saatkamp 2014, Awasthi et al 2015, Gaire and Ghimire 2019). The seed size has also a potential impact of germination and survival of seedlings of Sal (Patnaik et al 2015). The change in elevation shows the change in survival pattern of different plant communities (Devi et al 2019). The regeneration patterns of tree species within the communities showed altitudinal shift changing pattern of community compositions. Seedling density increased by the open canopy areas which favored regeneration of some tree species and increasing the alimentation of species diversity (Sapkota 2009).

CONCLUSIONS

The seed amount and distance of seed dispersed is related to germination and survival of seed. The higher distance seed dispersed is not vulnerable for germination. The medium elevation is suitable for the establishment of germination and survival of the seedling.

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Soil Chemical Properties and Nutrient Status as Influenced by Different Spacings of Poplar under Agroforestry in Semi-Arid Region

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Abstract: A field experiment was carried out during 2018–19 to study the effect of $2^{1/2}$ year old poplar based agroforestry system on soil chemical properties and nutrient dynamics. Present study was conducted in an already established two and half-year-old plantation of *Populus deltoides* with six different spacings *viz.,* 3×3 , 4×3 , 5×3, 6×3, 7×3 and 8×3m. During rainy season cowpea (var. RC-19) and sorghum (var. HC-171) were sown during first week of July and Oat (var. HJ-8) and Berseem (var. HB-1) were sown in interspaces of poplar spacing's as winter crops and sole crops of both rainy and winter seasons were sown in open condition (devoid of tree) as control. Intercropping of both winter and rainy season crops in poplar spacing's showed better available of macronutrients than sole cropping. Soil pH decreased considerably under different poplar spacing and maximum decrease of 13.60 percent was recorded in 3x3 m spacing over control. However, soil organic carbon (SOC) increased with the decrease in poplar spacing and maximum SOC (0.45%) was observed under 3×3m followed by 4×3m (0.42%), 5×3m (0.40%) and least under sole cropping (0.30 %) after harvesting of rainy season fodder crops. The available soil N, P and K increased significantly under different spacings of poplar based cropping system from initial values. The higher available nitrogen (158.5 kg/ha), phosphorous (16.8 kg/ha) and potassium (343.8 kg/ha) was recorded under 3×3 m spacing as compared to other spacings and sole cropping (open environment) after harvesting of both season fodder crops.

Keywords: Agroforestry, Nutrient status, Poplar, Spacing, Soil chemical properties

Trans Indo-Gangetic plain with deep fertile alluvium witnessed green revolution but now the productivity becomes stagnant. However, unremitting ecological degradation, especially of water, plant life and soil assets has resulted in loss of soil health due to over exploitation of natural indigenous resources. Ultimately crop production is decreasing due to the various intangible factors like declining efficiency of nutrient-use, soil degradation, inadequate availability of water, etc (Sirohi and Bangarwa 2017). The need has been realized for conservation of natural resources and protection of the deteriorating environment so that the required growth in agriculture is maintained with sustainability (Chauhan et al 2012). In agricultural lands, planting of trees improves the ecosystem services, enhances soil fertility through addition and decomposition of leaf litter on sustainable basis (Zhang et al 2013). For such scenario, agroforestry is considered as a natural resource conserving system (Shehnaz and Singh, 2014). Introduction of agroforestry systems improved the soil fertility and micro-environment (Giri et al 2019). Agroforestry systems help to reduce the use of chemical fertilizer, reduce surface run off, nutrient leaching and can maintain the nutrient requirement for the plant by nutrient recycling (Gliessmann 2007). Agroforestry systems are generally considered to be sustainable and to improve soil properties. Multifunctional agroforestry systems improve soil physical and chemical properties, maintain soil organic matter, and promote nutrient cycling (Bisht *et al*., 2018). Agroforestry affects nutrient status of soils via addition of leaf litter and also by plant uptake, and is considered an important option for C sequestration (Albrecht and Kandji 2003). Nutrient availability in soils is influenced by the addition of organics (Sui and Thompson, 2000, Singh et al 2010). In poplar based agroforestry system soil organic matter (SOM) increased significantly through the addition of above and below-ground biomass to soil (Dhillon et al 2020).

Populus deltoides Bartr. based agroforestry system is one of the realistic alternate land use system in semi-arid and dry sub-humid agro-ecosystem of North-Western states of India. Poplar has become most popular among the farmers as poplar based agroforestry systems are economically viable and more profitable than many of the other crop rotations. The information on the effect of addition of leaf and root biomass of poplar on SOC, soil chemical properties and available nutrients is known to lesser extent. Therefore, present study was conducted to investigate the effect of poplar based cropping system on soil chemical properties in semi-arid region.

MATERIAL AND METHODS

The present investigation was carried out at CCS Haryana Agricultural University, Hisar during 2018-19. Experimental site is situated at 29° 09' N latitude and 75° 43' E longitude at an altitude of 215.2 m above the mean sea level situated in the semi-arid region of north-western India. The climate is subtropical-monsoonic with an average annual rainfall of 350-400 mm, 70-80 per cent of which occurs during July to September. The summer months are very hot with mean maximum temperature ranging from 40 to 45 °C in May and June whereas December and January are the coldest months (lowest temperature reaches as low as 0 °C). However, Hisar region consists of plain land, 90% of its cultivated area is irrigated, whether under crop-growing or agroforestry systems, and the source of irrigation is good quality canal or tube-well water.

The present study was conducted in an already established two and half-year-old plantation of *Populus deltoides* with six different spacings of 3×3 , 4×3 , 5×3m, 6×3, 7×3 and 8×3m. During rainy season cowpea (var. RC-19) and sorghum var. (HC-171) were sown during first week of July and oat (var. HJ-8) and berseem (var. HB-1) were sown during first fortnight of November under different poplar spacings under study and also in open (control) with the recommended cultural practices of the university. The soil samples were taken before sowing and after harvesting of fodder crops of both rainy and winter seasons under different spacings of poplar and also from control field under study for the analysis of various soil chemical properties (pH, electrical conductivity (EC) and soil organic carbon (SOC)) and available nutrients content (nitrogen, phosphorus and potassium). Four soil samples were collected randomly at different depths (0-15 and 15-30 cm) from the experimental field in three replications from different spacing of poplar. The soil samples were first air-dried, ground in a wooden pestle with mortar, passed through a 2 mm stainless steel sieve and stored for subsequent analysis. The soil pH and electrical conductivity were determined in soil: distilled water suspension (1:2). The soil pH was determined using glass electrode pH meter (Jackson 1973), electrical conductivity (dS m^{-1}) was determined using conductivity meter (Jackson, 1973) and soil organic carbon content (%) was determined using partial oxidation method (Walkley and Black 1934). The available nitrogen (kg ha^{-1}) was determined by alkaline permanganate distillation method (Subbiah and Asija1956), phosphorus (kg ha^{-1}) by sodium bicarbonate method (Olsen et al 1954) and potassium (kg ha^{-1}) by neutral normal ammonium acetate method (Jackson 1973).

RESULTS AND DISCUSSION

Soil pH: Soil pH decreased slightly under different spacings of poplar as well as in control (sole crop) from July 2018-April 2019. The magnitude of decline in soil pH was greater under different poplar spacings than control. However, the soil pH increased with increase spacing and depth of soil profile. The maximum soil pH of 7.94 and 7.98 at 0-15 and 15-30 cm soil depth, respectively were recorded in 8×3m spacing followed by 7×3 m spacing (Fig. 2) while, the minimum pH of 7.75 and

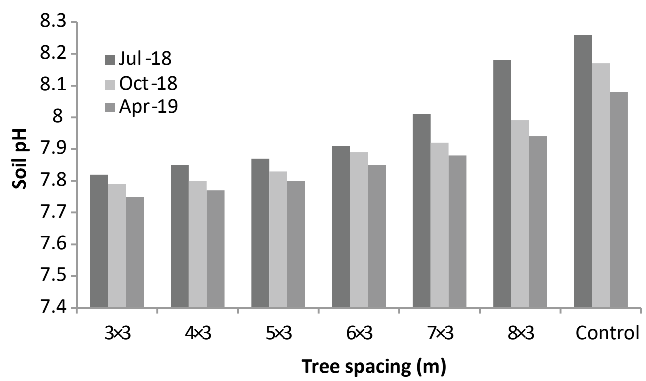

Fig. 2. Effect of different spacings of poplar on soil pH before sowing and after harvest of *Kharif* and *Rabi* crops (2018-19)

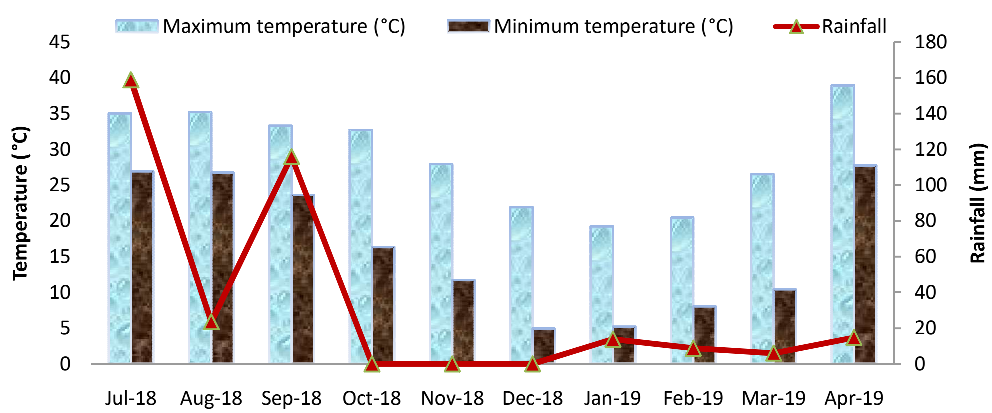

Fig. 1. The monthly mean meteorological data of the experimental site from July, 2018 to April, 2019

7.76 at 0-15 and 15-30 cm soil depth, respectively was recorded in closer (3×3m) spacing. However, in control, the soil pH at 0-15 cm and 15-30 cm was 8.08 and 8.12, respectively. The lesser value of soil pH under different spacings of poplar than sole cropping may be due to the acidic nature of litter humus of poplar after decomposition which decreased the soil pH. Similar results were reported by Pandey et al (2000) under *Acacia nilotica* based agroforestry system, Yadav et al (2011) under *Prosopis cineraria* (L.), *Dalbergia sissoo* (Roxb.) ex DC, *Acacia leucophloea* (Roxb.) and *Acacia nilotica* (L.) Del. based agroforestry system and Bisht et al (2018) under poplar based agroforestry system.

Soil electrical conductivity: The electrical conductivity of soil decreased from 0.22 to 0.19 dS/m under 3×3 m spacing of poplar and from 0.34 dS/m to 0.33 dS/m in control during the course of investigation (Table 1). The decrease in EC was maximum (13.60%) under 3×3 m spacing followed by 4×3 , 5×3 , 6×3 , 7×3 and 8×3 m spacing with a reduction of 13.04, 12, 7.69, 7.14 and 6.89% respectively. The rate of decrease in electrical conductivity was comparatively more under poplar based cropping system than control (2.94%) and may be due to substantial addition of organic matter under trees and release of weak organic acids during litter decomposition (Kumar et al (2020) under *Eucalyptus* based agroforestry system.

Soil organic carbon (SOC): The soil organic carbon was significantly influenced by tree spacing and it also increased from its initial status under different spacings of poplar and control as well (Fig. 3). The organic carbon in soil increased with the decrease in tree spacing and was recorded maximum (0.47 %) under 3×3 m spacing and followed by 4×3 m, 5×3 , 6×3 , 7×3 and 8×3 m after the harvesting of winter season fodder cops at 0-15 cm soil depth during 2019-20. High organic matter content in the intercropping treatment could be ascribed to the fact that heavy leaf fall in poplar occurs during winter and easily decomposed in soil. In control, continuous cropping with subsequent removal of plant residues leads to less accumulation of organic content in soil. Secondly may be due to lack of lignified cells in agricultural residues. Soil organic carbon under sole cropping may be reduced due to full exposure to the sun and thereby burning of organic carbon. Gill and Burman (2002) reported that the soil enrichment in organic carbon content under tree based systems could be due to several factors such as addition of litter, annual fine root biomass recycled and root exudates and its reduced oxidation of organic matter under tree shades. Agroforestry system recorded higher increase in soil organic carbon than open farming due to total leaf fall of poplar trees in winter season and leaves are easily decomposed in the soil. Addition of litter fall and fine-root in the soil turnover increased soil organic matter content. The changes in pH, EC and soil organic carbon of soil under the intercropping system could be due to the adequate plant

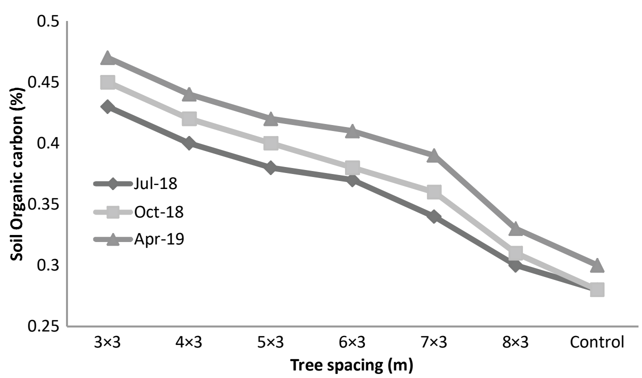

Fig. 3. Effect of different spacings of poplar on soil organic carbon content before sowing and after harvest of *kharif* and *rabi* crop

Table 1. Soil electrical conductivity (dsm^{-1}) under different spacings of poplar before sowing and after harvesting of cowpea, sorghum berseem and oat

Spacings (m)	EC (dsm^{-1})					
	Before sowing of fodder crops		After harvesting of cowpea and sorghum		After harvesting of berseem and oat	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
3×3	0.22	0.24	0.20	0.22	0.19	0.20
4×3	0.23	0.25	0.21	0.23	0.20	0.21
5×3	0.25	0.27	0.23	0.25	0.22	0.24
6×3	0.26	0.28	0.25	0.26	0.24	0.25
7×3	0.28	0.29	0.27	0.28	0.26	0.27
8×3	0.29	0.31	0.28	0.30	0.27	0.29
Control	0.34	0.34	0.34	0.33	0.33	0.32
CD (p=0.05)	0.017	0.019	0.020	0.021	0.014	0.020

cover on the soil which helped in the least crust formation, increased porosity and reduced bulk density. Dhillon et al (2018) and Dollinger and Jose (2018) also reported that the incorporation of trees in agroforestry system enhances the soil organic matter by adding litter both above and belowground. The results are in conformity with work of earlier researchers (Raj et al 2016, Kumar et al 2019, Bisht et al 2018, Dhillon et al 2020).

Available N, P and K: The available soil nitrogen increased significantly under different spacings of poplar from its initial values (Fig. 4). Like organic carbon, available nitrogen was also considerably influenced by different spacing because availability of nitrogen in soil depends upon organic matter. In present study, that available N content was maximum (158.5 kg/ha) under 3×3 m spacing and it decreased with the increase in the poplar spacings and minimum (142.5 kg/ha) under 8×3 m after harvesting of winter season fodder crops. The magnitude of increase in available N was highest under 3×3 m spacing and lowest in control. The increase in N content of soil under poplar based agroforestry systems may be due to addition of organic matter in soil in the form of litter fall and fine root biomass and availability of adequate moisture level associated with more moderate temperature in shade may result in a faster rate of mineralization, breakdown of litter, and turnover of N than occurs in open. The mineralization of organic matter releases nutrient into the soil (Osman et al 2001). Non N- fixing trees can also enhance soil physical, chemical and biological properties by adding significant amount of organic matter and releasing and recycling of nutrients in agroforestry systems (Yadav et al 200, Antonio and Gama-Rodrigues 2011**).** Similar findings of improvement in the nutrient status of soil due to intercropping in an agroforestry have been reported by Pardon et al (2017), Bisht et al (2018), Kumar et al (2019) and Dhillon et al (2020).

Similarly, available phosphorus in soil also exhibited similar trend as of soil nitrogen (Table 2). Available phosphorus in sole crop was 12.3 kg/ha while, it ranged from 15.5 to 16.8 kg/ha in different spacings of poplar. Among different spacings of poplar, the highest available soil P (16.8 kg/ha) was in 3×3 m spacing. Maximum (343.8 kg/ha) available potassium was under 3×3 m and minimum (332.9 kg/ha) under 8×3 m spacing after culmination of the experiment (after harvesting of winter season fodder crop) at 0-15 cm soil depth. However the least (330.3 kg/ha) available potassium was found under control /sole cropping (Fig. 5).

There was improvement in the available phosphorus and potassium content in the soil may be due to the increase in the humus content of soil after decomposition of litter fall of poplar. Similar findings were also reported by Githae et al (2011) in an Acacia-based agroforestry system. Similar findings of improvement in the nutrient status of soil due to intercropping in an agroforestry have been reported by Chen et al (2017) under rubber based agroforestry system and

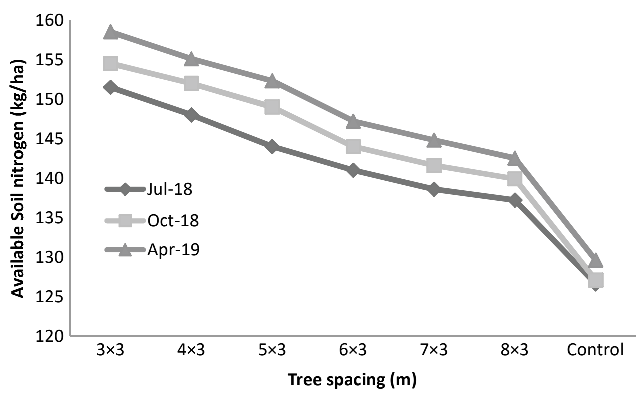

Fig. 4. Nitrogen content in the soil under different spacings of poplar before sowing and after harvesting of cowpea, sorghum berseem and oat

Table 2. Effect of different spacings of poplar on available soil phosphorus content before sowing and after harvest of *kharif* and *rabi* season crops

Spacings (m)	Available phosphorus (kg/ha)					
	Before sowing of fodder crops		After harvesting of cowpea and sorghum		After harvesting of berseem and oat	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
3×3	13.6	11.2	14.9	12.4	16.8	14.8
4×3	13.4	11.5	14.7	12.3	16.5	14.4
5×3	13.1	11.0	14.4	12.0	16.2	14.2
6×3	13.0	10.9	14.1	11.8	16.0	14.0
7×3	12.8	10.6	14.0	11.5	15.9	13.7
8×3	12.5	10.3	13.8	11.1	15.5	13.2
Control	11.3	9.7	12.0	10.2	12.3	11.8
CD (p=0.05)	0.75	0.67	0.92	1.22	0.73	0.99

Kumar et al (2019) under *Eucalyptus* based agroforestry system.

Yield of fodder crops : Among different spacings of poplar, significantly higher fodder yield of both rainy season (cowpea and sorghum) and winter season (berseem and oat) fodder crops was observed in wider spacing (8×3 m) as compared closer spacing (3×3 m) in poplar based agroforestry system and it follows increasing trend with increasing interspaces (Fig. 6). The maximum fresh fodder yield of both rainy and winter season fodder crops was recorded in control (crop devoid of trees) and it varied significantly under different spacings of poplar. The reduced yield of fodder crops under poplar plantation in present study may be ascribed to competition between tree and fodder crops for light, moisture and nutrients in a poplar based agroforestry system. Competition for light has a large influence in intercropping system than either moisture or nutrients and fodder production bears an almost linear relationship with the quantum of intercepted energy. Photosynthetic efficiency of crops increased due to more light intensity in control, resulting in better growth. The lesser availability of solar radiation and higher competition for growth resources in the silvi-pastoral system (poplar + fodder crops) may be responsible for lesser biomass production by fodder crops over control (fodder crops in open). Thus, competition for utilization of growth resources adversely affected the fodder yield of rainy (cowpea and sorghum) and winter (berseem and oat) season fodder crops under different spacings of poplar. Bhati et al (2004) revealed the similar result of fodder yield of cowpea and other fodder crops under the canopy of different agroforestry trees of arid regions of Rajasthan. Earlier researchers also reported reduction in grain yield of cowpea due to higher shade under *Eucalyptus tereticornis* based agroforestry system over open condition (Prasad et al 2010, Chesney et al 2010, Ratan et al 2015, Ranjan et al 2016). Sharma et al (2000) also reported that crop growth of wheat was inhibited under closer spacing of poplar.

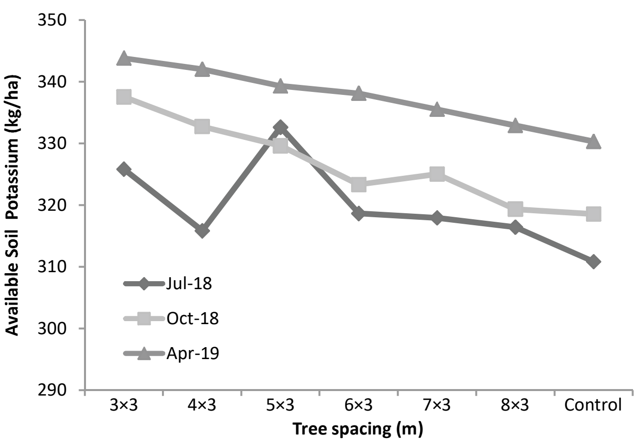

Fig. 5. Effect of different spacings of poplar on available soil potassium content before sowing and after harvest of *kharif* and *rabi* crops

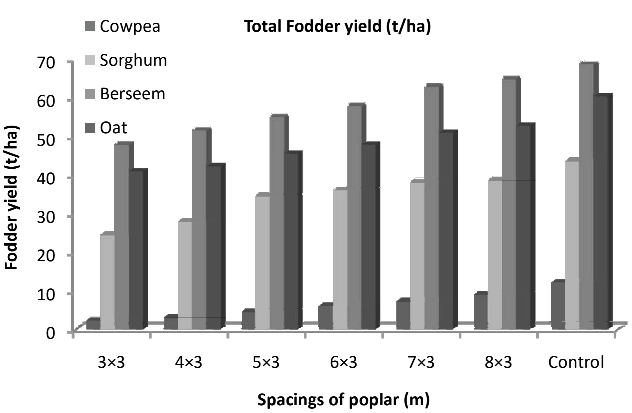

Fig. 6. Effect of different spacings of poplar on total fodder production of both rainy (cowpea and sorghum) and winter (berseem and oat) season

CONCLUSION

A considerable improvement in soil chemical properties (pH, EC, SOC, N, P and K) was observed under different spacings of poplar-based agroforestry system over control (sole crop). The soil pH and EC decreased more under different spacings of poplar than control (sole crop). The soil organic carbon and available soil N, P and K increased significantly under poplar spacings than control (sole crop) both at 0-15 and 15-30 cm soil depths. The effect was more pronounced under 3×3 m spacing, therefore this is more suitable for improving soil fertility by the accumulation of leaf litter with the advancement of tree age.

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Study on Frequency Distribution of Lianas on Trees in Dry Deciduous Forests of Guvvalacheruvu, Southern Eastern Ghats, Andhra Pradesh, India

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***Abstract*:** In the dry deciduous forest of Guvvalacheruvu, 43% of trees (>30 cm gbh) are colonized by at least one liana. Majority of lianas featured twining climbing mode in the forest. Liana infestation rate was higher among medium girth trees and a positive relation was observed between tree girth and liana infestation rate. No relation between a specific tree species and liana infestation was observed, but multiple stem trees were with high infestation rate. Spatial occurrence of lianas seemed to influenced the liana colonization rate on trees in these dry deciduous forests.

***Keywords*:** Dry deciduous forests, Lianas, Liana infestation rate, Stem twiners

Lianas are woody climbing plants that rely on trees for physical support in order to reach the tree canopy to avail better light conditions (Schnitzer and Bongers 2002) and represent nearly 25% of woody species in varied forest areas, but occur in high abundance as well as species rich in seasonal dry forests (Vivek and Parthasarathy 2015). The ability to grow and acquire nutrients in the dry season may allow lianas to occur in high abundance in seasonal forests (Tang et al 2012). In these dry forests also, lianas predominantly occur in canopy gaps and forest edges. In addition, for lianas, host finding and colonizing the suitable hosts form an important role in their life history (Perez-Salicrup 2005). Different lianas evolved different climbing modes and strategies that help in climbing the host trees (Vivek and Parthasarahty 2015). Tree-liana association studies provided inputs to both hypotheses that trees provide variety of niches and varied morphological features that could lead to specific relation between trees and lianas (Perez-Salicrup 2005, Vivek and Parthasarathy 2016) or trees just act as neutral support structures so that lianas near to trees will colonise them (Carse et al 2000, Cai et al 2009, Malizia and Grau 2006). Trees constituting rough bark, medium tree diameter and high tree or bole height may increase the liana frequency on trees and features like fast growth rates, evergreen foliage, stem flexibility may correspond to lower liana frequency (Vivek and Parthasarathy 2017). Further, small sized trees could be more negatively affected in their biological fitness in comparison with larger trees as they can balance the liana loads with their own big size and biomass (Carse et al 2000). Liana specific attributes like clonal growth, dispersal mechanisms, high abundance in tree fall gaps will determine the spatial distribution of lianas (Nabe-Nielsen 2001). The dry forests in the study sites have relatively lower tree species richness but comprise of higher tree individuals per unit area (Ramana and Reddy 2021) and this can form a well replicated unit for studying liana association on trees. Hence the study intends to inventory the number of lianas on trees to know specific tree-liana associations by recording the trees with and without the presence of lianas.

MATERIAL AND METHODS

Study area: The enumeration of the number of lianas on trees fieldwork was carried out in Guvvalacheruvu forest of Palakonda hill ranges 14^0 16'02.1''N latitude and 78^0 45'26.4''E and 14^0 16'25'25.9''N and 78^0 52'33.5''E longitude at an elevation of 330-470 msl. The forest hill ranges receive an annual average rainfall of 688mm and annual temperature range of 33^0C to 46^0C. In order to estimate the presence of lianas per tree, a total of 190 tree individuals (≥30 cm gbh) were observed in a random manner by laying a total of eight (10 X 100m) belt transects with atleast 100m distance between them and 20m distance from the edge of the forest. The field work effort was made to select atleast 33 tree individuals in each of the tree girth categories namely 30-50 cm, 51-70 cm and >70 cm gbh. The number of trees with and without lianas per girth classes were counted. All lainas (≥3 cm girth at 1.3 m distance from the root) either present in the tree canopy or liana climbed by taking the support of tree bole or branches were counted and identified. A correlation test was carried out to know the relation between tree girth and liana infestation rate. A chi-square test was applied to evaluate whether each girth class and the probability of being colonised by liana was independent or not. The observed frequency distribution of the number of lianas per trees was compared with the expected poison distribution with chi-square goodness test.

RESULTS AND DISCUSSION

A total of 190 tree individuals that belong to 36 tree species and 12 families was enumerated. Among them, 43% of trees have carried at least one liana individual. These lianas belong to 19 species and 11families; of them 12 species showed twining climbing mechanism, two species featured both twining and hook climbing mechanisms, three species have twining climbing mode and spines and two species are stragglers (Table 1). The frequency distribution of lianas on trees revealed a mean (lianas per tree) of 1.37 and a range of 0-7 liana individuals per tree. The majority of enumerated liana species were stem twiners as also recorded in Kolli hills (Chitti Babu and Parthsarathy 2001), southern Eastern Ghats of Tamil Nadu (Muthperumal and Parthasarathy 2010), dry evergreen forests (Reddy and Parthsarathy 2006), Sri Lankamalleswara wild life sanctuary (Mastan et al 2015) and Anamalais (Muthuramkumar and Parthsarathy 2001). In addition, the present study liana infestation rate (43%) is lower than the percent of liana infestation rate on trees in dry evergreen forests of point Calimere wild life sanctuary (57.9%; Vivek and Parthasarathy 2016) and profoundly higher than the liana infestation observed in Kolli hills (10%; Chitti Babu and Parthsarathy 2001) and Anamalais (28%; Muthuramkumar and Parthsarathy 2001).

The liana frequency distribution on trees was significantly different from the expected poison distribution (λ^2 = 77.03, P<0.05) indicating a clumped distribution of lianas on the trees (Table 2). The Chi –square test revealed a significant difference in liana infestation among the three different gbh class trees (λ^2 = 14.25, P<0.05. It suggests that liana presence on trees was found to be dependent on the tree gbh as tree individuals with 51-70 cm gbh showed high infestation rate (51.6%) followed by lower gbh trees (39%) and trees with higher gbh featured lower infestation rates (26.6%; Fig. 1). In addition, the correlation between trees (on log scale) and number of lianas was positive (0.646) revealing a considerable effect of tree gbh on the probability of liana occurrence on trees (Fig. 2). These observations are in line with the reports from Calimere (Vivek and Parthasarathy 2016), Los Tuxlas forest of Mexico (Perez-Salicrup (2005) and sub-tropical montane forest of Argentina (Malzia and Grau 2006) that higher gbh class trees provide longer exposure period for lianas to not only locate them and as well use them as supports to reach the top canopy and further venture to another tree canopy.

These dry deciduous forests constitutes trees with typical leafless period of around 4-6 months and in majority of trees leaf initiation occurred in the dry period, flowering peaked in summer period and got synchronized with leaf flushing activity (Mastan et al 2020). Similarly the large girthed lianas

Table 1. Liana species in the eight transects of dry deciduous forests of Guvvalacheruvu, Southern Eastern Ghats, Andhra Pradesh, India

Species	Family	Climbing mechanism
Abrus precatorius L.	Fabaceae	Twiner
Acasia caesia (L.) Wild.	Mimosaceae	Twiner
Aganosma cymosa (Roxb.) G.	Apocynaceae	Twiner
Calycopteris floribunda Lam.	Combretaceae	Twiner
Canavalia virosa (Roxb.) Wight & Arn.	Fabaceae	Twiner
Capparis zeylanica L.	Capparidaceae	Stragler
Carissa spinarum L.	Apocyanaceae	Stragler
*Combretum albidum*G.Don.	Combretaceae	Twiner
Decalepis hamiltonii Wight &Arn.	Asclepiadaceae	Twiner
Derris scandens (Roxb.) Benth.	Fabaceae	Twiner
Hugonia mystax L.	Liniaceae	Stragler
*Jasminum angustifolium*Vahl.	leaceae	Twiner
Mucuna pruriens (L.) DC.	Fabaceae	Twiner
Pterolobium hexapetalum (Roth) Sant. &Wagh	Caesalpiniaceae	Twiner
Rivea hypocrateriformis (Desr.) Choisy	Convolvulaceae	Twiner
Sarcostemma acidum (Roxb.) Voight Hort.	Asclepiadaceae	Twiner
Ventilago maderaspatana Gaertner	Rhamnaceae	Twiner
Wattakaka volubilis (L.f.) Stapf	Asclepediaceae	Twiner
Ziziphus oenoplia (L.) Mill.	Rhamnaceae	Scrambler

Table 2. Frequency distribution of lianas on observed trees in Guvvalacheruvu dry deciduous forest area

Frequency	Observed frequency distribution of Lianas on tree	Expected frequency by poison distribution
0	108	70
1	20	80
2	24	46
3	16	18
4+	8	6

like *Combretum albidum,* and *Calycopteris floribunda* shed their leaves in January and produced flowers in leafless period and winged fruits got matured for dispersal in May before the rains. Lianas with twining and spines like *Acacia caesia, Pterolobium hexapetalum, Ziziphus oenoplia* also have initiated the leaf fall in January/February and stragglers like *Carissa spinarum* and *Capparis zeylanica* have also promulgated their leaf fall in January indicating that lianas, like trees, also feature typical deciduousness of four-five months to overcome the water shortage and harsh dry conditions. Thus typical variation between trees and lianas in leaf phenology was not observed and indicates that leafless period among trees will not either deter or promote the lianas to reach the illuminated canopy and thus may not influence the liana infestation rate as also observed in Argentianian Montane forests (Malizia and Grau 2006). The lianas that have girth >10 cm gbh like *Combretum albidum*, *Calycopteris floribunda, Acacia caesia, Pterolobium hexapetalum, Ziziphus oenoplia* grow steady upright without structural support up to 1.0 to 1.5m height in the forest edges and then grow onto the canopy of moderate to large girthed trees. *Ventilago maderaspatana* with stem twining and hook structure gain girth increment even on small girthed trees. These lianas flower during the dry season and produce fruits to be matured with wind dispersed seeds at the beginning of the rainy season as it could result in seed set during the rainy season for better opportunities for germination and seedling survival rates, *Rivea hypocrateriformis, Wattakaka volubilis, Mucuna pruriens* with stem twining climbing mode reached the canopy by using the main tree trunks (below 1.3 m height) of low and medium girth trees. Stragglers like *Carissa spinarum* and *Hugonia mystax* were associated with low girthed trees along the rocky boulders. In the study, except for the observations of *Jasminum angustifloium* on *Albizia amara* trees with high mean infestation rate, no particular liana species and tree association was observed. These observations indicate that trees are acting as neutral supports by neither facilitating nor impeding the liana infestation. But trees with multiple stems (≥2 stems) were having high probability of liana occurrence on trees (λ^2= 9.22; P<0.05) than single stem trees as the possession of multiple stems may increase the probability of infestation from boles as well as provide larger area of tree canopy at lower height itself. These observations are in line with observations made in in tropical forests of Mexico and Ecuador, (Nabe Neilsen (2001) and Malizia and Grau (2006) that lianas are able to disperse or colonise along forest edges or canopy openings, then may try to infest whatever the tree individuals are available in the surrounding. In these dry deciduous forests trees with moderate girth and height are in high abundance and they may provide favourable light conditions on their lower canopy itself so that lianas can optimize their growth activity

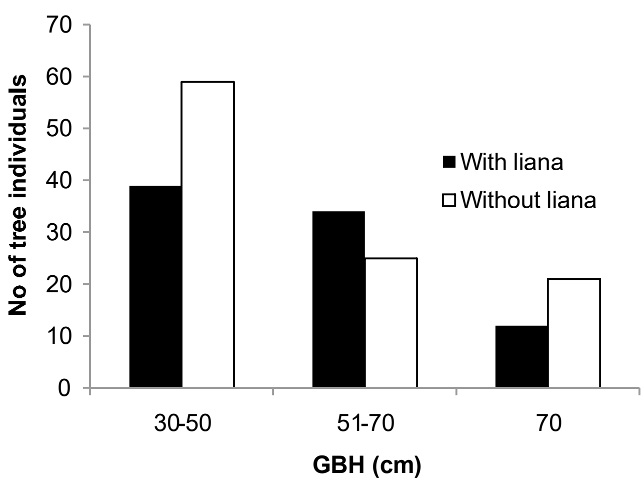

Fig. 1. Number of trees with and without lianas in three different gbh classes

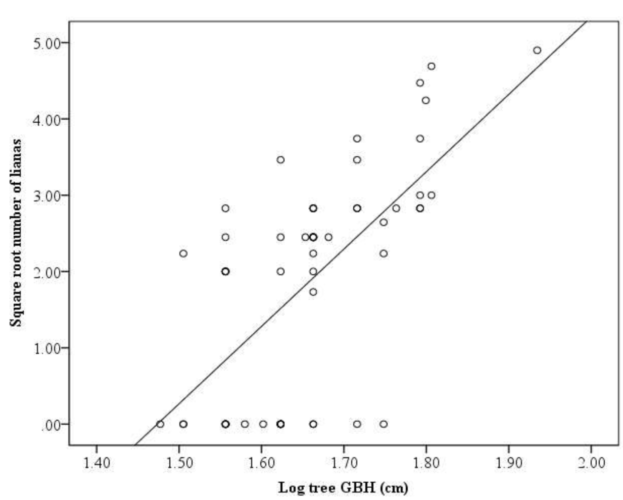

Fig. 2. Positive relationship between tree gbh and liana infestation

CONCLUSION

The study in dry deciduous forest reveal that stem twiners are the dominant liana types and liana spatial distribution, abundance, climbing mode influence the tree-liana interactions. The results are in line with the observations made in other studies that trees mostly act as passive neutral supports and spatial distribution of lianas which tends to occur more in suitable light conditions edges and can climb on to the trees mainly with medium gbh classes.

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Variability of Peach Cultivars for Growth, Yield and Fruit Quality Traits

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Abstract: The aim of this study was assessment of variability among fifteen peach cultivars. The evaluation was carried out at Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP) during 2014-2016 and several morphological and biochemical characters were recorded. Tree height ranged between 1.28 m (Suncrest) and 3.78 m (Shan-i-Punjab).Trunk girth was maximum in Flordaprince and minimum in Saharanpur Prabhat. Tree yield per plant varied from 4.50 kg (Early Redhaven) to 10.83 kg (Saharanpur Prabhat) and yield efficiency from 0.18% (Early Redhaven) to 0.54% (Pratap), respectively. Paradelux recorded maximum inter-nodal length whereas, July Elberta recorded minimum. Fruit length was maximum in Early Redhaven (53.69 mm) and minimum (37.37 mm) in Saharanpur Prabhat, whereas, fruit breadth was maximum (54.38 mm) in Flordaprince and minimum (42.62 mm) in July Elberta. The TSS content in fruits ranged between 8.65^{0}B to 13.02^{0}B. The variability observed in these genotypes excelling in one or more horticulturally desirable characters can further be utilized for the genetic improvement of peach genotypes.

Keywords: Peach, peach genotypes, fruit quality

Peach is an important fruit crop of temperature region all over the world. The peach cultivars are divided into three groups namely, Northern, Southern and European or Persian group according to their geographical distribution. Furthermore they can also be divided on the basis of their chilling requirements as high chilling and low chilling. The chilling requirement of most of the peach cultivars lies between 500 to 1000 hours below 7.2° C to foliate and bloom, however cultivars with less than 100 hours of chilling requirement are known (Joshi et al 2017). Low chilling peach cultivars were developed in Florida during last three to four decades and have become very popular in the sub-mountainous Himalayan region. In India, Peach is generally grown over an area of 18,000 hectares with a production of 1,23,000 MT (Anonymous 2019). Low-chilling cultivars viz. Flordasum, Flordared, Shan-e-Punjab, Sharbati and Sunred (nectarine) have become popular in subtropical belts of U.P. and Punjab States. Most of the peach cultivars are exotic introduction acquiring local name or synonyms at different agroclimatic conditions. Over the year, a large number of cultivar have been evolved through breeding in many countries to take best advantage of its diverse climatic adaptability and to make available the fresh fruits over a longer season. However, in recent times, peach production has a declining trend mingled with a number of factors such as diseases, overdependence on a selective cultivars and global warming (Jana 2015). Keeping in view above factors, variability in different tree growth, yield and fruit parameters were recorded to determine the possessed qualities that are desirable to consumers and growers with context to fruit quality characteristics and yield for commercial cultivation.

MATERIAL AND METHODS

In the present experiment, 6-7 years old bearing trees of fifteen peach accessions were evaluated in a randomized block design with three replications during 2014-2016 (Table 1). The data on tree growth, yield, foliage, floral and fruit quality parameters were recorded. Tree height was measured with the help of calibrated staff from ground level to the tip of tallest branch of the tree and it was expressed in meters (m). The spread of the tree was measured by calibrated staff in two directions i.e. NorthSouth (N-S) and East-West (E-W) and expressed in meters (m). Thirty leaves from the middle portion of the current growth were selected during the last week of July for recording leaf characters. Leaf length was measured by a scale from tip of the apex to the base, whereas width of the leaf was measured with a scale at its broadest expand. The data on inter-nodal length was recorded at the end of growing season from the middle third of shoot and expressed in cm. Yield efficiency was calculated by the method suggested by Westwood (1993) as under:

$$\text{Yield efficiency (kg/cm}^2\text{)} = \frac{\text{Yield (kg)}}{\text{Tree Trunk cross-sectional area}}$$

To study physico-chemical fruit parameters, 15 fruit samples were taken randomly in three replicates from all directions at optimum maturity. Fruit length, diameter, depth of stalk cavity and width of stalk cavity were measured. Fruit firmness was determined with the help of effigi penetrometer using 7/16" plunger in kg/cm^2. Erma hand refractometer (0–32° Brix) was used to work out total soluble solids content in fruits. Titratable acidity was determined with the standard alkali solution and expressed in terms of malic acid (%).

RESULTS AND DISCUSSION

Tree growth and yield parameters: Tree height in peaches ranged between 1.28 m in Suncrest and 3.78 m in Shan-i-Punjab with mean tree height of 2.58 m (Table 1) suggesting considerable variation and genotypic diversity in vigour of the tree. Such variation in growth characters has also been reported by Singh et al *(*2005) and Yepthomi (2011). Maximum (21.95 cm) trunk girth was in Flordaprince and minimum (12.80 cm) in Saharanpur Prabhat. All the peach accessions were statistically different from each other in respect of trunk girth. Maximum tree spread in North-South (N-S) direction was 1.26 m in Vallegrande and corresponding minimum was 0.90 m in Earligrande. Maximum tree spread in East-West (E-W) direction was 1.43 m in Vallegrande whereas minimum was 0.91m in Flordaglo. Tree yield per plant was maximum (10.83 kg) in Saharanpur Prabhat which was statistically at par with Tropic Beauty and minimum (4.50 kg) in Early Redhaven. Maximum (0.54%) yield efficiency was in Pratap and minimum (0.18%) in Early Redhaven (Table 1). The comparatively low yield efficiency can be attributed to young age of the plants under study.

Foliage parameters: Length of the stipule was maximum (1.25 cm) in July Elberta whereas, minimum (0.98 cm) in Earligrande. Statistical difference was found among all accessions for leaf length with value ranging between 13.8 cm (Glohaven) and 17.1 cm (TropicSweet and TropicBeauty). Leaf width was maximum (4.59 cm) in TropicSweet whereas minimum (2.32 cm) in Saharanpur Prabhat. Variation in width of peach leaves has been reported earlier also by Wolfe and Strang (2010). The length of the petiole was maximum in TropicBeauty (1.12 cm) and minimum in Earligrande (0.65 cm). Similarly, these characters are used by Bodh et al (2019) in order to distinguish between related varieties of the peach.

Floral parameters: The flowering shoot thickness was maximum (4.96 m) in TropicSweet and minimum (2.93 cm) in Suncrest (Table 3). Variation in inter-nodal length ranged from 2.39 cm (July Elberta) to 3.88 cm (Paradelux) and is in accordance, by and large with PPVFRA guidelines (Anonymous 2015). Similar variation in inter-nodal length has been observed in the past by Devi et al (2018). However, Pandey et al (2019) reported inter-nodal length to vary from 1.87 cm to 2.40 cm. Inter-nodal length of shoots is considered as a good indicator of darkness/compactness in a genotype.

Table 1. Variation in tree growth and yield parameters of peach accessions

Accessions	Tree height (m)	Tree spread (m)		Trunk girth (cm)	Tree yield (kg plant^{-1})	Yield efficiency (%)
		N-S	E-W			
Earligrande	2.36	0.90	1.13	20.67	9.17	0.45
Early Redhaven	2.67	1.11	1.00	16.00	4.50	0.18
Flordaglo	2.70	1.09	0.91	16.43	8.50	0.40
Flordaprince	2.25	1.07	1.21	21.95	8.67	0.22
Glohaven	3.21	0.95	1.22	16.27	6.33	0.30
July Elberta	2.90	1.17	1.05	18.70	8.03	0.29
Paradelux	2.60	0.98	1.30	16.47	5.83	0.42
Pratap	2.40	1.24	0.99	17.60	9.33	0.54
Suncrest	1.28	1.06	1.16	17.80	7.67	0.38
Saharanpur Prabhat	2.42	1.09	0.92	12.80	10.83	0.32
Shan-i-Punjab	3.78	1.01	1.17	14.70	7.20	0.29
TropicBeauty	2.21	0.97	1.17	18.60	10.50	0.38
TropicSnow	2.81	0.92	1.05	18.33	8.23	0.31
TropicSweet	2.70	1.21	1.04	18.93	7.65	0.27
Vallegrande	2.20	1.26	1.43	19.67	8.50	0.28
Mean	2.58	1.08	1.13	17.2	8.06	0.33
CD (p=0.05)	0.19	0.15	0.18	0.95	0.73	0.03

The width of petal was highest (1.51 cm) in Earligrande and lowest (1.03 cm) in Suncrest. All the accessions were statistically different from each other.

Biochemical parameters: In general, the domestic market has a likeness toward peach fruits which are large in size, sweet in taste, less acidic, juicy and flesh is easily separable from the stone. Several workers have worked on the physical aspects of peach fruits (Cantin et al 2010, Forcada et al 2014) in the past and have reported considerable variation in fruits of different peach cultivars. Length of the fruit was maximum (53.69 mm) in Early Redhaven and minimum (37.37 mm) in Saharanpur Prabhat, whereas, fruit breadth was maximum (54.38 mm) in Flordaprince and minimum (42.62 mm) in July Elberta (Table 4). These results are in accordance with the

Table 2. Variation in foliage parameters of peach accessions

Accessions	Stipule length (cm)	Leaf blade length (cm)	Leaf blade width (cm)	Leaf blade length/ width ratio	Petiole length (cm)
Earligrande	0.98	14.3	2.89	3.98	0.65
Early Redhaven	1.15	15.1	2.81	3.90	0.93
Flordaglo	1.15	14.3	3.27	3.95	1.09
Flordaprince	0.99	14.1	3.12	3.89	0.98
Glohaven	1.01	13.8	3.68	4.15	1.01
July Elberta	1.25	15.2	2.67	3.89	1.12
Paradelux	1.03	14.6	3.08	3.80	1.04
Pratap	1.12	14.2	3.65	4.21	0.97
Suncrest	1.02	14.4	2.74	3.91	0.72
Saharanpur Prabhat	1.13	15.6	2.32	4.18	0.83
Shan-i-Punjab	1.02	16.1	2.38	4.14	0.95
TropicBeauty	1.18	17.1	4.03	3.99	1.12
TropicSnow	0.99	14.1	3.88	4.23	1.03
TropicSweet	1.03	17.1	4.59	3.74	1.07
Vallegrande	1.04	15.3	2.69	3.86	1.03
CD (p=0.05)	0.03	0.80	0.11	0.18	0.10

Table 3. Variation in floral parameters of peach accessions

Accessions	Flowering shoot thickness (cm)	Length of the inter-nodes (cm)	Petal width (cm)	Number of petals
Earligrande	4.49	3.42	1.51	5
Early Redhaven	4.67	3.35	1.25	5
Flordaglo	4.74	2.58	1.48	5
Flordaprince	4.66	3.36	1.37	5
Glohaven	4.74	3.50	1.23	5
July Elberta	4.85	2.39	1.20	5
Paradelux	4.33	3.88	1.39	5
Pratap	4.68	3.39	1.09	5
Suncrest	2.93	3.52	1.03	5
Saharanpur Prabhat	4.68	3.09	1.21	5
Shan-i-Punjab	4.69	3.51	1.34	5
TropicBeauty	4.58	3.28	1.16	5
Tropic Snow	4.53	2.48	1.36	5
Tropic Sweet	4.96	2.93	1.28	5
Vallegrande	4.58	3.31	1.29	5
CD (p=0.05)	0.58	0.59	0.08	-

Table 4. Variation in biochemical parameters of peach accessions

Accessions	Fruit length (mm)	Fruit breadth (mm)	Depth of stalk cavity (cm)	Width of stalk cavity (cm)	Titratable acidity (%) as malic acid	Total soluble solids (°B)	Firmness (psi)
Earligrande	39.84	49.30	0.41	0.93	0.64	9.32	13.33
Early Redhaven	53.69	44.09	0.50	1.29	0.59	12.03	13.01
Flordaglo	38.49	49.04	0.21	0.77	0.65	9.99	13.85
Flordaprince	48.61	54.38	0.59	1.57	0.73	10.89	13.47
Glohaven	43.76	50.36	0.55	1.37	0.54	10.77	13.65
July Elberta	42.99	42.62	0.47	1.21	0.55	10.42	13.45
Paradelux	41.90	49.20	0.74	1.43	0.52	10.34	13.29
Pratap	47.25	51.30	0.81	2.01	0.80	8.65	13.19
Suncrest	44.55	47.02	0.48	1.09	0.57	9.94	13.07
Saharanpur Prabhat	37.37	50.26	0.47	1.43	0.48	8.98	13.59
Shan-i-Punjab	48.71	50.25	0.53	1.78	0.56	9.93	13.89
TropicBeauty	47.17	51.27	0.37	1.34	0.49	11.11	13.23
TropicSnow	47.13	50.35	0.56	1.40	0.62	10.21	14.01
TropicSweet	46.39	50.54	0.48	1.04	0.56	13.02	13.41
Vallegrande	44.00	52.50	0.39	1.13	0.71	10.56	13.18
CD (p=0.05)	2.43	1.71	0.05	0.10	0.04	0.41	0.51

study of Devi et al (2012) in which fruit length and breadth of different peaches varied from 43.64 to 58.10 mm and 43.88 to 61.34 mm, respectively. However, Singh et al (2014) reported maximum fruit length (56.18 mm) and breadth (59.30 mm) in 'Flordaglo', whereas, minimum fruit length (36.01 mm) and breadth (40.52 mm) in 'Vallegrande'. Fruit firmness varied between 13.01 psi and 14.01 psi among different peach accessions (Table 4.). This is an important factor taken into consideration where the fruit has to be transported over long distances and local markets are not available. However, Singh et al (2014) reported fruit firmness varied a little between 1.02 kg/cm^2 and 1.18 kg/cm^2 in different peach accessions. The TSS content in fruits was found to be ranging between 8.65°B in Pratap to 13.02°B in TropicSweet (Table 4) Singh et al (2014) observed TSS content in TropicSweet was 15.33 °B, whereas Jana (2015) reported 13.8°B TSS in Pratap. The highest acidity (0.80%) was in Pratap and lowest in Saharanpur Prabhat (0.48%). Rouse et al (2006) recorded highest acidity in TropicBeauty followed by UF Gold and lowest in Flordaprince. However, Singh et al (2009) observed highest acidity in Flordaprince followed by Earligrande and least in in Saharanpur Prabhat. Such variations may be due to different agro-climatic conditions influencing synthesis of biochemical constituents in the developing fruits and the duration of fruit development period.

CONCLUSION

The large amount of genetic variability was observed among peach cultivars for different morphological and physico-chemical traits. TropicSweet (High TSS and yield), Saharanpur Prabhat (thick flowering shoot, large sized fruit and firm), Flordaprince (High yield, trunk girth, large and firm fruits). These variations can further be utilized in future breeding and genetic improvement programmes for yield and fruit quality traits.

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Influence of Planting Density, Canopy Architecture and Drip Fertigation on Plant Growth and Productivity of Apple (*Malus × Domestica* Borkh.)

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Abstract: The experiment laid out in a randomized block design (factorial) had treatment combinations of three planting density, two training systems and fertigation doses of 100 and 75 per cent of AD (NPK). The results revealed that trees planted at a density of 2666 trees ha^{-1} registered highest annual shoot growth, increase in tree height, spread, scion girth, and volume. The lowest tree growth was recorded in high density planting of 5333 trees ha^{-1}. The results showed a positive relationship between productivity and planting density. The productivity increased with increasing density, while yield per trees decreased. Among the training systems, vertical axis trained trees reported the highest annual shoot growth, increase in tree height, spread, scion girth, and volume and the lowest tree growth was observed in vertical axis system. However, the maximum productivity was registered by the trees trained with Tall spindle system and the minimum was noted in vertical axis system. In case of fertigation, significantly highest tree growth parameters and productivity was registered by the trees subjected 100 % AD (NPK). Among three factors interaction, the highest tree growth parameters were recorded in trees planted at density of 2666 trees ha^{-1}, trained with vertical axis and subjected 100 % AD (NPK).

Keywords: Planting density, Tall spindle, Vertical axis, Fertigation, Plant growth, Productivity

Apple industry in the country is confronted with many problems contributing to low productivity, wide annual fluctuations in production and poor quality of produce. Several factors are associated with these problems but low density plantations with their improper canopy management are major. Most of the existing apple orchards in the state are on seedling rootstocks and planted under low density at a spacing of 7.5 x 7.5m (standard varieties) with a planting density of 178 trees ha^{-1} and at a spacing of 5.0 x 5.0m (spur type varieties) with a planting density of 400 plants ha^{-1}. High density planting (HDP) is one of the important methods to achieve high productivity per unit area.

High-density planting in fruit growing was first established in the apple in Europe in the early sixties (Robinson 2003). The continued decline in the availability of cultivable land, rising energy, and land costs together with the mounting demand for produce, has given a thrust to the adoption of HDP in fruit crops. High-density planting is more efficient since it is precocious, easily manageable, has higher yield potential with better quality fruits and higher returns/unit area. Training system and pruning are two important horticultural practices of canopy architecture that plays significant role in quality fruit production (Jackson and Palmer 1977). The aim of training system is to shape canopy architecture to improve light interception and distribution for optimizing fruit quality and yield. Thus, the choice of the training system plays a critical role in orchard profitability. Over the past two or three decades a large number of new intensive training systems for high-density apple orchards have been developed and recommended in Europe, America and New Zealand (Tall Spindle, Central Leader, Fruiting Wall, Palmette, Slender Pyramid, Slender Axis, Slender Spindle, Solaxe, Super Spindle, Vertical Axis, V-shaped and Y-trellis) (Robinson 2003).

Apart from managing crop loads (Meland 2011), balancing optimum plant nutrient contents and regulating water supply are important tools for influencing annual growth, productivity and fruit quality. Fertigation permits close synchronization of nutrient application with plant demand as the nutrients are delivered directly to the root system, therefore, the uptake of minerals is more efficient and nutrient leaching and run off are limited (Raina et al 2011). The rate of uptake and usage of different nutrients vary throughout the season in apple trees (Neilsen et al 2009).

MATERIAL AND METHODS

The present investigation was carried out in the experimental farm of Department of fruit science, University of Horticulture & Forestry, Nauni, Solan to ascertain the effect of different planting densities, training systems and fertigation levels on tree growth and productivity of apple under high density planting. The experimental site is situated at an elevation of 1256 m above mean sea level with 30° 51´ North latitude and 77° 88´ East longitude. Field trial was conducted during the years 2019 & 2020 on 4-years-old apple cultivar Jeromine grafted on M9 rootstock having uniform vigour and size. The apple plantation was done with three planting densities viz. 4000 trees ha^{-1} (2.5 × 1.0 m), 3200 trees ha^{-1} (2.5 × 1.25 m) and 2666 trees ha^{-1} (2.5 × 1.5m), trained to two training systems i.e., tall spindle and vertical axis. The experiment was laid out in a randomized block design (factorial) with treatment combinations of three planting density, two training systems and fertigation doses (50 and 37 per cent of RDF) and each replicated three times having two trees per replication.

The experimental trees were subjected under drip irrigation and fertigation system, consisting of two online emitters per plant, placed at a distance of 15 cm away from the tree trunk at an angle of 180°C to each other with a discharge rate of 4.2 l h^{-1}. The experimental trees were pruned every year during January to remove dead, diseased and unwanted branches and kept under uniform cultural practices with permanent support system during the course of investigation. Fertigation was done through venturi starting from third week of March of each experimental year and continued till July in fifteen equal split applications at weekly intervals. The water soluble fertilizer (WSF-19:19:19) was used for fertigation, fulfilling its phosphorus requirement and rest of the nitrogen and potassium requirement were supplemented with urea and muriate of potash (MOP).

Considering the solubility and compatibility of WSF, urea and MOP (Waterman, 2001), a 25 litres of stock solution having all the three fertilizers was prepared fresh for each fertigation. The quantity of WSF, urea and MOP was computed to be 24.56, 10.13 and 9.33 g $tree^{-1}$ $split^{-1}$, respectively with the application of 100 % of AD (NPK) and 18.13, 7.46 and 6.86 g $tree^{-1}$ $split^{-1}$, respectively with 75 % of AD (NPK) on 4-years-old plantation in the year 2019, whereas for 5-years-old plants (2020) the quantity came out to be 30.66, 12.68 and 11.68 g $tree^{-1}$ $split^{-1}$, respectively with 100 % of AD (NPK) and 22.69, 9.38 and 8.64 g $tree^{-1}$ $split^{-1}$, respectively with 75 % of AD (NPK). The observations were recorded on tree growth parameters and productivity and presented separately for two years of study.

Statistical analysis: The data obtained from this investigation were appropriately computed, tabulated and analysed using Randomized Block Design (Factorial). The statistical analysis was carried out for each observed character using MS-Excel and OPSTAT as per the design of experiment. The critical difference was calculated at a significance level of 5 per cent.

RESULTS AND DISCUSSION

Tree height: The perusal of data given in Table 1 indicates that planting densities, training systems and fertigation levels individually had a significant effect on tree height during both the years, however, their interactions were found non-significant. Considering the effect of planting densities, the highest increase in tree height (27.21 and 29.56 cm) was recorded in planting density of 2666 trees ha^{-1} and was found significantly superior to planting density of 4000 trees ha^{-1}) and 3200 trees ha^{-1}. However, the lowest increase in tree height (21.09 and 21.80 cm) was found in 4000 trees ha^{-1} planting density. Among the training systems, the maximum increase in tree height was observed in vertical axis training system, which was significantly higher than all spindle training system during both the years. Similarly, data of fertigation levels also showed significant effect on tree height and the highest increase in tree height (26.60 and 28.10 cm) was attained by trees subjected under F_1 fertigation level, which was significantly higher than F_2 level of fertigation (21.65 and 23.01 cm). The data also reveals that interactions between T×D×F were found non-significant. However, among three factors interaction, the highest increase in tree height (32.67 and 35.29 cm) was recorded in trees planted at D_3 density, trained with vertical axis and subjected under F_1 level of fertigation and the lowest (15.53 and 16.01 cm) was noticed in trees planted at D_1 density, trained with Tall spindle and subjected under F_2level of fertigation.

Tree spread: The perusal of the data given in Table 2 revealed that different planting densities, training systems, fertigation levels exhibited significant effect on tree spread during both the years. Among the planting densities, the highest increase in tree spread (19.23 and 21.60 cm) was observed in 2666 trees ha^{-1} planting density, which was significantly superior to D_2 and D_1 planting densities. Whereas, the lowest increase in tree spread of 13.96 and 14.69 cm was found in 4000 trees ha^{-1} density. In case of training systems, the maximum increase in tree spread (18.72 and 20.02 cm) was attained by trees trained with vertical axis training system, which was significantly higher than tall spindle training system during both the years. Similarly, the trees subjected to F_1 fertigation level showed significantly more increase in tree spread (18.14 and 19.65 cm) than F_2 level of fertigation (15.21 and 16.59 cm). It is apparent from the data that interactions between T×D×F were found non-significant, however, among three factors interaction, the highest increase in tree spread (22.82 and

25.07 cm) was recorded in trees planted at D_3 density, trained with vertical axis and subjected under F_1 level of fertigation.

Annual shoot growth: The data pertaining to the effect of different planting densities, training systems, fertigation levels and their interactions on annual shoot growth depicted in Table 3 reveals that planting densities, training systems and fertigation levels exhibited significant effect on annual shoot growth, during both the years, however their interactions were found non-significant. Among the planting densities, the highest annual shoot growth (34.81 and 38.14 cm) was recorded in 2666 trees ha^{-1} planting density, which was significantly higher than D_2 and D_1 planting densities. However, the lowest annual shoot growth of 25.73 and 28.19 cm was found in 4000 trees ha^{-1} planting density. The maximum annual shoot growth (31.95 and 35.21 cm) was observed in vertical axis training system and it was found statistically significant than Tall spindle system. Similarly, the trees subjected to F_1 fertigation level registered significantly more annual shoot growth (31.53 and 35.03 cm) than F_2 fertigation level. The data also reveals that interactions between T×D×F were found non-significant. However, among three factors interaction, the highest annual shoot growth (37.34 and 41.71 cm) was recorded in trees planted at D_3 density, trained with vertical axis and subjected under F_1 level of fertigation, whereas, the lowest annual shoot growth (22.87 and 24.80 cm) was noted in trees planted at D_1 density, trained with Tall spindle and subjected under F_2 level of fertigation.

Scion girth: The data presented in Table 4 reveals that planting densities, training systems and fertigation levels had marked influence on scion girth, during both the years, however their interactions were found non-significant. Among the planting densities, the highest increase in scion girth (6.93 and 5.20 mm) was registered in 2666 trees ha^{-1} planting density, which was significantly higher than D_2 and D_1 planting densities. On the contrary, the lowest increase in scion girth of 5.72 and 4.29 mm was found in 4000 trees ha^{-1} planting density. The maximum increase in scion girth (6.30

Table 1. Effect of different planting densities, training systems and fertigation levels on tree height and spread of apple cv. Jeromine during 2019 and 2020

T/D/F	Tree height 1st year (2nd year)						Tree spread 1st year (2nd year)					
	T_1			T_2			T_1			T_2		
	F_1	F_2	Mean (T×D)	F_1	F_2	Mean (T×D)	F_1	F_2	Mean (T×D)	F_1	F_2	Mean (T×D)
D_1	20.82 (21.45)	15.53 (16.01)	18.18 (18.73)	26.28 (27.21)	21.73 (22.51)	24.01 (24.86)	13.38 (14.31)	10.71 (11.56)	12.05 (12.94)	17.45 (18.13)	14.28 (14.76)	15.87 (16.45)
D_2	23.63 (24.76)	18.38 (19.33)	21.01 (22.05)	29.72 (31.18)	24.58 (25.97)	27.15 (28.58)	16.16 (17.62)	13.28(14.67)	14.72 (16.15)	20.42 (21.55)	17.53 (18.48)	18.98 (20.02)
D_3	26.48 (28.73)	21.86 (24.03)	24.17 (26.38)	32.67 (35.29)	27.83 (30.19)	30.25 (32.74)	18.63 (21.23)	15.52 (17.94)	17.08 (19.59)	22.82 (25.07)	19.96 (22.15)	21.39 (23.61)
Mean (T×F)	23.64 (24.98)	18.59 (19.79)		29.56 (31.23)	24.71 (26.22)		16.06 (17.72)	13.17(14. 72)		20.23 (21.58)	17.26 (18.46)	
Mean (T)		21.12 (22.39)			27.14 (28.73)			14.61 (16.22)			18.74 (20.02)	

F/D	F × D			CD (0.05)						
	F_1	F_2	Mean (D)	T	2.02 (2.09)	F_1	F_2	Mean (D)	T	2.10 (2.13)
D_1	23.55 (24.33)	18.63 (19.26)	21.09 (21.80)	F	2.02 (2.09)	15.42 (16.22)	12.50 (13.16)	13.96 (14.69)	F	2.10 (2.13)
D_2	26.68 (27.97)	21.48 (22.65)	24.08 (25.31)	D	2.47 (2.56)	18.29 (19.59)	15.41 (16.58)	16.85 (18.08)	D	2.57 (2.61)
D_3	29.58 (32.01)	24.85 (27.11)	27.21 (29.56)	T×F	NS(NS)	20.73 (23.15)	17.74 (20.05)	19.23 (21.60)	T×F	NS (NS)
Mean (F)	26.60 (28.10)	21.65 (23.01)		T×D	NS(NS)	18.14 (19.65)	15.21 (16.59)		T×D	NS (NS)
				F×D	NS(NS)				F×D	NS (NS)
				T×F×D	NS(NS)				T×F×D	NS (NS)

Training system (T):- T_1: Tall spindle; T_2: Vertical axis

Fertigation level (F):- F_1: 100 % of AD (NPK); F_2: 75 % of AD (NPK)

Planting density (D):- D_1: 4000 trees ha^{-1} (2.5 × 1 m); D_2: 3200 trees ha^{-1} (2.5 × 1.25 m); D_3: 2666 trees ha^{-1} (2.5 × 1.5 m)

Table 2. Effect of different planting densities, training systems and fertigation levels on annual shoot growth of apple cv. Jeromine during 2019 and 2020

T/D/F	Annual shoot growth (cm)- 2019						Annual shoot growth (cm)- 2020					
	T_1			T_2			T_1			T_2		
	F_1	F_2	Mean (T×D)	F_1	F_2	Mean (T×D)	F_1	F_2	Mean (T×D)	F_1	F_2	Mean (T×D)
D_1	25.63	22.87	24.25	28.74	25.67	27.21	28.21	24.80	26.51	32.25	27.49	29.87
D_2	29.82	27.43	28.63	33.37	31.26	32.32	33.09	29.91	31.50	37.45	33.85	35.65
D_3	34.28	32.29	33.29	37.34	35.34	36.34	37.47	34.86	36.17	41.71	38.52	40.12
Mean (T×F)	29.91	27.53		33.15	30.76		32.92	29.86		37.14	33.29	
Mean (T)		28.72			31.95			31.39			35.21	

F/D	F × D			CD (0.05)						
	F_1	F_2	Mean (D)	T	2.14	F_1	F_2	Mean (D)	T	2.22
D_1	27.19	24.27	25.73	F	2.14	30.23	26.15	28.19	F	2.22
D_2	31.60	29.35	30.47	D	2.61	35.27	31.88	33.58	D	2.72
D_3	35.81	33.82	34.81	T×F	NS	39.59	36.69	38.14	T×F	NS
Mean (F)	31.53	29.14		T×D	NS	35.03	31.57		T×D	NS
				F×D	NS				F×D	NS
				T×F×D	NS				T×F×D	NS

Training system (T):- T_1: Tall spindle
T_2: Vertical axis
Fertigation level (F):- F_1: 100 % of AD (NPK)
F_2: 75 % of AD (NPK)

Planting density (D):- D_1: 4000 trees ha^{-1} (2.5 × 1 m)
D_2: 3200 trees ha^{-1} (2.5 × 1.25 m)
D_3: 2666 trees ha^{-1} (2.5 × 1.5 m)

Table 3. Effect of different planting densities, training systems and fertigation levels on tree volume of apple cv. Jeromine during 2019 and 2020

T/D/F	Increase in tree volume (cm) - 2019						Increase in tree volume (m^3)- 2020					
	T_1			T_2			T_1			T_2		
	F_1	F_2	Mean (T×D)	F_1	F_2	Mean (T×D)	F_1	F_2	Mean (T×D)	F_1	F_2	Mean (T×D)
D_1	0.95	0.77	0.86	1.65	1.49	1.57	1.13	0.99	1.06	2.00	1.92	1.96
D_2	1.11	1.08	1.10	1.42	1.40	1.41	1.47	1.36	1.42	1.93	1.86	1.90
D_3	1.77	1.28	1.53	1.96	1.56	1.76	2.41	1.75	2.08	2.80	2.18	2.49
Mean (T×F)	1.28	1.04		1.68	1.48		1.67	1.37		2.24	1.99	
Mean (T)		1.16			1.58			1.52			2.12	

F/D	F × D			CD (0.05)						
	F_1	F_2	Mean (D)	T	0.013	F_1	F_2	Mean (D)	T	0.014
D_1	1.30	1.13	1.22	F	0.013	1.57	1.46	1.51	F	0.014
D_2	1.25	1.25	1.25	D	0.015	1.70	1.61	1.66	D	0.017
D_3	1.86	1.42	1.64	T×F	0.018	2.60	1.96	2.28	T×F	0.019
Mean (F)	1.47	1.27		T×D	0.022	1.96	1.68		T×D	0.024
				F×D	0.022				F×D	0.024
				T×F×D	0.031				T×F×D	0.033

Training system (T):- T_1: Tall spindle
T_2: Vertical axis
Fertigation level (F):- F_1: 100 % of AD (NPK)
F_2: 75 % of AD (NPK)

Planting density (D):- D_1: 4000 trees ha^{-1} (2.5 × 1 m)
D_2: 3200 trees ha^{-1} (2.5 × 1.25 m)
D_3: 2666 trees ha^{-1} (2.5 × 1.5 m)

Table 4. Effect of different planting densities, training systems and fertigation levels on scion girth of apple cv. Jeromine during 2019 and 2020

T/D/F	Increase in scion girth (mm) -2019 1st year (2nd year)						Increase in scion girth (mm) -2020 1st year (2nd year)					
	T_1			T_2			T_1			T_2		
	F_1	F_2	Mean (T×D)	F_1	F_2	Mean (T×D)	F_1	F_2	Mean (T×D)	F_1	F_2	Mean (T×D)
D_1	5.66	5.73	5.70	5.72	5.78	5.75	4.36	4.15	4.26	4.42	4.24	4.33
D_2	6.24	6.03	6.14	6.29	6.11	6.20	4.83	4.71	4.77	4.92	4.87	4.90
D_3	6.94	6.86	6.90	6.98	6.92	6.95	5.21	5.07	5.14	5.33	5.18	5.26
Mean (T×F)	6.28	6.21		6.33	6.27		4.80	4.64		4.89	4.76	
Mean (T)		6.24			6.30			4.72			4.83	

F/D	F × D			CD (0.05)						
	F_1	F_2	Mean (D)	T	0.05	F_1	F_2	Mean (D)	T	0.09
D_1	5.69	5.76	5.72	F	0.05	4.39	4.20	4.29	F	0.09
D_2	6.27	6.07	6.17	D	0.06	4.88	4.79	4.83	D	0.11
D_3	6.96	6.89	6.93	T×F	NS	5.27	5.13	5.20	T×F	NS
Mean (F)	6.31	6.24		T×D	NS	4.85	4.70		T×D	NS
				F×D	NS				F×D	NS
				T×F×D	NS				T×F×D	NS

Training system (T):- T_1: Tall spindle
T_2: Vertical axis
Fertigation level (F):- F_1: 100 % of AD (NPK)
F_2: 75 % of AD (NPK)
Planting density (D):- D_1: 4000 trees ha^{-1} (2.5 × 1 m)
D_2: 3200 trees ha^{-1} (2.5 × 1.25 m)
D_3: 2666 trees ha^{-1} (2.5 × 1.5 m)

and 4.83 mm) was observed in Vertical axis training system and it was found statistically significant than Tall spindle system. Similarly, the trees subjected to F_1 fertigation level registered significantly more increase in scion girth (6.33 and 4.85 mm) than F_2 fertigation level. The data also shows that interactions between T×D×F were found non-significant. However, among three factors interaction, the maximum increase in scion girth (6.98 and 5.33 mm) was recorded in trees planted at D_3 density, trained with Vertical axis and subjected under F_1 level of fertigation.

Tree volume: An inquisition of the data given in Table 5 clearly indicates that different planting densities, training systems, fertigation levels and their interactions produced a significant effect on tree volume during both the years. Considering the effect of planting densities, the highest increase in tree volume (1.64 and 2.28 m^3) was recorded in D_3 (2666 trees ha^{-1}) planting density the lowest increase in tree volume of 1.22 and 1.51 m^3 was found in D_1 (4000 trees ha^{-1}) planting density. Among the training systems, the maximum increase in tree volume of 1.58 and 2.12 m^3 was observed in Vertical axis system, which was significantly higher than Tall spindle system during both the years. Under fertigation, the highest increase in tree volume (1.47 and 1.96 m^3) was attained by trees subjected under F_1 fertigation level, which was significantly higher than F_2 level of fertigation (1.27 and 1.68 m^3). In case of interactions between planting densities and training systems, significantly highest increase in tree volume of 1.76 and 2.49 m^3 was recorded in trees planted at density D_3 and trained with vertical axis and the lowest increase in tree volume was observed in trees planted at density D_1 and trained with tall spindle (0.86 and 1.06 m^3). Among training systems and fertigation levels interactions, the maximum increase in tree volume of 1.68 and 2.24 m^3 was registered in trees trained with Vertical axis and subjected to F_1 fertigation level, whereas, the lowest in tall spindle trained trees subjected to F_2 fertigation level. Among planting densities and fertigation levels interactions, the highest increase in tree volume of 1.86 and 2.60 m^3 was observed in trees planted at D_3 density and subjected under F_1 level of fertigation, while the lowest was noticed in trees planted at D_1 density and subjected under F_2 level of fertigation. In case of three factors interaction, significantly highest increase in tree volume (1.96 and 2.80 m^3) was attained by the trees planted at D_3 density, trained with Vertical axis and subjected under F_1 level of fertigation.

Productivity: A critical appraisal of data presented in Figure 1, 2 and 3 indicates that different planting densities, training systems and fertigation levels and their interactions had significant effect on fruit yield per hectare (productivity). Considering the effect of planting densities (Fig. 1), the highest yield (35.81 and 39.39 t ha^{-1}) was recorded in 4000 trees ha^{-1} planting density, which was significantly higher than planting density of 3200 trees ha^{-1} and 4000 trees ha^{-1}. However, the lowest yield of 30.49 and 34.09 t ha^{-1} was found in 2666 trees ha^{-1} planting density. Among the training systems (Fig. 2), the highest yield of 35.58 and 38.61 t ha^{-1} was observed in tall spindle training system, which was significantly higher than vertical axis training system. Considering the effect of fertigation levels (Fig. 3), the maximum yield (35.07 and 38.38 t ha^{-1}) was attained by trees subjected under F_1 fertigation level, which was significantly higher than F_2 level of fertigation. The highest yield of 38.58 and 41.52 t ha^{-1} was recorded in trees planted at density of 4000 trees ha^{-1} and trained with Tall spindle, which was significantly superior to other interactions. However, the lowest yield was observed in trees planted at density of 2666 trees ha^{-1} and trained with Vertical axis. Among training systems and fertigation levels interactions, the maximum yield of 37.43 and 40.69 t ha^{-1} was recorded under trees trained with Tall spindle and subjected to F_1 fertigation level, however lowest under Vertical axis trained trees subjected to F_2 fertigation level. The significantly highest yield of 38.10 and 40.91 t ha^{-1} was obtained in subjected to F_1 level of fertigation, while lowest (28.80 and 32.38 t ha^{-1}) was noticed in trees planted at density of 2666 trees ha^{-1} and subjected under F_2 level of fertigation. Among three interaction, significantly highest yield (40.92 and 43.34 t ha^{-1}) was found in trees planted at D_1 density, trained with Tall spindle and subjected under F_1 level of fertigation, which was found superior to all the interactions. However, the lowest yield (26.74 and 31.08 t ha^{-1}) was recorded in trees planted at D_3 density, trained with Vertical axis and fertigated with F_2 level.

The results of present investigation revealed that vegetative growth traits such as tree height and spread, annual shoot growth, scion girth and tree volume was significantly influenced by planting density, training system and fertigation level. The study shows that the planting density had a strong negative effect on tree growth parameters. Tree height, spread and volume, annual shoot growth, scion and stock girth, tree volume, leaf area and pruning wood weight was found more in planting densities of 2666 trees ha^{-1} compared to the other planting densities. The larger tree size in lower densities might be due to the more availability of space for vegetative growth of the trees and lack of competition for the nutrients, water and light, while smaller tree size in higher densities may be due to the more competition for nutrients and water (Dhiman et al 2018). These results are in confirmatory with the studies of Robinson (2007), Lordan et al (2018) and Reig et al (2019), who reported maximum TCSA and crown volume per tree at wider spacing and minimum under close spacing in apple. Similarly, Dhiman et al (2018) recorded that the tree growth parameters like tree height spread, annual shoot growth, scion and stock girth, and tree volume was highest in low planting density of 2666 trees ha^{-1}.

In the present study, Vertical axis training system resulted in higher growth as compared to Tall spindle. The lesser vegetative growth in trees trained with tall spindle is due to bending of branches below horizontal and no heading back of branches and leader during dormant pruning, which reduced branch growth due to more accumulation of carbohydrates resulting in to small canopy (Robinson 2007). The present findings are similar to that observed by Robinson et al (2013)

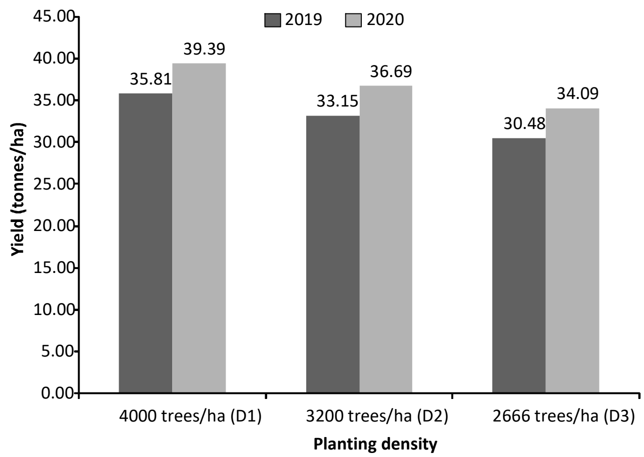

Fig. 1. Effect of different planting densities on yield of apple cv. Jeromine during 2019 and 2020

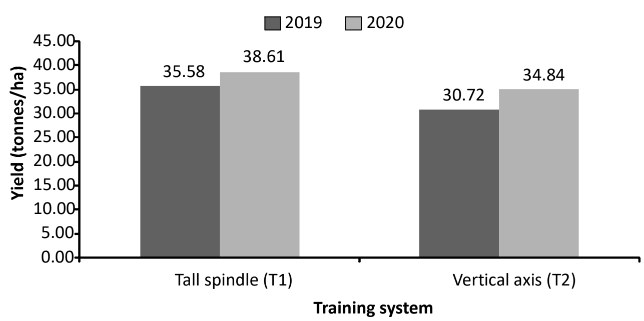

Fig. 2. Effect of different training system on yield of apple cv. Jeromine during 2019 and 2020

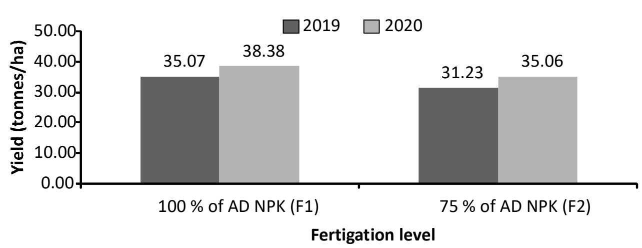

Fig. 3. Effect of different fertigation level on yield of apple cv. Jeromine during 2019 and 2020

and Reig et al (2019), who reported that the tall spindle trained trees were least vigorous and TCSA was 25 per cent lower than vertical axis.

Higher growth was reported under higher levels of fertigation, which declines with decreasing fertigation doses. The higher growth characteristics at higher fertigation levels may partially be assigned to the higher leaf nutrient content (NPK) in the tree foliage, which had a positive correlation with the level of fertilizer used (Treder 2006). The present findings are in confirmatory with the studies of Raina et al (2013), Kumar et al (2016) and Thakur et al (2020), who observed maximum tree height and spread, annual shoot growth, trunk girth and tree volume with the application of 100 per cent AD (NPK) fertigation dose.

CONCLUSION

It was concluded that planting of trees at 4000 trees per ha density and trained with tall spindle training system and subjected to 100 per cent AD (NPK), resulted in highest productivity.

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Effect of Application of Micronutrients on Yield and Economics of Ginger under Eastern Ghat High Land Zone of Odisha

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Abstract: A study was conducted in Eastern Ghat High Land Zone of Odisha to explore the effect of foliar application of micronutrients on ginger during 2017-18 to 2019-20.The treatments consisted of three cultivars viz. V1 (Supranha), V2 (Suravi) and V3 (Varada) and foliar application of IISR ginger micronutrients @ 5 g/litre of water at 60 and 90 days after planting. Ginger variety Suravi was superior over other varieties in terms of yield and economics. Application of micronutrient showed significant variation for all characters studied. Higher fresh rhizome yield of 4.11-8.39% was obtained with foliar application of IISR ginger micronutrients (5 g/litre of water at 60 and 90 days after planting). Higher economic benefit (5.67-11.78% more net return) was also obtained with application of micronutrient. The present experimental findings signify the importance of foliar application of micronutrient along with recommended dose of fertilizers in improving yield and economics of ginger.

Keywords: Economics, Ginger, Micronutrients, Rhizome yield

Ginger (*Zingiber officinale* Rosc.) is an herbaceous perennial and cultivated in many regions of the world as well as in India. In the world, India has largest ginger growing areas (37.18%) with highest production (38.15%) during 2019 (FAOSTAT 2021). The total cultivated area of ginger in India is 160 thousand ha with production of 1118 thousand tons (HSG 2018). Odisha occupies 16.6 thousand ha of ginger land with production of 32 thousand tons. Per unit production of ginger in Odisha is very less (1.93 t/ha) as compared to national average i.e. 6.98 t/ha (HSG 2018). There is a huge scope to intervene different ways to increase productivity of ginger. Different cultivars, sowing time, climatic condition, nutrient management and maturity time are the major dependable factors for variability in productivity of ginger (Behera et al 2020). Different nutrients are essential for growth and development of crop plants. Micronutrients are the essential elements required in smaller amount but importance of these in growth and development of a crop is very much necessary as of major nutrients. Application of micronutrients showed positive impact on different crops (Barbosa et al 2016, Sarker et al 2018, Alkarawi and Hasan 2021). In ginger, different treatments of micronutrients showed extra yield starting of 29.40 to 125.10% (Singh and Dwivedi 2007). Halder et al (2007) reported the beneficial impact of zinc and boron on ginger yield. Although a number of researchers have evaluated the positive effect of micronutrient application in different crops, but very few work has been done on ginger. Study was conducted to evaluating the effect of foliar application of micronutrients on ginger interms of production and economics in Eastern Ghat High Land Zone of Odisha.

MATERIAL AND METHODS

Field experimentation was carried out at High Altitude Research Station, Pottangi (latitude 18.564151, longitude 82.968756-'E and 943 m above msl) under Odisha University of Agriculture and Technology during 2017-18 to 2019-20. The initial soil of the experimental site was sandy loam in texture with pH and nutrients status as in Table 1. A total rainfall of 1435.7, 983.7 and 1717.4 mm with 76, 77 and 99 number of rainy days were observed in first, second and third year of experiment, respectively (Fig. 1). The monthly mean maximum and minimum temperature is presented in Table 2. The experiment consisted of three cultivars viz. V1 (Supranha), V2 (Suravi) and V3 (Varada) and application of micronutrients viz. M1 [Foliar application of IISR (Indian Institute of Spices Research) ginger micronutrients @ 5 g/litre of water at 60 and 90 days after planting] and M2 (control). Experiment was conducted in a factorial RBD design with four replications. Different ginger varieties were sown at 30 cm x 25 cm spacing in a net plot of 3 m X 1 m on 1^{st} week of April and the crop was harvested during first week of February to last week of March. FYM @ 15 t/ha was applied before sowing of ginger. A basal dose of 35 kg N, 100 kg P_2O_5

and 40 kg K_2O/ha were applied uniformly to each subplot during the final land preparation. Two top dressing of 45 kg N and 30 kg K_2O/ha were applied at 45 and 90 DAS. Data on number of leaves/plant and number of tillers/plant were collected from randomly selected five plants at 135 DAS. Observations on clump weight/plant, plot yield and fresh rhizome yield were recorded at harvest. Data were statistically analyzed using analysis of variance (ANOVA) as split-plot design in MS Excel 2010.

RESULTS AND DISCUSSION

Pooled data of three years showed significant variation in number of tillers/plant among different ginger cultivars (Fig. 2). However, a non significant variation was observed among the cultivars for number of leaves/plant (Fig. 2). Highest tiller numbers (13.3/plant) were obtained with ginger variety Suravi (V2) followed by Varada (V3). Differences in growth attributes among the varieties were perhaps due to the variation in genetic make-up within different varieties. Growth attributing characters like number of tillers and leaves per plant were significantly superior with application of IISR ginger micronutrients @ 5 g/litre of water at 60 and 90 days after plantingas compared to non-application of IISR ginger micronutrients mixture (Fig. 2). Recommended fertilizer application with supplementation of foliar nutrition enhances growth characters. Similar improvement reported by application of micronutrients in ginger (Sudha et al2020) and turmeric (Datta et al 2017).

Clump weight of ginger was significantly influenced by different cultivars during all years (Table 2). The maximum clump weight was recorded with Suravi (V2) followed by Varada (V3) and Suprabha (V1). In terms of clump weight, there was no significant difference between V2 and V3. Among the micronutrient, foliar application of IISR ginger micronutrients @ 5 g/litre of water at 60 and 90 days after planting (M1) recorded statistically highest clump weight as compared to control during all three years of experimentation.

During third year of experiment yield performance of ginger crop was better as compared to first and second year (Table 2) may due to be total of 1717.4 mm rainfall received in 99 rainy days in third year as against 1435.7 mm and 983.7 mm in first and second year, respectively during entire growing cycle (Fig. 1). Too much less rainfall in second year resulted in poor performance in terms of production of ginger during this year. Significant variation in plot yield and fresh rhizome yield among the different cultivars was found in all years (Table 2). Ginger cultivar Suravi (V2) recorded higher fresh rhizome yield of 22.03, 20.80 and 21.93 t/ha in first, second and third year of experimentation. In terms of yield, ginger variety Varada (V3) positioned second for all years. It was also observed that there was no significant variation

Table 1. Initial pH and nutrients status of studied soil

pH	Org. C (%)	Av. N (kg ha^{-1})	Av. P (kg ha^{-1})	Av. K (kg ha^{-1})	Av. Fe (mg kg^{-1})	Av. Mn (mg kg^{-1})	Av. Cu (mg kg^{-1})	Av. Zn (mg kg^{-1})	Av. B (mg kg^{-1})
5.96	0.88	300	20.16	180.0	16.0	15.12	1.10	0.45	0.35

Table 2. Monthly mean maximum and minimum temperature during experimentation

Months	2017-18		2018-19		2019-20	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
April	40.8	19.0	36.0	22.2	36.8	22.0
May	42.0	22.0	35.8	26.3	38.8	24.5
June	38.2	22.3	32.6	23.3	34.8	24.1
July	33.0	22.0	28.2	22.8	29.0	22.1
August	29.0	22.0	36.0	22.2	29.7	21.8
September	31.0	23.0	29.7	21.8	30.0	21.0
October	30.8	21.4	29.3	18.8	30.4	21.2
November	29.5	15.4	27.3	14.8	29.8	16.0
December	29.1	10.2	26.0	11.2	25.6	12.0
January	29.3	10.7	27.9	10.7	28.5	10.8
February	32.1	14.5	31.7	14.4	31.8	14.3
March	33.4	18.6	35.0	20.2	34.9	19.2

within V2 and V3.The fresh rhizome yield is the inherent capacity of the ginger variety and dependent on vigour of the plant and other plant characters (Kallappa et al 2015). Earlier studies also revealed significant variations for yield of ginger (Rani et al2019, Kallappa et al 2015). Application of micronutrient showed significant variation in both plot yield and fresh rhizome yield. Highest rhizome yield was found when the plant was sprayed with IISR ginger micronutrients @ 5 g/litre of water at 60 and 90 days after planting (M1) over control plot (M2). A higher fresh rhizome yield of 4.11-8.39% obtained when crop was cultivated with foliar application IISR ginger micronutrients as compared to control condition. This

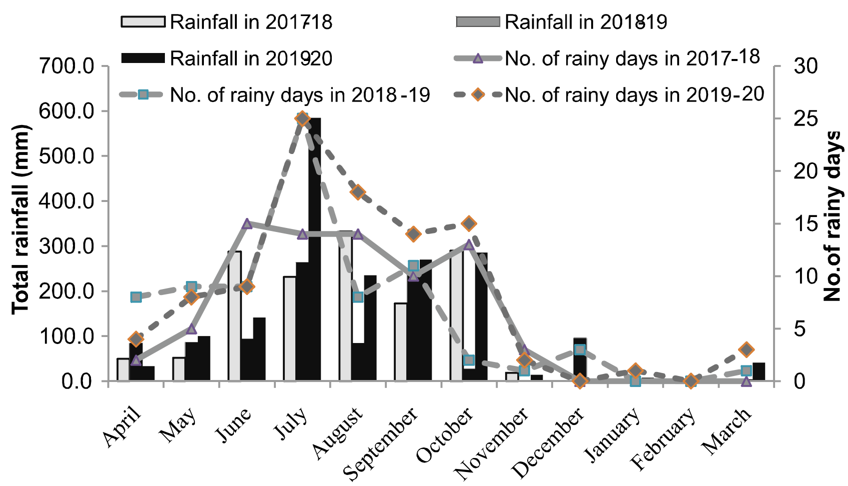

Fig. 1. Total rainfall and number of rainy days during experimentation

Table 3. Effect of cultivars and micronutrients on clump weight, plot yield and fresh rhizome yield of ginger

Treatments	Clump weight (g plant^{-1})			Plot yield (kg^{-3} m^{2})			Fresh rhizome yield (t ha^{-1})		
	2017-18	2018-19	2019-20	2017-18	2018-19	2019-20	2017-18	2018-19	2019-20
Cultivars									
V1	226.07	229.11	237.49	9.04	9.08	9.58	20.08	20.15	21.28
V2	248.03	234.81	243.28	9.92	9.37	9.88	22.03	20.80	21.93
V3	239.58	232.28	243.24	9.58	9.35	9.64	21.28	20.76	21.40
CD (p=0.05)	10.35	3.55	5.06	0.41	0.23	0.23	0.92	0.51	0.51
Micronutrients									
M1	245.12	241.65	246.52	9.80	9.64	9.89	21.77	21.40	21.97
M2	230.67	222.49	236.16	9.23	8.89	9.50	20.48	19.74	21.10
CD (p=0.05)	8.45	2.90	4.13	0.34	0.19	0.19	0.75	0.42	0.42

Table 4. Effect of cultivars and micronutrients on economics of ginger production

Treatments	Gross return (Lakh ha^{-1})			Net return (Lakh ha^{-1})			B;C ratio		
	2017-18	2018-19	2019-20	2017-18	2018-19	2019-20	2017-18	2018-19	2019-20
Cultivars									
V1	16.06	16.12	17.02	11.75	11.62	12.32	3.73	3.58	3.62
V2	17.62	16.64	17.54	13.31	12.14	12.84	4.09	3.70	3.73
V3	17.02	16.61	17.12	12.71	12.11	12.42	3.95	3.69	3.64
Micronutrients									
M1	17.41	17.12	17.57	13.10	12.62	12.87	4.04	3.80	3.74
M2	16.39	15.79	16.88	12.08	11.29	12.18	3.80	3.51	3.59

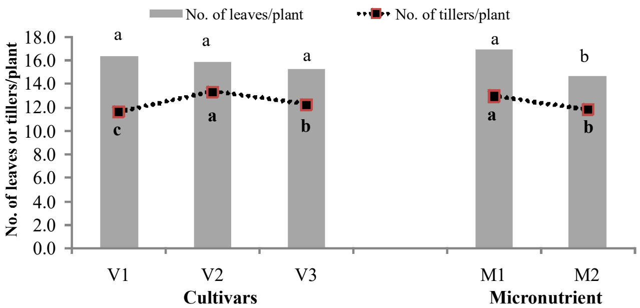

Fig. 2. Number of leaves and tillers per plant of ginger as influenced by cultivars and micronutrient application (pooled data of three years). [Means above the column bar followed by same letter do not differ significantly]

result suggests that recommended crop nutrition along with such foliar feeds could result in improved yield. Foliar sprays of IISR ginger micronutrient attributed to well balanced nutrition resulted in higher yield with this treatment. Improved production of ginger with application of micronutrients was also reported in other studies (Halder et al 2007, Sudha et al 2020).

Highest gross return, net return and B:C ratio was manifested by the cultivation of Suravi (V2) variety during all years because of the highest rhizome yield, the only economic part of the crop. B: C ratio of 3.73-4.09, 3.58-3.70 and 3.62-3.73 were obtained in first, second and third year, respectively by different cultivars with highest value with Suravi. In case of micronutrient application, gross return and net return was recorded higher with the application of micronutrients as compared to control. Higher B: C ratio was also recorded with application of micronutrients (M1). Application of micronutrients enhanced rhizome yield of ginger and ultimately reflected on highest economic return.

CONCLUSION

Ginger cultivar Suravi proved superior over other varieties in experimental region in terms of studied characters like yield and economics. Foliar application IISR ginger micronutrient (5 g/litre of water at 60 and 90 days after planting) with recommended dose of fertilizers helps in increasing ginger production and gave higher economic return.

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Climate Change impact on Water Requirement of Micro Irrigated Mango Orchard under Climatic Conditions of Udaipur, Rajasthan

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***Abstract*:** A study was carried out to analyze the impact of climate change on crop water requirement (CWR) of mango for Udaipur district of Rajasthan during five years (2014 to 2018). There was significant impact of climate change on daily reference evapotranspiration (ETo), rainfall pattern, crop evapotranspiration (ETc) and CWR. The uneven trend of ETo and Rainfall was for different year. The average daily ETo were maximum for May while, minimum for December months. The ETc was maximum for August, 2015. In this study most of the time, ETc was recorded minimum for month of December. The average CWR for mango tree ranges from 23.3 to 94.7 litre/plant/day during all five years. The trend of ETo, rainfall and CWR of mango crop was found uneven, due to temporal variation in climatic condition of Udaipur region by improper use of natural resources, over industrialization and mining interventions, which results improper future planning for establishment of mango orchard. The result of this study are capable for policy makers and planners of water resources for future planning and helps to conserve water in satisfying crop water requirement of orchard crops.

***Keywords*:** Climate change, Crop water requirement, Reference evapotranspiration, Crop coefficient and perennial crop

Climate change is likely to contribute substantially to food insecurity in the future, by increasing cost of food product and reducing crop yield. Climate changes have possible impactions on the water cycle, accessible fresh water resources and the water required for agriculture (Mansa and Anand 2016). Water required for crop production may become scarcer due to increased water requirement of crops and drought. Water is an important because it is needed for irrigation in agriculture sector. Drip irrigation and sprinkler irrigation are the different types of irrigation methods commonly adopted by the Indian farmers (Sharma and Yadav 2021) in order to mitigate the impact of climate change on water requirement of crop in agriculture sector. Adequate data on irrigation water requirements under drip irrigated orchard crops is not available in developing nations of the world. This is one of the reasons why for the failure of large scale irrigation projects in most developing countries of the world. Mango (*Mangifera indica* L.) belongs to the family Anacardiaceace, also called as the cashew family. It is a highly productive and popular tropical fruit crop which may have originated from India. Despite the Indian sub-continent it has spread to another part of world and is a very choicest fruits of India. The yield depends on the age and cultivar, but generally, about 500-1500 fruits per tree per year may be obtained at three to four years. Mango trees, considered medium-sized with comparatively high water requirements are usually irrigated by surface or drip irrigation methods and their water consumption varies based on many factors, including climate and soil. Crop water requirement may be define as the amount of water that is lost through evapotranspiration (Allen et al 1998). Reference evapotranspiration is the sum of two processes of evaporation from the soil and transpiration from the plants. The FAO-Penman Monteith a standard approach and provide consistent reference evapotranspiration (ET). Therefore a study was undertaken to estimate the crop water requirement of mango crops based on computed reference crop evapotranspiration using weather data of different year.

MATERIAL AND METHODS

Study area: A study on estimation of crop water requirement using CROPWAT model for mango crop under drip irrigation was carried out for Udaipur district of Rajasthan. The study area is located between 24°35'31.5" latitude 73°44'18.2" longitude and at an altitude of 582.17 m above mean sea level (MSL). Udaipur comes under dry, sub-humid agro-climatic region. The average annual rainfall of Udaipur is 637mm, most of the rain received during the period of July to Sep. The hottest month is s May and December is the coolest month. The daily meteorological data i.e. maximum temperature (Tmax, 0C) and minimum temperature (Tmin, 0C), maximum relative humidity (RHmax, %) and minimum relative humidity (RHmin, %), pan evaporation (Epan, mm), wind speed (WS, kmhr^{-1}) at height of 2.0 m, sun shine hours (SSHr, hr), rainfall (R, mm) were collected from meteorological observatory of CTAE, Udaipur.

Estimation of reference evapotranspiration using CROPWA Tmodel: The model used to calculate the reference evapotranspiration, irrigation requirements and crop water requirements under various management conditions. The modified Penman- Monteith method suggested by Allen et al (1998) was used to compute reference evapotranspiration (ETO). The FAO Penman-Monteith method to estimate ETo is given below:

$$\mathrm{ET}_0 = \frac{0.408\,\Delta(R_n - G) + \gamma \dfrac{900}{T-273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34 u_2)}$$

Where, ET_O = reference evapotranspiration [mm/day], Rn= net radiation at the crop surface [MJ/ m^2 day], T= air temperature at 2 m height [°C], G= soil heat flux density [MJ/ m^2day], u2=wind speed at 2 m height [m s^{-1}], e_a= actual vapour pressure [kPa],

e_s= saturation vapour pressure [kPa], e_s - e_a=saturation vapour pressure deficit [kPa],

Y=psychrometric constant [kPa°C^{-1}], Δ=slope vapour pressure curve [kPa°C^{-1}],

Estimation of crop water requirement under drip irrigation: The crop water requirement (CWR) is basically the function of evapotranspiration rate, crop coefficient and area covered by plants. The reference evapotranspiration (ETo) for all months during five were derived from CROPWAT model. The water requirement or volume of water to be applied for mango orchard was calculated by the following equation.

CWR = ETo × A × Kc × Wp

Where,

CWR = Water requirement of crop, litre/day/plant,ETo = Reference evapotranspiration, mm/day

A = Area of the crop (m2) = row to row (m) × plant to plant spacing (m),Kc = Crop coefficient;

Wp = Percentage wetted area, decimal.

The crop coefficient (Kc) varies by month based on phonological stages of plant and percentage of the growth shaded by the tree canopy. In the present study the phonological stage wise values of crop coefficient for perennial fruit crops were taken as given by Kisekka et al (2010) (Table 1). In this study mango crop was selected as perennial crop with row to row (m) × plant to plant spacing (m) 8×8 meters and percentage wetted area under drip irrigation was consider as 40% of area of crop.

Irrigation scheduling for mango orchard: In this study an irrigation scheduling for mango orchard was developed for a standard size of drip system on the basis mean of average daily crop water requirement for different months.

Specification of drip irrigation system: In this study crop water requirement of mango tree was estimated in order to developing irrigation scheduling (including number of irrigation and operating time of standard size of drip system for mango orchard). The specifications of drip system are as follows: Size of mainline-63 mm, Size of sub mainline-32 mm, Size of lateral-16 mm, Dripper type-Online dripper

RESULTS AND DISCUSSION

The maximum annual ETo was for year 2015 (1410 mm/year) and maximum annual rainfall was for 2016 (747 mm/year) (Fig. 1). The minimum annual ETo and annual rainfall were years 2017 (1259, mm/year) and 2018 (556 mm/year) respectively. The uneven trend of ETo and rainfall was for different year due to change in climatic conditions with respect to time. There are various factors responsible for this and replacement of agricultural land by urban land can be a major factor, evapotranspiration of moisture from soil and vegetation are often diminished, leading to decreased atmospheric humidity and potentially suppressing precipitation. Jha et al(2021) has reported uneven trend of rainfall with respect to time.

Reference evapotranspiration by CROPWAT Model: The daily meteorological data during all months for five year were used to estimate the average daily reference evapotranspiration (mm/day) for Udaipur district of Rajasthan through CROPWAT model. The average daily reference evapotranspiration were maximum for May, minimum were for December during all five year (Fig. 2). The maximum temperature (Tmax, ^{0}C) and minimum temperature (Tmin, ^{0}C), pan evaporation (Epan, mm), wind speed (WS, kmhr^{-1}) at height of 2.0 m, sun shine hours (SSHr, hr), rainfall (R, mm) was also minimum in winter season which results lowest value of average daily reference

Table 1. Crop coefficient (Kc) for perennial mango crop

Month	January	February	March	April	May	June
Crop coefficient (Kc)	0.60	0.50	0.45	0.45	0.50	0.50
Month	July	August	September	October	November	December
Crop coefficient (Kc)	0.60	0.80	0.80	0.70	0.70	0.60

evapotranspiration. It is also indicates that, during winter season less amount of water was evaporated from soil surface and plant canopy. It is probably due to lower intensity of solar radiation as well as more relative humidity in atmospheric air.

The crop evapotranspiration was almost highest for August months during all years. During year 2014 to 2018 the maximum crop evapotranspiration was recorded for August 2015 with of 3.698 mm/day (Fig. 3). In this study most of the time crop evapotranspiration was recorded minimum for month of December. Similar results were reported by (Morgan et al 2017). It shows that peak crop evapotranspiration due to high atmospheric temperature.

Crop water requirement of mango orchard: The result shows that daily CWR ranges from 23.3 l/p/day to 94.7 l/p/day during year 2014 to 2018 (Table 2). The maximum CWR was in August and September followed by May and June during all years. The rainfall mainly occurs during August and September months in Udaipur region consequently the irrigation requirement for mango tree were less as compared to irrigation requirement during May and June. In Udaipur region during water scarcity condition which occurred mainly in May and June, at this time mango tree feel water stress so it is very essential to irrigate the mango orchard as per crop water requirement obtained from the study. Similar finding was reported by Yadav et al (2017). The average daily CWR was minimum under December and January for all five year. It is probably due to less atmospheric temperature in winter season. The average daily CWR for perennial mango tree normally varies from 29 to 83 litre/plant/day under different months in a year (Fig. 4). The mean value of CWR was maximum for September

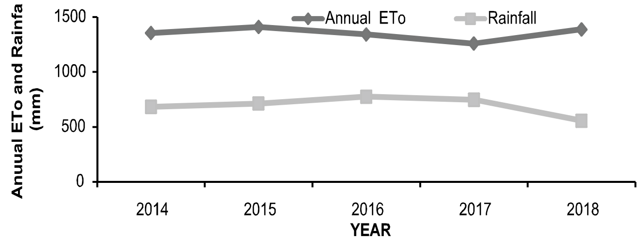

Fig. 1. Impact of climate change on Annual ETo and Annual Rainfall (mm/year) for 2014 to 2018 for Udaipur region

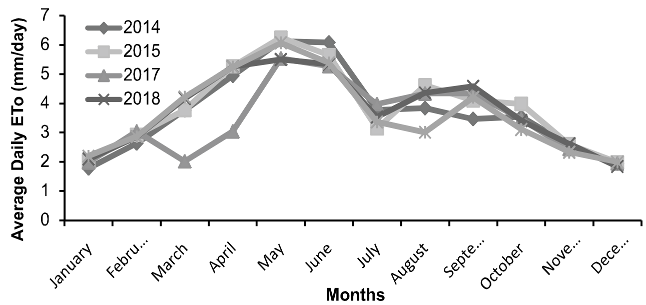

Fig. 2. Average daily reference evapotranspiration for different month during five year

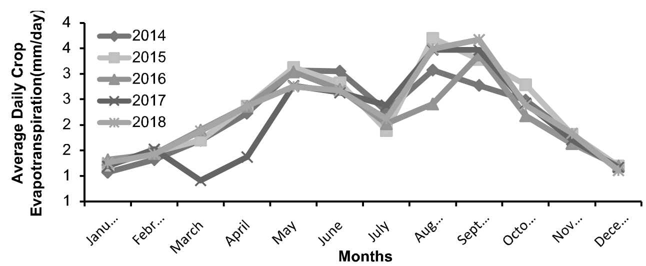

Fig. 3. Average daily crop evapotranspiration (ETc) for different month during five year

Table 2. Average daily crop water requirement (litre/plant/day) of mango orchard for different month

Months	Average daily crop water requirement (litre/plant/day)					
	2014	2015	2016	2017	2018	Mean
January	27.4	31.1	33.8	30.4	32.2	31.0
February	33.7	37.4	36.7	39.0	36.6	36.7
March	43.4	43.5	48.7	33.3	48.1	41.4
April	57.0	60.7	60.5	35.1	60.6	54.8
May	78.5	80.0	77.7	70.9	70.5	75.5
June	78.0	72.3	68.9	67.4	68.4	71.0
July	58.0	48.3	51.8	61.1	54.3	54.7
August	78.7	94.7	61.7	88.8	89.4	82.6
September	71.0	84.0	86.2	88.8	94.0	84.8
October	63.6	71.3	55.8	62.0	61.2	62.8
November	45.9	46.6	41.8	43.7	47.0	45.0
December	29.9	30.5	30.6	29.9	28.3	29.9

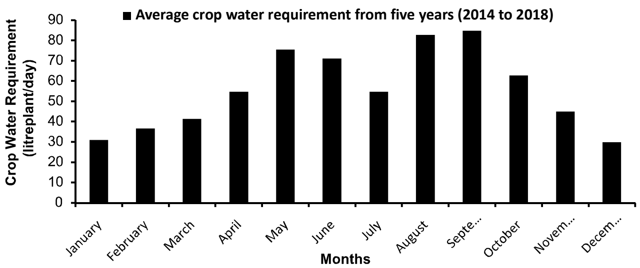

Fig. 4. Mean value of average daily crop water requirement (litre/plant/day) of mango orchard for different months

(84.8litre/plant/day) while, minimum CWR was for December (29.9 litre/plant/day).

CONCLUSIONS

The average daily reference evapotranspiration were maximum for May during all five year while, minimum average daily reference evapotranspiration were or December months during all five year. The average daily CWR ranges from 23.3 l/p/day to 94.7 l/p/day during 2014 to 2018. The maximum CWR was in August and September. In Udaipur region during water scarcity condition which occurred mainly May and June, it is very essential to irrigate the mango orchard as per crop water requirement. It has been concluded from the results and analysis that in future years crop water requirement will change unevenly in the study area. The reason for this may be due to uneven trend of maximum and minimum temperature and decrease in relative humidity in future years. The results of this study will be useful for policy makers and planners of water resources for the future planning and suggest water saving techniques to satisfy varying crop water requirement for orchard.

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Photo-period Benefit and Climate Resilient Practices on Performance and Economics of Transplanted Pigeonpea and Sunflower Intercropping in *Vertisol* of Northern Karnataka

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Abstract: Field experiment was conducted during *Kharif* 2019 to evaluate transplanted pigeonpea and sunflower intercropping system at different dates of sowing and fertilizers. Treatment combinations consists of two sowing time and eight intercropping system replicated thrice. Pigeonpea transplanted in second fortnight of July recorded significantly higher grain yield, equivalent yields of pigeonpea (PEY) and sunflower (SEY), over second fortnight of August. However, higher sunflower seed yield and oil content was recorded in second fortnight of August sowing. Among intercropping systems, sole pigeonpea and sunflower recorded significantly higher seed yield (2254 and 1781 kg ha^{-1}) over sole drilled pigeonpea as well as intercropping systems. Transplanted pigeonpea + sunflower (1:2) with RDF for both crops recorded superior pigeonpea grain yield (1936 kg ha^{-1}), PEY (2914 kg ha^{-1}), SEY (3816 kg ha^{-1}), gross returns (Rs. 157680 ha^{-1}) and net returns (Rs. 108880 ha^{-1}). Drilled pigeonpea + sunflower (1:1) considered as farmers practice recorded significantly greater sunflower seed yield (1382 kg ha^{-1}), oil content (37.7 %) and B: C (3.49) over rest of the combinations Transplanted pigeonpea was superior over drilled sown pigeonpea.

Keywords: Sowing time, Transplanted pigeonpea, Drilled sown pigeonpea

Among pulses, pigeonpea [*Cajanus cajan* (L) Millsp.] holds first place in Karnataka both in area (1.48 m ha) and production (0.94 m t) with a productivity of 647 kg ha^{-1} (Anonymous 2018). Among major production constraints, erratic and scanty rainfall that resulted low soil moisture was reducing pigeonpea productivity in *Vertisol* of N-Karnataka. Sunflower (*Helianthus annuus* L.) is grown as a source of vegetable oil; Karnataka shares 52% of the area and 40% of the production in the country. Amongst factors responsible for yield improvement in both the crops, optimum fertilizers application and timely sowing were of prime importance. Wide yield gap can be minimized by use of adequate and balanced fertilization (Umesh et al 2018, Umesh et al 2020). Crop nutrient removal far exceeds than the addition through fertilizers and manures. Balanced application of major fertilizers can increase sunflower growth and yield substantially. Long duration pigeonpea can adjust to a wide range of population and spacing. It is cultivated on marginal lands in mono/mixed cropping system without or suboptimal fertilizers under rainfed conditions. Delayed sowing shortens the time for growing period, hastens maturity and ultimately reduces the yield. The early sowing may encourage the vegetative growth which may make the crop prone to different insect pests and diseases. Thus, the need for timely planting through alternate feasible means such as transplanting is felt essential. The farmers are adopted different row proportions in pigeonpea based intercropping rather than the recommended optimum. Hence, it was felt necessary to study the different row proportions of pigeonpea and sunflower intercropping and assess the economics of the system. Transplanting of seedlings and seed hand dibbling in pigeonpea is recently adopted techniques in the region. It has been proved that transplanting of pigeonpea would be one of the better agronomic practices to avoid delayed sowing and also maintain desired plants (Priyanka et al 2013, Praharaj et al 2015). Raising pigeonpea seedlings well in advance and transplanting in the field on receipt of good rains would help in reaping the benefits of early sowing. Keeping these facts, present investigation was carried out with an objective to evaluate feasibility of pigeonpea and sunflower intercropping in a changed sowing window and fertilizers supply in *Vertisol* of N Karnataka.

MATERIAL AND METHODS

Field experiment was conducted in *Kharif* 2019 at Main Agricultural Research Station, Raichur. The soils of the site was medium black having soil reaction 7.74, organic carbon 0.6 % and EC of 0.21 dS m^{-1}. The available N, P_2O_5 and K_2O before initiation of experiment were 293.3, 39.7 and 365.9 kg ha^{-1}. The experiment was carried out in split plot design replicated thrice. Main plots were sowing both the crops in July second fortnight and August second fortnight, sub-plot treatments were transplanted pigeonpea + sunflower (1:1) with RDF for main crop, transplanted pigeonpea + sunflower (1:1) with RDF for both the crops, transplanted pigeonpea + sunflower (1:2) with RDF for main crop, transplanted pigeonpea + sunflower (1:2) with RDF for both the crops, drilled sown pigeonpea + sunflower (1:1) sole sunflower, sole transplanted pigeonpea, sole drilled sown pigeonpea (Table 1). Fertilizer application was applied as per recommendation for the region by considering the plant population occupied by each crop. Fertilizers were applied for pigeonpea @ 25-50 kg N-P_2O_5 and sunflower @ 90-90-60 kg N-P_2O_5-K_2O ha^{-1}. Under intercropping system, the fertilizers were applied according to the component crops population in each treatment. The entire quantities of fertilizers were applied to pigeonpea at basal. For sunflower half of recommended N and entire dose of P and K were applied at basal and remaining N was top dressed at 30 DAS. Foliar application of 19:19:19 @ 1% and Pulse magic @ 10 g/l was done at flowering stage. Pigeonpea variety BSMR-736 and sunflower hybrid RSFH-1887 were selected for the trial.

Pigeonpea seeds were sown on first week of June and July, in polythene bags (8'' x 5'') having 3/4th of soil and 20 g of vermicompost. Polythene bags were watered regularly. After one month, the seedlings were transplanted in the main field at 1.2 m x 0.6 m. For drill sown treatment seeds were sown at 1.2 m x 0.6 m spacing on the day of transplanting. In main field 30 days old pigeonpea seedlings were transplanted after removing the polythene cover without disturbing the soil at the root zone. Sunflower under intercropping was sown exactly at the center of pigeonpea rows in 1:1, whereas two rows of sunflower at 60 cm apart in 1:2 row proportion. Rest of the production practices for both the crops was followed as per the regional recommendation by the University. Irrigation was provided during long dry spells to avoid stress. Pigeonpea and sunflower equivalent yields were calculated based on market price and yield levels as per the standard procedure (Umesh et al 2002). The cost includes expenditure on seeds, fertilizers, weed management and plant protection chemicals. At maturity, the crop was harvested and plot wise yields were recorded. The data recorded at different stages of crop was subjected to statistical analysis.

RESULTS AND DISCUSSION

Pigeonpea grain yield: Pigeonpea transplanted in July recorded 35.3 per cent higher grain yield (2130 kg^{-1} ha) over August transplanting (Table 1). The sole transplanted pigeonpea was recorded significantly higher grain yield (2253 kg ha^{-1}) over rest of the intercropped treatments and drilled pigeonpea. Application of fertilizers to both the crops in transplanted pigeonpea + sunflower (1:2) recorded

Table 1. Effect of sowing time and row proportions on seed yield, protein content, oil content and equivalent yields of pigeonpea (PEY) and sunflower under (SEY) intercropping system

Treatment	Pigeonpea						Sunflower					
	Grain yield (kg ha^{-1})		Protein content (%)		PEY (kg ha^{-1})		Seed yield (kg ha^{-1})		Oil content (%)		SEY (kg ha^{-1})	
Sowing time												
July	2130		21.4		2570		1124		36.1		3354	
August	1574		20.5		2122		1300		38.3		2778	
CD (p=0.05)	305		0.6		180		186		2.1		82	
Row proportions and fertilizers												
Transplanted pigeonpea + sunflower (1:1) RDF for main crop	1819		20.9		2453		831		36.8		3213	
Transplanted pigeonpea + sunflower (1:1) RDF for both crops	1926		21.3		2635		929		37.0		3451	
Transplanted pigeonpea + sunflower (1:2) RDF for main crop	1778		20.7		2592		1066		36.0		3394	
Transplanted pigeonpea + sunflower (1:2) RDF for both crops	1936		21.0		2914		1280		36.5		3816	
Drill sown pigeonpea + sunflower (1:1)	1593		20.0		2648		1382		37.7		3468	
Sole sunflower	-		-		1360		1781		39.1		1781	
Sole transplanted pigeonpea	2252		22.2		2252		-		-		2949	
Sole drill sown pigeonpea	1659		20.4		1659		-		-		2457	
S.Em. ±	58		0.1		59		34		0.4		92	
CD (p=0.05)	170		0.4		173		101		1.3		267	
Interaction	I×S	S×I	I×S	S×I	I×S	S×I	I×S	S×I	I×S	S×I	I×S	S×I
CD (p=0.05)	NS	NS	277	275	277	275	NS	NS	NS	NS	383	360

significantly greater grain yield (1937 kg ha^{-1}) over drilled pigeonpea + sunflower (1593 kg ha^{-1}). Combined effect of sunflower intercropped with different row proportions and RDF along with time of transplanting found non-significant. Yield advantage in the early sown crop was mainly due to increased growth, yield attributes and physiological characters. It also favored by climatic conditions, temperature during growth, development and maturity stages. Efficient utilization of nutrients and moisture as well as greater light interception had resulted in higher growth and yield attributes. Similar results on yield advantage in sown crops were reported earlier worker (Reddy et al 2012, Yu 2014, Uprikar 2017). Another study at the same site by Channabasavanna et al (2015) observed that the growth and yield benefits in early sown pigeonpea due to long growing period consequently accumulated greater dry matter enhanced leaf area and phenological potential. Intercropping has reduced the yields of both pigeonpea and sunflower. Sole transplanted pigeonpea recorded higher seed and dry matter yield over intercrop with sunflower. It was mainly attributed to enhanced growth in terms of plant height, primary branches, LAI and TDMP and yield parameters *viz*., pods per plant, seed yield per plant, seeds per plant and 100 seed weight. Impact of intercropping on growth and yield was reported by Pal et al (2016).

Sunflower seed yield: The sunflower has photoperiod benefit in terms of improved yield (15.66%) either by sowing or transplanting in second fortnight of August (1300 kg ha^{-1}) over second fortnight of July (Table 1). Sole sunflower was out yielded (1781 kg ha^{-1}) over rest of the intercropped combinations. Drilled pigeonpea + sunflower (1:1) considered as farmer practice has recorded greater sunflower seed yield (1382 kg ha^{-1}) over sunflower as intercrop with transplanted pigeonpea (1:1) with RDF for main crop (831 kg ha^{-1}). Intercropping systems has reduced the sunflower yield 22.4-55.3% owing to population difference. It was compensated by contribution from pigeonpea indicated in terms of equivalent yield. Combined effect of sowing time and intercropping with row proportions and fertilizers application has no significant effect on sunflower seed yield. An uninterrupted availability of various resources *viz*., solar interception, soil moisture and nutrients for sole crop might have helped the crop to utilize the resources to a great extent resulting in enhanced values of varied growth parameters and yield attributes (Umesh et al 2017, Suresh et al 2019). Ahmed et al (2015) reported that sunflower yield was significantly influenced by date of sowing. The productivity of sunflower was mainly determined by the weather and soil moisture throughout its life cycle and the imposed cultural practices. Delayed sowing increased the yields. The timely sown crop experienced moisture stress during early stage of the crop which adversely affected its growth and yield attributes. However, well distributed rainfall and adequate moisture in late sown might be responsible for enhanced sunflower yield. Additionally, sunflower is a thermo insensitive and it adjust to the late sowing but most important factor is moisture availability for their luxuriant crop growth and yield (Kaleem et al 2011, Demir 2019). Sole sunflower gave higher yields than intercropped has a result of more plant populations in the sole than intercropping (Sandeep Kumar et al 2019). Row proportion of sunflower and pigeonpea had significant effect on seed and stalk yield of sunflower. The higher seed and stalk yield of sunflower was recorded under 2:1 row proportion than 1:1 row proportion.

Seed protein content: Crops sown in second fortnight of July recorded significantly higher protein content (21.36 %) than second fortnight of August (20.50 %) (Table 1). Greater accumulation of nutrients in seed and better crop performance might be responsible for enhanced protein content. Sole transplanted pigeonpea recorded higher protein (22.2%) content than intercropped as well as drilled pigeonpea. Further supply of fertilizers to both the crops in transplanted pigeonpea + sunflower (1:1) also recorded greater protein content (21.32 %) over rest of the treatment combinations. Drilled pigeonpea + sunflower (1:1) and sole drilled pigeonpea was on par to each other but recorded lower than the sole transplanted and intercropped pigeonpea. Combined effect of time of sowing and intercropping system with different row proportion and fertilizer application on protein content was found non-significant. Vishwanatha (2009) reported sole pigeonpea recorded higher protein content than intercrops. In intercropping supply of balanced nutrients to the crop especially phosphorus may be due to the biochemical role of phosphorus in protein synthesis (Udikeri 2013). The protein content in seed is a function of its N concentration; therefore, higher concentration of N in seed under the superior treatments seems to be the only reason for attaining higher protein content in pigeonpea (Kumar and Paslawar 2017).

Sunflower seed oil content: Sunflower seed oil content was significantly greater in second fortnight of August sown (38.3%) over July (36.1%) (Table 1). Intercropping combinations were reduced the oil content over sole sunflower (39.1 %). Combined effect of time and row proportions and fertilizers application was found non-significant in respect to oil content. It was mainly due to enhanced vegetative growth and development of sunflower in August sown than July. Late-sown plants increases the seed oil might be due to the fact that the temperature increases during seed development (Allam et al 2003).

Supply of RDF to both the crops recorded higher oil content. It might be due to lower fatty acid synthesis through the pentose phosphate pathway in seeds of without fertilizer application to intercrop owing to increased competition and non-availability of required quantity of nutrients but the treatment which received RDF had better synthesis of fatty acid owing to optimum nutrient availability (Vishwanatha, 2009).

Equivalent yields: Pigeonpea transplanted in second fortnight of July recorded 21.1% higher PEY (2570 kg ha^{-1}) over August (Table 1). Further it was greater in sole transplanted pigeonpea (2252 kg ha^{-1}) than drilled pigeonpea and sunflower alone. It also improved by application of fertilizers to both the crops in transplanted pigeonpea + sunflower (1:2) (2914 kg ha^{-1}). Application of RDF to main crop in transplanted pigeonpea + sunflower (1:1) recorded significantly lower PEY (2453 kg ha^{-1}). Prevalence of favorable climatic weather and resources availability has resulted higher yield of both main and intercrop yield with early sowing than delayed sowing (Ravindra 2019). Murali et al (2014) also showed increased PEY with transplanting 4-5 weeks old pigeonpea seedlings than drilled sown pigeonpea as sole crop. It might be due to efficient utilization of natural resources and additional yields of component crops and their better performance in transplanted pigeonpea with higher market prices which intern resulted in higher PEY and net returns. Praharaj et al (2015) reported that significantly higher PEY over direct sown in pigeonpea and soybean intercropping and sole cropping. Intercropping system had a significant influence in obtaining higher PEY over either of sole cropping except unfertilized control. This was due to higher seed yields of component crops owing to optimum nutrient availability (RDF to both the crops) coupled with higher price of both the crops contributed to higher PEY (Poornima 2009). Sunflower equivalent yield was 20.7% greater in crops sown in second fortnight of July (3354 kg ha^{-1}) over August sown (Table 1). It might be due to better sunflower productivity under timely sowing than delay sowing coupled with higher market price. Intercropped treatments were recorded significantly greater SEY than the sole. Further it also enhanced by application of RDF to both the crops in transplanted pigeonpea + sunflower (1:2). It was significantly reduced under sole sunflower (1781 kg ha^{-1}). The interaction effect between sowing time, intercropping system, row proportions and fertilizers application have significant effect on the SEY. The pigeonpea and sunflower 1:2 row proportion was recorded higher SEY as compared to other row proportions.

Economic returns: Crops sown/transplanted in second fortnight of July has registered higher gross returns (Rs. 136769 ha^{-1}) net returns (Rs. 94421 ha^{-1}) and B: C ratio (3.2) over August sown crops (Table 2). Sole transplanted pigeonpea recorded significantly higher gross returns (Rs. 123860) and net returns (Rs. 83560 ha^{-1}) over drilled pigeonpea and sunflower. Sole Application of RDF for both crops in transplanted pigeonpea + sunflower (1:2) higher gross returns (Rs.157680 ha^{-1}) and net returns (Rs.108880 ha^{-1}) over 1:1 row proportions and RDF to main crop. Whereas drilled pigeonpea + sunflower (1:1) considered as farmers practice recorded higher BC ratio (3.49) over

Table 2. Economic returns of pigeonpea and sunflower intercropping system sown in different months and row proportions

Treatments	Cost of cultivation (Rs. ha^{-1})	Gross returns (Rs. ha^{-1})	Net returns (Rs. ha^{-1})	B:C ratio
Sowing /transplanting time				
July	42339	136769	94421	3.20
August	39811	114163	74361	2.85
CD (p=0.05)	-	10454	10079	0.28
Row proportions				
Transplanted pigeonpea + sunflower (1:1) RDF for main crop	43850	133285	89435	3.11
Transplanted pigeonpea + sunflower (1:1) RDF for both crops	48800	143090	94290	2.99
Transplanted pigeonpea + sunflower (1:2) RDF for main crop	43850	140430	96580	3.28
Transplanted pigeonpea + sunflower (1:2) RDF for both crops	48800	157680	108880	3.30
Drill sown pigeonpea + sunflower (1:1)	44000	142895	98895	3.49
Sole sunflower	26500	71240	44740	2.69
Sole transplanted pigeonpea	40300	123860	83560	3.07
Sole drill sown pigeonpea	32500	91245	58745	2.72
CD (p=0.05)	-	8616	7126	0.23

Market price of Pigeonpea: Rs. 55/kg, Sunflower price: Rs. 42/kg transplanted pigeonpea + sunflower in (1:1) and 1:2. Higher grain yield which results in higher gross return same results were reported by Uprikar (2017). Higher net returns realized from intercropping was due to higher complementarity between component crops which produced higher yield. Higher returns from drilled pigeonpea + sunflower (1:1) were due to additional cost was incurred towards nursery raising and transplanting in transplanting method. Superior performance in transplanted pigeonpea treatments might be due to efficient utilization of natural resources and additional yields of component crops and their better performance in transplanted pigeonpea with higher market prices which intern resulted in higher pigeonpea equivalent yield and net returns. Similarly higher remunerative returns were observed in intercropping system over sole cropping by several workers (Vishwanatha et al 2012). Sole transplanted pigeonpea recorded higher BC ratio over sole sunflower (2.69) and drilled sown pigeonpea (2.72) (Table 2). Dibbled pigeonpea and sunflower intercropping has recorded higher BC ratio was due to higher cost of cultivation in transplanting method than of dibbling method. Similar results were reported by Priyanka et al (2013) and Ramanjaneyalu et al (2016).

CONCLUSION

The pigeonpea either sown or transplanted was out yielded and greater economic returns. Whereas second fortnight August was benefitted to sunflower. Drill sown pigeonpea and sunflower combination in 1:1 had greater yield advantage over sole drilled pigeonpea. Yield and economic benefit were greater in second fortnight of July sown crop as compared second fortnight of August sown crop. Higher net returns was realized in transplanted pigeonpea and sunflower intercropping system with 1:2 row proportion and application of RDF based on both the crops. Transplanted pigeonpea has the greater potential and productive than drilled sown pigeonpea.

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Growth, Dry Matter Partitioning and Productivity of Wheat (*Triticum aestivum* L.) under Different Establishment Methods and Inter-Culture Practices

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Abstract: A field experiment was carried out under Randomized Block Design at Norman. E. Borlaug Crop Research Centre of GBPUA&T, Pantnagar, Uttarakhand to evaluate the effect of establishment methods and inter-culture practices on growth and productivity of wheat (cv. HD 2967). Seven treatments including five crop establishment methods viz., conventional tillage (CT), reduced tillage (RT), zero tillage (ZT), raised bed 40/20 cm and raised bed 60/20 cm and two inter-culture practices viz. conoweeding (CW) and alternate ridging (AR) at 35days after sowing were evaluated. Wheat grown under ZT, RT and CT conditions produced significantly higher plant height compared to raised bed. Effective tillers (423 m^{-2}) and total dry matter (DM) production (1478.3 g m^{-2}) were higher in RT condition. Tiller mortality was highest (56.0%) with CT practice and lowest in RB 40/20 cm land configuration. Wheat grown on raised bed 40/20 cm recorded significantly higher root weight density compared to remaining treatments followed by raised bed 60/20 cm. Partitioning of DM towards sink (spike) was highest in raised bed method. Wheat grown under reduced tillage condition resulted highest grain yield (5.44 t ha^{-1}) that was statistically similar to CT, and ZT, however it was 17.6 and 11.6% higher than raised bed 40/20 and 60/20cm, respectively. Both CW and AR inter-culture practices resulted less yield than CT.

Keywords: Dry matter, Wheat, Raised bed, Reduced tillage, Zero tillagety

Wheat (*Triticum aestivum* L.) covers about one-fifth of the total area under food grains and contributes to about one-third (40%) of the total food grain production in India (Mukherjee and Mandal 2021). Among different agronomic management practices, the planting method is of great significance as it not only determines optimum plant stand due to proper resource utilization but also enhances the yield potential of the individual plant through minimizing inter plant competition and facilitating the harvest of light energy into economic form. Conventionally, wheat establishment is resource intensive and needs friable seed bed which is obtained by multiple tillage passes consuming huge energy, increasing the cost of cultivation besides taking long turnaround period (Sah et al 2014). Accelerated rate of decomposition of organic matter under conventional tillage without its addition leads to degradation of the soil health and environmental pollution threatening the system stability and sustainability. Thus, there is a need to find out an alternate management strategy which can sustain the crop productivity. Wheat production can be improved through judicious input use; better production technology and tillage practice (Leghari et al 2015). Resource conservation technologies like reduced tillage, zero tillage, raised bed planting etc. have been considered to be the alternatives to conventional method. Retaining residues in conservation tillage has the potential to conserve the soil moisture and reduce soil erosion and runoff (Singh et al 2021). It ensures timely sowing of wheat when harvesting of rice is delayed. Minimizing soil agitation by reduced tillage improves physical, chemical and biological properties of soil (Lopez-Garrido et al 2012). Sowing of wheat on beds provide better solar radiation penetration and aeration within the crop canopy due to more open space available to plants on either sides of the bed. It can save 50% seed, 25% water, reduces lodging, minimizes loss of nitrogen, helps in rain water conservation resulting in nearly 25% yield enhancement (Alwang et a l2018). Piling up of fertile top soil in the form of bed also helps in vigorous root system enabling the plant to explore more soil volume and resist against lodging. Ahmad et al (2010) found that bed planted wheat consumed approximately 35.6% less water as compared to the conventional row planting in flat beds with flood irrigation.

Post-sowing inter-culture soil operations like conoweeding and alternate ridging can also sustain crop growth and development through soil manipulation and resource conservation in terms of water. Mechanical manipulation of soil through conoweeder in the widely spaced wheat crop may enhance root growth and tillering ability of the crop. It not only incorporates the weeds and adds the organic matter in the soil but may also help in effective recycling of the depleted nutrients which in turn could have augmented the nutrient pool of the rhizosphere together with aeration of the root zone. Alternate ridging in relatively wider spaced standing crop provides better anchorage to the plants. Besides saving irrigation water and better aeration for root expansion, post sowing ridging also helps in checking weed growth, conserving soil moisture, mixing of applied fertilizers thoroughly with the soil. Hence, the present study was conducted to assess the impact of establishment methods and inter-culture practices on wheat growth and productivity.

MATERIAL AND METHODS

The field experiment was initiated at Norman. E. Borlaug Crop Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand during *rabi* season, 2017. Pantnagar comes under humid sub-tropical climate with cool winter. During the crop period, the average weekly maximum temperature ranged between 12.9 ºC in January to 33.6 ºC in April while the minimum temperature ranged from 4.2 ºC in January to 18.7 ºC in April.

The total rainfall received during crop growing period was 55.8 mm. Soil of the experimental field was sandy loam texture with medium organic carbon (0.65%). Soil bulk density was 1.54 g cm^{-3} with neutral in reaction (pH 7.2), low in available nitrogen (149.6 kg ha^{-1}) and high in available phosphorus (26.9 kg ha^{-1}) and available potassium (286.1kg ha^{-1}). The moisture content of soil at FC and PWP were 20.5 and 8.4%, respectively. The experiment consisting of seven treatments viz. conventional tillage (CT), zero tillage (ZT), reduced tillage (RT), raised bed land configurations of 40/20 cm (RB 40/20) and 60/20 cm (RB 60/20) and two inter-culture practices conoweeding (CW) and flat sowing followed by alternate ridging (AR), was laid out in randomized block design with three replications. In CT, land was prepared by 4 harrowings and 4 plankings that was reduced to 2 harrowings and 2 plankings in RT and totally omitted in ZT. In case of CT, ZT and RT wheat (cv. HD 2967) was sown in rows 20 cm apart. In conoweeding treatment, land preparation was similar to conventional tillage treatment except the widening of row spacing (25 cm). Conoweeder was used manually at the time of second and third irrigations in the field along the rows in single direction. For conoweeding, crop should attain sufficient growth and crop canopy should not be mudded during conoweeding. Hence, conoweeding was done during second and third irrigations. For alternate ridging, sowing was done on flat bed at 30 cm row spacing followed by alternate ridging at 35 DAS. After first irrigation, flat bed was converted in to alternate ridge and furrows by removing soil between two rows and placing it in adjacent two rows in the form of ridge using a small spade and water was applied in the furrows formed. In both raised bed treatments, after tillage operations, the land form was changed into raised beds and furrows. Two and three wheat rows were accommodated on the bed in RB 40/20 and RB 60/20 cm, respectively. The crop was uniformly fertilized with 150 kg N, 60 kg P_2O_5 and 40 kg K_2O per hectare with one third dose of nitrogen and the entire amount of phosphorus and potassium were applied as basal. The remaining two third nitrogen was top dressed in three equal splits at CRI, tillering and booting stage after first, second and third irrigation, respectively. After CRI irrigation, subsequent irrigations were scheduled as per IW:CPE ratio 1.0. Total five irrigations were given. A composite soil sample was taken with the help of auger from0-15 cm soil layer before each irrigation and soil moisture content was calculated by thermo-gravimetric method. Volumetric soil moisture content was worked out by multiplying the gravimetric soil moisture content with bulk density. The growth parameters, yield and yield attributes were recorded at specified stages with standard procedure. The economic yield was expressed at 14% moisture content. Tillers mortality was worked out by using the following formula:

$$\text{Tillers mortality (\%)} = \frac{\text{Maximum tillers} - \text{Effective tillers}}{\text{Maximum tillers}} \times 100$$

Dry matter accumulation was recorded at 60 DAS, 100 DAS and at harvest. The plant samples were sun dried for 3-4 days to lose excess moisture, then kept in dryer at 70 ± 2°C temperature for 48 to 78 hours till the samples attained a constant weight. Dry weight of leaf, stem and spike was taken separately to record dry matter partitioning at different stages. Root sampling was done up to a depth of 13 cm along with soil mass with the help of core sampler of diameter 10 cm from the 0.5 m sampled area already used for plant dry matter study. The root samples along with soil mass were kept in fine meshed nylon net bag and washed under running water to get rid of soil and avoid the loss of finer roots. After cleaning, the roots were cut off from the shoots. Roots were separately placed in brown paper bags and were dried in plant drier at 70 ± 2°C till constant weight. The root weight density was worked out as follows:

$$\text{Root weight density (mg cm}^{-3}\text{ of soil)} = \frac{\text{Total root weight (mg)}}{\text{Total soil volume sampled (cm}^{3}\text{)}}$$

The experimental data collected at different stages were analyzed by using analysis of variance technique appropriate to Randomized Block Design as per the statistical programme OPSTAT, developed by CCS Hisar Agricultural University, Haryana.

RESULTS AND DISCUSSION

Plant growth, tiller count and tiller mortality: Wheat grown under ZT, RT and CT conditions produced significantly higher plant height (Table 1) compared to raised bed mainly because of better moisture availability (Fig. 1) facilitating better root growth and utilization of available resources. Wheat sown on raised beds produced shortest plants because of low moisture retention in top soil due to increased surface area of the changed land configuration under raised bed (Zhang et al 2007). On the basis of the availability of natural resources *viz.* light, water and nutrients tillering may affect wheat yield either positively or negatively (Elhani et al 2007). Tiller count at maximum tillering stage (55 DAS), was highest (945 per m^2) in wheat grown under CT condition being statistically at par with the RT and ZT treatments might be because of better soil moisture availability in CT, RT and ZT facilitating effective utilization of resources (Fig. 1). Wheat sown on raised bed produced less number of effective tillers because of its wider row spacing leading to reduced population. Tiller mortality was highest (56.0%) with CT practice and lowest in RB 40/20 cm land configuration. Higher tiller number in CT might have resulted increased intra- plant competition for partitioning of photosynthates, however less production of side tillers in RB resulted in low mortality. Tillers mortality in reduced tillage was 2.4 and 1.9 percent lower as compared to conventional and zero tillage, respectively. Alternate ridging had 3.9 per cent less tillers mortality than conoweeding. Raised bed with three rows per bed showed 1.9 per cent higher tiller mortality than two rows of wheat per bed. This can be attributed to the fact that at lower densities, plants can use different resources (solar radiation, water, soil, nutrients etc.) more efficiently, and also competition among tillers is reduced (Evers et al 2006).

Root weight density: At peak period, root growth is a key factor determining acquisition of nutrients and water from soil, stem elongation, sturdiness, crop growth and productivity. Root weight density measured at 60 DAS varied significantly due to different treatments (Table 1). Wheat

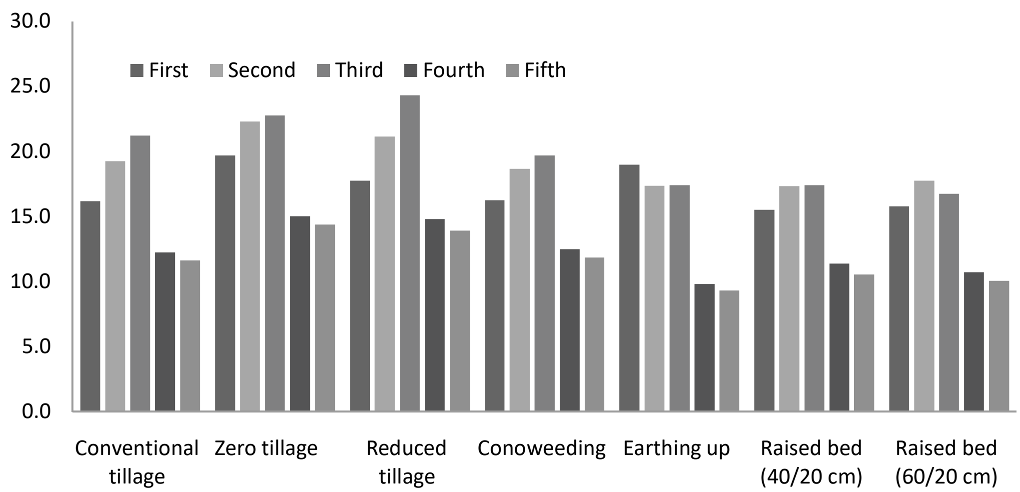

Fig. 1. Volumetric soil moisture content (%) before irrigations under different treatments

Table 1. Wheat growth and yields under different establishment methods and inter-culture practices

Treatment	Plant height (cm)	Tillers per m² at maximum tillering stage	Effective tillers per m²	Tiller mortality (%)	Root weight density ($mg\ cm^{-3}$)		Thousand grain weight (g)	Grain weight perear (g)	Grain yield ($t\ ha^{-1}$)	Straw yield ($t\ ha^{-1}$)
					60 DAS	80 DAS				
CT	101.2	945	416	56.0	0.75	1.80	40.8	1.48	5.20	7.70
ZT	98.9	908	404	55.5	0.58	1.34	39.4	1.49	5.20	7.55
RT	100.5	911	423	53.6	0.80	1.41	40.7	1.55	5.44	7.86
CW	100.5	783	374	52.0	0.84	1.87	40.2	1.49	5.00	7.39
AR	99.6	755	388	48.1	0.81	1.61	35.8	1.45	4.51	6.83
RB 40/20cm	97.1	669	344	47.6	1.20	2.32	39.3	1.54	4.48	6.26
RB 60/20cm	96.5	757	363	49.5	1.03	2.10	39.7	1.47	4.81	6.79
CD (p= 0.05)	3.1	150.0	45	NS	0.17	NS	2.5	NS	0.35	0.61

grown on RB 40/20 cm land configuration recorded significantly higher root weight density compared to remaining treatments followed by RB 60/20 cm land configuration. RB 40/20 cm land configuration recorded 51.9 and 28.% higher root dry weight than conventional tillage at 60 and 80 DAS, respectively. Better root development under raised bed configuration was due to less penetration resistance impedance (Naresh et al 2012). Du et al (2021) also explained that raised bed modified the rhizosphere that resulted in better root growth of wheat. Zero till wheat recorded minimum root weight density which was lower by 29.3 and 34% than conventional tillage at both 60 and 80 DAS, respectively. Soil type is also an important consideration for no tillage especially in areas subjected to excessive precipitation and high levels of crop residue. Generally, soils with an imbalance in particle size distribution (*i.e.*, high clay and sand content) are susceptible to compaction and thus demand tillage.

Leghari et al (2015) also reported that conventional tillage recorded higher root dry weight followed by reduced and zero tillage. At both the stages of observation, conoweeded treatment had higher root weight density than conventional, zero and reduced tillage treatments. Mechanical weeder improves aeration of the soil during weeding that allows oxygen to circulate within the soil. This might facilitate emergence of new roots from the roots which got pruned during this operation.

Dry matter yield and its partitioning: Total dry matter yield under various plant growth stages along with dry matter partitioning into leaf, stem and spike were significant for different establishment methods except for leaf and stem at harvest and stem at 100 DAS (Table 2).

Stem dry weight: Stem dry weight (g m^{-2}) increased from 60 DAS reaching maximum at 100 DAS and declined thereafter. Initially (60 DAS), dry weight of stem (m^2) differed significantly but later on differences became non-significant (Table 2). At 60 DAS, conventional tillage attained highest stem dry weight (77.7 g m^{-2}) that might be due to more tillering as well as taller plants and was statistically at par with reduced tillage (71.5 g m^{-2}) but significantly higher than other treatments because of more number of tillers. The stem dry weight was lowest in alternate ridging (45.8 g m^{-2}). Dry weight of stem (m^2) was more in raised bed having three rows per bed (50.6 g m^{-2}) compared to two rows of wheat per bed (47.4 g m^{-2}). At harvest, maximum stem dry weight was noticed with reduced tillage (451.3 g m^{-2}).

Leaves dry weight: Leaves dry weight (g m^{-2}) increased till 100 DAS and declined thereafter and attaining the lowest value at harvest. Leaves dry weight (m^2) showed significant variations up to 100 DAS. Initially, at 60 and 100 DAS, wheat grown under reduced till condition gained highest dry weight of leaves/m^2 (Table 2) because of more number of leaves per m^2 and better moisture availability that might result in more photosynthates accumulation. At 60 DAS, it was at par with conventional tillage, however, at 100 DAS, it was also statistically at par with dry weight of leaves (m^2) obtained under zero till condition. At all the growth stages, leaves dry weight was less in raised bed treatments than conventional tillage that could be attributed to low rate of photosynthesis under low soil moisture (Quanqi et al 2007). Conoweeding

Table 2. Impact of different treatments on dry matter partitioning of wheat at different growth stages

Treatment	60 DAS			100 DAS				At harvest			
	Leaf dry weight (g m^{-2})	Stem dry weight (g m^{-2})	Total dry weight (g m^{-2})	Leaf dry weight (g m^{-2})	Stem dry weight (g m^{-2})	Spike dry weight (g m^{-2})	Total dry weight (g m^{-2})	Leaf dry weight (g m^{-2})	Stem dry weight (g m^{-2})	Spike dry weight (g m^{-2})	Total dry weight (g m^{-2})
CT	155.3 66.7*	77.7 33.3*	233.0	358.7 28.6*	657.7 52.4*	237.7 19.0*	1254.0	137.8 9.5*	442.8 30.5*	872.2 60.0*	1452.8
ZT	141.7 70.1*	60.3 29.9*	202.0	345.0 31.0*	561.2 50.4*	207.2 18.6*	1113.3	132.2 9.2*	440.1 30.6*	867.8 60.3*	1440.1
RT	164.7 69.7*	71.5 30.3*	236.2	403.8 30.0*	681.0 50.7*	259.5 19.3*	1344.3	143.3 9.7*	451.3 30.5*	883.7 59.8*	1478.3
CW	115.5 68.6*	52.9 31.4*	168.4	305.5 27.1*	609.6 54.1*	211.5 18.8*	1126.5	135.9 9.5*	419.1 29.3*	876.8 61.2*	1431.7
AR	110.6 70.7*	45.8 29.3*	156.4	279.4 27.7*	541.3 53.7*	187.4 18.6*	1008.0	119.1 10.1*	347.1 29.5*	710.5 60.4*	1176.6
RB 40/20 cm	114.1 76.7*	47.4 31.9*	148.7	317.3 28.4*	595.7 53.4*	215.9 19.3*	1116.2	127.8 9.2*	399.5 28.7*	863.5 62.1*	1390.9
RB 60/20 cm	101.4 61.6*	50.6 30.7*	164.7	304.6 28.6*	525.0 49.3*	222.7 20.9*	1065.1	118.3 8.5*	397.3 28.7*	869.8 62.8*	1385.5
CD (p= 0.05)	18.7	15.3	30.6	70.6	NS	41.3	169.3	NS	NS	72.7	152.1

* Percent share of the component with respect to total dry weight

treatment recorded higher leaves dry weight than flat bed sowing followed by alternate ridging might be due to better tillering. Wheat grown on raised bed accumulated lower dry weight of leaves than conventional. At harvest, differences were found to be the non-significant among all the treatments.

Spike dry weight: At 100 DAS and harvest, reduced tillage produced highest dry weight of spike. At 100 DAS, it was statistically at par with the conventional tillage and raised bed 60/20 cm. At harvest, it recorded significantly higher dry weight of spike compared to alternate ridging treatment by 24.4%. At both the stages, the lowest dry weight of spike (187.4 and 710.5 g m^{-2}) was noticed with alternate ridging treatment. Conoweeding showed their superiority over alternate ridging with 19.0% higher spike dry weight. Both RB 40/20 cm and 60/20 cm configuration attained almost equal dry weight of spike that was at par with CT. Partitioning of dry matter towards sink (spike) was considerably higher *i.e.,* nearly 62 per cent in raised bed condition which might be due to better light penetration, air circulation and optimized phenological pattern leading to higher total assimilate, thereby, better grain filling and maximum translocation of assimilates towards the wheat spikes (Reynolds et al 2009).

Total dry matter accumulation: Dry matter accumulation increased with time and reached the maximum value at harvest. At 60 DAS, dry matter yield was highest under reduced tillage (236.2 g m^{-2}) due to more leaf and stem dry weight followed by conventional (233 g m^{-2}) and zero tillage (202 g m^{-2}). Hofmeijer et al (2019) also observed higher above ground biomass yield of wheat in reduced tillage with respect to conventional tillage. Total dry matter yield was significantly lower (13.3%) in zero till condition as compared to conventional tillage because of less stem and leaf dry weight. Dry matter yield in raised bed 40/20 cm was 9.7% lower than raised bed 60/20 cm although both of them were at par and superior to alternate ridging treatment mainly due to better root development and aeration owing to free space available at both sides of bed resulted in faster growth (Kumar et al 2010). At harvest, dry matter accumulation was the highest in reduced tillage condition (1,478.3 g m^{-2}) might be due to having higher number of green leaves and tillers (per m^2). Accumulation of dry matter in zero till wheat was at par with conventional tillage. At all the three stages, dry matter of wheat was the lowest in treatment with 30 cm row spacing with alternate ridging. At 60 and 100 DAS, dry weight of leaf and stem was more; respectively than at harvest due to greater dry matter accumulation in those parts, but later on, it reduced slightly. It might be due to more translocation of assimilates towards spike at the time of grain filling. Under high soil moisture condition, dry matter partitioning along with its accumulation were more which improves the yield. Plaut et al (2004) also reported reduced grain weight under moisture stress condition.

Yield and yield attributes: Wheat under RT produced the maximum number of spikes (423 m^{-2}) due to more number of leaves as well as leaf dry weight that might lead to better photosynthesis. Under raised bed condition number of spikes m^{-2} was significantly lower than CT (Table 1) because of increased row spacing. Conservation tillage practice had the highest thousand grain weight (40.8 g) which was closely followed by RT (40.7 g). Higher dry matter accumulation in these treatments might have led to better grain filling in turn the 1000 grain weight got increased. Leghari et al (2015) also found greater thousand grain weight in CT followed by RT and ZT treatments. In the experiment, wheat grown under RT condition produced the maximum grain yield (5.44 t ha^{-1}) which was statistically similar with CT and ZT condition but significantly superior than other treatments. In a similar way, Lopez-Garrido et al (2014) also found more wheat grain yield under reduced tillage than conventional tillage system. Reduced tillage, a form of mulch tillage conserves more moisture on surface due to residues remaining as mulch. Relatively larger clod size than conventional tillage also improve water infiltration, lower bulk density (*i.e.,* soil compaction), increasing root growth, uptake of water and nutrients leading to more tillering, dry matter production and yield attributes and ultimately the grain yield. Conventional and zero tilled wheat also produced identical grain yield of 5.2 t ha^{-1}. Zhang et al (2009) and Martinez et al (2008) found no significant difference in wheat yield under zero and conventional tillage. Zero tillage stored more moisture (Sah et al 2014) than other treatments that might favor tillering and heavier spike. Jat et al (2017) also found improved crop production under no-tilled condition compared with CT. Han et al (2020) explained that straw from previous crop improved wheat crop production. Multiple factors were responsible for the improved wheat performance. Firstly, straw-return increased available plant nutrients in the soil (Bartaula et al 2020). Secondly, addition of organic matter from the left over residue of previous crop contributes to superior soil quality leading to better crop performance (Yang et al 2016). Thirdly, improved hydrological and physical soil conditions (Han et al 2020) under reduced tillage was congenial for increased wheat yield and yield components. Conoweeding on wheat resulted significantly higher grain yield with respect to alternate ridging treatment as it recorded more thousand grain weight and grain weight per spike. Between the raised bed treatments, RB 60/20 cm land form produced 7.4% higher grain yield than RB 40/20 cm (4.48 t ha^{-1}). Kamboj et al (2017) also found better wheat yield realization with three rows per bed with respect to two rows per bed mainly due to lower weed infestation and more efficient utilization of space and nutrient under three rows of wheat per bed. Although, grains per spike and grain weight per spike were higher in RB 40/20 cm than conventional tillage but these were not sufficient to bridge the gap of reduced plant population. Both the raised bed treatments were significantly inferior as compared to conventional tillage. In conventional plots, wheat seeds were spaced 20cm apart while in bed planting, row to row distance was 30 cm and 26.7 cm, respectively. This reduction in number of rows per unit area resulted in reduced wheat crop stand at planting and less number of effective tillers per m^2contributed towards lower yield of bed planted wheat. The results are in accordance with the findings of Kilic (2015) who also obtained lower grain yield in bed planting than flat planting.

CONCLUSION

The present study in sandy loam soil belonging to Mollisols of *Tarai* of Uttarakhand, indicate that for growth parameters and productivity, wheat grown under reduced tillage performs better with highest number of effective tillers, dry matter accumulation that ultimately leaded towards highest grain yield. The grain yield in reduced tillage was 17.6 and 11.6% higher than raised bed 40/20 and 60/20 cm. Conoweeding and alternate ridging practice did not attain comparable yield with other treatments, however tiller mortality was 4- 8% less than conventional method.

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Effect of Integrated Nutrient Management on Plant Growth, Yield and Quality of Papaya (*Carica papaya* L.) cv. Red Lady

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Abstract: Continuous use of chemical fertilizers poses soil health issues, whereas organic manures alone are not able to fulfil the requirements of highly nutrient-demanding crops. Indian soils are deficient in NPS, leading to low yield potential, so the only option left is to concentrate on alternate strategies like Integrated Nutrient Management (INM) to maintain the soil fertility. In this view, research was carried out to study the effect of integrated nutrient management on plant growth, yield and quality of papaya (*Carica papaya* L.) cv. Red Lady under Punjab conditions. The main objectives of this study were to adopt organic manures instead of relying only on chemical fertilizers or to find the appropriate combination of organic manures along with chemical and bio-fertilizers. The dose of chemical fertilizers was kept constant (50% for all treatments), whereas different concentrations of organic manures and bio-fertilizers were added. Among all the treatments, the application of 50% NPK + 20 kg FYM + 100 g *Azospirillum* + 100 g PSB resulted in the maximum plant height, stem girth, fruit length, fruit width, and yield. Likewise, all the fruit quality parameters such as TSS, firmness, total sugars, reducing sugars, non-reducing sugars and ascorbic acid were also improved by the same treatment. Hence, integrated nutrient management significantly improved the vegetative growth, yield and quality of papaya cv. Red Lady when 20 kg of FYM and 100g of *Azospirillum* were applied along with 50% NPK.

Keywords: Integrated nutrient management, Papaya, Organic manures, Bio-fertilizers, Fruit yield, Fruit quality

Papaya (*Carica papaya* L.) belongs to the Caricaceae family. In Punjab, papaya was earlier considered a backyard crop or it was mainly grown as a filler crop in the main orchard like mango. But nowadays, farmers in Punjab are more concerned about cultivating papaya as a major crop because of its numerous health and commercial benefits. Among several varieties of papaya, Red Lady is gaining popularity due to its continuous flowering habit and unlike other dioecious varieties there is no need to maintain a female and male plant ratio. The nutritional requirements of papaya vary from those of other fruit crops. If sufficient nutrients are not provided during the growth and flowering phases, a papaya crop will be vulnerable to various biotic and abiotic stresses (Kanwar et al 2020). Use of chemical fertilizers is a primary choice of farmers in order to maximize the productivity. Sole application of chemical fertilizers in huge amounts creates soil health problems, while organic sources alone are not sufficient to fulfil the requirements of high nutrient-demanding crops, as organic manures have low nutrient content and are slow releasing into the soil. Though organic manures are easily available and eco-friendly, the major drawback of adopting organic agriculture is that it would not be possible to feed billions of people solely through organic agriculture. Thus, integrated nutrient management is the only sustainable approach to strengthen fruit production without posing detrimental effects on human health and the environment. INM could be an option where a portion of nutrients are supplied through inorganic fertilizers and the remaining portion with organic manures along with microbial inoculations. It also gives a good return on the economy (Bakshi et al 2018). The main objectives of this study were to assess the effect of integrated nutrient management on vegetative growth, flowering and yield of the papaya.

MATERIAL AND METHODS

Experimental site and planting material: The present investigation was carried out at the Horticultural Farm, School of Agriculture, Lovely Professional University, Phagwara Punjab during the year of 2020-2021. The experimental area is located at 31.2498° North latitude and 75.7084° East longitude. It has an altitude of 232 m above mean sea level (MSL). The soil of the experimental site was sandy loam, well fertile, rich in organic matter and free from perennial weeds. The soil sample was collected from 0 to 15.0 cm depth of the soil from the exploration site before the start of the experiment and analysis of the sample was done physically and chemically.

Treatment details: One-month-old (10-15cm) healthy seedlings of papaya cv. Red Lady were taken from the nursery of Punjab Agriculture University (PAU), Ludhiana and transplanted at Lovely Professional University's horticultural farm with a spacing of 1.8 m x 1.8 m. The experiment was laid out in a Randomized block design with 13 treatments and 3 replications, each studying 2 units of plants per replication. 200:250:250 g NPK/plant was used as a recommended dose of fertilizers and applied as per details of all thirteen treatments including bio-fertilizers and organic manures *viz.* T_0(100% RDF + Control), T_1(50% RDF + 20 kg FYM/plant), T_2 (50% RDF + 20 kg FYM + 100g *Azospirillum*/plant), T_3 (50% RDF + 20 kg FYM + 100g PSB/plant), T_4 (50% RDF + 20 kg FYM + 100g *Azospirillum* + 100g PSB/plant), T_5 (50% RDF + 10 kg Vermicompost/plant), T_6 (50% RDF + 10 kg Vermicompost + 100g *Azospirillum*/plant), T_7 (50% RDF + 10 kg Vermicompost + 100g PSB/plant), T_8 (50% RDF + 10 kg Vermicompost + 100g *Azospirillum* + 100g PSB/plant), T_9 (50% RDF + 2 kg Neem cake/plant), T_{10} (50% RDF + 2 kg Neem cake + 100g *Azospirillum*/plant), T_{11} (50% RDF + 2 kg Neem cake + 100g PSB/plant), T_{12} (50% RDF + Neem cake + 100g *Azospirillum* + 100g PSB/plant). The well-rotted farmyard manure, vermicompost and neem cake along with bio-fertilizers were applied before transplanting according to the details of treatments. Inorganic fertilizers like phosphorus and potash were applied along with the organic manures. Nitrogen was applied in three equal splits, with the first dose applied with organic manures, the second dose applied during flowering and the third dose applied during fruit set. The sources of NPK were Urea, SSP and MOP, respectively.

Estimation of vegetative and reproductive growth: The vegetative growth *viz.* plant height, stem girth, the number of leaves, petiole length, plant spread (N-S) and plant spread (E-W) while reproductive growth *viz.* days taken to first flowering and days from flowering to fruit maturity were determined following the conventional methods previously used by Yadav (2017).

Estimation of fruit quality: Fruits are harvested from the experimental field to determine the fruit weight, fruit length and fruit width and was measured in centimeter (cm). A digital hand refractometer was used to determine the TSS (total soluble solids) of fruit pulp. Physicochemical analysis of fruits for evaluation of quality was done as per standard procedures (AOAC 1980). To determine ascorbic acid content fresh fruit pulp was used by following the methods recently detailed by Baswal et al (2020) and expressed in mg/100 g pulp.

Statistical analysis: The recorded mean of all the field and lab parameters for each replication was subjected to statistical analysis for analysis of using OP Stat software (Sheoran 1998).

RESULTS AND DISCUSSION

Plant height and stem girth: At 210 days after transplanting (DAT), the application of 50% RDF + 20 kg FYM + 100g *Azospiriilum* + 100g PSB/plant was better in increasing the plant height (179.17cm) and stem girth (30.83cm) as compared to the other treatments and control (Table 1). The increase in plant height and stem girth may be due to an improvement in soil physical properties or an increase in nutrients uptake, growth of beneficial soil microorganisms and released growth factors such as auxins, cytokinins and gibberellins in the root zone (Godara and Bakshi 2021). However, the results of the present study are moderately different from the results of Singh and Varu (2013) who reported that in papaya cv. Madhubindu, the application of 50% NPK + 50g *Azotobactor* + 2.5g/m^2 PSB was better in increasing the plant height.

Number of leaves, leaf length, petiole length, plant spread (North-South) and plant spread (East-West): Application of Integrated Nutrient Management significantly influenced the number of leaves, leaf length, petiole length, plant spread (North-South) and plant spread (East-West). The application of 50% RDF + 20 kg FYM + 100g *Azospiriilum* + 100g PSB/plant recorded the significantly highest mean number of leaves (32.50), leaf length (66.83cm), petiole length (73.83cm), plant spread in the North-South (172.00cm) and plant spread in the East-West (180.83cm). The inoculation of plant beneficial microorganisms through organic formulations in the root zone and nitrogen content available in inorganic fertilizers may be responsible for the increase in vegetative growth and other parameters (Verma et al 2021, Wani et al 2021). Moreover, might be due to the continuous supply of nutrients and also that organic manures have the capability to restore the fertility of the soil. When organic manures are applied to the soil in addition to inorganic fertilizers, complex nitrogenous compounds slowly degrade and provide a constant source of nitrogen throughout the cropping period. Though organic manures release nutrients very slowly, their residual effect remains available in the soil for a longer time, resulting in improved soil structure, fertility, porosity and physiological activity inside the plants. Moreover, beneficial microorganisms influence cell division and cell elongation, which increase vegetative growth and eventually increase plant height, plant spread and number of leaves per plant. The results of the present investigation are in close conformity to the study done by Ennab *et al* (2016), where application of 50% of NPK + 55 kg of FYM + bio-fertilizers increased the growth parameters of Eureka lemon trees. The variation in concentration might have happened because of variations in different fruit crops, as Eureka lemon required more nutrients compared to papaya.

Days taken to initiation of flowering and from flowering to fruit maturation: During the investigation, the use of Integrated Nutrient Management had a significant impact on the flowering parameters of papaya. The minimum days (135.83) taken for first flowering and for fruit maturation (184.50) were obtained with the combined application of organic manures, inorganic and bio-fertilizers (Table 2). Among all the treatments, the application of 50% NPK + 20 kg FYM + 100 g *Azospirillum* + 100 g PSB was better in terms of enhancement of flowering parameters as compared to control. The earliness in flowering might be due to the higher net assimilation rate on account of better growth, leading to

Table 1. Effect of integrated nutrient management on growth parameters at 210 days after transplanting

Treatment	Plant height (cm)	Stem girth (cm)	Number of leaves per plant	Leaf length (cm)	Petiole length (cm)	Plant spread (N-S)	Plant spread (E-W)
T_0	138.67	20.58	24.00	50.42	56.17	132.50	125.17
T_1	144.17	22.50	26.33	53.33	59.83	143.50	136.17
T_2	156.17	26.67	28.67	58.83	65.33	153.83	146.17
T_3	150.75	27.33	27.50	58.42	63.93	151.67	144.17
T_4	179.17	30.83	32.50	66.83	73.83	172.00	180.83
T_5	141.17	25.58	24.17	58.17	64.58	147.50	144.83
T_6	152.50	26.67	26.00	57.25	63.33	149.50	150.50
T_7	158.67	26.67	26.00	56.67	63.17	148.83	151.50
T_8	170.83	29.17	29.50	64.08	70.75	167.00	172.83
T_9	143.75	24.08	25.67	55.17	61.42	139.67	135.83
T_{10}	149.33	25.17	28.83	58.92	65.00	154.50	154.50
T_{11}	148.83	25.42	26.50	57.42	63.42	148.67	147.50
T_{12}	161.92	27.83	29.17	60.42	66.83	160.83	166.50
Mean	153.53	26.04	27.29	58.15	64.43	151.54	150.50
CD (p=0.05)	5.52	1.86	2.58	3.33	3.23	5.62	6.21

T_0(100% RDF + Control), **T_1**(50% RDF + 20 kg FYM/plant), **T_2**(50% RDF + 20 kg FYM + 100g *Azospirillum*/plant), **T_3** (50% RDF + 20 kg FYM + 100 g PSB/plant), **T_4** (50% RDF + 20 kg FYM + 100 g *Azospirillum* + 100 g PSB/plant), **T_5** (50% RDF + 10 kg Vermicompost/plant), **T_6** (50% RDF + 10 kg Vermicompost + 100 g *Azospirillum*/plant), **T_7** (50% RDF + 10 kg Vermicompost + 100 g PSB/plant), **T_8** (50% RDF + 10 kg Vermicompost + 100 g *Azospirillum* + 100 g PSB/plant), **T_9** (50% RDF + 2 kg Neem cake/plant), **T_{10}** (50% RDF + 2 kg Neem cake + 100 g *Azospirillum*/plant), **T_{11}** (50% RDF + 2 kg Neem cake + 100 g PSB/plant), **T_{12}** (50% RDF + Neem cake + 100 g *Azospirillum* + 100 g PSB/plant)

Table 2. Effect of integrated nutrient management on flowering and yield parameters

Treatment	Initiation of flowering (days)	From flowering-maturity (days)	Number of fruits per plant	Fruit length (cm)	Fruit width (cm)	Fruit weight (kg)	Fruit yield (kg)
T_0	159.33	213.17	19.50	13.42	8.86	0.64	20.84
T_1	150.33	204.83	21.33	15.07	11.38	0.72	22.36
T_2	145.50	198.17	22.17	19.02	14.43	0.97	24.24
T_3	144.33	200.33	22.00	18.43	15.01	0.94	25.74
T_4	135.83	184.50	28.17	24.23	22.11	1.69	37.26
T_5	151.67	207.00	20.67	14.92	11.67	0.72	22.85
T_6	147.17	198.67	22.50	18.21	16.19	0.96	25.48
T_7	145.83	200.83	23.17	17.54	15.57	0.93	24.18
T_8	140.33	188.67	26.33	22.64	20.46	1.40	33.93
T_9	155.00	209.83	20.33	14.92	11.30	0.67	21.58
T_{10}	146.00	203.67	22.33	18.14	15.68	0.95	23.64
T_{11}	149.17	206.50	22.00	16.81	14.58	0.89	24.48
T_{12}	142.83	192.17	24.33	21.17	19.36	1.00	29.32
Mean	147.18	200.64	22.68	18.04	15.12	0.96	25.84
CD (p=0.05)	3.17	2.77	1.99	1.28	1.53	0.10	2.16

See Table 1 for details

the production of endogenous metabolites earlier than optimum level, enabling an early flower (Yadav et al 2011). Similarly, Khalid et al (2013) reported that in strawberry cv. Chandler the combination of Soil + Silt + Vermicompost 200g/kg was beneficial in inducing earliness in flowering. However, in contrast to the present results, Kanwar et al (2020) observed that in papaya cv. Red Lady, the minimum days taken for first flowering was 70.41, which was very early compared to the results obtained under the treatment containing 75% RDF + 10 kg Vermicompost + 100g *Azotobacter* + 100g PSB/plant. As temperature fluctuations under Punjab conditions is very common, variation in flowering might be possible due to the variation in environmental conditions or because of the increased application of inorganic fertilizers and organic manures.

Number of fruits per plant, fruit length, fruit width, fruit weight and fruit yield: The use of integrated nutrient management had a significant impact on the number of fruits produced per plant, fruit length, fruit width, fruit weight and fruit yield (Table 2). Among all the treatments, the application of 50% NPK + 20 kg FYM + 100 g *Azospirillum* + 100 g PSB was better in increasing the number of fruits (28.17), fruit length (24.23 cm), fruit width (22.11 cm), fruit weight (1.69 kg) and fruit yield (37.26 kg/plant). Yield characteristics were enhanced when organic manures were used along with chemical fertilizers, as opposed to when these fertilizers were applied alone. Enhancement in the quality content of fruit might be associated with an increase in phenotypic characters like stem girth, plant height and leaf length also because of better consumption and utilization of major nutrients. This might also be possible due to a rise in the number of leaves, which ultimately enhanced photosynthetic activity, resulting in a greater buildup of carbohydrates and higher carbohydrate levels, which aided in the acceleration of growth and thus increased the yield. The result of the present investigation is in agreement with Singh (2009) on papaya cv. Ranchi dwarf, where the highest yield was achieved with the of 50% of NPK +*Azotobacter* + PSB + FYM was better.

Fruit firmness, total sugars, TSS, ascorbic acid and titrable acidity: Maximum fruit firmness (9.50kg/cm^2) and TSS (10.43%) were obtained with the application of 50% RDF + 20 kg FYM + 100 g *Azospiriilum* + 100 g PSB/plant (Table 3). The higher firmness may be due to the higher microbial population and better uptake of nutrients as organic manures make the nutrients mobile for the plants. Ravishankar and Karunakaran (2008) also observed that in papaya cv. Coorg Honey Dew, the application of 20 kg FYM increased the total soluble solids under organic farming, whereas Ghosh et al (2012) reported that the total soluble solids increased with the application of 20 kg FYM + 400:100:300g NPK in pomegranate.

The total sugars (10.90%), reducing sugars (9.54%), non-reducing sugars (1.26%) and ascorbic acid (44.58mg) were maximum with minimum acidity (0.01%) through the application of 50% RDF + 20 kg FYM + 100 g *Azospiriilum* + 100 g PSB/plant (Table 3). This might be due to the addition of organic manure supplements that are high in moisture content, nutrients and growth-promoting substances, which

Table 3. Effect of integrated nutrient management on quality parameters

Treatment	Fruit firmness (kg/cm^2)	Total soluble solids (^{0}Brix)	Titrable acidity (%)	Total sugars (%)	Reducing sugars (%)	Non-reducing sugars (%)	Ascorbic acid (mg/100g of pulp)
T_0	5.57	6.1	0.13	6.55	5.8	0.71	35.75
T_1	6.52	6.83	0.03	7.36	6.4	0.92	37.83
T_2	7.45	8.47	0.03	9.2	8.1	1.07	40.33
T_3	7.63	8.07	0.03	9.334	8.18	1.1	41.25
T_4	9.5	10.43	0.01	10.9	9.54	1.26	44.58
T_5	6.15	6.7	0.08	7.09	6.32	0.92	37.5
T_6	7.42	8.2	0.03	9.12	7.98	1.05	39.5
T_7	7.32	7.77	0.03	9.01	8.02	1.06	39.5
T_8	8.73	9.47	0.02	10.26	8.98	1.22	43.17
T_9	6.08	7.73	0.1	6.91	6.04	0.82	36.83
T_{10}	7.25	8.33	0.04	8.06	6.95	1.04	38.17
T_{11}	7.28	8.1	0.04	8.46	7.35	1.05	38.5
T_{12}	8.28	9.03	0.03	9.59	8.43	1.18	42.58
Mean	7.32	8.09	0.05	8.6	7.54	1.03	29.4
CD (p=0.05)	0.37	0.36	0.04	0.42	0.46	0.28	2.76

See Table 1 for details

may help to increase the hormonal and metabolic activity of the plants, allowing them to produce more photosynthates, which are then stored in the fruits as starch and carbohydrates form. Secondly, this could also be possible due to the insoluble carbohydrates, such as reserved starch and other insoluble carbohydrates in papaya fruits, being converted into soluble sugars, which results in an increase in total sugars and ascorbic acid in papaya fruits. Similar results were obtained by Shukla *et al* (2009) with the application of 50% NPK + FYM + bio-fertilizers in order to improve the fruit quality characteristics in guava.

CONCLUSION

Chemical fertilizers can be used in combination with economic and eco-friendly organic manures to achieve substantial productivity. The integrated nutrient management of papaya is one of the important factors in boosting the yield and improving the quality of fruits. In the present study, increase in crop performance among different treatments, the application of 50% NPK + 20 kg FYM + 100 g *Azospirillum* + 100 g PSB was better in terms of increasing growth, flowering, yield and fruit quality parameters in papaya. Therefore, it is recommended to supply 50% of the nutrients through inorganic fertilizers and the remaining 50% from organic sources.

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Trait Association Analysis for Yield and Attributing Traits in Sesame (*Sesamum indicum* L.)

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Abstract: Correlations and path coefficient were studied in 60 genotypes of sesame (*Sesamum indicum* L.) in Randomized Complete Block Design (RCBD) in *Kharif* season, 2017 at Chaudhary Charan Singh Haryana Agricultural University, Hisar to evaluate the association of seed yield and yield attributing traits and to determine the direct and indirect effects of yield-related traits on seed yield.Seed yield per plant had significantly positive correlation with plant height, number of primary branches per plant, number of secondary branches per plant, number of capsules per plant, number of seeds per capsule, 1000 seeds weight capsule length while, with significantly negative correlation with days to 50% flowering and days to maturity.Path coefficient analysis revealed that number of capsules per plant had the highest direct and positive effect on seed yield per plant, thus indicates the selection based on these traits could be more effective to maximize the seed yield in sesame breeding programmes.

Keywords: Sesame, Correlation coefficient, Path coefficient, RCBD, Replication

Sesame (*Sesamum indicum* L.) is one of the most important oilseeds crops due to its high degree of stability to oxidation and rancidity (Yadava et al 2012) .The sesame seeds contains good quality proteins, carbohydrates, fibers, phenolic compounds and also possess considerable quantity of mineral nutrients, amino acids and different vitamins (Chang et al 2002, Ojiako et al 2010). The crop is highly drought tolerant and cultivated in tropical and subtropical regions of Asia, Africa and South America. However, it is well suited to tropical climates with moderate rainfall and humidity (Chakraborthy et al 2008). India ranks first in world with 1.95 Million ha area and 0.87 Million tonnes production. The average yield of sesame in India is 413 kg/ha, low as compared with other countries in the world is 535 kg / ha (Singh et al 2022). In Indian subcontinent the crop is mainly cultivated on discarded land with poor fertility. Genotypes grown in India produce inferior yield in terms of both quality and quantity due to lack of efficient crop improvement programmes (Tadele 2019). Yield improvement depends on the use of improved genotypes. In present scenario, more aggressive breeding efforts are required to harness the untapped potential of this crop for further yield enhancement (Furat and Uzun 2010). Seed yield is one of the most complex characters that results due to the actions and interactions of various attributes or traits which are highly correlated, thus these traits have potential to influence seed yield either directly or indirectly (Rauf et al 2004). Thus, by keeping the above facts in view present investigation was carried out to understand the genetic association between yield and its component traits. It helps to determine direct and indirect effects of different yield and its attributing traits on seed yield of sesame.

MATERIAL AND METHODS

Field experiment was conducted at, Chaudhary Charan Singh Haryana Agricultural University, Hisar, during the *kharif*) 2017, under normal conditions with proper irrigation. The experimental plant material consists of sixty genotypes of sesame (*Sesamum indicum* L.) from the Oilseeds Section, Department of Genetics & Plant Breeding, CCS Haryana Agricultural University, Hisar (Table 2). All the genotypes were evaluated in Randomized Complete Block Design (RBD) with three replications. Each genotype was sown in rows of 3-meter length with spacing of 30 x 15 cm (row-to-row and plant-to-plant). All the agronomic packages and practices such as seed treatment, fertilizer application, timely irrigation, weeding and pesticides application were followed to raise healthy crop.

Morphological data: The observations on morphological traits such as days to flowering (50%) and days to maturity were recorded on the plot basis whereas remaining characters were recorded from five randomly selected plants from each genotype in each replication. Mean values over replications were used for statistical data analysis.

Statistical analysis: Correlation coefficients analysis among all possible character combinations at phenotypic and genotypic level was done by using formulae developed by Johnson et al. (1955). Path coefficient analysis was worked out as per the formula suggested by Wright (1921) and further adopted by Dewey and Lu (1959). Genotypic correlation coefficients of eleven morphological characters with seed yield per plant were used to estimate the path coefficients for the direct and indirect effects of various independent characters on yield. Statistical analysis of Correlation and Path coefficient data between yield and its attributing traits was done by Statistical Package for Social Sciences (SPSS) Version 11.0.

RESULTS AND DISCUSSION

The seed yield in sesame as well as in most of the crops is a complex character, which results from the multiplicative interaction of several other characters that are termed as yield components. The genetic makeup of seed yield in sesame is based on the overall net effect produced by various yield components directly or indirectly by interacting with one another. Therefore, selection for seed yield *per se* alone would not matter much as such unless accompanied by the selection for various component characters responsible for conditioning it. Thus, identification of important component characters and information about their association with yield and also with each other is useful for developing efficient breeding strategies for developing high yielding varieties. The magnitudes of genotypic correlation coefficients were higher than corresponding phenotypic correlation coefficients indicating the presence of genetic association among various characters (Table 2).

Correlation analysis : Seed yield per plant had significant and positive correlation with plant height , number of primary branches per plant, number of secondary branches per plant, number of capsules per plant, number of seeds per capsule and 1000 seeds weight at both genotypic and phenotypic level. It shown significant and positive correlation with capsule length at genotypic level. Hence, such characters should be given priority during selection for increasing seed yield in sesame. It was found to have significant and negative correlation with days to 50% flowering and days to maturity at both genotypic and phenotypic level. These results implies that selection based onplant height, number of primary branches per plant, number of secondary branches per plant, number of capsules per plant, number of seeds per capsule, 1000 seeds weight and capsule length could be more efficient in contributing towards more seed yield of sesame because the selection practiced for improving such traits individually or simultaneously is likely to bring improvement in others due to correlated response. The characters contributes more or less towards seed yield because of seed yield is a complex character, which results from the interaction of several other characters that are termed as yield components as we discussed. Similar findings have also been reported earlier for one or more characters by earlier researchers (Shekhawat et al 2013, Hika et al 2014, Mahmoud et al 2015, Saxena and Bisen 2016).

Path coefficient analysis: The evaluation of path coefficient in which diagonal values are have direct effects and off-diagonal indirect effects of yield attributing character on seed yield. The highest positive direct effect and significant association with seed yield per plant was exhibited by number of capsules per plant followed by days to 50%

Table 1. Sixty genotypes of sesame (*Sesamum indicum* L.) used in the present investigation

S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes
1	NIC-7834	16	SI-2139	31	PCU-39	46	RJS-44
2	NIC-7925	17	SI-1865-B	32	PCU-129	47	Julander
3	NIC-8025	18	SI-3281	33	PCU-136	48	MIS-8526
4	NIC-8165	19	SI-119-2-84	34	EC-3034190	49	TC-154
5	NIC-8254	20	IS-85	35	EC-31045	50	TC-173
6	NIC-8339	21	IS-113	36	EC-310427	51	TC-176
7	NIC-8394	22	IS-120-A	37	ES-71-A	52	TC-177-A
8	NIC-8414	23	IS-136	38	ES-120-1-84-A	53	TC-182
9	NIC-16104	24	IS-154	39	KMR-13	54	TC-190
10	NIC-16214	25	IS-207	40	KMR-60	55	TC-191
11	NIC-16347-1	26	IS-8480-A	41	SP-41	56	TC-206
12	NIC-17257	27	IC-1634-3	42	S-0253-A	57	TC-208
13	NIC-17311	28	IS-750-1-84	43	S-0268-C	58	TC-318
14	SI-44	29	IC-14160-1	44	SO-516-A	59	HT-1
15	SI-212	30	PCU-34	45	GSM-21	60	HT-2

Table 2. Phenotypic (above diagonal) and genotypic (below diagonal) correlation coefficients among morphological characters in sesame

Characters	DF	DM	PH	PBP	SBP	NCP	NSC	CL	CW	1000 SW	OC	SYP
DF		0.967**	-0.168*	0.133	-0.209**	-0.265**	-0.022	-0.006	0.023	-0.066	-0.056	-0.158*
DM	0.977**		-0.186*	0.105	-0.219**	-0.253**	-0.011	0.023	-0.019	-0.043	-0.088	-0.167*
PH	-0.299**	-0.312**		-0.097	0.011	0.128	0.242**	-0.047	-0.073	0.182*	0.032	0.219**
PBP	0.158*	0.127	-0.169*		0.347**	0.200**	0.014	0.041	0.118	0.087	0.140	0.210**
SBP	-0.221**	-0.230**	0.023	0.451**		0.379**	-0.074	0.100	0.034	0.161*	0.154*	0.273**
NCP	-0.286**	-0.273**	0.226**	0.250**	0.405**		0.129	0.120	-0.125	0.280**	0.074	0.819**
NSC	-0.013	-0.011	0.395**	0.013	-0.070	0.180*		0.215**	0.073	0.117	-0.102	0.446**
CL	-0.035	-0.003	0.103	0.030	0.154*	0.197**	0.300**		-0.104	0.219**	-0.139	0.144
CW	0.006	-0.040	-0.253**	0.235**	0.049	-0.170*	0.138	-0.282**		-0.087	0.148*	-0.029
1000 SW	-0.091	-0.055	0.354**	0.183*	0.185*	0.428**	0.196**	0.421**	-0.179*		0.054	0.452**
OC	-0.124	-0.150*	0.191*	0.210**	0.205**	0.058	-0.132	-0.231**	0.296**	0.077		0.062
SYP	-0.178*	-0.187*	0.381**	0.283**	0.298**	0.863**	0.512**	0.233**	-0.041	0.634**	0.122	

* Significant at 5 % ** Significant at 1 %
DF = Days to flowering (50%), DM = Days to maturity, PH= Plant height, PBP = Primary branches per plant, SBP = Secondary branches per plant, NCP = No. of capsules per plant, NSC = No. of seeds per capsule, CL = Capsule length, CW = Capsule width, 1000SW = 1000 Seeds weight, OC = Oil content, SYP = Seed yield per plant

Table 3. Path coefficient analysis for morphological characters in sesame

Traits	DF	DM	PH	PBP	SBP	NCP	NSC	CL	CW	1000 SW	OC	PC with SYP
DF	0.365	-0.313	-0.001	0.004	0.004	-0.195	-0.007	0.000	0.001	-0.015	0.000	-0.158*
DM	0.353	-0.324	-0.001	0.003	0.005	-0.186	-0.004	-0.001	-0.001	-0.010	-0.001	-0.167*
PH	-0.061	0.060	0.006	-0.003	0.000	0.094	0.081	0.002	-0.003	0.042	0.000	0.219**
PBP	0.048	-0.034	-0.001	0.028	-0.007	0.148	0.005	-0.002	0.004	0.020	0.001	0.210**
SBP	-0.076	0.071	0.000	0.010	-0.021	0.280	-0.025	-0.005	0.001	0.037	0.001	0.273**
NCP	-0.097	0.082	0.001	0.006	-0.008	0.737	0.043	-0.006	-0.004	0.064	0.001	0.819**
NSC	-0.008	0.003	0.001	0.000	0.002	0.095	0.334	-0.011	0.003	0.027	-0.001	0.446**
CL	-0.002	-0.007	0.000	0.001	-0.002	0.088	0.072	-0.050	-0.004	0.050	-0.001	0.144
CW	0.008	0.006	0.000	0.003	-0.001	-0.092	0.024	0.005	0.035	-0.020	0.001	-0.029
1000 SW	-0.024	0.014	0.001	0.002	-0.003	0.207	0.039	-0.011	-0.003	0.229	0.000	0.452**
OC	-0.020	0.028	0.000	0.004	-0.003	0.054	-0.034	0.007	0.005	0.012	0.008	0.062

See Table 2 for details

flowering, number of seeds per capsule and 1000-seed weight, capsule width, primary branches per plant (Table 3). The oil content and plant height also exhibited positive direct effect on the grain yield but association was low. However, days to maturity, capsule length and number of secondary branches per plant showed negative direct effect on seed yield per plant. These results are in accordance with the earlier finding (Gangadhara et al 2012, Shekhawat et al 2013, Meena et al 2016, Saxena and Bisen 2016).

CONCLUSION

Seed yield per plant showed a significant and positive correlation with plant height, number of primary branches per plant, number of secondary branches per plant, number of capsules per plant, number of seeds per capsule, 1000 seeds weight at both genotypic and phenotypic level. Genotypic correlation coefficients were higher in magnitude than phenotypic correlation coefficients for all the traits under study. This indicates the presence of inherent association among various characters. Path coefficient analysis for different characters revealed that number of capsules per plant, days to 50% flowering and number of seeds per capsules had the highest direct and positive effect on seed yield per plant and inferred that there is true relationship between respective traits and grain yield. Consequently, these traits should be considered as important selection criteria in sesame breeding programmes for getting higher seed yield.

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Yield Maximization of Rice Varieties under Varying Fertility Levels in Northern Himalayas

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Abstract: A field experiment was conducted during *kharif* 2018 at CSKHPKV, Rice and Wheat Research Centre (RWRC), Malan with the objective of fine-tuning fertility levels for maximizing yield of rice varieties in Northern Himalayas. The experiment consisted of 5 main-plot treatments comprising different fertility levels {50% recommended dose of fertilizer (RDF), 100% RDF (90:40:40), 150% RDF, 50% RDF + Azolla and 100% RDF + Azolla} and 4 varieties as sub-plot treatments {Vivekdhan 65, HPR 2143, HPR 2720 (red rice) and AZ 6508 (hybrid)}. The soil of the experimental site was silty clay loam in texture, acidic in reaction, medium in available nitrogen, phosphorus and potassium. Azolla was applied @ 20-25 g m^{-2} at 10 DAT, which was allowed to multiply *in-situ* for about 2-3 weeks and subsequently trampled twice. The increase in fertility level and Azolla application increased the growth, yield attributes and yield of rice. Application of Azolla increased the rice productivity significantly both at 50 and 100% RDF by 399 and 481 kg ha^{-1}, respectively. Application of 100% RDF + Azolla recorded productivity (5319 kg ha^{-1}) and profitability (INR 79,490 ha^{-1}) which was at par with productivity and profitability achieved with 150% RDF (5192 kg ha^{-1}, INR 75,738 ha^{-1}). Similarly, 50% RDF + Azolla recorded productivity and profitability which was at par with 100% RDF thus revealing a saving of 50% RDF with the use of Azolla. Hybrid 'AZ 6508' recorded maximum grain yield of 6924 kg ha^{-1} followed by Vivekdhan 65 (4822 kg ha^{-1}), red rice 'HPR 2720' (3924 kg ha^{-1}) and HPR 2143 (3740 kg ha^{-1}). The maximum net return (INR 93,924 ha^{-1}) and benefit cost (BC) ratio (2.13) were recorded by the hybrid AZ 6508. Though the productivity of Vivekdhan 65 was more than red rice 'HPR 2720', but due to higher price of red rice it fetched more net return (INR 76,248 ha^{-1}) and B:C ratio (1.94). Interaction effect revealed that the application of 100% RDF + Azolla to the hybrid AZ 6508 produced grain yield of 7430 kg ha^{-1} with net return of INR 101,630 ha^{-1} and BC ratio of 2.23. Thus, farmers of mid-hills of Himachal Pradesh can maximize the rice productivity by growing hybrid AZ 6508 and applying 100% RDF + Azolla @ 20-25 g m^{-2} 10 DAT. Next to hybrid, red rice HPR 2720 is the most profitable choice.

Keywords: Azolla, Rice, Varieties, Fertility levels, Red rice

Rice is one of the most important cereal crop that hold the key towards food security of the world. Rice plays a vital role in ensuring nation's food security and is a means of livelihood for millions of rural households. The population in India is increasing at a rate of 2.2 per cent per annum and is expected to stabilize only around 2050. Vast majority of the farmers in the country derive their livelihood from rice cultivation. Total food grain demand of the country will increase to about 291 million tonnes by 2025 (Kumar and Shivay 2010) and country has to produce about 130 million tonnes of rice by 2025 to feed the growing population (Anonymous 2012). Meeting the targeted demand of food grains is a challenging task for the policy makers, researchers and all other stakeholders. The problem is still confounded as the targeted increase has to be met in the background of declining resource base (land, water, soil productivity, labour, etc.) and increasing environmental concerns. To safeguard and sustain the food security in India, it is therefore, imperative to explore and evaluate such technologies which may increase the productivity of rice under situations of dwindling resource base particularly when there is little scope of horizontal or lateral expansion. Thus, the increase in production has to be vertical and should come from the same cultivated land by way of increased crop productivity. Low yield of rice at farmers' field can be ascribed mainly to the non-availability of thermo-insensitive, early maturing, high yielding varieties, non adoption of new agro technologies. Substantially high rainfall, most of which is concentrated within four monsoon months, results in the loss of the applied nutrients and reduction in the night temperatures also contributes to the low yield of crop.

Therefore, need for approaches for vertical growth in agricultural productivity on sustainable basis, so as to feed this ever increasing population. This would require better planning and resource management besides intensification of cropping. Fertilizers have played a predominant role in increasing the productivity of crops and would continue to do so, provided these are used judiciously as per crop requirement and soil status. However, imbalanced fertilizer use not only results in lower yields but also deteriorates the soil health (This imbalanced nutrient use has resulted in wide gap between crop removal and fertilizer application. Thus, balanced NPK fertilization has received considerable attention in India (Ghosh et al 2004). It is therefore, necessary to apply fertilizer elements, particularly N, P and K through inorganic sources in optimal quantity to improve and sustain the productivity. In nitrogen, inputs in agricultural systems may be in the form of nitrogen fertilizers, or be derived from atmospheric nitrogen through biological nitrogen fixation (BNF). Therefore, Azolla is a free floating water fern which fixes atmospheric nitrogen in association with the cyanobiont *Anabaena azollae*. The heterocyst of symbiont *Anabaena* is site of nitrogen fixation. Nitrogen fixation associated with high growth rate can enable Azolla to accumulate more than 10 kg N $ha^{-1}day^{-1}$ under optimal growth conditions. In general, a single crop of Azolla is known to provide 20-40 kg ha^{-1} nitrogen and also increases the availability of both macro and micro nutrient but this is insufficient to meet the total nitrogen requirement of the target crop. Therefore, the use of Azolla in combination with chemical nitrogen fertilizers affords a feasible alternative practice (Rao 1999).

In addition to the use of Azolla as a nitrogen source for rice crop, it can be used for reclaiming saline soils, reducing evapotranspiration and to control weed infestations in rice crop and can also be used to purify waste water as it can accumulate P and some heavy metals from water). Azolla is capable of tolerating a wide range of temperature from 5-45°C, so can be used from temperate to tropical environment. Sufficient work has been done on exploitation of Azolla as a bio-fertilizer in rice in other states of country, however limited systematic studies has so far been made in Himachal Pradesh about the usefulness of Azolla in rice production (Thakur 2013).

Besides the inadequate and imbalanced use of fertilizer nutrients as well as non adoption of the recently released rice varieties, is also one of the reasons for low productivity of rice crop. In Himachal Pradesh, many new rice varieties have been released with more productivity, but the farmers are not aware of them. Among them, red rice is acquiring a place in market, because of its high nutritive and medicinal properties. Hybrid rice has become a potential technology for meeting increased global demand of rice as it can outyield all other rice varieties. The hybrid rice is bridging yield gaps in many areas. Doubling the farmer's income by 2022 has been kept as a national goal. To achieve the same, many states in India have documented/implemented multi-pronged strategies; one of them is to increase the crop productivity, as the horizontal expansion is not possible. Rice soils do respond to the organic matter application but on account of the increased demand of FYM for vegetables its availability for rice crop is reducing. Azolla has been found to be promising bio-fertilizer as well as green manure for rice crop.

MATERIAL AND METHODS

The research was carried out during *kharif* 2018 at CSK Himachal Pradesh Krishi Vishvavidyalaya, Rice and Wheat Research Centre, Malan. The experimental field was silty clay loam in texture and acidic in reaction. The soil was rated as high in organic carbon and medium in available nitrogen, phosphorus and potassium. The trial consisted of twenty treatments (5 fertility levels and 4 varieties) which were tested in split plot design with three replications. The experiment consisted of 5 main-plot treatments comprising varying fertility levels {50% recommended dose of fertilizer (RDF), 100% RDF (90:40:40 kg N-P-K ha^{-1}), 150% RDF, 50% RDF + Azolla and 100% RDF + Azolla} and 4 cultivars as sub-plot treatments {Vivekdhan 65, HPR 2143, HPR 2720 (red rice) and AZ 6508 (hybrid)}. Nutrients *viz.,* N, P, and K were applied through urea, di-ammonium phosphate (DAP), and muriate of potash (MOP) in all the plots as per the treatments. Half dose of N and full dose phosphorus and potassium were applied as basal application just before transplanting. The remaining half dose of nitrogen was applied in two split doses at tillering and panicle initiation stages. Plant observations such as plant height, number of tillers, dry matter accumulation, effective panicles, number of grains $panicle^{-1}$, panicle length & weight and 1000-grain weight were recorded at harvest. The crop from each net plot was harvested with the help of sickle after removing the border and sample plant rows. The net plot produce was dried thoroughly for 5 days. When most of the straw in a handful bundle broke up on folding, then total produce was weighed and recorded as biological yield (grain + straw). After the threshed grains were cleaned, the weight of the grains was recorded on electronic balance and converted to kg ha^{-1}. Moisture content in the grains was recorded with moisture meter and the grain yield was adjusted at 14 per cent moisture content. The following formula was used to adjust yield at 14 per cent moisture content

$$\text{Grain yield at 14 per cent moisture} = \frac{100 - \text{moisture (\%) in grain}}{100 - 14} \times \text{Grain yield}$$

The grain yield so recorded from each net plot was subtracted from the biological yield of each net plot to get the straw yield. This straw yield per net plot was then converted to kg ha^{-1}. Treatment-wise cost of cultivation was worked out. The yield of the crop obtained from each plot in the experiment was converted into gross returns in rupees based on prevailing market price of grains and straw. The treatment-wise net returns were obtained by subtracting the cost of cultivation from gross returns. Benefit: cost ratio was calculated by dividing net returns with cost of cultivation as follow:

$$\text{B:C ratio} = \frac{\text{Net returns from treatment (INR } ha^{-1})}{\text{Cost of cultivation of the treatment (INR } ha^{-1})}$$

The statistical analysis of data was done by using OPSTAT online statistical analysis software (Sheoran 2010).

RESULTS AND DISCUSSION

The shoot elongation continued with the advancement in crop age and reached to its maximum at harvest stage (Table 1). Significantly taller plants were recorded with the application of 150% RDF though it was at par with 100% RDF. Application of 50% RDF resulted in significantly shortest plants. Similar results were obtained by Verma et al (2017) where application of 150% RDF recorded significantly tallest plants. Azolla being biological nitrogen fixer, helped in the crop growth. Application of Azolla increased the plant height of rice at both the fertilizer levels of 50 and 100% though the increase was significant only at 50% RDF. Thus, the contributing effect of Azolla was equivalent to 50% RDF. Baktash et al (2021) obtained similar results where application of 50 kg N ha^{-1} + 8 t Azolla recorded highest plant height. Averaged over the fertility levels, main effects of varieties revealed significant variation in plant height at all the dates of observation. Vivekdhan 65 was observed to be significantly taller though it was at par with HPR 2720 at harvest whereas hybrid 'AZ 6508' produced significantly dwarf plants. This was due to genetic makeup of genotypes. Significantly more number of tillers were recorded with the application of 100% RDF + Azolla at all the stages of observation though it was at par with the application of 150% RDF. Application of 50% RDF resulted in significantly least tillers. Application of Azolla benefitted the crop and number of tillers per unit area increased significantly at both 50 & 100% RDF. The improvement in the formation of tillers with increase in fertility level and Azolla application might be due to increase in nutrient availability which enhanced tillering. Razavipour et al (2018) have also reported similar findings. The number of tillers per square meter were significantly more in Vivekdhan 65 being at par with the hybrid AZ 6508, revealing that tiller mortality was less in hybrid. The other treatments with respect to tiller count were in order of HPR 2143>HPR 2720. Application of 100% RDF + Azolla recorded significantly higher dry matter accumulation compared to 150% RDF. This may be attributed to the fact that increase in fertility level and Azolla application might have continuously supplied the nitrogen and helped in increasing the dry matter accumulation because nitrogen is directly involved in vegetative growth. Application of Azolla saved the 50% RDF. These findings are in agreement with Razavipour et al (2018). Taller variety Vivekdhan 65 recorded significantly highest dry matter. The other varieties with respect to dry matter were in order of AZ 6508>HPR 2143>HPR 2720. The increase in fertility level increased the number of effective panicles, grains $panicle^{-1}$, panicle weight and 1000-grain weight consistently and significantly. Significant increase in number of effective panicles, grains $panicle^{-1}$, panicle weight and 1000-grain weight were observed with Azolla application at both 50 and 100% RDF. Significantly highest number of effective panicles were recorded with the application of 100% RDF + Azolla (F_5) which was statistically same as with 150% RDF (F_3). Baktash et al (2021) also observed that application

Table 1. Effect of varying fertility level and varieties on growth, yield attributes and of rice at harvest

Treatment	Plant height (cm)	Dry matter accumulation (kg m^{-2})	Number of tillers m^{-2}	Number of effective panicles m^{-2}	Number of grains $panicle^{-1}$	Panicle length (cm)	Panicle weight (g)	1000 grain weight (g)	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})
Fertilizer level										
F_1: 50% RDF	106.7	1016.2	9764	163.5	129	24.02	2.82	22.5	4257	5508
F_2: 100% RDF	112.0	1123.7	10899	185.4	146	25.25	3.06	22.9	4838	6061
F_3: 150% RDF	115.1	1261.3	11624	198.2	156	25.05	3.20	23.6	5192	6432
F_4: 50% RDF + Azolla	111.9	1103.6	10542	174.4	139	24.56	2.96	22.9	4656	5886
F_5: 100% RDF + Azolla	115.1	1318.9	11798	201.2	165	25.33	3.33	23.9	5319	6479
CD (P=0.05)	3.7	54.8	769	6.2	11.7	NS	0.32	0.4	350	423
Varieties										
V_1: Vivekdhan 65	124.7	1340.2	12033	196.9	123	24.05	2.72	24.9	4822	7211
V_2: HPR 2143	105.6	1123.5	9462	179.0	131	25.17	2.61	24.5	3740	5723
V_3: HPR 2720	122.4	1050.0	9709	169.3	150	25.54	2.69	16.5	3924	5785
V_4: AZ 6508	95.9	1145.4	12498	192.8	184	24.59	4.29	26.8	6924	5574
CD (P=0.05)	2.9	49.2	278	4.4	6.1	0.67	0.23	0.2	126	167

of 100 kg N ha^{-1} + 4 t Azolla increased the effective panicle m^{-2} of rice. Srivastava et al (2014) and Gohain (2014) also found similar trend. Vivekdhan 65 produced significantly higher number of effective panicles m^{-2} being statistically at par with AZ 6508 while HPR 2720 produced significantly lowest panicles. Number of grains $panicle^{-1}$ were significantly highest in AZ 6508 followed by HPR 2720 and HPR 2143 whereas Vivekdhan 65 recorded significantly lowest grains $panicle^{-1}$. HPR 2720 recorded significantly higher length of panicle than Vivekdhan 65 and AZ 6508 but was at par with the variety HPR 2143. Significantly highest panicle weight was recorded by hybrid AZ 6508. Significantly lower panicle weight was obtained in the variety HPR 2143 being at par with HPR 2720 and Vivekdhan 65. The hybrid AZ 6508 recorded significantly more 1000-grain weight (26.8 g) followed by Vivekdhan 65 (24.9 g), HPR 2143 (24.5 g) and HPR 2720 (16.5 g). These are the varietal characters which are varied from variety to variety. Similar results have also been reported by Razavipour et al (2018). The grain yield of rice increased with increasing fertility level with significantly higher yield recorded with the application of 150% RDF while significantly lower yield recorded with application of 50% RDF. The lowest yield obtained with the application of lowest dose was due to the inability of the soil to provide adequate quantity of nutrients to the rice crop while adequate supply of nutrients in treatments where higher dose of fertilizers were applied which led to higher yields. Similar results have been reported by Patel and Mishra (2014).

Application of Azolla also had a positive effect on grain yield of rice. The grain yield obtained with the application of 100% RDF was at par with the yield obtained with the application of 50% RDF along with Azolla inoculation. Similarly yield recorded with the application of 150% of RDF was at par with yield recorded with 100% RDF along with Azolla inoculation. Azolla application increased the grain yield over both the fertility levels of 50 and 100% RDF and the magnitude of increase was 8.57 (399 kg ha^{-1}) and 9.04 per cent (481 kg ha^{-1}), respectively. The results are indicative of the possibility of economizing 50% RDF with the use of Azolla in transplanting rice. The increase in yield as a result of Azolla use was due to the nitrogen fixed by the bio-fertilizer which was made available to rice crop during the late vegetative or reproductive phase. The organic matter added to the soil as a result of Azolla incorporation improved the physical condition of the soil which provided better environment to the crop for achieving higher yield. Yao et al (2017) substituted 25% nitrogen through Azolla bio-fertilizer. Baktash et al (2021) observed highest grain yield with the application of fertilizer with Azolla. The results are in conformity with earlier researchers (Kumar et al 2017, Razavipour et al 2018 , Grace et al 2018). Significant variation was also observed amongst varieties in term of grain yield with hybrid 'AZ 6508' producing significantly higher grain yield followed by Vivekdhan 65, HPR 2720 and HPR 2143, in that order, each variety differing significantly from one another. The highest yield was in AZ 6508 was due to significantly higher number of effective panicles per unit area, number of grains per panicle and 1000-grain weight recorded in this variety. Srivastava et al. (2014) reported similar results of hybrid rice. The lowest grain yield was in HPR 2143. Patel and Mishra (2014) also mentioned similar trend. Straw yield was significantly more with the application of 100% RDF + Azolla (6479 kg ha^{-1}) being at par with the treatment 150% RDF (6432 ha^{-1}) and 100% RDF (6061 kg ha^{-1}). This may be attributed to the fact that increase in fertility level and Azolla application might have continuously supplied the nitrogen and helped in boosting the vegetative growth. Srivastava et al (2014) observed significantly higher grain and straw yield with increase in fertility level. Baktash et al (2021) found that application of chemical fertilizers with Azolla gave significantly higher grain and straw yield. The taller plants and more tiller count resulted in significantly higher straw yield in Vivekdhan 65 (7211 kg ha^{-1}). The significantly lower straw yield was obtained from dwarf 'AZ 6508' (5574 kg ha^{-1}) being at par with HPR 2143 (5723 kg ha^{-1}).

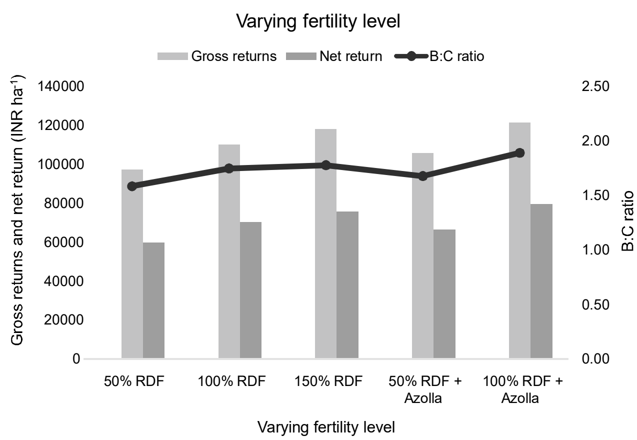
Interaction effects of varying fertility level and varieties on grain yield: At all the levels of fertility, hybrid 'AZ 6508' produced significantly highest grain yield followed by Vivekdhan 65 (Table 2). The yield from other two varieties HPR 2143 and HPR 2720 was at par at all the fertility levels

Table 2. Interaction effect of varying fertility level and varieties on grain yield of rice

Treatment	F_1: 50% RDF	F_2: 100% RDF	F_3: 150% RDF	F_4: 50% RDF + Azolla	F_5: 100% RDF + Azolla
Vivekdhan 65	4283	4815	5257	4610	5143
HPR 2143	3273	3680	4020	3693	4032
HPR 2720	3293	3873	4247	3537	4671
AZ 6508	6177	6983	7243	6785	7430
CD (P=0.05)					
For comparison of two varieties at same fertility level					281
For comparison of fertility levels at same or different varieties					464

except at treatment in which 100% RDF was applied along with Azolla. Varieties also behaved differentially in their response to added fertilizers with the yield increasing significantly with increasing fertilizer application from 50 to 100% RDF in all the varieties except HPR 2143 where the increase was not significant. Further increasing the fertilizer application from 100 to 150% RDF also increased the yield in all the varieties though the increase was not significant. Additional inoculation of the rice crop with Azolla also increased the yield at both the fertility levels of 50 and 100% RDF though the increase was significant only for AZ 6508 when the crop was fertilized with 50% RDF along with Azolla application. Azolla application saved the 50 per cent of fertilizer application. AZ 6508 recorded productivity level of 7430 kg ha^{-1} with 100% RDF + Azolla.

Economics of rice: The increase in fertilizer level from 50 to 100% RDF, significantly increased the gross returns and net return (Fig. 1). The gross returns and net return was significantly higher with the application of 100% RDF + Azolla being at par with 150% RDF. AZ 6508 recorded significantly higher gross returns and net return (INR 137,886 ha^{-1} & INR 93,924 ha^{-1}, respectively) followed by HPR 2720 (INR 115,460 ha^{-1} & INR 76,248 ha^{-1}). Significantly higher value of BC ratio was observed with the application of 100% RDF + Azolla being at par with the 150% RDF. Hybrid AZ 6508 (2.13) recorded significantly higher BC ratio followed by HPR 2720 (1.94). The interaction showed that significantly higher B:C ratio was recorded from AZ 6508 at all the fertility levels being at par with HPR 2720 when fertilized with 150% RDF except at 100% RDF + Azolla at which significantly higher BC ratio was recorded from variety HPR 2720 being at par AZ 6508.



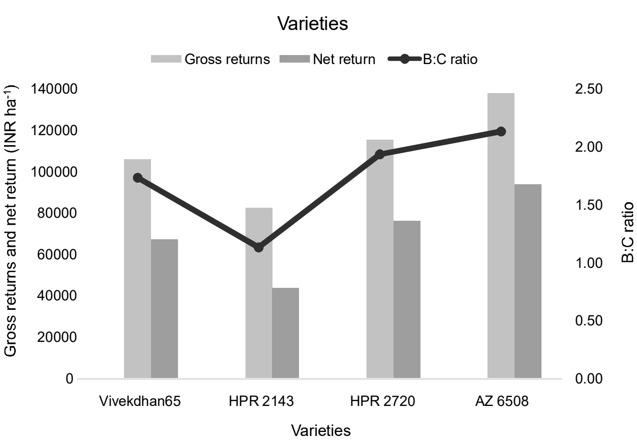


Fig. 1. Effect of varying fertility level and varieties on economics of rice

CONCLUSION

Hybrid 'AZ 6508' with the application of 100% RDF (90:40:40) + Azolla @ 20-25 g m^{-2} 10 DAT achieved maximum productivity and profitability of rice in North-Western Himalayas. The next best option is red rice variety 'HPR 2720' with the same fertility management. For resource poor farmers, Hybrid 'AZ 6508' can also be a good option even at lower fertility level (50% RDF).

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Performance of Rice Establishment Methods and Nutrient Management Practices on Growth, Yield and Nutrient uptake of Rice (*Oryza sativa* L.)

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***Abstract*:** A field experiment was conducted during *Kharif* seasons of 2018 and 2019 at Rice Research Farm of Birsa Agricultural University, Ranchi, Jharkhand to evaluate the performance of rice establishment methods and nutrient management practices on growth, yield attributes, yield and total nutrient uptake of rice. The wet direct seeding of rice using drum seeder manifested higher number of total tillers/m^2(267), leaf area index (3.58), dry matter accumulation (1292.20 g/m^2), crop growth rate (8.53g/m^2/day), panicles/m^2 (251), panicle weight (3.38g), total grains/panicle (117), grain yield (42.19 q/ha), straw yield (65.22 q/ha) and nutrient uptake of nitrogen (96.13 kg/ha), phosphorus (16.47 kg/ha) and potassium (95.42 kg/ha) which were comparable with normal transplanting but establishes its distinct superiority over aerobic rice. Among the different nutrient management practices, RDF + 25% N through FYM produced maximum growth, yield attributes, yield and nutrient uptake which was comparable with LCC based N application. However, both of the nutrient management practices viz. RDF + 25% N through FYM and LCC based N application led to significantly higher total tillers^2 leaf area index, dry matter accumulation, crop growth rate, panicle/m^2, panicle weight, total grains/panicle, grain yield, straw yield, nitrogen, phosphorus, and potassium uptake than other nutrient management practices viz RDF and 75% RDF + 25% N through FYM and control.

***Keywords*:** Aerobic rice, Crop growth rate, Leaf Colour Chart, Rice establishment method

Rice (*Oryza sativa* L.), a high calorie *kharif* cereal is an essential diet constituent for more than half of the world's production and a basic food crop in the world which directly supply more than 50% of the calories consumed by the entire human population. Human consumption accounts for 85% of total production of rice which provides 21% of global human per capita energy and 15% of per capita protein. Rice also provides minerals, vitamins and fibre, although all constituents except carbohydrates are reduced by milling. Rice production in India is an important part of the national economy. The world's total rice area is 167 million ha and production are about 782 million tons with productivity of 4.67 Mt/ha. The productivity of rice in Southeast Asia is 4.41 tons/ha and China is the largest producer (FAOSTAT 2018). India ranks first in acreage (43.1 m ha) and second in production (96.43 mt) only after China (Directorate of Economics Statistics 2017).Rice is the most important crop to millions of small farmers grown in millions of hectares throughout the region, and to the many landless workers who derive income from working on these farms. Since, the rice production area either remain steady or declining, reflecting a huge gap between the projected demand and present-day production level. The only way to reduce this gap is through enhancing vertical production level by applying scientific approach to farming. The rice establishment methods and appropriate nutrient management practices is one of the practically feasible technologies to enhance rice production. In Jharkhand, more than 80 percent of agricultural area is under rainfed and the late arrival of monsoon usually delays the seedling graising and transplanting operation resulting in lower yield. Under these circumstances, wet direct seeding of rice through drum seeder may be an alternative to transplanting in boosting the rice production. Wet direct seeding of rice through drum seeder is a successful cultivation method which not only boosts the productivity but also conserves water along with reducing the labour requirement thereby proved more economical as compared to normal transplanting. Nutrient management is a major component of soil and crop management system in rice**.** Excessive use of inorganic fertilizers has apparently depleted the fertility status of the soil so appropriate nutrient management practices in rice establishment methods are of utmost importance. Nitrogen is one of the most volatile nutrients presents in the soil subjected to different type of losses. For need based nitrogen management, plant-based tools such as leaf colour chart (LCC) is gaining popularity for nitrogen management in rice which suggests the dose and time of nitrogen application as per the need of the crop. The intensity of leaf colour is directly proportional to chlorophyll content of leaf which indirectly related to status of leaf nitrogen (Alam et al 2005). However, among the different tools available for measurement of leaf greenness, the non-destructive measurement of green colour intensity of leaf using leaf colour charts (LCC) is most suitable. Thus, adoption of suitable establishment methods and appropriate nutrient management practices can bring tremendous change in agricultural scenario through enhancing the rice productivity and improvement of rural economy.

MATERIAL AND METHODS

A field experiment was undertaken at Rice Research Farm of Birsa Agricultural University, Kanke, Ranchi for two consecutive years during *kharif* season of 2018 and 2019. The soil was clay loam in texture with 37.1, 29.7 and 33.2% sand, silt and clay composition and slightly acidic (6.2) in reaction , medium in organic carbon (4.3 g/kg), available nitrogen (230.00 kg/ha), available phosphorous (36.80 kg/ha), available potassium (161.20 kg/ha) of content. The experiment was laid out in split plot design replicated thrice with rice variety Naveen as test crop. The treatments combination consists of three different rice establishment methods *viz.,* normal transplanting, wet direct seeding of sprouted seeds in puddle field using drum seeder and aerobic rice assigned to main plots and five nutrient management practices *viz.,* Recommended dose of fertilizer (RDF) (80:40:20 Kg NPK/ha), 75% RDF + 25% N through Farm yard manure (FYM), RDF + 25% N through FYM, LCC based nitrogen application and control (no fertilizer) assigned to sub plots. Good quality seeds of cultivar Naveen 50 kg/ha were sown on well-prepared nursery bed for transplanting method of establishment. Twenty-one days old 2-3 seedlings/hill were transplanted manually in the puddled field at a depth of 2-3 cm with a spacing of (20 cm× 15 cm). There were 20 rows in each transplanted plot. Direct sowing of the sprouted seeds through drum seeders under puddled condition is known as drum seeding**.** In the present investigation, the field preparation and puddling matched that of transplanted rice. On pulling the seeder, seeds were placed on the soil surface at a distance of 20 cm between rows. The IRRI 8 row plastic drum seeder was used in the experiment which consisted of four rotating drums with circular holes at the two edges. In aerobic rice treatment, seeds were soaked in water for 10 hours followed by incubation for another 12 hours. After those seeds were treated with carbendazim @ 2 g/kg seed before sowing. Then, line sowing of rice was done with 20 cm row spacing. Irrigate the field immediately after sowing. The field was maintained near saturation without stagnation of water.

Well rotten farm yard manure was applied prior to sowing or transplanting of rice crop, as per the treatment and thoroughly mixed in soil. Full dose (80:40:20 Kg NPK/ha) of phosphorus and potassium and 25% of nitrogen was applied as basal in wet-direct seeded and aerobic rice while 50% of nitrogen with full dose of phosphorus and potassium was applied as basal in transplanted rice plots through urea, diammonium phosphate and murate of potash as per treatment dose. The remaining quantity of nitrogen was top dressed in two equal splits at tillering and at panicle initiation stage in all the establishment methods. In LCC based nitrogen application, full recommended dose of phosphorus, potassium and half of the recommended dose of nitrogen (40 kg/ha) was applied as basal and remaining nitrogen was top dressed at the rate of 13.33 kg N/ha applied thrice on 23rd, 45th and 61st days after sowing or transplanting when color of 6 out of 10 leaves fall below a threshold level of shade 4 on the leaf colour chart. Nominee Gold (bispyribac sodium) was applied @ of 25 g ai/ha (10% SC) at 20 days after sowing/transplanting in the experimental plots as post emergence herbicide to control the weed population. Need based plant protection measure were used for crop protection from insect and pests.

Plant sampling was done at 30, 60, 90 days after sowing or transplanting and at maturity to record the growth parameters such as total tillers/m^2, leaf area index and dry matter accumulation /m^2 while, the yield attributes viz. panicles/m^2, panicle weight, grains/panicle and 1000 grain weight and yield parameters viz. grain and straw yield were recorded at harvest. The crop growth rate was calculated.

$$Crop\ growth\ rate\ (CGR) = \frac{W2 - W1}{t2 - t1} \times \frac{1}{G}$$

Where W_1 and W_2 represent the dry weight of the plant at the beginning and end of the time interval $t_1$andt$_2$respectively. The G represent the ground area.

The plant samples collected at harvest were dried at 70^{0}C, powdered in willey mill and digested to analyze the nutrient compositions. The nitrogen, phosphorus and potassium content in plants were estimated by Kessler's reagent method, HNO_3:$HClO_4$ (9:4) digestion, colour development by Vandomolybdate solution followed by spectrophotometer determination and flame photometric determination after digestion in HNO_3:$HClO_4$(9:4) solution respectively. The nutrient uptake was estimated by multiplying the nutrient concentration with grain and straw yield. Addition of grain and straw nutrient uptake gave the total nutrient uptake by the plants.

RESULTS AND DISCUSSION

Total tillers/m^2: Total tillers per meter square increased with the crop age and reached peak at 60 Days after sowing or transplanting after which declined till maturity (Table 1). The reduction in tillers after 60 DAS/DAT was due to intra species competition for higher space and nutrients which are responsible for degeneration of late formed tillers (Chatterjee 2016). The establishment methods failed to cause significant difference in total tillers/m^2 at the early growth stages i.e., 30 DAS/T of rice. At later growth stages *i.e.* 60and 90 DAS/T and at maturity, wet direct seeding using drum seeder recorded maximum total tillers per meter square (276, 270 and 267) which was statistically at par with normal transplanting (267, 262 and 257). However, both of establishment methods viz. wet direct seeding through drum seeder and normal transplanting produced significantly higher tillers than aerobic rice (237, 231 and 227) at respective stages. Among the nutrient management practices, the maximum tillers per meter square was with RDF + 25% N through FYM (294, 291 and 284) followed by LCC based N application (291, 287 and 283) which was significantly higher than rest of the treatments. However, at 30 DAS/DAT, the application of nutrient under different nutrient management practices establishes significantly higher tillers number than control but, failed to cause significant variation in total tillers/m^2 among themselves.

Dry matter accumulation: Dry-matter accumulation of rice plant increased with the crop age and the maximum value was observed at harvest (Table 2). The rice establishment methods were unable to cause significant differences in dry matter accumulation at the initial period of growth i.e., 30 DAS/T. At subsequent growth stages i.e.60 and 90 DAS/DAT and at maturity, wet direct seeding using drum seeder recorded maximum dry- matter accumulation (512, 1022 and 1292 g/m^2) in plant but, remained at par with normal transplanting method. However, both of establishment methods viz. wet direct seeding using drum seeder normal transplanting method led to significantly higher dry matter accumulation than aerobic rice. The maximum dry matter accumulation with wet direct seeding using drum seeder might be owing to the fact that rice established through drum seeder leads to vigorous initial growth, optimum plant population and better weed control leads to availability of more resources to plants (Gangwar et al 2009).Among nutrient management practices, RDF + 25% N through FYM and LCC based N application due to higher tiller number and leaf area index led to higher dry matter accumulation than other nutrient management practices and control at 60 and 90 DAS/T and maturity. However, the nutrient management practices failed to cause significant variation among themselves except control at 30 DAS/DAT.

Leaf area index: The leaf area index increased successively as the growth progressed up to 90 DAS/DAT (Table 1). Leaf are index was not significantly influenced by establishment methods at 30 DAS/DAT while, at other stages viz. 60 and 90 DAS/DAT, wet direct seeding using drum seeder (2.68 and 3.58) being at par with normal transplanting method. However, both of these methods were superior in leaf area index as compared to aerobic rice establishment method. The higher leaf area index was recorded in transplanted rice

Table 1. Total tillers of rice and leaf area index as influenced by establishment methods and nutrient management practices

Treatments	Tillers count / m^2				Leaf area index		
	30 DAS/T	60 DAS/T	90 DAS/T	Maturity stage	30 DAS/T	60 DAS/T	90 DAS/T
Establishment methods							
Normal transplanting	122	267	262	257	1.31	2.62	3.48
Wet direct seeding using drum seeder	142	276	270	267	1.45	2.68	3.58
Aerobic rice	134	237	231	227	1.38	2.30	3.29
CD (p=0.05)	NS	22.36	13.75	24.40	NS	0.30	0.18
Nutrient management practices							
RDF (80:40:20 kg NPK/ha)	143	271	259	259	1.37	2.48	3.51
75% RDF + 25% N through FYM	139	262	257	254	1.35	2.43	3.52
RDF + 25% N through FYM	153	294	291	284	1.48	2.78	3.77
LCC based Nitrogen application	150	291	287	283	1.46	2.76	3.75
Control (No fertilizer)	78	181	179	172	1.23	2.21	2.71
CD (p=0.05)	16.44	18.76	22.18	23.77	0.15	0.26	0.22
CV%	12.74	7.42	8.96	9.76	11.10	10.40	6.62

which was significantly higher over dry seeded rice at harvest stage (Rajvanshi et al 2021). Among different nutrient management practices, there was no significant difference observed in leaf area index between nutrient management practices at 30 DAS/DAT except control which is significantly lower than rest of the treatments. At 60 and 90 DAS/DAT, nutrient management through application of RDF + 25% N through FYM (2.78 and 3.77) and LCC based N application (2.76 and 3.75) were at par among themselves and recorded significantly more leaf area index than RDF and 75% RDF + 25% N through FYM and control. The higher leaf area index with RDF + 25% N through FYM and LCC based N application might be due to higher number of tillers putting forth more leaves resulting in higher leaf area index.

Crop growth rate ($g/m^2/day$): Crop growth rate (CGR) of rice increased with the crop age up to 60-90 DAS/DAT and thereafter declined (Table 2). The rice establishment methods through wet direct seeding using drum seeder recorded maximum crop growth rate at all the growth stages which was superior to aerobic rice at 30-60 DAS/DAT (10.65 $g/m^2/day$) and 90 DAS-at maturity (8.53 $g/m^2/day$) but, statistically similar to normal transplanting at the respective growth stages. However, at 60-90 DAS/DAT the different rice establishment methods were unable to cause significant statistical differences in crop growth rate and remained at par between themselves. Among nutrient management practices, RDF + 25% N through FYM (12.15 $g/m^2/day$) and LCC based N application (12.14$g/m^2/day$)were at par among themselves but, proved their significant superiority over other nutrient management practices and control at 30-60 DAS/DAT while, at 60-90 DAS/DAT all of the nutrient management practices were statistically similar in respect of crop growth rate but, recorded significantly higher crop growth rate than control. However, the significance of nutrient management practices and control were found to be non-significant at 90 DAS/DAT-at maturity. The higher crop growth rate with RDF + 25% N through FYM and LCC based N application might be due better vegetative growth at all the growth stages.

Yield attributes: Significant improvement in yield attributes (Table 3) i.e., number of panicles per meter square (251), panicle weight (3.38 g) and number of grains/panicle (117) was recorded under wet direct seeding using drum seeder as compared with aerobic rice. The wet direct seeding using drum seeder also registered significantly higher number of grains/panicle than normal transplanting (111). However, wet direct seeding using drum seeder and normal transplanting method being *at par* among themselves in respect of number of panicles and panicle weight. The 1000 grain weight of rice was found to be unaffected by different rice establishment method. Among the nutrient management practices, RDF+ 25% N through FYM (277, 3.60 g and 120)was significantly superior to RDF and 75%RDF+ 25% N through FYM and control in respect of number of panicles per meter square, panicle weight and number of grains/panicles but remained at par with LCC based N application. The higher yield attributing characters with application of RDF+ 25% N through FYM might be due to integration of farm yard manure with inorganic sources would have resulted in slow release of nutrient and increased availability which in turn might have

Table 2. Dry-matter accumulation in plant and crop growth rate as influenced by establishment methods and nutrient management practices

Treatments	Dry matter accumulation (g/m2)				Crop growth rate (g/m2 /day)		
	30 DAS/T	60 DAS/T	90 DAS/T	Maturity stage	30 DAS/T	60 DAS/T	90 DAS/T
Establishment methods							
Normal transplanting	184.42	501.80	1008.00	1249.40	10.58	17.34	7.58
Wet direct seeding using drum seeder	192.44	512.00	1022.20	1292.20	10.65	17.47	8.53
Aerobic rice	189.70	458.40	955.40	1149.80	8.96	17.03	6.01
CD (p=0.05)	NS	41.71	50.41	46.34	1.45	NS	2.01
Nutrient management practices							
RDF (80:40:20 kg NPK/ha)	190.90	517.33	1094.00	1338.33	10.88	20.39	6.98
75% RDF + 25% N through FYM	200.26	512.67	1075.67	1333.33	10.41	19.93	7.42
RDF + 25% N through FYM	203.20	567.67	1158.67	1406.67	12.15	19.70	8.27
LCC based Nitrogen application	200.61	564.67	1153.33	1403.67	12.14	19.62	8.34
Control (No fertilizer)	149.30	291.33	494.33	670.33	4.73	6.77	5.87
CD (p=0.05)	25.90	40.64	55.37	64.29	1.17	2.43	NS
CV%	14.10	8.51	5.72	5.37	11.90	14.44	44.73

Table 3. Yield attributing characters, yield and total N, P and K uptake of rice as influenced by establishment methods and nutrient management practices

Treatments	Panicles /m^2	Panicle wt. (g)	Total grains/ panicle	1000 grain wt.(g)	Yield (q/ha)		Total nutrient uptake (kg/ha)		
					Grain	Straw	Nitrogen	Phosphorou	Potassiun
Establishment methods									
Normal transplanting	248	3.29	111	24.22	39.49	61.89	88.52	15.23	88.84
Wet direct seeding using drum seeder	251	3.38	117	24.26	42.19	65.22	96.13	16.47	95.42
Aerobic rice	219	3.24	102	24.05	32.65	52.40	72.04	12.57	73.82
CD (p=0.05)	15.88	0.13	4.08	NS	4.43	5.21	9.01	1.74	9.74
Nutrient management practices									
RDF (80:40:20 kg NPK/ha)	251	3.35	111	24.15	41.32	66.32	92.17	16.11	94.57
75% RDF + 25% N through FYM	241	3.29	106	24.21	38.98	61.39	84.42	14.85	86.89
RDF + 25% N through FYM	277	3.60	120	24.36	46.33	71.53	107.34	18.46	105.28
LCC based nitrogen application	273	3.57	118	24.23	45.10	68.72	106.38	17.89	100.05
Control (No fertilizer)	154	2.70	94	23.95	18.80	31.22	37.51	6.48	43.33
CD (p=0.05)	19.22	0.12	4.71	NS	2.93	4.61	5.97	1.46	6.82
CV%	8.25	3.59	4.41	3.20	7.90	7.92	7.17	10.18	8.15

enhanced more photosynthates production and the translocation from source to sink and improved the yield attributing characters (Ramamoorthy et al 2000). Various nutrient management treatments also did not influence the 1000 grain weight.

Grain and straw yield: The rice establishment methods through wet direct seeding using drum seeder recorded pronounced effect on grain and straw yield of rice over aerobic rice (Table 3). However, the grain (42.19 q/ha) and straw yield (65.22 q/ha) was obtained with wet direct seeding using drum seeder was statistically at par with normal transplanting. The higher yield under wet direct seeding using drum seeder was due to adequate supply of resources which contributed towards higher dry matter accumulation and better portioning of photosynthate resulting in higher yield traits and ultimately the yield. Among the nutrient management practices, treatment with RDF +25% N through FYM produced highest grain (46.33 q/ha) and straw yield (71.53 q/ha), which was at par with LCC based N application. These two treatments produced significantly higher grain and straw yield than RDF (41.32 q/ha and 66.32 q/ha), 75% RDF + 25% N through FYM) and control. This might be due to favourable soil conditions and synchronized release of nutrients throughout the crop growth period resulted in enhanced growth and yield attributes which in turn increased grain and straw yield (Murali and Setty 2004).

Nutrient uptake: The rice establishment through wet direct seeding using drum seeder being comparable to normal transplanting led to significantly higher uptake of nitrogen, phosphorus and potassium than aerobic as the establishment of rice through drum seeding and transplanting removed higher amount of nutrients because of better environment available around the eco-rhizosphere as a result of pulverization of soil under a film of water and transplanting of rice seedlings in such an ideal environment might have enabled the crop to absorb native as well as applied nutrients incessantly to give an early lead to the growth of individual plants as well as higher nutrient content that resulted in higher nutrient uptake by transplanted and drum seeded rice (Kanthi et al 2014). Total nitrogen, phosphorus and potassium uptake were higher when the rice crop was raised with RDF + 25% N through FYM (107.34 kg/ha, 18.46 kg/ha and 105.28 kg/ha) and LCC based N application (106.38 kg/ha, 17.89 kg/ha and 100.05 kg/ha) which were statistically at par between themselves but, recorded significantly more total nitrogen, phosphorus and potassium uptake than rest of the nutrient management practices and control. Increase uptake of nutrient with higher nutrient doses was owing to increased availability of nutrients facilitating better root growth and as such better nutrient uptake (Singh et al 2011)

CONCLUSION

The wet direct seeding of rice using drum seeder is the most suitable method of rice establishment and application of RDF (80:40:20 kg NPK/ha) + 25% N through FYM or LCC based N application are the most suitable nutrient management practices having the potential to enhance the rice productivity and serve as a better alternative to rice growing farmers.

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Impact of Phosphate Solubilizing Bacteria on Soil Enzyme Activity and Plant Vigor in Four Wheat Genotypes

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Abstract: Soil microbial communities interact with roots, affecting plant nutrient acquisition. In the present study, attempts were made to analyze the effect of 18 Phosphate solubilizing bacteria (PSB) recovered from *Dalbergia sissoo* Roxb. forest soil on wheat vigor and prove statistically that these PSB inoculants are wheat genotypes specific. For this, a pot trial in a Completely Randomized Design (CRD) with four superior wheat genotypes (UP 262, PBW 502, HDV 2329, and CBW 38) was carried out. The response of soil enzyme activities and plant vigor parameters were taken and analyzed by R-software at the p-value of 0.5. Results confirmed that PSB showed a significant positive response on soil enzyme activities e.g. FDA (Fluroscein di acetate htdrolysis), AP (Alkaline phospatase) and urease which promote plant vigor in all genotypes. FDA activity for plants inoculated with L3 (333002 nmol^{-1}gm^{-1}) and P2 (334002 nmol^{-1}gm^{-1}) was higher as compared to control (28791 nmol^{-1}gm^{-1}) after final harvesting. A similar trend was observed for AP and urease activities. The quantitative phosphate solubilizing index of bacteria was highest in P2 (811.32 µgm L^{-1}) followed by L3 (628.32 µgm L^{-1}). A positive correlation (r= +0.8) was observed between plant vigor and soil enzyme activities. R- software model analysis confirms that the quantitative response of each PSB was wheat genotype-specific.

Keywords: Phosphate solubilizing bacteria, R-Software, Plant vigor, Quantitative, Genotypes

After nitrogen (N), phosphorus (P) is the second most important plant nutrient required at a constant rate throughout the early stages of development. P is not only a major structural component but also an active metabolic compound present in plants. Besides, it plays a significant role in the various important biological processes e.g., photosynthesis, energy transfer and genetic activities of the plant (Theodorou and Plaxton 1993). P solubility and its concentration are low in soil due to their interacting nature with other inorganic compounds (Setia and Sharma 2007) and thus phosphate reaction in the soil plays a critical role in crop development and fertilizers use efficiency. Plants take up P in two forms; primary ($H_2PO_4^-$) and secondary (HPO_4^{2-}). But the availability of soil P to the plants is limited by its precipitation due to the presence of magnesium (Mg^{2+}), aluminum (Al^{3+}), and calcium (Ca^{2+}) ions. Thus most of the free available P is present in immobilized form and hence becomes unavailable to plants. The P unavailability affects around two billion hectares of land worldwide (Oberson et al 2001). Phosphorous deficiency in plants can cause some serious symptoms which include reddish leaves and the appearance of necrotic spots in old leaf tips (Luiz et al 2018). Moreover, it can cause around a 5-15% reduction in crop yield (Shenoy and Kalgudi 2005). It has been estimated that there will be a drastic shortage in phosphorus supply from the industry sector in the future perspective as most of the agriculture phosphorus is getting depleted by soil erosion (Alewell et al 2020). Considering this, the role of phosphate solubilizing bacteria (PSB) in mitigating soil p unavailability becomes prominent. PSBs have the potential to solubilize immobilized forms of phosphorous into a plant-available form. The inoculation with PSB reportedly enhanced solubilization of fixed and immobilized phosphates (Selvi et al 2017). Amongst soil-dwelling micro-organisms, about 1-50% of bacterial and 0.1-0.5% of fungal communities possess phosphate solubilizing potential (Mehrvarz et al 2008). The most common phosphate solubilizing bacterial genera are *Bacillus*, *Pseudomonas,* and *Rhizobium*. Wheat (*Triticum aestivum* L.) is the world's most important cereal crop contributing about 30% of total protein and 45% of digestible energy to the human diet and is also a useful livestock feed (Shewry 2009). To meet the increasing wheat demand of the growing world population amidst low P availability in soils its production and productivity need to be enhanced. However, several biotic factors that include rodents (Neena and Babbar 2010) and insects (Dhaliwal et al 2010) damage the crops which result in high yield loss**.** The eco-friendliest and cheap alternative include inoculation of PSBs as biofertilizers in agricultural production systems. Studies are reporting an increase in wheat production upon inoculation of phosphate solubilizing *Bacillus*, *Pseudomonas,* and *Streptomyces* (Rana et al 2011). Several studies reported a positive correlation between major PSB inoculated groups (*Pseudomonas*, *Streptomyces)* and plant agronomic parameters e.g. inoculation effect of one variable (inoculated strains) positively influences second variable (plant agronomic parameters) (Yadav et al 2017). The inoculation of *Rhizobium, Pseudomonas* and *Flavobacterium* individually or in co-inoculation was reported to increase P solubilization and hence resulted in higher crop yield (Satyaprakash et al 2017). The inoculation of PSB resulted in increased phosphorous mobilization as well as phosphorous content in several legumes plants (Singh et al 2005). The possible reason for the increase in agronomic performance is the development of an extended root network around the plants' rhizosphere by the inoculated strains of PSB. This enhanced the absorption of phosphorous which directly promotes plant development (Mehrvarz et al 2008). These studies provide an insight into a solution to soils that are deficit in phosphorous and other plant nutrients. In this study, we aim to maximize plant-available soil soluble phosphorous through the use of PSB as bioinoculants. PSB provides the dual advantage of providing cheap biofertilizers and environmentally friendly techniques to promote plant development. In our study statistical analysis is the central tool for all the outcomes and the testing of the hypothesis is interpreted by the R-software tool. Although several tools like SPSS and Excel are available for data analysis, R-software provides a greater visualization in statistical analysis as we can create the specific model by analyzing variables (Schandry 2017). On applying ANNOVA model in the R-software tool we can simply hypothesize our data and in-depth analysis was done. In this study, we also created a model in the R-software tool to analyze whether the response of eighteen PSB is wheat genotype-specific or not.

MATERIAL AND METHODS

Isolation and screening of PSB: All the eighteen P solubilizing bacterial strains were retrieved from culture collection maintained at the Department of Microbiology, G.B. Pant University of Agriculture & Technology, Pantnagar. These strains were previously isolated from *Dalbergia sissoo* rhizosphere soils from three different regions of Uttarakhand such as Lachhiwala (30.2099°N latitude, 78.1342° E longitude)**,** Pantnagar (29º3´0´´N latitude and 79°31´0´´ E longitude) and Tanakpur (29.0722°N latitude, 80.1066° E longitude) (Joshi et al 2019).

Quantitative estimation of phosphorous: Inorganic phosphate solubilizing ability of bacterial isolates was assessed by spectroscopic determination of reduced phosphomolybdic acid (blue color) by α-aminonapthosulphonic acid. Phosphorous content was directly proportional to the amount of blue color developed on reduction. Absorbance was taken at 640nm (Fieske and Subbarow 1925).

Pot experiment: A pot experiment was conducted to evaluate the effect of 18 phosphate solubilizing bacteria on the growth of wheat seedlings in four wheat varieties separately. The experimental design was a completely randomized block design with three replicates for each treatment and three pots per replicate (Fig. 1). In all, there were 19 treatments including 18 bacterial treatments plus one uninoculated control for each wheat variety. Experiment was set up in a glass house at Agroforestry Research Center (AFRC) G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India according to CRD design under controlled conditions of temperature and relative humidity. The seeds of four superior wheat genotypes viz., UP 262, PBW 502, HDV 2329 and CBW 38 obtained from Seed Processing Center (SPC), G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India were used for the experiment. The seeds were surface sterilized with 75% of ethanol for 1 min, followed by 1% sodium hypochlorite. Thereafter thoroughly washed with sterile distilled water. Each of the 18 PSB was grown in nutrient broth (NB) medium at 28^0C and 120 rpm on incubator shaker till bacterial population reached 10^8 CFU mL^{-1}. The surface sterilized seed of four wheat varieties was separately dipped in 20 mL bacterial suspension containing 1% carboxymethyl cellulose for 30 min under gentle shaking and then dried for at least 3h. Bacterial density (CFU mL^{-1}) on the coated seeds was determined using the dilution plating method. The 10 coated seeds from each of the four different wheat genotypes and 18 PSB were washed in distilled water

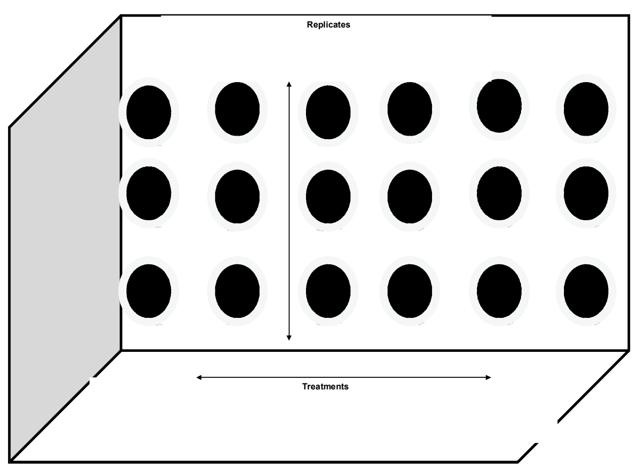

Fig. 1. Pot trial lay out with treatments and replications lane mentioned

before serial dilution in phosphate buffer saline (Sagervanshi et al 2012). Seeds were carefully sown in pots to avoid cross-contamination. All the pots were regularly watered during crop growth as per agronomic practices. After 45 and 90 days post-inoculation, the wheat seedlings were uprooted separately to determine plant biomass indices (shoot length, shoot fresh weight, root length and root fresh weight). Rhizospheric soil was carefully collected as 3 random soil cores from pots, and the samples were pooled to yield one composite sample per replicate. The soils were passed through a 2 mm sieve, thoroughly homogenized, and stored at -80º C for soil enzyme analysis after final harvesting.

Soil enzyme assays: The three main soil enzyme activities e.g. urease, fluorescein di-acetate and alkaline phosphatase were done. To test Fluorescein di-acetate (FDA) hydrolysis activity 1g of moist soil was taken in Erlenmeyer flask and was drenched with 1 mL of FDA solution and 15 mL of phosphate buffer. The flasks were shaken for 20 min on a rotary shaker at 25°C after which 10 mL of acetone was added for extraction. The absorbance of filtered samples was measured at 490 nm (Inbar et al 1991). For AP activity 1g of moist soil was placed in a 50 mL Erlenmeyer flask, to which 4 mL of Modified Universal Buffer (MUB), 0.25 mL of toluene; 1 mL of p- nitrophenyl phosphate (PNPP) solution was added, swirled and incubated at 37° C. After 1h, 1 mL of 0.5M calcium chloride and 4 mL of 0.5M sodium hydroxide were added. The thoroughly mixed soil suspension was filtered and absorbance was measured at 400nm on a spectrophotometer (Tabatabai and Bremner 1969). Urease activity was measured according to Kandeler and Gerber (1998). 5g of moist soil was taken and mixed with 2.5ml 0.08M of urea solution and incubated for 2h at 37° C. 1N KCL was then added and kept on the shaker for 30 min. The developed suspension was then filtered. 1mL of filtered suspension was diluted with distilled water which was followed by the addition of 5 mL sodium salicylate and 2 mL of 0.1% sodium dichloroisocyanurate. Then after 30 min of incubation period absorbance of the sample taken at 690 nm**.**

Statistical analysis: All the experiments were done in triplicates and all analysis was performed using SPSS and R-software. SPSS was used to evaluate variation within treatments in all four varieties separately. Differences in mean values were considered significant when P<0.05. The R-software was used for testing the hypothesis and drawing a correlation between plant vigor parameters and soil enzyme activities.

Selection of hypothesis: To evaluate the response of 18 PSB on four wheat varieties we proposed, two hypotheses. First is the null hypothesis, H_0= Response of 18 PSB on four different genotypes is same (Accepted if p-value>0.05 and rejected if p-value <0.05) and second is Alternative hypothesis, H1 = Response of 18 PSB on four different genotypes is not same (Accepted if p-value<0.05 and rejected if p-value >0.05). The hypothesis is drawn at 95% confidence level.

Correlation analysis: R-software was used to carry out a correlation analysis between two variables e.g. plant vigor parameters versus enzymatic activity.

RESULTS AND DISCUSSION

Characterization of PSBs: All 18 PSB isolates have been previously identified using 16S RNA sequence analysis (Joshi et al 2019) (Table 1).

Estimation of phosphate solubilization index: The quantity of phosphate solubilized by all 18 strains in NBRI-BPB medium was measured as phosphate solubilizing index. Most of the PSB bio-inoculants shows positive results after inoculation but the maximum quantification of phosphorus was observed in P2 (811.32) followed by L3 (560) and T3 (545.49 μgmL^{-1}) (Fig. 2).

Soil enzymes analysis: The measure of soil enzyme activity is considered a soil health indicator. Rhizospheric soil was assessed for fluorescein diacetate, urease, and alkaline phosphatase, activities to evaluate the impact of PSB inoculation on soil health. Overall, from the results, it was

Table 1. Phosphate solubilizing potential and 16SrDNA identity of the isolates along with NCBI Gen bank accession numbers

Treatments	Isolate	NCBI Gen Bank accession no.
L1	*Pseudomonas simiae*	MG966339
L2	*Staphylococcus petrasii*	MG966340
L3	*Pseudomonas paralactis*	MG966341
L4	*Klebsiella variicola*	MG966342
L5	*Pseudomonas paralactis*	MG966343
L6	*Streptomyces curacoi*	MG966344
L7	*Streptomyces cellostaticus*	MH031699
L8	*Pantoea conspicua*	MG966345
P1	*Pseudomonas hunanensis*	MG966346
P2	*Pseudomonas aeruginosa*	MG966347
P3	*Pseudomonas putida*	MG966348
P4	*Pseudomonas plecoglossicida*	MG966349
T1	*Kitasatospora kifunensis*	MG966350
T2	*Klebsiella singaporensis*	MG966351
T3	*Streptomyces antibioticus*	MG966352
T4	*Micrococcus yunnanensis*	MG966353
T5	*Streptomyces griseoruber*	MG966354
T6	*Staphylococcus pasteuri*	MG966355

discovered that the majority of the 18 PSB showed a positive response when compared to uninoculated control. Since FDA is a measure of total microbial activity present in soil e.g FDA directly correlates with other enzyme activity stimulated by microorganisms present in soil because if the total functional microbial population is higher then it will positively influence the activity of other enzyme activity like AP and urease in soil. Significantly higher FDA activity was reported in wheat seedlings treated with T3 (34602.33) followed by P2 (34002.33), L3 (34002), and T4 (33802.33 nmol $g^{-1}h^{-1}$) respectively. This increase in FDA activity signifies that inoculation of PSBs directly influenced the functional microbial community in the rhizospheric soil. Similarly, urease activity was significantly higher for wheat seedlings treated with L3 (221.6), L4 (244.66), T4 (410.73), T5 (426.53) and T6 (552.33 nmol $g^{-1}h^{-1}$). This trend was similar for the alkaline phosphatase activity. But the maximum response of AP activity was shown in seedlings treated with T2 (43462), T3 (41021) and T4 (42261 nmol $g^{-1}h^{-1}$) respectively. An increase in AP activity signifies the increased availability of phosphorous upon application of PSB as bioinoculants. The positive correlation is depicted between FDA e.g. total microbial activity and other two soil enzyme activity e.g. AP and urease (Fig. 3).

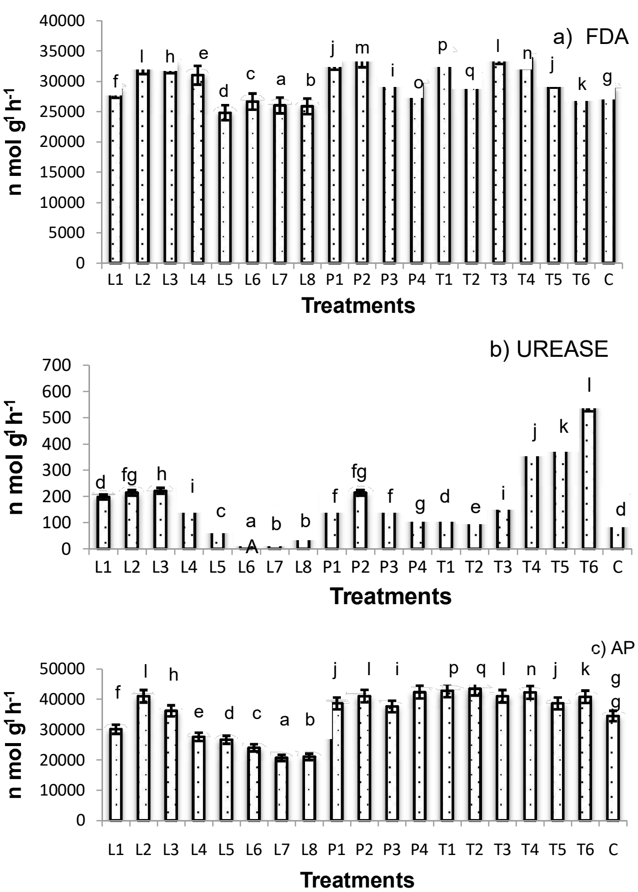

Fig. 2. Response of 18 PSB bacteria along with uninoculated control on soil health measured via three soil enzyme assay a)FDA (Fluorscein di acetate hydrolysis), b) Urease and c) AP (Alkaline phosphatase)

Growth promoting effect of PSB inoculation on wheat genotypes: The growth promoting efficacy of eighteen phosphate solubilizing bacteria on wheat was monitored at 45 and 90 days post inoculation (DPI). The growth of young wheat plants was substantially promoted by the application of the 18 PSB and all four wheat genotypes used in this study. However, the effect was significant in plants treated with two PSB strains namely L3 and P2. The predominant influences observed were enhanced plant biomass at 45 and 90 DPI. At 45 DPI, all plant vigor parameters were already positively influenced by majority of the PSB strain in all four wheat genotypes. The shoot fresh weight/biomass of wheat seedlings of all four genotypes was already increased by all PSB. At 90 DPI, in wheat genotype UP262 majority of the PSB strain show positive response with about 1.5 to 2-fold increase shoot fresh biomass. Maximum two-fold increase was observed in P2 (Table 2). In wheat genotype PBW 502 maximum shoot fresh biomass was also observed in P2 (0.48 g) with around 1.8-fold increase as compared to control (0.25g) (Table 3). In wheat CBW 38 maximum shoot fresh biomass was recorded in T4 inoculated wheat seedlings with 3.3 fold (3.0 g) increase as compared to control (0.9 g) followed by 3-fold increase in P2 (2.7g) inoculated wheat seedlings (Table 5). In HDV 2329 maximum fold increase in shoot fresh biomass of about 1.43 was observed in P2 (1.86g) as compared to control (1.3g) followed by L3 inoculated wheat seedling with 1.3-fold increase (1.76g) (Table 4).

Although the majority of PSB strains seem to promote all the plant vigor parameters in all four genotypes by positively influencing soil enzyme activities quantitative response of all the PSB bioinoculants occurs in a genotype-specific manner which is verified from further R-software analysis. For example, at 90 DPI, P2 treated wheat seedlings of genotypes

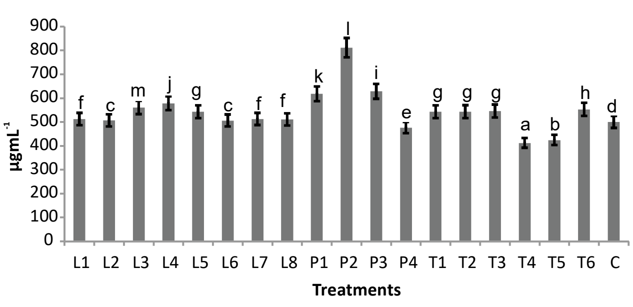

Fig. 3. Measurement of phosphorous quantification on inoculation of 18 PSB

Table 2. Plant vigor parameters of 18 different isolates of PSB on wheat genotypes UP262

UP 262	45 DAP				90 DAP			
	SL	RL	SFW	RFW	SL	RL	SFW	RFW
C	11.66±.27^{ab}	9±.47^{a}	0.15±.004^{a}	0.16±.007^{ab}	17.66±.72^{ab}	12.33±.27^{a}	0.71±.01^{ab}	0.16±.004^{abc}
L1	11±.47^{a}	11±.47^{ab}	0.53±.02^{bc}	0.12±.004^{ab}	21.66±1.24^{cde}	11.4±.94^{ab}	1±.09^{abc}	0.18±.004^{bcd}
L2	13±.47^{abc}	14±.94^{bcd}	0.8±.09^{bcd}	0.14±.01^{bc}	22±.94^{cdef}	16±.94^{abc}	1.2±.09^{bcd}	0.14±.004^{a}
L3	17±.47^{def}	15.33±1.44^{cde}	0.7±.14^{bcd}	0.16±.01^{cd}	28±.72^{hi}	21±1.41^{defg}	1.06±.16^{abc}	0.16±.01^{abc}
L4	14±.94^{abcd}	14±.94^{bcd}	0.56±.14^{cd}	0.15±.01^{cd}	22.3±3.72^{defg}	24±1.88^{fg}	1.4±.18^{cde}	0.15±.004^{ab}
L5	12.66±.54^{abc}	12±.47^{abc}	.4±.09^{ab}	0.19±.004de	22.66±.47^{defgh}	20±.47^{cdef}	1±.04^{abc}	0.19±.004^{cd}
L6	15±.47^{bcde}	9±.47^{a}	0.6±.04^{bc}	0.14±.004^{bc}	20±1.24^{bc}	20±.94^{cdef}	1.2±.18^{bcd}	0.16±.01^{abc}
L7	13±.94^{abc}	13±.94^{bcd}	0.8±.09^{bcd}	0.15±.004^{bc}	15±.72^{a}	19±1.4^{cde}	1.1±.04^{abcd}	0.15±.004^{ab}
L8	14±.47^{abcd}	11±.9^{ab}	0.6±.04^{bc}	0.17±.01^{cd}	20.33±.72^{bcd}	18±.47^{bcde}	1.4±.14^{cde}	0.17±.01^{abc}
P1	13.33±.1.08^{abc}	12±1.41^{abc}	0.93±.05^{cd}	0.17±.004^{cd}	22.66±.47^{defgh}	22±.94^{efg}	1.6±.14^{cd}	0.17±.01^{abc}
P2	19.66±.98^{fg}	16±1.41^{de}	1±.09^{cd}	0.16±.01^{cd}	27±.72^{i}	17±1.41^{bcd}	1.8±.09^{d}	0.16±.01^{abc}
P3	18±.1.4^{efg}	18±.94^{ef}	1±.12^{d}	0.14±.005^{a}	22.66±.98^{defgh}	20.66±1.65^{defg}	1.46±.10^{bcd}	0.14±.005^{ab}
P4	20±.1.88^{fgh}	20±.47^{f}	0.8±.14^{bcd}	0.15±.004^{bc}	24.33±.72^{efghi}	25±.47^{g}	1.4±.14^{abc}	0.15±.004^{ab}
T1	16±.47^{cde}	16±1.4^{de}	0.4±.04^{ab}	0.21±.01^{ef}	25.66±.98^{ghi}	24±1.41^{fg}	.6±.04^{a}	0.21±.01^{d}
T2	18±.94^{efg}	18±.47^{ef}	0.6±.09^{bc}	0.19±.004de	25.33±.47^{fghi}	24±.94^{fg}	.8±.04^{ab}	0.19±.004^{cd}
T3	23±.47^{h}	16±.47de	0.7±.09^{bcd}	0.16±.01^{bc}	27±.94^{i}	24±1.41^{fg}	1.1±.14^{abcd}	0.16±.01^{abc}
T4	20.66±27^{gh}	16.6±1.44^{ef}	0.76±.15^{bcd}	0.16±.01^{cd}	26±1.18^{hi}	22±.47^{efg}	1.2±.04^{bcd}	0.16±.01^{abc}
T5	14±.94^{abcd}	14±.47^{bcd}	0.9±.04^{cd}	0.16±.002^{f}	21.66±.72^{cde}	22±.47^{efg}	1.2±.08^{abcd}	0.16±.002^{abc}
T6	13.33±.72^{abc}	12±.94^{abc}	0.4±.09^{ab}	0.16±.004^{bc}	17.66±.72^{ghij}	19±.94^{cde}	1.4±.04^{cde}	0.16±.004^{abc}

Table 3. Plant vigor parameters of 18 different isolates of on wheat genotypes PBW502

PBW 502	45 DAP				90 DAP			
	SL	RL	SFW	RFW	SL	RL	SFW	RFW
C	17.33±.54^{a}	16±.94^{b}	0.75±.00^{cd}	0.15±.00^{a}	24.66±.47^{bc}	13.66±.54^{cde}	0.25±.0^{b}	0.1±.00^{a}
L1	11±.47^{abcd}	17.66±.27^{bc}	0.48±.00^{a}	0.22±.00^{bcd}	29±.94^{a}	11±.47^{abcd}	0.29±.0^{b}	0.14±.01^{bc}
L2	16±1.88^{abc}	18±.47^{bcd}	0.53±.01^{ab}	0.21±.01^{bc}	28±.47^{b}	8±.94^{a}	0.33±.0^{c}	0.16±.0^{cd}
L3	22.66±.72^{e}	17.47^{bc}	1.23±.02^{fg}	0.4±.01^{i}	36±1.8^{fghi}	15±.47de	0.45±.01^{efg}	0.25±.0^{g}
L4	22±.47^{a}	18±.1.4^{bcd}	0.44±.01^{a}	0.23±.01^{bcde}	25±.47^{efgh}	10±.47^{abc}	0.26±.0^{b}	0.15±.0^{c}
L5	21±.47^{abc}	18±.94^{bcd}	0.86±.01de	0.23±.00^{cdef}	28±.94^{defg}	11±.47^{abcd}	0.39±.0^{d}	0.15±.0^{c}
L6	20±.94^{a}	12±1.41^{a}	0.76±.00^{cd}	0.25±.00^{efg}	25±1.88^{cdef}	12±.94^{abcde}	0.34±.0^{c}	0.17±.0^{cd}
L7	19±.94^{ab}	17±1.41^{bc}	0.67±.00^{bc}	0.27±.00^{g}	26±1.41^{bcde}	9±.94^{ab}	0.2±.0^{a}	0.21±.0^{ef}
L8	18±.47^{ab}	16±.47^{b}	0.65±.00^{bc}	0.2±.00^{b}	26±.47^{bcd}	11±1.41^{abcd}	0.19±.0^{a}	0.15±.0^{c}
P1	22±.94^{bcd}	21±.47^{cde}	0.66±.04^{bc}	0.23±.01^{cdef}	30±.94^{efgh}	14±.94^{cde}	0.347±.0^{c}	0.14±.01^{bc}
P2	27±.47^{e}	25±.47^{fg}	1.4±.11^{g}	0.34±.00^{h}	36±1.65^{j}	15.66±.72^{e}	0.48±.0^{g}	0.22±.0^{fg}
P3	25.33±.72^{bcd}	21.66±.98^{def}	1.23±.04^{e}	0.27±.00^{fg}	27.33±.94^{ij}	13±.47^{bcde}	0.41±.0de	0.19±.0de
P4	19.33±.72^{ab}	24.33±1.6^{efg}	1.2±.09^{f}	0.25±.0^{efg}	27±1.41^{cde}	20±2.3^{f}	0.27±.0^{b}	0.14±.01^{bc}
T1	24±.47^{abc}	18±1.41^{bcd}	1.4±.03^{g}	0.25±.00^{defg}	28±1.41^{ghij}	12±1.41^{abcde}	0.47±.0^{fg}	0.19±.0de
T2	22±.94^{ab}	21±.47^{bcd}	1.01±.04^{e}	0.2±.00^{b}	26±1.41^{efgh}	14±1.41^{cde}	0.45±.0^{ef}	0.14±.01^{bc}
T3	24.66±.54^{e}	26±.47^{g}	1.4±.01^{g}	0.32±.01^{h}	36±.94^{hij}	14±1.88^{cde}	0.33±.0^{c}	0.16±.0^{cd}
T4	25±.47de	17±.47^{bc}	1.2±.04^{f}	0.35±.00^{h}	33±.72^{hij}	16±.94^{e}	0.57±0^{h}	0.15±.0^{c}
T5	20.66±.72^{cde}	17±1.41^{bc}	1.3±.00^{fg}	0.2±.01^{b}	32.33±.72^{def}	12±.47^{abcde}	0.42±.0de	0.11±.0^{ab}
T6	19±1.24^{abcd}	20±.94^{bcd}	0.98±.00^{e}	0.2±.01^{b}	29.33±.27^{bcde}	14±.94^{cde}	0.34±.0^{c}	0.16±.01^{bc}

Table 4. Plant vigor parameters of 18 different isolates of PSB on wheat genotypes PBW502

HDV 2329	45 DAP				90 DAP			
	SL	RL	SFW	RFW	SL	RL	SFW	RFW
C	27±.47^{a}	9±.47^{a}	0.45±.01^{a}	0.17±.00^{abc}	27±.47^{a}	15±.47^{a}	1.3±.04^{abc}	0.17±.00^{cd}
L1	28±.47^{ab}	14.66±.94^{bcde}	0.56±.02^{a}	0.16±.00^{ef}	34±.94^{bcde}	19±.54^{bc}	1.4±.18^{bcd}	0.26±.01^{ef}
L2	28.66±3.03^{ab}	14.33±.94^{bcd}	0.7±.04^{abc}	0.11±.01^{a}	34.66±1.08^{cdef}	20±.27^{bcd}	1.2±.1^{ab}	0.1±.00^{a}
L3	32.66±3.06^{bcd}	21±.47^{fg}	0.96±.02^{bcd}	0.4±.02^{g}	37±.47^{def}	29±.47^{fg}	1.76±.01^{ef}	0.4±.02^{i}
L4	31±.47^{abcd}	21±.94^{fg}	0.6±.04^{a}	0.27±.05^{f}	37±.47^{def}	20±.47^{bcd}	1.57±.01^{cdef}	0.27±.05^{cde}
L5	31±.47^{abcd}	15±.47^{bcde}	0.55±.02^{a}	0.18±.00^{bcd}	30±.94^{ab}	20±.47^{bcd}	1.8±.1^{ef}	0.18±.00^{def}
L6	32.66±.54^{ab}	12±.47^{ab}	0.58±.01^{a}	0.34±.01^{f}	31±.94^{ab}	19±.47^{bc}	0.58±.01^{cdef}	0.34±.01^{cdef}
L7	36±.94de	16±.47^{cde}	0.59±.00^{a}	0.12±.00^{ab}	32±1.88^{bc}	18±.94^{b}	0.59±.00^{ab}	0.12±.00^{ab}
L8	33±1.24^{bcd}	17±.94de	0.64±.01^{ab}	0.2±.01^{cde}	32±.47^{bc}	19±1.41^{bc}	0.64±.01^{cde}	0.2±.01^{cd}
P1	27±1.41^{a}	12±.94^{ab}	1±.1^{cd}	0.23±.00^{cdef}	36±1.88^{cdef}	18±.47^{b}	1±.1^{cdef}	0.23±.00^{cd}
P2	36±.47de	21±.47^{fg}	1.2±.18de	0.51±.02^{g}	30±1.41^{ab}	31±1.41^{g}	1.86±.01^{h}	0.51±.02^{h}
P3	34±.94^{cd}	15±.72^{bcde}	.76±.01^{abc}	0.17±.02^{abc}	39±.47^{f}	22.33±.47^{d}	1.41±.1^{ab}	0.17±.02^{ab}
P4	36±.94de	22±.54^{g}	1.1±.04de	0.21±.00^{cdef}	37±1.41^{def}	21.66±.94^{cd}	1.30±.00^{ab}	0.21±.00^{ab}
T1	31±.94^{abcd}	16±1.41^{cde}	1.2±.18de	0.16±.01^{abc}	36±1.88^{cdef}	25±.94^{e}	1.4±.14^{abc}	0.16±.01^{a}
T2	34.66±1.9^{ab}	21±.94^{fg}	1.4±.18^{e}	0.17±.00^{abc}	38±.94^{ef}	26±1.41^{e}	1.29±.04^{abc}	0.17±.00^{bc}
T3	40±.47^{e}	18±.47^{ef}	1.16±.1de	0.35±.01^{g}	38.33±1.78^{ef}	27±.47^{ef}	1.84±.02^{g}	0.35±.01^{h}
T4	36±.94de	16±.47^{cde}	1.43±.1^{e}	0.37±.00^{g}	34±1.88^{bcde}	26±.94^{e}	1.8±.01^{g}	0.37±.00^{h}
T5	30±1.24^{abc}	13±.47^{bc}	0.59±.02^{a}	0.21±.00^{cdef}	38±.94^{bc}	22±.47^{cd}	1.68±.01^{cdef}	0.21±.00^{fg}
T6	34±.94^{cd}	15±.72^{bcde}	0.73±.03^{abc}	0.23±.01^{def}	33±1.41^{bcde}	17.33±.94^{ab}	1.48±.01^{def}	0.25±.00^{g}

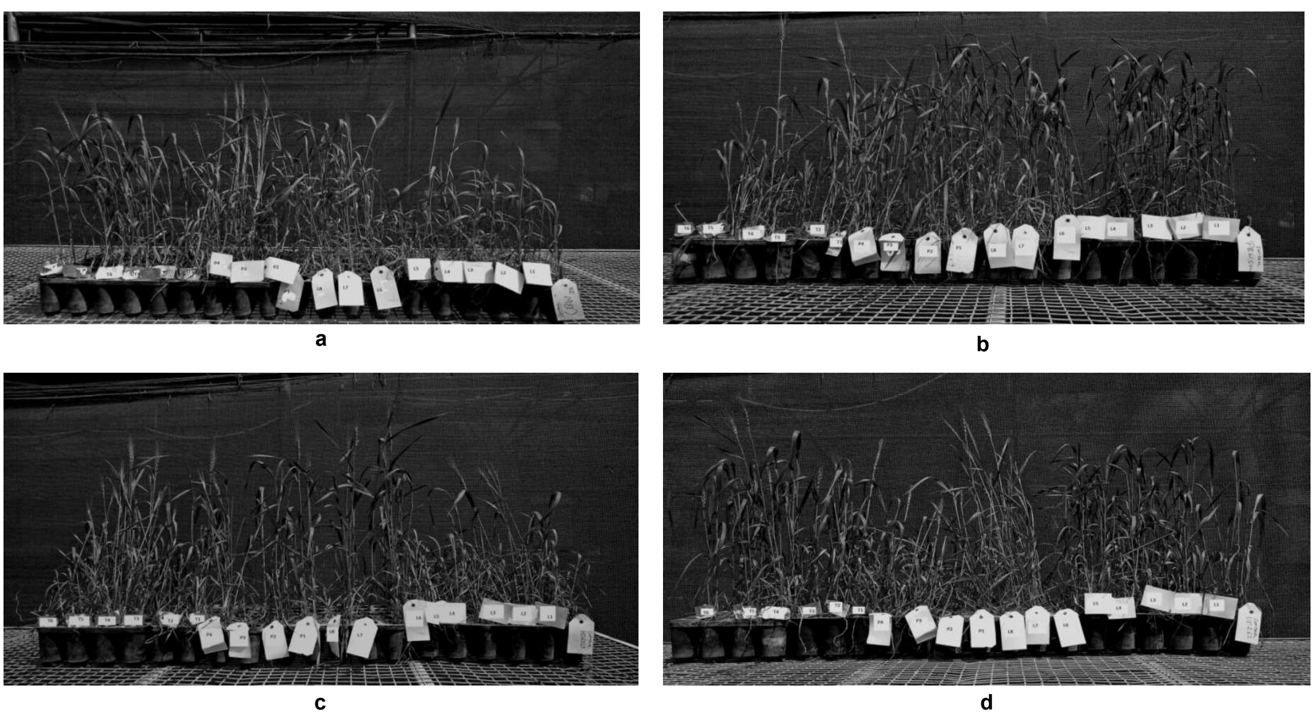

Fig. 4. Response of four different wheat genotypes during pot trial experiment treated with PSB along with uninoculated control a) CBW 38, b) PBW 502, c) HDV 2329, and d) UP 262

UP 262, PBW 502, HDV 2329 and CBW 38 recorded 0.52, 0.45, 0.11, 0.5-fold increase in shoot length respectively. A similar pattern of increase was observed for other plant vigor parameters also (Fig. 4).

Correlation between Plant Growth Parameters and Soil Enzyme Activities

Correlation analysis: The positive correlation has been observed when two variables e.g. total microbial activity (FDA) and plant vigor parameters are plotted against each other in UP 262 wheat genotype and a similar positive was also observed in the other three wheat genotypes. It was clear from the results that an increase in one variable e.g. FDA positively influenced the second variable e.g. to plant vigor parameters (Fig. 5).

Evaluation of hypothesis using R-software: R-software analysis of plant growth parameters for all four wheat genotypes UP262 CBW 38, PBW 502 and HDV 2329 (Supplementary material 1) showed that at a p-value less than 0.05, the null hypothesis is rejected and alternative hypothesis accepted. Plant shoot length and root length of all four genotypes treated with bioinoculants when tested by applying ANNOVA model in R-software revealed that quantitative response of each of the 18 PSB varied wheat genotypes at $P<0.05$ (Table 6) and thus response was genotype-specific. Since PSB can act as biofertilizers, their inoculation alone or in combination with inorganic/organic phosphate helps to solubilize the unavailable soil phosphorous (Kalayu 2019). In the present study, we hypothesized that inoculation of PSB positively influences soil health indicators which further promoted plant growth and development. To evaluate this, we determined three major soil enzyme activities e.g. FDA, AP and urease upon inoculation of PSB. A significant increase in soil enzyme activity was recorded on adding 18 PSB as bioinoculants as compared to uninoculated control. Response of each of 18 PSB was strictly monitored and results were statistically verified and the null hypothesis was rejected and we concluded that quantitative response of PSB strains was genotype-specific. Although the response of the majority of 18 PSB was positive in all four wheat genotypes quantitative response varied for four wheat genotypes. In the present study to evaluate mobilization of phosphates and other nutrients upon PSB inoculation, we performed soil enzyme assays and found that PSB treated soil showed a 1-1.5-fold increase in total enzyme activity (Fluorescent di acetate hydrolysis) which directly influenced and increased soil alkaline phosphatase and urease activity. This increase in soil enzymatic activities could be the main factor for mobilizing free phosphorous to plant upon and PSB inoculation. Correlation analysis between soil FDA and plant vigor parameters shows a positive correlation which clearly suggests that increase in FDA activity positively influenced plant vigor. Similarly, the positive correlation was observed between enzymatic activity (FDA and AP) and plant vigor traits in case of *Pseudomonas koreensis* AS15 treated *Dalbergia sissoo* seedlings (Dasila et al 2018). Higher biomass along with enhanced soil urease and alkaline phosphatase activity was observed in wheat

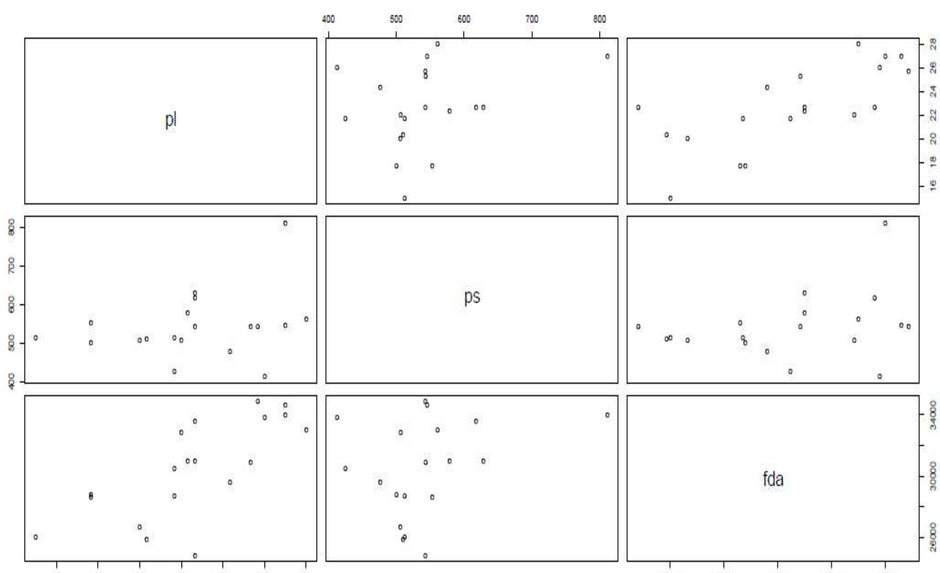

Fig. 5. Correlation analysis done by R-software to analyze the interaction between plant vigor parameters e.g. pl (plant root length), ps (shoot fresh weight and shoot length) versus FDA which was found to be r=+0.8

Table 5. Plant vigor parameters of 18 different isolates of PSB bacteria on wheat genotypes PBW502

CBW 38	45 DAP				90 DAP			
	SL	RL	SFW	RFW	SL	RL	SFW	RFW
C	24±.94^{a}	12±1.41^{a}	0.6±.04^{a}	0.12±.00^{abc}	30±.47^{a}	15±1.41^{ab}	0.9±.04^{a}	0.15±.01^{a}
L1	30±.47^{bcd}	14±.94^{ab}	0.6±.04^{a}	.26±.00^{a}	35±1.88^{ab}	14.66±1.44^{ab}	0.8±.09^{a}	0.2±.04^{a}
L2	32±.94^{def}	18±.47^{bcd}	0.8±.09^{abc}	.38±.21^{ab}	37±1.41^{bcd}	18±1.41^{bc}	1.2±.09^{ab}	0.19±.01^{a}
L3	38±.47^{g}	24±1.41^{f}	2.1±.14^{ij}	.36±.01^{fg}	48±.47^{f}	33±1.41^{e}	2.4±.04^{c}	0.57±.13de
L4	35±1.88^{efg}	20±.47^{cde}	0.7±.04^{ab}	0.21±.00^{cdef}	41±.47^{cde}	16±.47^{ab}	1.6±.09^{b}	0.67±.1^{bc}
L5	26±.47^{ab}	9.33±1.44^{bc}	0.9±.047^{abcd}	0.2±.01^{fg}	36±.94^{bc}	18±.94^{bc}	1.4±.04^{b}	0.11±.00^{ab}
L6	28±.94^{abcd}	10±.94^{bcd}	1.5±.09^{fgh}	0.34±.01^{bcde}	37±1.88^{bcd}	21±1.41^{cd}	1.4±.14^{b}	0.12±.01^{ab}
L7	26±.47^{ab}	12±1.88^{bcd}	1.6±.14^{gh}	0.12±.00^{defg}	37±.47^{ab}	16±.47^{ab}	1.2±.14^{ab}	0.12±.01^{a}
L8	28±.94^{abcd}	11±1.41^{de}	1.2±.18^{cdefg}	0.34±.18^{bcde}	42±2.94^{a}	15.33±1.44^{ab}	1.5±.04^{b}	0.12±.01^{ab}
P1	27±.1.41^{abc}	16±1.41^{bc}	1.1±.14^{bcdef}	0.35±.18^{abcd}	36.33±2.12^{bc}	18±1.41^{bc}	1.6±.09^{b}	0.1±.01^{bc}
P2	36±.47^{fg}	27±1.41^{ef}	1.63±.14^{hi}	0.27±.01^{g}	45±.47^{ef}	33±.47^{e}	2.7±.04^{ab}	0.36±.01^{e}
P3	27±1.88^{abc}	14±.94^{bc}	1.3±.09^{defg}	0.18±.00^{bcdef}	36±.47^{bc}	21±.94^{cd}	1.43±.28^{a}	0.12±.01^{a}
P4	32±.47^{def}	12±1.41^{ab}	0.8±.14^{abc}	0.21±.00^{ab}	34±1.88^{ab}	22±1.41^{cd}	1.2±.18^{ab}	0.2±.04^{a}
T1	31±.94^{cde}	10±.94^{ab}	1±.18^{abcde}	0.12±.00^{abc}	34±.47^{ab}	24±.94^{d}	1.7±.09^{b}	0.11±.00^{bc}
T2	32±1.41^{def}	9.66±.98^{bc}	1.4±.14^{efgh}	0.14±.00^{cdef}	36±.94^{bc}	22±1.41^{cd}	1.6±.04^{b}	0.7±.1^{bc}
T3	38±.94^{g}	27±.47^{f}	2.1±.04^{ij}	0.47±.00^{h}	50±.94^{f}	30±1.41^{e}	2.7±.14^{ab}	0.54±.01^{cd}
T4	39±1.41^{g}	24±1.41^{ef}	2.4±.14^{j}	0.39±.00^{g}	45±1.41^{f}	33±1.41^{e}	3±.14^{d}	0.42±.01^{f}
T5	27±1.41^{h}	14±.94^{ab}	1.2±.04^{cdefg}	0.16±.00^{efg}	33±1.41^{ab}	14±.94^{ab}	1.4±.14^{b}	0.14±.01^{ab}
T6	30±.47^{fg}	12±.94^{ab}	0.96±.01^{abcde}	0.17±.00^{cdef}	33±.47^{ab}	12±.94^{a}	1.2±.04^{ab}	0.7±.04^{bc}

Table 6. R-software analysis of plant vigor parameters in wheat genotype UP262 rejected null hypothesis and accepts alternative hypothesis. The similar response was observed in other three wheat genotypes

Wheat genotypes	R- software output	Comment
Shoot length: I sampling	> mod=aov(r~t+b) > summary(mod) Df Sum Sq Mean Sq F value Pr(>F) t 3 3554 1184.6 90.273 <2e-16 *** b 18 540 30.0 2.285 0.0101 * Residuals 54 709 13.1 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	Since p < 0.05 Hence, null hypothesis was rejected and an alternate hypothesis accepted. Hence, impact of inoculation of 18 strain of Phosphate solubilizing bacteria on four different wheat varieties is not same and is genotype specific.
root length I sampling	> summary(mod) Df Sum Sq Mean Sq F value Pr(>F) t 3 908.2 302.72 29.107 2.47e-11 *** b 18 728.5 40.47 3.891 5.46e-05 *** Residuals 54 561.6 10.40 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	Since p value is less than 0.05 thus our null hypothesis rejected and alternate hypothesis accepted hence impact of inoculation of 18 strain of Phosphate solubilizing bacteria on four different wheat varieties is not same e.g genotype specific.
shoot length II sampling	> mod=aov(r~t+b) > summary(mod) Df Sum Sq Mean Sq F value Pr(>F) t 3 2606.9 869.0 93.665 < 2e-16 *** b 18 727.7 40.4 4.357 1.33e-05 *** Residuals 54 501.0 9.3 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	Since p value is less than 0.05 thus our null hypothesis rejected and alternate hypothesis accepted hence impact of inoculation of 18 strain of Phosphate solubilizing bacteria on four different wheat varieties is not same e.g genotype specific.
root length II sampling	> mod=aov(r~t+b) > summary(mod) Df Sum Sq Mean Sq F value Pr(>F) t 3 236.3 78.77 5.893 0.001484 ** b 18 855.1 47.51 3.554 0.000157 *** Residuals 54 721.8 13.37 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	Since p value is less than 0.05 thus our null hypothesis rejected and alternate hypothesis accepted hence impact of inoculation of 18 strain of Phosphate solubilizing bacteria on four different wheat varieties is not same e.g genotype specific.

seedlings treated with bacterial inoculants (Mader et al 2011). The growth of young wheat plants was substantially promoted by the application of the 18 PSBs and all four wheat genotypes used in this study. The plant vigor parameters such as shoot length, root length, shoot fresh weight and root fresh weight were significantly higher in all the four wheat genotypes particularly in L3 and P2 treatments. The increase in plant vigor parameters could be due to increased mobilization of phosphorous into the plant due upon inoculation of PSB. A similar increase in phosphorous uptake in wheat plants with PSB application has been observed earlier also (Rezakhan 2019). PGPR strains isolated from Jujibe (*Ziziphus lotus*) enhance wheat growth (Fahsi et al 2021). Wheat grain yield was significantly increased upon introduction of phosphate solubilizing arbuscular mycorrhizal (VAM) fungus *Glomus* sp.88 and *Bacillus circulans* which promoted root colonization aiding in increased nutrient uptake (Singh and Kapoor 1999). Not only in wheat but in other crops also phosphate solubilizing microorganisms promote plant development. For example, inoculation of p solubilizing *Pseudomonas putida* increased root and shoot length in sorghum (Ali et al 2011) further the inoculation phosphate solubilizing micro-organisms increased chickpea yield by 70% (Verma et al 2013). The possible explanation for this could be an increase in soil fertility after the application of bioinoculatants which is evident in the form of enhanced soil enzyme activities. The increased value of soil enzyme activities indicates that treatment of PSBs resulted in enhanced phosphorous mobilization across the soil and this resulted in increased plant growth (Leifheit et al 2015, Meena et al 2014) or by mobilizing soil P by mediating biogeochemical P cycle (Tian et al 2021).

CONCLUSION

This study established the role of inoculated PSB in stimulating soil enzyme activities which in turn promotes the plant vigor parameters. An increase in soil FDA upon inoculation of PSB stimulates alkaline phosphatase activity which results in greater mobilization of free phosphorus in soil and thus provides greater availability of phosphorus to plants. Applying ANNOVA (analysis of variance) model in R-software revealed that though the impact of 18 PSB was positive for not only influencing the growth of wheat genotypes however the quantitative response is genotype-specific which signifies that broad-spectrum PSB inoculants can be used for a large range of wheat varieties. This kind of study could be significant for developing single inoculants for wheat cultivation in different agroclimatic zones of the country.

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Influence of PGPR on Growth and Yield of Oat (*Avena sativa* L.) under Field Conditions

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***Abstract*:** India has 15% of the world's livestock population. Currently, livestock rearing for animal goods such as dairy products, meat, eggs, wool and their byproducts, which have become a huge source for livelihood. But insufficient availability of quality fodder affects animal health and productivity. Oat is a unique cereal fodder crop, which is utilized for animal fodder as well as for the human diet. Despite having such potential of the crop, the area and yield are still not much more increasing in India. Concerning this problem, the present investigation was performed in search of potential plant growth-promoting rhizobacteria to enhance the crop growth and productivity of forage oat. During the study, the bacterial isolates were screened for plant growth-promoting properties such as siderophore production, zinc, potassium and phosphate solubilization. Afterward, a field trial was conducted with selected potential bacterial isolates. The results of the study demonstrated that among all bacterial isolates PAU 12 and CHM 7 were performed superior as they improved the green forage, dry matter yield, plant height, grain yield and weight of oat grains. The correlation coefficient analysis also showed that the plant growth-promoting properties proportionally enhanced fodder productivity, seeds yield and other agronomical parameters. Moreover, cluster and principal component analysis also confirmed that among all PGPR, the performance of PAU 12 and CHM 7 was remarkable. The present investigation confirmed the potential of bacterial isolate PAU 12 and CHM 7 and could be utilized with oat as potential bioinoculants to enhance its productivity in an eco-friendly manner.

***Keywords*:** Forage oat, PGPR, Growth and yield, Animal nutrition

Fodder production has attained significance in recent years. The sustainability and survival of our livestock depend upon the quality of feed and fodder resources available to the livestock. The production of livestock in India has taken a new leap by venturing itself on a commercial basis. Oat (*Avena sativa* L.) ranks sixth in world cereal production, following wheat, maize, rice, barley and sorghum. Oat is an important forage crop as it is grown in winters and is used for animal feed, human nutrition as well as industrial purposes. Oat has multiple uses as it is grown for grains, which is a source of high protein and is also widely used for haymaking. It is considered good fodder as its straw is soft and palatable. Moreover, its grains have high value for nutritional and medicinal purposes. Animals use forage as green fodder but it is also preserved in the form of hay and silage and is known as dry fodder. Day to day rising population of the world simultaneously increasing the demand of milk, butter, meat and their derivates products. India has one of the world's largest livestock populations and is known as a billion-dollar industry but the demand for animal goods is still unable to fulfill. As the worth of animals and their goods are associated with their genetics, nutrition and environment, hence adequate fodder with sufficient nutrients is obligatory. But currently cultivating fodder is unable to accomplish the rising fodder demand because crops residues are burned by the farmers and among all cropping areas, only a small fraction (4.4%) of land is devoted for forage production, moreover, their fodder and seed yield is also low, which is fails to fulfill the forage demand (Dikshit and Birthal 2010). The low nutrient content in forage crops is a major reason cause weight loss, low milk production, the occurrence of disease in animals. Yadav et al (2017) estimated that approximately an additional 219.2 MT of green fodder and 226.53 MT of dry fodder is required, which is beyond the production of fodder currently cultivated in India. Inspite of the requirement and importance of fodder and grain, research is being more focused on the improvement of wheat, rice and maize cultivation. Hence, to cope with this problem, efficient alternative strategies and government policies should be devised and formulated to augment fodder production and their grain yield.

Chemical fertilizer application is an easy approach to improve forage oat production but their extensive use causes soil infertility, soil water pollution, destroys native microflora and ultimately makes the land barren. The root rhizosphere is the junction between bacteria and plant which support the growth and developmental activity of both plant and bacteria (Singh et al 2017, Joshi et al 2018). Plant growth-promoting rhizobacteria (PGPR) are classified as bacteria, closely associated with the root zone and are capable to improve plant growth, physiology and ultimately crop yield by facilitating nutrient availability and soil moisture without affecting environmental geochemical cycles (Khan et al 2020). Hence, exploitation of plant growth-promoting rhizobacteria (PGPR) could be efficient, eco-friendly, cost-effective and safer approach to augment the production of oat crops. PGPR possesses several incredible mechanisms such as atmospheric nitrogen fixation, phosphorous, zinc and potassium solubilization, iron chelation, exopolysaccharide and phytohormones production to preserve the soil health, which is the core quality and yield determination factor for crops (Khan et al 2019). Considering the importance of oat, the present investigation was carried out to find a potential PGPR to augment the oat productivity sustainably.

MATERIAL AND METHODS

Bacterial cultures and growth conditions: A total of twenty bacterial isolates were obtained from the Department of Microbiology culture collection, College of Basic Sciences and Humanities, GBPUA&T, Pantnagar, India. Bacterial cultures were grown on a nutrient agar medium at 28°C and purity was confirmed through Gram's staining followed with microscopic analysis. All the bacterial isolates were preserved in slants at 4°Cand in glycerol stocks at -20°C for for further use.

Plant growth-promoting traits assessment: The plant growth-promoting properties of the bacterial isolates were assessed through siderophores production, solubilization of potassium, and zinc as well as by nitrogen fixation.

Siderophore production: Siderophore production was determined on chrome azurol S test as described by Schwyn and Neilands (1987). In brief, actively grown bacterial culture was spot inoculated on Petri plate containing nutrient agar medium amended with CAS dye then plates were incubated at 28±2°C for 48 to 72 hours. The appearance of the yellow zone around the bacterial colony indicating the positive results for siderophore production and its efficiency was calculated by the following formula.

Siderophore production efficiency%= (Diameter of halo zone/diameter of colony) x 100

Zinc and potassium solubilization potential: The zinc solubilization potential of bacterial isolates was tested on minimal agar medium supplemented with 0.1 % ZnO and $ZnCO_3$(Ramesh et al 2014) and potassium solubilization was determined through Aleksandrow agar medium (Parmar and Sindhu 2013). Actively grown bacterial cultures were spot inoculated on respective medium and plates were incubated at 28±2°C for 48-96 hours. The appearance of halo zone around the bacterial colony was designated as zinc and potassium solubilization and solubilization efficiency were calculated by the following formula.

Solubilization efficiency %= (Diameter of halo zone/diameter of colony) x 100

Nitrogen fixation: The nitrogen fixation ability of bacterial isolates was tested by spot inoculation on Burk medium. The appearance of bacterial growth on Burk's medium was considered for positive results.

Field study: A field trial was conducted to compare plant growth promotory potential of selected six bacterial isolates on the growth of forage oat (*Avena sativa* cv. UPO 10-2) at an instructional dairy farm, Nagla, Pantnagar. For effective delivery of bacterial isolates, the bioformulation of each bacterial isolate was prepared with activated charcoal as an inert carrier material. The oat seeds were bacterized by each bacterial bioformulation separately and allowed to adhere to seeds by drying under shade for 1 hour. Subsequently, seeds were sown in the respective field plot. The whole experiment was performed in randomized block design with three replications.

Plant growth promotion assessment: At about 50% of the flowering stage of the crop five representative plant samples were taken for estimation of green forage yield and samples were dried in an oven at 80˚C for 48h and their dry weight was measured. Moreover, at the time of maturity, plant height was measured. After crop harvesting, spike length, number of grains/spike, 1000grain weight, biological and grain yield, and harvest index were calculated.

Statistical analysis: To determine the significance and variance between the treatments one-way ANOVA followed by Duncan was performed at P<5%. Pearson's correlation coefficient analysis was performed to determine the link among the PGP traits, grain, biological yield and harvest index. Further, to confirm the effect of relative bacterial isolates on the plant's agronomical parameters, principal component analysis and cluster analysis were performed.

RESULTS AND DISCUSSION

PGP potential of bacterial isolates: Among all bacterial isolates, PAU 12 followed by CHM 7 and HRC 23 showed the highest siderophore production efficiency (Table 1). Further, maximum efficacy of zinc solubilization exhibited by CHM4 and CHM7 i.e 333.33% (Table 1). Parveen et al (2018) also observed the siderophore production and zinc solubilization potential of bacterial isolates ranged from 72.7-250% and 150-600%, respectively. Moreover, Roshani et al (2020) also screen the PGPR for consortium preparation by investigating their siderophore and zinc solubilization potential ranging 66.66 – 70.66 % and 260-325%, respectively and further assessed them for plant growth promotion on wheat and found. In addition, among all bacterial isolates, PAU 12 showed the highest potassium solubilization efficiency i.e. 225% followed by CHM7 (i.e. 200%). All the bacterial isolates except CHC 4, BW 12, and DHB 8 were able to grow on nitrogen-deficient Burk's Medium and confirmed their nitrogen fixation ability (Table 1) Parmar and Sindhu (2013) and Ahemad and Kibret (2014) documented that potassium solubilization and nitrogen fixation ability are the crucial mechanisms of PGPR to support plant growth and productivity.

Yield and growth performance: The current investigation demonstrated the remarkable influence of all bacterial isolates on the growth promotion and yield attributing parameters of oat crop over control. Green forage and dry matter yield are the important attributes for oat production. The highest green forage (621.33 and 624.00 q/ha) and dry matter yield (98.27 and 95.10 q/ha) were observed under treatment of PAU 12 and CHC 4 bacterial isolates, respectively. Likewise, Deva et al (2014) also reported the enhancement in fodder yield when oat seeds were treated with potential PGPR. In a present study when concerning the plant height, seeds primed with PAU 12 exhibited maximum plant height i.e. 129.00 cm followed by CHM7 and CHC 4 (Table 2). Almaghrabi et al (2013) also reported 60% increment in tomato plant height when primed with potential PGPR over uninoculated control. Among all treatments, the utmost spike length (37.97 and 37.13 cm) and the number of grains per spike (50.33 and 49.33) were observed in PAU 12 and CHM 7 treated plants, respectively (Table 2). Naeem et al (2018) also observed 22 and 20% increase in spike length and the number of grains per spike, respectively over un-inoculated control, when wheat seeds were primed with PGPR. The present study results confirmed the grain weight enhancement upon priming of each PGPR over control, but a

Table 1. Plant growth-promoting traits of bacterial isolates

Bacterial Isolates	Siderophore production efficiency%	Potassium solubilization efficiency%	Zinc solubilization efficiency%	Nitrogen fixation
PAU 12	133.33	225.00	185.71	+
CHC 4	-	175.00	333.33	-
CHM 7	116.66	200.00	333.33	+
HRM 29	112.50	-	166.66	+
BH 7	110.00	180.00	200.00	+
PIT 4	100.00	150.00	260.00	+
J 28	100.00	180.00	160.00	+
HRC 23	116.66	140.00	150.00	+
BW 12	-	-	-	-
DHB 8	-	-	-	-

Table 2. Influence of PGPR on plant growth promotion and yield attributing traits of oat

Treatment	Forage yield (q ha^{-1})	Dry matter yield (q ha^{-1})	Plant height (cm)	Spike length (in cm)	Number of grain per spike	1000 grain weight (in g)	Grain yield (q ha^{-1})	Straw yield (q ha^{-1})	Biological yield (q ha^{-1})	% Harvest index
Control	566.40±3.33^{a}	86.76±0.77^{a}	119.33±0.88^{a}	32.00±0.94^{a}	43.00±1.52^{a}	45.21±0.55^{a}	15.93±0.37^{a}	179.63±2.33^{a}	195.56±4.09^{a}	8.15±0.22^{a}
PAU 12	621.33±2.66^{c}	98.27±0.48^{d}	129.00±1.15^{d}	37.97±0.61^{c}	50.33±0.88^{c}	50.73±1.23^{b}	19.31±0.08^{e}	207.87±1.92^{C}	227.18±3.39^{c}	8.50±0.10^{a}
BH7	581.33±11.6^{a}	91.38±0.77^{b}	125.00±0.85^{c}	35.57±0.97^{c}	47.00±0.57^{b}	49.99±1.11^{b}	18.15±0.16^{c}	196.76±0.96^{b}	214.91±1.59^{b}	8.45±0.11^{a}
PIT 4	589.33±2.66^{b}	89.53±0.43^{b}	124.67±2.02^{c}	35.15±0.20^{b}	47.00±1.00^{b}	48.74±0.36^{b}	17.64±0.13^{c}	193.52±0.26^{b}	211.16±0.54^{b}	8.35±0.05^{a}
J2 8	576.00±9.23^{a}	88.48±0.66^{a}	123.33±2.18^{c}	35.20±0.43^{b}	46.33±1.45^{a}	48.19±1.47^{a}	17.27±0.12^{b}	193.06±6.07^{a}	210.32±10.3^{b}	8.26±0.47^{a}
HRC23	581.33±11.6^{a}	88.90±1.33^{a}	121.67±1.20^{b}	34.80±1.08^{a}	46.00±0.57^{a}	46.75±0.36^{a}	17.08±0.08^{b}	187.96±2.79^{a}	205.05±4.86^{a}	8.34±0.18^{a}
HRM29	584.00±4.61^{a}	93.91±0.78^{C}	126.67±0.33^{c}	35.43±0.28^{b}	47.33±1.85^{b}	47.15±0.62^{a}	18.24±0.12^{d}	198.15±0.70^{b}	216.39±1.10^{b}	8.43±0.09^{a}
CHM7	598.93±3.73^{b}	94.57±0.82^{C}	127.00±1.15^{d}	37.33±0.95^{b}	48.00±0.57^{b}	50.06±1.20^{b}	18.59±0.30^{d}	201.39±0.92^{b}	219.98±1.70^{b}	8.45±0.12^{a}
CHC4	624.00±923^{c}	95.10±0.54^{C}	127.67±0.88^{d}	37.13±0.72^{b}	49.33±1.20^{b}	49.62±1.91^{b}	18.56±012^{d}	200.93±0.53^{b}	219.49±0.81^{b}	8.46±0.08^{a}

Data were analyzed at $P<0.05$ level of significance. Mean±SE is shown in the table; each value is the mean of three replicates

Table 3. Correlation between PGP traits of bacterial isolates and oat agronomical parameters

Characters	Dry matter yield	Plant height	Straw yield	Biological yield	Forage yield	Harvest index	Grain yield	Number of grain per spike	Spike length	Weight of 1000 grain weight	Siderophore production efficiency	potassium solubilization efficiency	Zinc solubilization efficiency
Dry matter yield		0.000296	0.000258	0.0002	0.01119	0.002918	5.22E-05	0.000717	0.001869	0.095258	0.1089	0.28022	0.39202
Plant height	0.95029		6.69E-05	5.70E-05	0.01590	0.009339	6.91E-05	0.001111	0.004364	0.054586	0.33731	0.30049	0.2364
Straw yield	0.95254	0.96987		2.37E-11	0.02070	0.012726	1.21E-05	0.000786	0.000975	0.022173	0.18701	0.12301	0.35363
Biological yield	0.95643	0.97144	0.99979		0.02005	0.010548	5.95E-06	0.00075	0.000973	0.022243	0.18197	0.12778	0.34872
Forage yield	0.82767	0.80512	0.7861	0.78851		0.052772	0.018444	0.000337	0.003928	0.083933	0.43811	0.17404	0.14548
Harvest index	0.89177	0.83824	0.81978	0.83123	0.70093		0.001725	0.019877	0.027912	0.084685	0.15227	0.42246	0.3293
Grain yield	0.97227	0.96954	0.98304	0.9866	0.79468	0.90958		0.000966	0.00164	0.026174	0.15343	0.17825	0.31748
Number of grain per spike	0.93293	0.92218	0.93079	0.93188	0.94807	0.78918	0.92577		0.000871	0.040906	0.24595	0.13343	0.29227
Spike length	0.90708	0.8757	0.92555	0.92559	0.88014	0.76219	0.91113	0.92834		0.017006	0.19342	0.042068	0.1659
Weight of 1000 grain weight	0.6283	0.69723	0.78086	0.78062	0.64539	0.64421	0.76757	0.72727	0.80047		0.49405	0.006559	0.17328
Siderophore production efficiency	0.60919	0.39164	0.51949	0.52458	0.32105	0.55618	0.55488	0.46474	0.51314	0.28488		0.35662	0.5275
Potassium solubilization efficiency	0.43598	0.41978	0.59082	0.58489	0.53271	0.33151	0.52837	0.578	0.7245	0.85692	0.37746		0.452
Zinc solubilization efficiency	0.35233	0.47311	0.37962	0.38321	0.56386	0.39764	0.4066	0.42628	0.54129	0.5335	-0.26401	0.31191	

maximum 12.20% increment in 1000-grain weight was recorded for PAU 12 inoculated plants. The highest grain and straw yield was in PAU 12 and CHM 7 primed plants, respectively (Table 2). Akbar et al (2019) and Yadav et al (2014) also mentioned the increase in grain weight, grain yield and straw yield upon priming with potential PGPR. In addition to these findings, the present investigation demonstrated a significant harvest index (8.50) of oat in PAU 12 primed plants. The present study was also supported by numerous earlier studies conducted with potential PGPR and reported a remarkable increment in plant agronomical and yield attributes in many crops (Kalita et al 2015, Roychowdhury et al 2016, Nawaz et al 2020).

Correlation coefficient analysis: Statistical analysis between PGP properties and oat yield parameters demonstrated a highly positive correlation among them and illustrated that amplitude of PGPR activity significantly enhanced the yield and other agronomical parameters of oat (Table 3). The findings of Singh et al (2011) also supports the present study with a highly positive correlation among phosphate solubilizing bacteria, growth and yield attributes of lentil. TshibuyiKasu-Bandi et al (2019) also recorded the positive correlation of PGPR on agronomical parameters of soybean.

Cluster and Principal component analysis: The relative effect of PGPR on yield attributes of oat plants was performed through principal component and cluster analysis and results of these analyses revealed that PAU 12 and CHM 7 are fall on the right side which confirmed their maximum positive influence on plant yield attributes (Fig. 1). The cluster analysis also revealed that PGPR PAU 12, CHC4 and CHM 7 were the potential PGPR as they had clustered together on the left side (Fig. 2). Similarly, TshibuyiKasu-Bandi et al (2019) also determined the best PGPR that had maximum

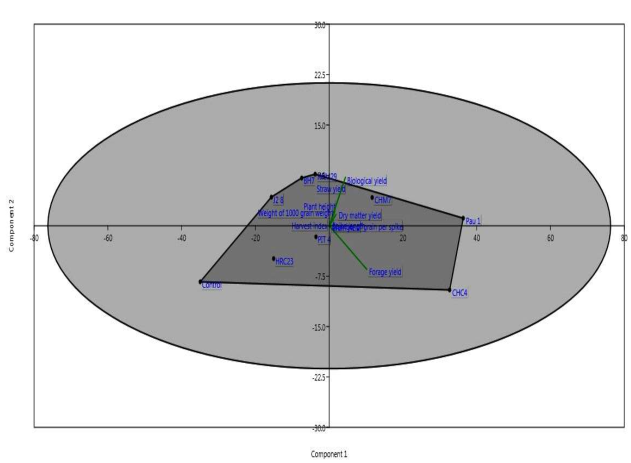

Fig. 1. Principal component analysis depicting the influence of PGPR on growth and yield attributes of forage oat

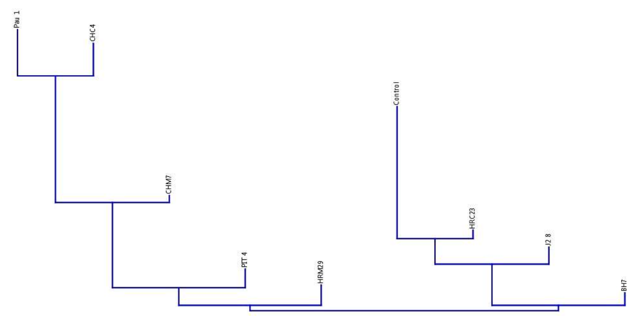

Fig. 2. Cluster analysis of treatments depicting grouping on the basis of their plant growth promotion potential

effect on agronomical parameters through principal component analysis (PCA).

CONCLUSION

PAU 12 and CHM 7 were exhibited utmost potential for plant growth promotion, as they significantly improved yield and other agronomical attributes of forage oat. Positive effects and their relative amplitude on yield were also supported by the correlation, principal component and cluster analysis. Hence, after further field evaluations, these PGPR could be utilized as plant probiotics to augment the forage and seed yield of oat to maintain sustainable, economical and eco-friendly agriculture.

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Standardisation of Assay of L-glutaminase Activity in Arid and Semi Arid Tropical Soils of South India

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Abstract: L-glutaminase (L-glutamine amidohydrolase E.C. 3.5.1.2) is the soil enzyme that catalyzes the hydrolysis of L-glutamine and produces L-glutamic acid and ammonia. Thirty surface soil samples of varying physico-chemical properties representing, various cropping systems were collected from the Rajendranagar campus of Hyderabad. The procedure for assay of L-glutaminase activity in soils was standardized and quantification was compared between the modified indophenol blue method and the steam distillation method. A linear regression analysis (R^2 = 0.997) was carried out for the activity of enzymes obtained by the modified indophenol method against steam distillation. Assay its activity is described, which involves determination of the NH_4^+ released by L-glutaminase activity when 10 grams of soil is incubated with L-glutamine, THAM buffer (pH 8) and toluene at 37°C for 4 h. Incubation was terminated by the addition of 2.5 M KCl containing a L-glutaminase inhibitor (100 ppm Ag_2SO_4). The NH_4^+-N released soil suspension is determined modified indophenol method. Correlation analysis between the soil properties and L-glutaminase activity indicated that L-glutaminase was significantly and positively correlated with organic carbon, total nitrogen, clay content (and available nitrogen for red soils. Similarly, in black soils, these were positive and significant also, coefficient correlation with O.C, total N, clay content and available N. For all the soils (red and black soils together) highly significant correlation was with O.C, total nitrogen, clay content and available nitrogen. However, there was no significant correlation between L-glutaminase activity and pH, total soluble salt content.

Keywords: L-glutaminase, Modified indophenol blue method, O.C, Total N, Clay content

Soil is the medium for crop growth through which nitrogen is provided to the plants. The nitrogen in the organic forms is mineralized and made available to crop growth through the action of microorganisms and soil enzymes. The organic form of N is a major component of soil organic matter and may account for greater than 95% of the total N in most of the surface soils. About half of this organic N has not yet been identified. It has been estimated that about 20-40% of the total N in soils is present in the form of amino acids, but only a small portion of the amino acids are present in a "free" state and the major portion is bound to soil organic matter (Bremner 2006). The amino acids bound to the soil organic matter are most likely in the form of proteins or associated with the clay-organic matter complexes as peptides, amides or arylamides. These amino acids are released from the soil organic matter by the activities of enzymes such as arylamidase, and later the "free" amino acids are hydrolyzed by specific enzymes, producing NH_4^+, which in turn is nitrified for plant uptake. Among the aminohydrolases, L-glutaminase (L-glutamine amidohydrolase E.C. 3.5.1.2) is one that acts on free amino acids in soils. L-glutaminase is the enzyme that catalyzes hydrolysis of L-glutamine to glutamic acid and ammonium, thus it is important in making the amide form of nitrogen available to plants (Hojjati and Nourbakhsh 2007). For the assessment of amidase enzyme activity, the quantification of ammonical nitrogen is an essential requirement. Quantification of NH_4^+-N in KCl extracts of soils has been attempted by the steam distillation method (Frankenberger and Tabatabai 1991a) and the modified indophenol blue method (Dorich and Nelson 1983). A number of soil properties like organic carbon, pH, total and available N, CEC, clay content, and nutrient content have influenced L-glutaminase activity. Studies on the distribution of L-glutaminase in soil profile samples have shown that the activity decreased with soil depth, which was accompanied by decreasing O.C content. A significant relationship among soil L-glutaminase and L-asparginase activity and O.C has been observed by Frankenberger and Tabatabai (1991b) in diverse soils of Iowa State. Further, they observed a strong relationship between the soil nitrogen content and the activity of enzymes, mainly because of their association with O.C (Nahidan and Nourbakhsh 2018). Increasing soil enzyme activity is important to increasing plant and microbial access to nutrients, leading to more efficient cycling of nutrients. The available literature on L-glutaminase activity is scanty and no systematic investigation seems to have been carried out on the L-glutaminase activity, its determination and methodology, especially under Indian conditions (low organic matter content soils), and hence the present research work was carried out.

MATERIAL AND METHODS

Thirty soil samples (10 red soils and 20 black soils) of varying physico-chemical properties representing, various cropping systems were collected from the Rajendranagar campus of Hyderabad by the quartering method. These soil samples were air dried and passed through a 2 mm sieve before use. The samples were analyzed for soil properties, viz., physical, physico-chemical and chemical properties, by using standard procedures (Jackson, 1973). Soil pH, electrical conductivity (dSm^{-1}), available potassium (kg ha^{-1}) and total nitrogen (%) were determined as described by Jackson (1973). The mechanical composition (particle size analysis) of soils was determined by the Bouyoucos hydrometer method. The relative proportions of sand, silt and clay soils were determined to describe their textural classes (Singh, 1980). Organic carbon (%) in soil was determined by the chromic acid wet digestion method given by Walkley and Black (1934). The available nitrogen was determined by a Macro Kjeldahl distillation method using alkaline potassium permanganate as described by Subbiah and Asija (1956) and expressed in kg ha^{-1}. The available phosphorus was determined by Olsen's method (Olsens et al 1954). The intensity of the blue colour was developed by L-ascorbic acid and was measured using a UV-1800 spectrophotometer at 660 nm and expressed in kg P_2O_5 ha^{-1}.

Assay of soil L-Glutaminase (L-glutamine amidohydrolase E.C. 3.5.1.2): L-glutaminase activity was assayed by incubating the soil samples with L-glutamine as described by Frankenberger and Tabatabai (1991a), with some modifications. For the quantification rate of release of NH_4^+-N estimated by the ammonical distillation method and the modified indophenol method. Soil sample (10 g) was taken in a 150 ml conical flask and adds 0.4 ml of Toluene, to which 12 ml of 0.1 M THAM buffer of pH 8 was added. The flasks were gently swirled to mix the contents, followed by the addition of 8 ml of 0.125 M L-glutamine (pH 8) was added, so that the concentration substrate was 50 mM. The flasks were gently shaken for a few seconds and covered with polythene paper. Then the contents were incubated at 37±0.5°C for 4 hours in the BOD incubator. After incubation, the reaction was terminated by the addition of 30 ml of KCl-Ag_2SO_4 solution. The contents were agitated on a mechanical shaker for 30 min to release all NH_4^+ formed and the suspension was allowed to settle and filter. In the controls, the same procedure described above was followed, but the L-glutamine solution was added after deactivating with the KCl-Ag_2SO_4 reagent.

Steam distillation of KCl extract: The activity of L-glutaminase was assayed by the steam distillation method, which was followed as given by Frankenberger and Tabatabai (1991a).

Modified Indophenol method: This method was followed as given by Dorich and Nelson (1983). One ml of supernatant from the soil suspension after incubation with L-glutamine and deactivation with KCl-Ag_2SO_4 was transferred to a 25 ml volumetric flask. To this, 1 ml of 6% EDTA (EDTA complexes di and trivalent cations present in the extract and prevents precipitate formation) was added, followed by the addition of 2 ml of phenol-nitroprusside and 8 ml of buffered hypochlorite reagent (this was prepared by dissolving 14.8 g of NaOH and 49.8 g of Na_2HPO_4 in 400 ml of distilled water, adding 400 ml of NaOCl (4-5%), adjusting the pH to 11.8 and making up to 1 liter). The volume was then made up to the mark with double distilled water, mixed thoroughly by inverting several times and placed in a water bath for 30 min at 40 °C for color development. The flasks were removed and brought to room temperature and the absorbance of the blue color was measured at 636 nm using UV-1800 spectrophotometer. The L-glutaminase activity was measured with respect to the amount of NH_4^+ liberated and expressed as µg of NH_4^+ released g^{-1} soil $4h^{-1}$.

RESULTS AND DISCUSSION

Thirty soil samples (10 red soils and 20 black soils) of varying Physico chemical properties representing various cropping systems were collected from the Rajendranagar campus of Hyderabad. The details of soil samples collected are presented in Table 1. In Table 2, the physical, physico-chemical, and chemical properties of soil samples were presented.

Standardization of assay of L-Glutaminase: In the present investigation, an attempt was made to standardize the assay of L-glutaminase activity and compare the amount of NH_4^+ liberated by the steam distillation method (Frankenberger and Tabatabai, 1991a) and the modified Indophenol method as given by Dorich and Nelson (1983). The preliminary studies carried out in the laboratory indicated that 10 grams of soil, 4 hours of incubation and incubation of soil at pH 8 were optimum for the assay of this enzyme. Another problem associated with the assay of L-glutaminase was the elimination of enzymes produced by the growing population of soil microorganisms and the assimilation of reaction products during the assay of abiontic enzymes, as they don't include the enzymes accumulated by growing microorganisms. To overcome this problem, the use of toluene was recommended (Frankenberger and Tabatabai 1991b). Hence, in the present study, toluene was used as a microbial biostatic agent. However, the results reported on the use of toluene were contradictory, with several authors showing increased activity of enzymes by the addition of toluene and a decrease in activity, especially for soil urease and phosphatase (Tabatabai and Bremner 1972, Skujins 1978). However, in the present study, toluene was used as a microbial biostatic agent because of its incubation period of 4 hours, which might induce proliferation of microbial cells during assaying.

The L-glutaminase activity determined by the above two methods is presented in Table 3. The activity of L-glutaminase by steam distillation varied from 3.13 to 23.41 µg of NH_4^+ released g^{-1} soil $4h^{-1}$ and for the modified Indophenol method, the values varied from 3.25 to 23.50. A linear regression analysis (R^2 = 0.997) was carried out for the activity of enzymes obtained by the modified indophenol method against steam distillation. The paired t-test was carried out between these two methods and results indicated no significant difference between the values obtained by these two methods, suggesting that both these methods can be used for the assay of L-glutaminase activity. However, considering the sensitivity of the modified indophenol blue

Table 1. Details of soil samples collected

Sample number	Crop	Soil type	Location of sample collected
V1	Forest	Black	Agricultural Research Institute
V2	Mango	Black	Agricultural Research Institute
V3	Paddy	Black	Rice Section (ARI)
V4	Sapota	Black	Agricultural Research Institute
V5	Maize	Black	All India Coordinated Research Project Maize (ARI)
V6	Maize	Black	Soil Test Crop Response field (ARI)
V7	Paddy	Black	Agricultural Research Institute
V8	Maize	Black	Integrated Farming Systems, ARI
V9	Aerobic paddy	Black	College Farm
V10	Paddy	Black	College farm
V11	Cotton	Black	College farm
V12	Agro forestry	Black	AICRP on Agro Forestry
V13	Paddy	Black	Student farm
V14	Sesame	Black	Indian Institute of Oil Seeds Research, Rajendranagar
V15	Safflower	Black	Indian Institute of Oil Seeds Research, Rajendranagar
V16	Sunflower	Black	Indian Institute of Oil Seeds Research, Rajendranagar
V17	Castor	Black	Indian Institute of Oil Seeds Research, Rajendranagar
V18	Korra	Black	College Farm
V19	Cauliflower	Black	College Farm
V20	Paddy	Black	Indian Institute of Rice Research, Rajendranagar
A1	Teak block	Red	Bio diversity park, Rajendranagar
A2	Pomogranate	Red	Horticultural university, Rajendranagar
A3	Guava	Red	Horticultural university, Rajendranagar
A4	Capsicum	Red	Shade net (ARI)
A5	Mahu block	Red	Bio diversity park, Rajendranagar
A6	Ground nut	Red	Seed Research and Technology Center, Rajendranagar
A7	Brinjal	Red	Horticulture Garden
A8	Red gram	Red	Student farm
A9	Custard apple	Red	Horticultural university, Rajendranagar
A10	Mango	Red	NAARM

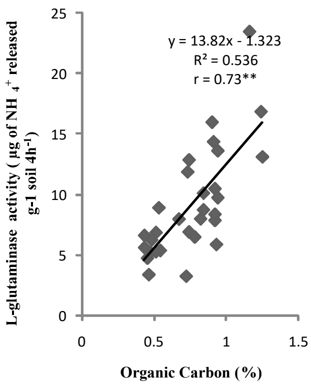

Fig. 1. Relationship between L-glutaminasc activity and % organic C in soils

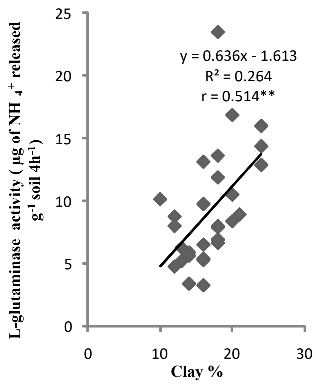

Fig. 2. Relationship between L-glutaminasc activity and clay

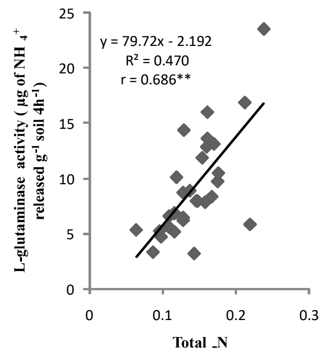

Fig. 3. Relationship between L-glutaminasc activity and Total-N

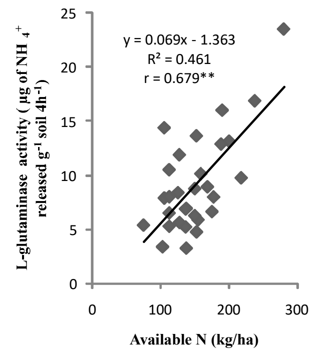

Fig. 4. Relationship between L-glutaminasc activity and Available N

Table 2. Physical, physico-chemical and chemical properties of surface soil samples

S.No.	pH	EC (dsm^{-1})	OC%	Available N kg ha^{-1}	Available P_2O_5 kg ha^{-1}	Available K_2O kg ha^{-1}	Total N (%)	Clay (%)	Silt (%)	Sand (%)	Texture
V1	7.98	0.152	1.25	200.70	81.24	734.72	0.170	16	24	60	Sandy loam
V2	7.81	0.154	1.16	280.70	68.23	675.36	0.238	18	28	54	Sandy loam
V3	8.23	0.479	0.84	150.50	92.30	659.68	0.128	12	16	72	Loamy sand
V4	7.86	0.136	1.24	238.30	53.76	704.48	0.212	20	44	36	Loam
V5	7.93	0.490	0.94	218.50	72.36	607.04	0.175	16	22	62	Sandy loam
V6	7.64	0.149	0.74	137.98	61.50	323.68	0.116	18	18	64	Sandy clay loam
V7	7.67	0.343	0.43	175.61	35.84	211.68	0.109	18	12	70	Sandy loam
V8	8.32	0.127	0.92	112.90	71.80	331.52	0.176	20	26	54	Sandy clay loam
V9	7.86	0.116	0.93	155.30	71.80	272.16	0.219	14	21	65	Sandy loam
V10	7.96	0.302	0.48	150.50	87.20	189.28	0.128	13	25	62	Sandy loam
V11	7.80	0.174	0.51	137.00	51.29	374.08	0.116	18	16	66	Sandy clay loam
V12	7.58	0.066	0.72	138.00	51.29	812.00	0.143	16	8	76	Sandy loam
V13	7.74	0.664	0.46	103.20	56.50	610.40	0.087	14	16	70	Loamy sand
V14	7.92	0.272	0.91	105.60	46.17	775.04	0.129	24	22	54	Sandy clay loam
V15	7.52	0.294	0.94	152.80	51.30	680.96	0.161	18	36	56	Sandy loam
V16	8.06	0.312	0.92	105.80	66.70	482.72	0.158	18	12	70	Sandy loam
V17	7.69	0.291	0.92	125.80	61.50	465.92	0.167	20	14	66	Sandy loam
V18	6.80	0.179	0.43	128.00	44.57	312.00	0.109	14	16	70	Sandy loam
V19	7.20	0.182	0.47	137.00	45.14	298.00	0.116	13	12	75	Sandy loam
V20	7.90	0.240	0.53	169.00	44.80	346.00	0.137	21	24	55	Sandy clay loam
A1	6.98	0.080	0.90	190.53	56.40	514.08	0.161	24	10	66	Sandy clay loam
A2	7.56	0.153	0.51	113.00	41.03	285.60	0.096	16	4	80	Sandy loam
A3	7.58	0.163	0.82	113.00	41.03	294.50	0.146	12	8	80	Loamy sand
A4	7.58	0.722	0.45	153.07	85.30	1043.84	0.098	12	24	64	Sandy loam
A5	7.18	0.064	0.74	189.00	66.68	445.76	0.160	24	6	70	Sandy clay loam
A6	6.98	0.375	0.78	113.00	61.50	618.24	0.128	16	18	66	Sandy loam
A7	7.18	0.325	0.84	159.30	66.68	450.24	0.119	10	14	76	Loamy sand
A8	7.55	0.174	0.73	87.80	56.50	564.48	0.154	18	22	60	Sandy loam
A9	7.62	0.196	0.54	75.26	46.16	303.52	0.064	16	4	80	Sandy loam
A10	6.92	0.167	0.67	178.00	46.20	321.00	0.148	18	21	61	Sandy loam

method, its accuracy and its capability to carry out the assay of a large number of samples at a given time, was higher.

Distribution of L-glutaminase and its correlation with physico-chemical properties of soils: The L-glutaminase activity of surface soils (NH_4^+ released g^{-1} soil $4h^{-1}$) varied from 3.25 to 23.50 with an average value of 9.15. Simple correlation analysis (Table 4) indicated that L-glutaminase was significantly and positively correlated with organic carbon, total nitrogen and clay content and available nitrogen for red soils. Similarly, in black soils, there was also a positive

Table 3. L–glutaminase activity (µg of NH_4^+ released g^{-1} soil $4h^{-1}$) in soil samples determined by Modified indophenol Blue method and Steam distillation method

Soil samples	Modified indophenol blue method	Steam distillation method
V1	13.13	13.41
V2	23.5	23.41
V3	8.75	8.92
V4	16.87	16.53
V5	9.75	10.20
V6	6.92	6.78
V7	6.63	6.76
V8	10.50	10.81
V9	5.88	6.13
V10	6.25	6.41
V11	6.87	6.73
V12	3.25	3.13
V13	3.38	3.91
V14	14.38	14.50
V15	13.63	13.72
V16	7.88	8.00
V17	8.38	8.13
V18	5.62	5.50
V19	5.21	5.41
V20	8.92	9.14
A1	16.00	16.00
A2	5.28	5.41
A3	8.00	7.80
A4	4.75	4.72
A5	12.87	13.12
A6	6.50	6.88
A7	10.13	10.50
A8	11.88	11.88
A9	5.38	5.50
A10	7.98	8.43
Mean	9.15	9.26

Table 4. Coefficient of correlation between different soil properties and soil L-glutaminase enzyme activity in different soils

Soil properties	Coefficient of correlation for L-glutaminase		
	Red soils	Black soils	Red + Black soils
OC %	0.75*	0.74**	0.73**
Total N	0.78**	0.688**	0.686**
Available N	0.664*	0.69**	0.679**
Clay %	0.664*	0.475*	0.514**
pH	0.11	0.1	0.1
E.C	-0.55	-0.27	-0.3

and significant coefficient correlation with O.C, total N, clay content and available N. For all the soils (red soils and black soils together) highly significant correlation was noticed with O.C, total nitrogen, clay content and available nitrogen. However, there was no significant correlation among L-glutaminase activity, pH and total soluble salt content. Soil enzyme activities are, in general, significantly correlated with soil organic carbon because of possible immobilization on soil organic matter (Boerner et al 2005). The enzyme L-glutaminase might be immobilized on humus and organic polymers or entrapped in their polymeric networks. In the present study, though the organic matter content was low to medium, it played a predominant role in enhancing the activity of L-glutaminase (Frankenberger and Tabatabai 1991c, Hu and Cao 2007, Zimmermanet and Ahn 2011). The significant and positive correlation with clay content indicates that the tropical soil, which contains large quantities of clay, could play an important role in immobilizing and entrapping the enzymes either on the edges or clay surfaces, and sometimes getting entrapped in between layers of the crystal lattice. Further, there could be a possibility of the formation of stable clay and humus complexes on which the immobilization of enzymes occurs and expresses their activity. The significant and positive correlation of L-glutaminase with total nitrogen and available nitrogen indicates that the considerable portions of nitrogen present in the amide or amine form are easily subjected to hydrolysis. Similar results were reported for other amidases (Frankenberger and Tabatabai 1991b, Frankenberger and Tabatabai 1991c, Vandana et al 2012).

CONCLUSION

For the assay of L-glutaminase 10 grams of soil, 4 hours of incubation and incubation of soil at pH 8 were optimum. Linear regression analysis indicated no significant difference between values obtained by these two methods, suggesting that the modified indophenol blue method was simple, accurate and the number of samples analysed can be increased at any given time. Correlations between the soil properties and L-glutaminase activity indicated that L-glutaminase activity was significantly and positively correlated with OC, clay content, total nitrogen and available nitrogen. The high correlation between enzyme activity and organic carbon content is because the increase in organic carbon and total nitrogen content could serve as a basis for an increase in soil enzyme activity. Therefore, it can be concluded that the organic matter content of the soil is the main factor controlling variations in enzyme activity. So, this study helps to find some cause-effect relationship between soil properties and enzyme activities. However, there was no significant correlation with pH and EC.

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Land use Classification and Change Detection of Bastar District, Chhattisgarh State, India by using GIS and Remote Sensing Techniques

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Abstract: Change detection is one of the landscape ecological objectives to prepare land use land cover. Change detections were performed by using remote sensing and GIS techniques. The study has been done through multitemporal satellite data set by LISS-III Multispectral scanner (MSS), LISS-III (2005 and 2015) matching topographic map. The land use and land cover classification maps were prepared through remote sensing and GIS technology. Ground truth was also performed to check the accuracy of the land classification. There was a significant change in land use and their dynamics. Built up urban (201.07%), rural (2156.23%), agricultural plantation (22.20%), cropland (22.79%) and barren/unculturable/wastelands/sandy area (300.00%) were increased in land use of 2015 as compared to 2005. Similarly agricultural fallow, deciduous forest, forest scrub and barren/unculturable/wastelands scrubland were negative as like -74.43%, -15.23%, -43.23% and -38.10%, respectively. The highest positive change was observed in built up rural area (2156.23%), whereas the lowest change in water body (4.93%)

Keywords: LULC, Change detection, Image processing, GIS and remote sensing

The land is the most dynamic natural resource, which includes soil and water together simultaneously associates the flora and fauna, thus expressing the total earth ecosystem. Information on spatial distribution of land use and land cover is essential for the future planning and management (Riebsame et al 1994). The term land cover describes the types of features present on the surface of the earth. Land use refers to 'man's activities on land, which are directly related to land' (Yuan et al 2005). Land use is characterized by the arrangements, activities and inputs in a certain land cover type to produce change or remain unchanged. Kumar et al (2013) expressed the land cover that establishes a direct link between land cover and the anthropogenic activities in their environment. Land cover does not focus on economic function which is essential to the perception of land use. Land use and land cover changes is a widespread and accelerating process, mainly driven by natural phenomena and anthropogenic activities, which in turn drive changes that would impact natural ecosystem (Ruiz-Luna and Berlanga-Robles, 2003, Turner and Ruscher 2004). Since from beginning of invention of remote sensing (RS) and geographical information system (GIS) techniques, land resource mapping has given useful tools and opened the ways to improve the areas designed for agriculture and other uses of a region (Selcuk et al 2003). El Gammal et al (2010) have used several Landsat images of different time periods i.e. 1972, 1982, 1987, 2000, 2003 and 2008 and processed these images in ERDAS and ARC-GIS software to analyze the changes in the shores of the lake and in its water volume. It was explained that the change analysis based on the statistics extracted from the four land use/cover maps of the Kathmandu Metropolitan by using GIS. Land use is influenced by different factors (economic, cultural, political, historical and land-tenure) at multiple scales. On the other hand, biophysical attributes of the land that affect how ecosystems function (Turner et al 1995). Pontius and Batchu (2003) calibrated on land use change pattern and also validated the disturbance in the Western Ghats of India (1920-1990). IIRS (2004a) has clearly brought out 36 LULC classes with description of 17 vegetation cover and/or other land uses. Inter-institute level collaborative work was also initiated by Indian Institute of Remote Sensing (IIRS), Dehradun, India for study for South Central Asian Region as part of this programme. Earlier studies have demonstrated substantial contribution of RS and GIS techniques to mapping habitats (Roy and Tomar 2001, Amarnath et al 2003, Roy and Behera 2005). Gagas watershed has been quantified the land use and land cover of Almora district using survey of India topographic sheet of the year 1965 and LISS III satellite data for the year 2008 over a period of 43 years (Pooja et al 2012). Rawat *et al.* (2013) have done a study on land use and land cover of Ramnagar, Nainital, Bhimtal, Almora and Haldwani of Kumaun Himalaya in Uttarakhand state of India. Similar study of mapping Srinagar city in

Kashmir Valley with observing the Srinagar city significant changed during 1990 to 2007done by Amin et al (2012). Mehta et al (2012) presented an integrated approach of remote sensing and GIS for land use and land cover study of arid environment of Kutch region in Gujarat during 1999 and 2009. Kumar *et al* (2013) estimated the biomass of Sariska Wildlife Reserve with forest inventory and geospatial approach to develop a model based on the statistical correlation between biomass measured at plot level and the associated spectral characteristics. The data on land use included forest, forest scrub, Barren scrub, water body, agriculture and built up were taken in study for temporal change over the district. The district having 1047000 hectares area covering seven blocks. The different blocks have the range of area from 366.51 to 899.26 sq km as lowest and the highest areas.

MATERIAL AND METHODS

Study area: The study area (Fig. 1) viz., seven blocks (*Bakwand, Bastar, Tokapal, Darbh, Bastanar, Jagdalpur* and *Lohandiguda*) lies in the southern most part of Chhattisgarh, India. It extends between 81°27'03 N to 82°7'58 N latitudes and 18°40'25 E to 19°38'45 E longitudes and encompasses an area of 6795 km^2. Geologically, the blocks are made-up of laterite, basalt, sandstone, shale, limestone, granite, quartzite, charnokite, and gneiss. On an average, the study area receives about 1404.80 mm of rainfall annually. The annual maximum, minimum and average temperature of the study area stands at 42°C, 18°C and 28°C, respectively. The master stream of blocks is Indrawati River which flows from east to west and divides the blocks into two halves. The total length of streams in the Bastar district is about 535 km. The district comprises of 606 villages in seven blocks with total

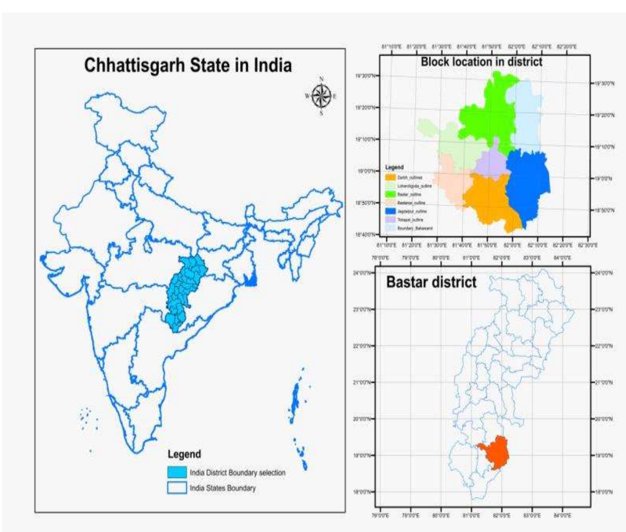

Fig. 1. Location map of Bastar district

population of 1,411,644 (Anonymous 2011). In the present study, nine land use and land cover (LULC) categories were identified namely agriculture field, agriculture field (double crop), forest scrub, barren scrub, moderate dense forest, very dense forest, concrete, river and water body. Description of these land cover classes are presented with the main objective of the study was to analyse and identify the nature and extent of land use and land cover changes.

Database preparation: These data sets were imported in image processing software *i.e.* ERDAS Imagine version 9.3 (Leica Geosystems, Atlanta, U.S.A.) to create a false colour composite (FCC). The layer stack option in image interpreter tool box was used to generate FCCs for the study areas. The sub-setting of satellite images were performed for extracting study area from both images by geo-referenced out line boundary. Multi- temporal satellite data set observed by LISS - III, Multi spectral scanner (MSS), LISS - III, were used for the analysis. The remote sensing data used for this study include: LISS - III (2005) and LISS-III (2015) and other materials used were topographic maps. The change detection techniques by using multi-temporal satellite imagery improve in knowing landscape dynamics. The present study illustrates the spatio-temporal dynamics of land use and land cover of seven blocks of district Bastar, Chhattisgarh, India. Supervised classification technique has been chosen using maximum likelihood technique in ERDAS 9.3 Software. The images of the study area were categorized into nine different classes namely agriculture field, agriculture field (double crop), forest scrub, barren scrub, moderate dense forest, very dense forest, concrete, river and water body.

Land use and land cover detection and analysis: For the land use and land cover classification, supervised classification, method having maximum likelihood algorithm was applied in the ERDAS Imagine 9.3 Software. MLC (Maximum likelihood algorithm) is one of the wide used methods like supervised classification with remote sensing data which is based on the probability means a pixel belongs to a particular class. The spectral distance method calculates the spectral distance between the measurement vector for the candidate pixel and the mean vector for each signature. The misclassified areas were corrected using recode option in ERDAS Imagine after ground truthing. Erdas imagine and Arc GIS software were constructive tools for getting out the land use and land cover layers, toposheets and satellite imageries of Survey of India also used in study of land use and land cover classes including agriculture field, agriculture field (double crop), forest scrub, barren scrub, moderate dense forest, very dense forest, concrete, river and water body. Image processing techniques was used to make visual explanation of land use geometric correction, radiometric correction, mosaicking and clipping of the images.

Land use and land cover change detection and analysis: For performing land use and land cover change detection; a post-classification detection method was employed. A pixel-based comparison was used to produce change information on pixel basis. Thus, interpret the changes more efficiently taking the advantage of "-from, -to" information. A change matrix (Weng 2001) was produced with the help of ERDAS Imagine software. Quantitative aerial data of the overall land use and land cover changes as well as gains and losses in each category between 2005 and 2015 were then compiled. Analysis of remote sensing imagery includes the identification of different marks in image. Visual interpretation using elements (tone, shape, size, pattern, texture, shadow, and association) was used for interpretation. Digital image classifications techniques were grouped pixels represent to land cover features. This area was classified into nine classes: agriculture field, agriculture field (double crop), forest scrub, barren scrub, moderate dense forest, very dense forest, concrete, and river and water body.

RESULTS AND DISCUSSION

Image analysis and accuracy assessment was corrected contract amongst a standard assumed to be correct and a classified image of unknown class accuracy assessment of image classification was approved, using 150 points, 100 points field survey data and 50 points current topographic maps and ISRO Bhuvan land use and land cover maps. The land cover and land use representing of the LISS-III image, auxiliary data and the result of visual explanation was combined with the classification outcome using GIS in instruction to progress the classification accuracy of the classified images.

Land use and land cover of district: Accuracy assessment of the land use and land cover classification results obtained exhibited an overall accuracy of 89.25 for 2005 and 93.51% for 2015. In 2005, area of seven blocks were under unban built up 0.05% (3.10s q km) followed by rural built agricultural plantation, agricultural crop land agricultural fallow, very dense forest (Table 1). The decreasing trend of land use was observed in agricultural fallow land (-187.29 sq km), very dense forest (-582.31 sq km), moderate forest (220.63 sq km) and barren scrub-I (-19.06 sq km) and remaining land uses were increased. The higher degree of increment in rural built up area and less increment was seen under wetlands/water body. The decreasing trend was in land use during ten year span due to conversion of vacant lands into different human activities increasing size and separation of family (Fig. 2-5).

Land use and land cover change: During the last one decade, built up area both in urban (6.23 sq km) and rural (143.05 sq km), followed by agricultural plantation, cropland and barren scrub-II supposed to be increased showing more intense in agricultural crop lands but same time agricultural fallow (-96.34 sq km), very dense forest (-582.31 sq km), moderate forest (-102.10 sq km) and barren scrub-I (-19.05 sq km) were decreased due to harvesting of forest illegally and encroachment with felling activities expanded the existing land covers. Many of the abandoned lands are being converted in commercial crop and vegetable farming which resulted as increased in land use for Built up area both in urban and rural, agricultural plantation, crop land and barren scrub. To assess land encroachment for different land categories during the last two decades, a change detection were given in Table 1.

Built up urban as well as rural, agricultural plantation and cropland and barren/unculturable/wastelands/sandy area were increased in land use of 2015 as compared to 2005, Agricultural fallow, very dense forest, moderate forest and barren scrub-I were decreased due to harvesting of forest

Table 1. Land use change in different classes of Bastar district

LULC	Area (sq km)		Difference	Change as compared to 2005
	2005	2015		
Built up urban	3.10	9.33	6.23	83.16
Built up rural	6.63	149.68	143.05	891.82
Agriculture, plantation	3.54	4.33	0.79	9.18
Agriculture 1	1905.52	2339.70	434.18	9.43
Agriculture 2	251.63	64.34	-187.29	-30.78
Very dense forest	3822.30	3239.99	-582.31	-6.30
Moderate forest	510.35	289.72	-220.63	-17.88
Barren scrub 1	50.02	30.97	-19.06	-15.76
Barren scrub 2	0.20	0.81	0.61	124.08
Water body	42.60	44.70	2.10	2.04

illegally and encroachment with felling activities expanded the existing land covers. The highest positive change in built up rural and the lowest change was recorded in water body. The higher degree of increment in rural built up area and less increment was seen under wetlands/water body. The decreasing trend was analysed in barren/unculturable/wastelands, agricultural fallow, forest deciduous and forest scrub in 2015 as compared to 2005.

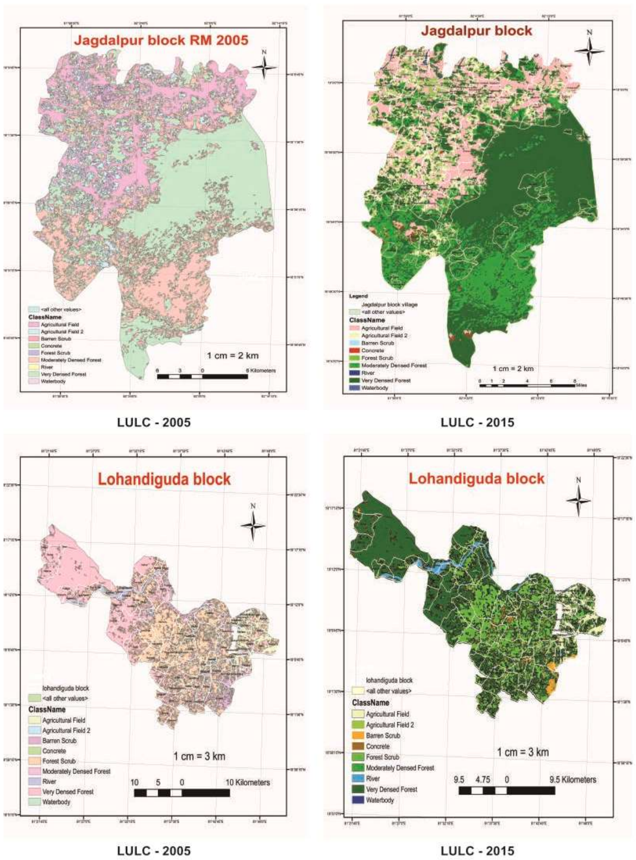

Fig. 2. LULC map of Jagdalpur and Lohandiguda blocks in 2005 and 2015

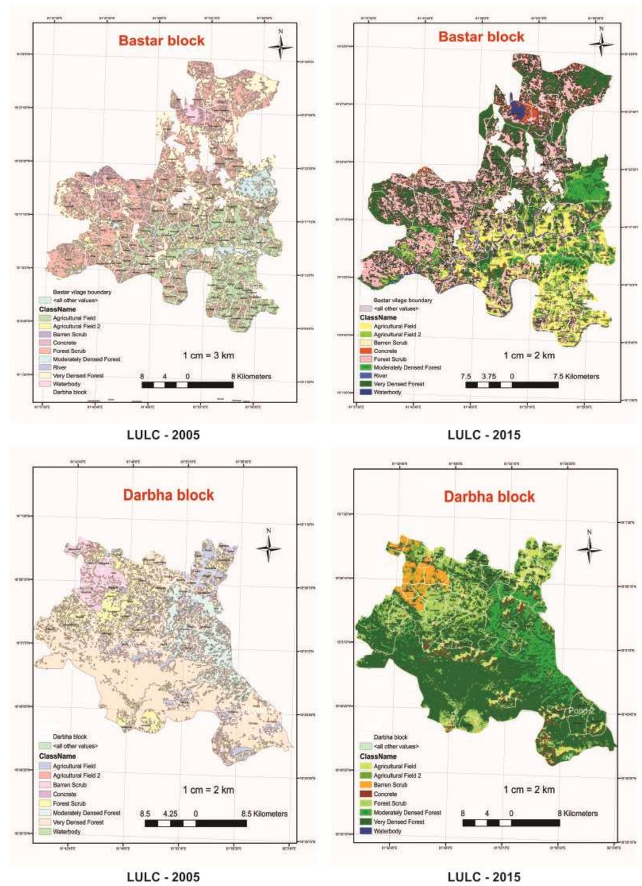

Fig. 3. LULC map of Bastar and Darbha blocks in 2005 and 2015

Table 2. Relative percentage of LULC of Bastar District

Class	LULC (Sq km) 2005		LULC (Sq km) 2015		Differences (Sq km)	
	Area	Percentage	Area	Percentage	2005	2015
Built up, urban	3.10	0.05	9.33	0.14	6.23	300.97
Built up rural	6.63	0.10	149.68	2.27	143.05	2257.62
Agriculture, plantation	3.54	0.05	4.33	0.07	0.79	122.32
Agriculture 1	1905.52	28.89	2539.7	38.52	634.18	133.28
Agriculture 2	251.63	3.81	165.29	2.51	-86.34	-65.69
Very dense forest	3822.3	57.95	3239.99	49.14	-582.31	-84.77
Moderate forest	510.35	7.74	408.25	6.19	-102.10	-79.99
Barren scrub	50.02	0.76	30.97	0.47	-19.05	-61.92
Barren scrub	0.20	0.00	0.81	0.01	0.61	405.00
Wetlands/water body/river/stream/canals	42.6	0.65	44.7	0.68	2.10	104.93

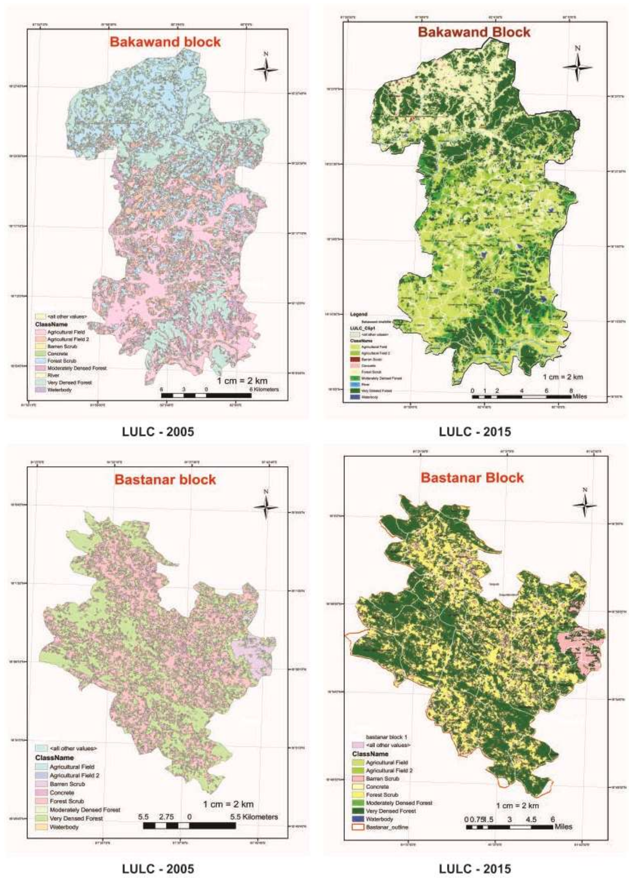

Fig. 4. LULC map of Bakawand and Bastanar blocks in 2005 and 2015

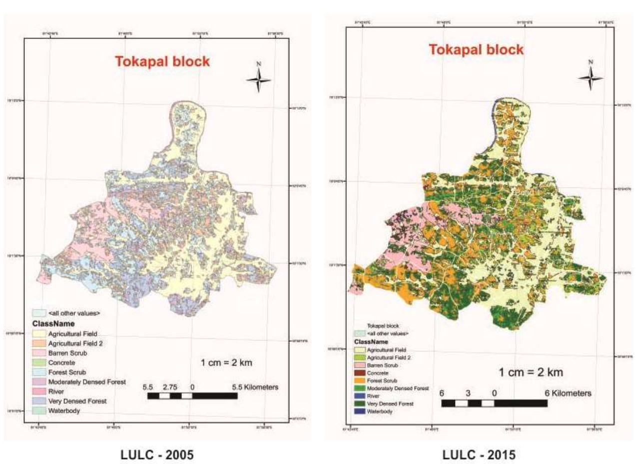

Fig. 5. LULC map of Tokapal block in 2005 and 2015

CONCLUSION

The land use and land cover through remote sensing and GIS can be an easier and fast assessing tool for assessment of land use. The higher degree of increment was recorded in settlement because ever increasing population is driving force for converting maximum available land resource that needs special attention in policy making, otherwise the land resource will shift towards settlement with other associated problems encounters regularly.

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Soil Test Crop Response Based Nutrient Management Modules for Enhancing Growth, Productivity, Profitability and Nutrient Uptake of Maize in an Acid Alfisol of North-Western Himalayas

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***Abstract*:** Soil test crop response based nutrient management modules could be extremely useful for prescribing fertilizer doses to achieve desired productivity and better soil health. The present Soil Test Crop Response (STCR) study was carried out on hybrid maize where in seven approaches of fertilization were evaluated in RBD. The experiment was conducted at the experimental farm of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, India during *kharif*, 2019. The maize growth, yield, nutrient uptake, and economics was computed. The maize growth, yield and its attributes, nutrient uptake, profit, and grain quality improved under the influence of different treatments and maximum value for measured variables was recorded in STCR based IPNS treatment of 40 q ha^{-1} and minimum in control. It was concluded from the current study that IPNS-STCR approach improved maize productivity and profitability in the western Himalayan region. This will immensely help resource poor farmers not only increasing yield but also reduce the cost of cultivation by increasing fertilizer use efficiency and saving costly fertilizers.

Keywords: Soil test crop response, Growth, Productivity, Profitability, Nutrient uptake, Maize

Rapidly increasing population and emerging production vulnerabilities spell out an urgent need for enhancing and sustaining productivity of land through cereal food production systems (Pooniya et al 2015). Under such situation, maize appears to be a potential cereal crop because of its highest genetic yield potential over other cereals and its suitability to diverse climates and management practices that is why it is known as queen of cereals (Kumar et al 2015). Maize is a major a *kharif* crop of Himachal Pradesh with an average yield of 25.5 q ha^{-1} (Choudhary et al 2013a) and Shivalik and Himalayan foothill region constitute main conventional maize production areas. Although the maize productivity is quite higher than the national and state averages, but still there is a scope to increase its yield to desired level, which may be achieved by the adoption of recommended farm technology (Choudhary et al 2015). Fertilization of crops based on generalized recommendation not only leads to under fertilization or over fertilization, but also before results in lower productivity, profitability with after along environmental pollution. Under these circumstances, the need of the day is to sustain agriculture without harming the delicate balance of soil ecology, soil fertility as well as unlocking the mystery of biota influencing plant growth by integration of fertilizers and organic manure (Chaterjee et al 2005). Among the various scientific methods of fertilizer recommendation, soil test based nutrient management approach has been found most effective to develop recommendations for potential productivity of crops and maintaining soil health. It provides a scientific basis for balanced fertilization and balance between applied nutrients and soil available nutrients (Ramamoorthy and Velayutham 2011). Use of soil test-based fertilizer adjustment equations could be extremely useful for prescribing fertilizer doses to achieve desired productivity and improving soil health. Based on this concept, soil test crop response correlation studies were undertaken in different parts of India and fertilizer prescriptions have been derived for desired yield targets of various major field and horticultural crops on different soil types and agro-climatic zones (Dey and Bhogal 2016). However, such studies have not yet been carried out for maize in most of the soil types, particularly in acid alfisols.

MATERIAL AND METHODS

Experimental site: The present study was undertaken as a part of an ongoing long-term fertilizer experiment initiated from *kharif* 2007 in a maize-wheat sequence at the Experimental Farm of Department of Soil Science, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur. The experiment was conducted on maize for one year during *kharif* 2019. The experimental farm is situated at 32° 6' N latitude and 76° 3' E longitude at an elevation of 1290 m above mean sea level.

Climate and weather: The experimental site represents the mid-hill wet temperate agro-climatic zone of Himachal

Pradesh. The region receives an average annual rainfall of 2500 mm to 3000 mm per annum with 75% of the showers occuring mainly during monsoon months (June to September). The study area received a total rainfall of 200.2 mm with a mean maximum temperature of 27.6 °C in the month of June 2019 and minimum temperature of 17.9°C during October 2019.

Soil characteristics: The soil of the experimental site belonged taxonomically to the order alfisol under the sub-group *Typic Hapludalf*. Initially, the soils under study were acidic in nature with silty clay loam texture, low in available nitrogen, high in available phosphorus and medium in available potassium and organic carbon and had sufficient micronutrients.

Field experimentation and operations: The field experiment was laid out in a RBD comprising different treatment combinations (Table 1). Each treatment was replicated thrice. Sowing of maize crop was done on June 20, 2019 and the recommended seed rate of 20 kg ha^{-1} was used. The row to row and plant to plant spacing was kept as 40 cm and 60 cm, respectively. Full doses of P_2O_5 and K_2O along with $1/3^{rd}$ dose of N as per treatments was applied at the time of sowing as a basal dose. The remaining $2/3^{rd}$ dose of nitrogen was applied in two equal splits ($1/3^{rd}$ at knee height and $1/3^{rd}$ at tasseling stage). All the recommended cultural practices were followed during the entire crop growth. After attaining the physiological maturity, maize was harvested on October 22, 2019 and grain as well as stover yield was recorded. FYM was applied @ 5 t ha^{-1} (dry weight basis) in STCR treatment with IPNS. Fertilizer doses in case of yield targeted treatments were worked out using following equations given by Verma et al. (2007):

$FN = 5.88\,T - 0.23\,SN – 0.9\,ON$ $FP_2O_5 = 4.87\,T – 1.22\,SP – 0.81\,OP$ $FK_2O = 3.66\,T – 0.49\,SK – 0.51\,OK$

In above equations, FN, FP_2O_5, FK_2O are doses of N, P_2O_5 and K_2O, respectively in kg ha^{-1}. T is yield target (q ha^{-1}), SN, SP and SK are soil available N, P and K, respectively in kg ha^{-1}. ON, OP and OK are N, P and K that were supplied through FYM (kg ha^{-1}), respectively.

Table 1. Treatment details of the experiment

Sr. No.	Treatments
T_1	Control
T_2	Farmers' practice (25% recommended dose of fertilizers + 5t ha^{-1} FYM)
T_3	General recommended dose of fertilizers (GRD)
T_4	Soil test-based fertilizer application (STB)
T_5	Target yield 30q ha^{-1} without FYM (T_{30})
T_6	Target yield 30q ha^{-1} with FYM @ 5t ha^{-1} (T_{30} IPNS)
T_7	Target yield 40q ha^{-1} without FYM (T_{40})
T_8	Target yield 40q ha^{-1} with FYM @ 5t ha^{-1} (T_{40} IPNS)

Field studies: Five randomly selected plants in each plot were tagged for various periodic observations such as growth and yield attributing characters The grain yield from each plot was recorded separately and then converted to q ha^{-1}. After removal of the cobs, the stalks were weighed to determine the stover yield (q ha^{-1}) on dry weight basis.

Laboratory studies: Representative grain and stover samples collected after maize harvest were air dried and later kept in the hot air oven at 60-70°C for eight hours. The oven dried grain and stover samples were powdered separately with Wiley milling machine and stored in paper bags and were subjected to wet digestion for further analysis as per standard procedures. The nutrient uptake was calculated by multiplying per cent concentration of a nutrient with grain and stover yield (dry weight basis) as per following formula:

Uptake (kg ha^{-1}) = [% nutrient concentration x yield in q ha^{-1} (dry weight basis)]

The uptake of the nutrients obtained in respect of grain and stover was summed up to compute the amount of total nutrients removed by the crop.

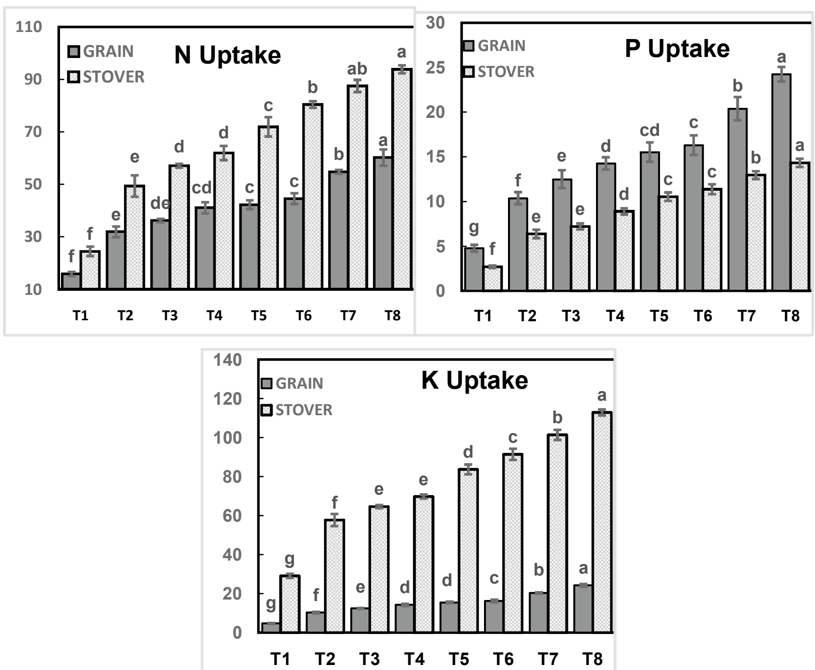
Total uptake = stover uptake + uptake by grains

Economic analysis: The economic analysis of the experiment in terms of net returns and B:C ratio was carried out by taking into consideration the prevailing prices of the inputs and outputs in the market during the study. Gross returns were calculated by multiplying the maize grain yield (q ha^{-1}) by price of maize grains and expressed in (₹ ha^{-1}). The net returns (₹ ha^{-1}) were calculated by deducting the cost of cultivation from gross returns (₹ ha^{-1}). The benefit–cost ratio was calculated by dividing gross returns with cost of cultivation.

RESULTS AND DISCUSSION

Growth parameters: In general, all the growth parameters were significantly influenced due to IPNS treatments as compared to non-IPNS treatments (Table 2). The tallest plants, maximum number of rows per cob, number of grains in a single cob, maximum 100 grain weight, longest cob length existed in the treatment corresponding to target yield of 40 q ha^{-1} with FYM (T_8), whereas least values of these parameters prevailed in T_1 (control). Application of organic and inorganic nutrient sources improved synergism and synchronization between nutrient release and plant recovery thus resulted in better crop growth (Huang et al. 2010). The increase in plant height might be since nitrogen being an essential constituent of plant tissue favours rapid cell division and its enlargement, which together with the adequate quantity of phosphorus and potassium helps in the rapid cell division and better development of the cell size ultimately produced taller plants (Meena et al 2013). These findings are in conformity with and Singh et al (2017). The maximum number of grains per row might be due to availability of N at proper time, which was required for better growth and development of plants, improved moisture retention and soil structure by organic manures. The increase in hundred grain weights could be due to balanced supply of food nutrients both from inorganic and organic manure throughout the grain filling and development period of plant (Khaliq 2004).

Uptake of major (N, P, K) and secondary nutrients (Ca, Mg, S): The uptake of major nutrients was significantly higher in all the treatments as compared to control (Fig. 1). The maximum N, P and K uptake by maize grains was recorded under T_8 (STCR based IPNS treatment for target yield of 40 q ha^{-1} and minimum in T_1(Control). Similarly, N, P and K uptake by maize stover followed the same trend and as depicted by grains, which was minimum in T_1(Control)and maximum in T_8 (target yield of 40 q ha^{-1} with FYM). Likewise, the Ca, Mg and S uptake by maize grain and stover was recorded highest in T_8 and minimum in control (T_1) (Fig. 2). The reason for



Error bars denote ± 1SE. Bars with similar lowercase letters are not significantly different with respect to least significant difference (LSD) values at p=0.05

Fig. 1. Effect of IPNS-STCR module on nitrogen, phosphorus and potassium uptake (kg ha^{-1})

Table 2. Effect of IPNS-STCR module on maize growth

Treatments	Plant height (m)		No. of rows/cob	No. of grains/row	No. of grains/cob	100 Seed weight (g)	Cob length (cm)	Cob diameter (cm)
	Tasseling	Harvest						
T1	1.1e±0.10	1.4d±0.16	12.8f±0.38	22.9f±0.78	294.6g±17.15	19.0c±1.15	11.4c±1.66	3.4g±0.03
T2	1.7d±0.08	2.2c±0.04	13.4e±0.26	37.0e±1.31	498.5f±9.32	26.6b±1.66	13.8b±0.62	3.7f±0.06
T3	1.8cd±0.05	2.3c±0.08	13.6de±0.08	38.4de±1.76	523.6ef±25.26	27.1b±0.48	14.2b±0.22	3.8ef±0.08
T4	1.9bcd±0.10	2.4bc±0.21	13.9cde±0.15	40.2cd±1.05	559.5de±20.0	27.6b±1.57	14.6b±0.11	3.9de±0.11
T5	2.0bc±0.03	2.5abc±0.12	14.1bcd±0.08	41.7bc±0.96	591.3cd±16.1	28.4ab±1.13	15.1ab±0.7	4.0cd±0.08
T6	2.1abc±0.09	2.7abc±0.14	14.3abc±0.06	43.2ab±0.63	619.7bc±10.66	29.3ab±0.33	15.5ab±0.36	4.1bc±0.04
T7	2.1ab±0.03	2.8ab±0.14	14.6ab±0.15	43.3ab±0.97	633.4ab±20.2	29.6ab±0.54	16.0ab±0.35	4.2ab±0.01
T8	2.3a±0.07	2.9a±0.04	14.8a±0.08	45.1a±0.30	668.9a±6.03	31.0a±0.57	17.2a±1.01	4.4a±0.08

Treatments with similar lowercase letters within a column are not significantly different with respect to least significant difference (LSD) values at p=0.05. The results are presented as mean ± SE increased N uptake could be ascribed to slow and continued supply of the nutrients, coupled with reduced N losses through denitrification or leaching, resulting in improved synchrony between plant N demand and supply from the soil (Haile et al 2012, Tilahun et al 2013). The form of orthophosphate ion might have converted from PO_4^{3-} to HPO_4^{2-} or even $H_2PO_4^-$ for short periods, resulting higher concentration of P in the various parts of maize plants (Siddaram et al 2011).The increased K-uptake in maize crop might be attributed to improved grain yield, better availability of potassium from organic sources and to solubility action of organic acids produced during degradation of organic materials that resulted into more release of native P and K in soil (Srinivas et al 2010). Thirunavukkarasu and Kousalya (2015) revealed that the nitrogen application through organic and inorganic sources increased the magnesium uptake indicating the synergistic effect of nitrogen on Ca and Mg. Increase in the uptake of sulphur, calcium and magnesium might also be attributed to the fact that organics are excellent sources of these nutrients and due to decomposition, mineralization and solubulization might have accelerated their availability and uptake by maize plants (Eghball et al 2002).

Uptake of micronutrients (Fe, Mn, Zn, Cu): Different treatments manifested a significant productive effect on uptake of micronutrients by maize over control (Fig. 3). The value of micronutrients uptake in grain and stover, respectively stretched from a minimum value in T_1 (control) to a maximum in T_8 (target yield of 40 q ha^{-1} with FYM). Organic manures play a dual role by adding the micronutrients to soil and increase the availability of native nutrients due to chelation, complex formation. etc. FYM is a good source of nutrients and growth promoting substances and higher uptake of these micronutrients in treatments supplied with FYM might be attributed to higher content of these micronutrients present in FYM, presence of higher microbial and enzymatic activity which stimulated the root growth and resulted in higher uptake Laxminarayana and Patiram (2006).

Productivity and profitability: The highest grain yield of 44.6 q ha^{-1} was observed in T_8 (IPNS based treatment for target yield 40 q ha^{-1}) and minimum (14.1 q ha^{-1}) in T_1 (Control) (Table 3). Among the STCR treatments, target yield of 40 q ha^{-1} with and without FYM established themselves as the superior most treatments when compared with their corresponding IPNS and non IPNS treatment with target yield of 30 q ha^{-1}, respectively. It was certainly perceptible from the data that like grain yield, stover yield also followed a similar trend with minimum value in T_1 (control) and maximum inT_8 (target yield 40 q ha^{-1} with FYM). The highest net returns of Rs. 63831 ha^{-1} were manifested by T_8 (target yield of 40 q ha^{-1} with FYM) as compared to other treatments. With respect to the benefit drawn per unit rupee invested on input, the highest B:C ratio (2.98) was perceived in the T_7 treatment (target yield of 40 q ha^{-1} without FYM), followed by the same treatment (T_8) with FYM (2.83) (Table 2). Many researchers described increased yield levels of different crops including maize in STRCR-IPNS approach due to their effect on root growth, nutrient uptake, simulation of many different enzymes related photosynthesis, efficient response to plant nutrient requirement, integrated supply of nutrients from different sources and improved nutrient supply (Suresh and Santhi 2018). The net returns and B:C ratio increased when FYM was included in the fertilizer prescription, which might be due to better use efficiency of applied NPK fertilizers at low yield target levels (Bera et al 2006). The

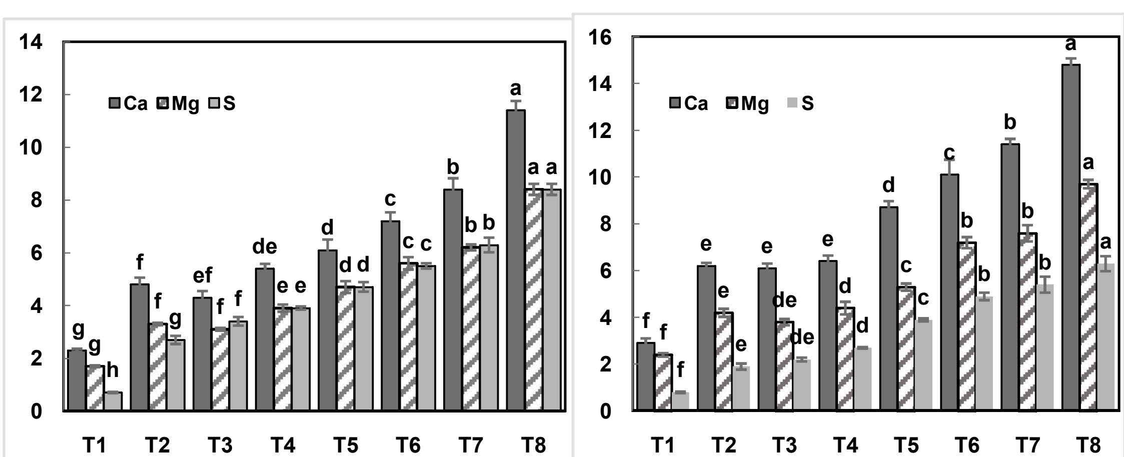


Fig. 2. Effect of IPNS-STCR module on Calcium, Magnesium and Sulphur uptake. Error bars denote ± 1SE. Bars with similar lowercase letters are not significantly different with respect to least significant difference (LSD) values at p=0.05

Table 3. Effect of IPNS-STCR module on productivity and profitability of maize

Treatments	Grain yield (q ha^{-1})	Stover yield (q ha^{-1})	Net returns (Rs ha^{-1})	B:C Ratio
T1	13.6f±0.59	23.0f±1.26	7859e	1.38e±0.05
T2	25.7e±0.98	42.2e±2.29	24801d	1.87d±0.07
T3	28.8d±0.56	47.2d±0.92	32547c	2.20c±0.05
T4	31.6c±0.69	50.3d±1.15	38565b	2.46c±0.05
T5	32.4c±1.08	57.9c±1.74	41337b	2.50b±0.03
T6	32.9c±1.24	61.2c±1.55	39214b	2.24b±0.04
T7	39.7b±0.45	69.4b±1.25	53199a	2.74ab±0.03
T8	42.7a±0.64	76.2a±0.8	55854a	2.60a±0.02

Treatments with similar lowercase letters within a column are not significantly different with respect to least significant difference (LSD) values at p=0.05. The results are presented as mean ± SE

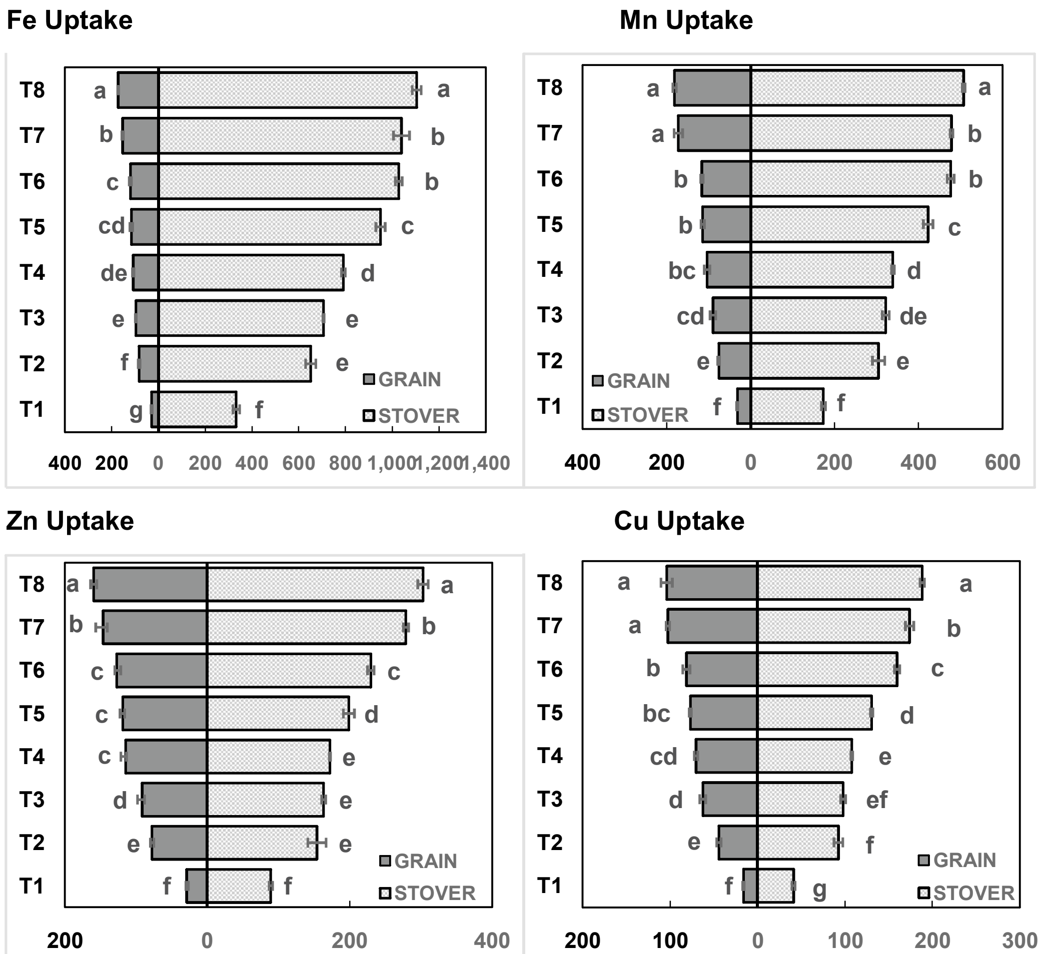


Fig. 3. Effect of IPNS-STCR module on Iron, Manganese, Zinc and Copper uptake (g ha^{-1}). Error bars denote ± 1SE.Bars with similar lowercase letters are not significantly different with respect to (LSD) least significant difference values at p=0.05

current findings are in conformity with the results obtained for different crops (Majumdar et al 2018, Choudhary 2019).

CONCLUSIONS

IPNS-STCR approach discussed in the present study will improve maize productivity and profitability in the Western Himalayan region. This will immensely help resource poor farmers not only increasing yield but also reduce the cost of cultivation by increasing fertilizer use efficiency and saving costly fertilizers.

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Impact of Nutrient Management Practices on Soil Physical and Biological Properties in an Acid Alfisol of Himachal Pradesh, India

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Abstract: The present investigation was carried out to assess the effect of nutrient management practices on soil physical and biological properties under maize crop in an acid Alfisol. The experiment consisted of eleven treatments comprising recommended NPK levels, NPK + FYM, NPK + lime, organic farming packages, NFS-*Desi* Cow, NFS-Crossbred Cow, NFS-Buffalo, and their supplementation with 25 per cent of recommended NPK. The highest water holding capacity, and mean weight diameter, while lowest bulk density were under organic farming+ 25 per cent NPK treatment. Among soil microbial properties, organic farming + 25 per cent NPK treatment showed the highest microbial biomass nitrogen, urease activity, viable bacterial, fungal, and actinomycetes count, however, all these parameters were found lowest under 100 per cent NPK treatment.

Keywords: Bulk density, Mean weight diameter, Microbial biomass nitrogen, Urease activity, Bacterial count

Productive soil is the fundamental base for harnessing the potential of intensified cropping system. In the last fifty years, globally, the size of the per capita arable landholding has significantly reduced from 0.44 ha to 0.18 ha per capita, and by 2050, it is likely to drop even lower to 0.1 ha (Mehra et al 2018). Furthermore, diminishing agricultural output, soil fertility, and mounting environmental issues add to the difficulties of agricultural sustainability and consequently, production is inadequate to meet the demands of growing population. Adoption of chemical fertilizers for increasing food grain production has largely replaced traditional practices, *viz.,* recycling of crop residues and application of organic manures. Intensive cultivation and declining soil organic carbon content results in deterioration of soil physical and microbial properties. Soil physical properties *viz.,* bulk density, porosity, and aggregate stability are the key components of soil quality, and their variation with time can reflect the agricultural sustainability. Maintenance of optimum soil physical properties is an essential component of soil fertility management. Sole application of inorganic fertilizers decreases the stability of macro-aggregates and capacity of moisture retention, however, increases the bulk density of soil. A number of management practices viz., integrated farming, organic farming and natural farming have been developed that are supposed to be more sustainable substitutes to traditional farming systems. Organic amendments in combination with inorganic fertilizers can improve aggregate stability as well as moisture retention capacity of soil. Another essential component of soil fertility management is soil microbes. Microorganisms regulate various important processes such as soil aggregate formation, humus formation, regulation of nutrient cycling, decomposition of various compounds etc. The alterations in soil organic carbon contents are directly linked with microbial biomass nitrogen (MBN), and biological activity in the soil. Microbial adaptation to environmental variabilities allows microbial analysis to be quite fruitful in soil health assessment, and therefore, microbial population dynamics may serve as an excellent indicator of change in soil health. The sole application of inorganic fertilizers significantly declined the total microbial activity, porosity, particle and bulk density of soil (Manivannan et al 2009). However, another study showed that the combined use of organic and inorganic fertilizers maintained the highest soil quality index followed by the application of 100% organic fertilizer (Schulz and Glaser 2012). Xu (2006 reported that natural farming practices can improve soil physical properties, biodiversity, and enzyme activities. As natural farming system generally involves use of formulations prepared using products of indigenous cow, therefore, little is known about the composition and characteristics of the microbial communities present in formulations prepared using products of crossbred cows and buffaloes. It is important to know the efficacy of formulations prepared from the dung and urine of these cattle (crossbred cows and buffaloes), as most of the farmers in our country are rearing these cattle as compared to indigenous one.

Therefore, this study was carried out to examine the comparative effects of different sources of nutrients on some soil physical and biological properties under maize based cropping system. We hypothesized that organic farming practices in conjunction with inorganic fertilizers might have a great beneficial impact on soil physical and microbial properties in comparison to other treatments.

MATERIAL AND METHODS

Study site: The present study was conducted in 2020 at CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur. The experimental site is located at an altitude of nearly 1290 meters above mean sea level ($32^0 6'$N latitude and $76^0 3'$ E longitude). The study site falls in the North-Western Himalayas of district Kangra and lies under mid hills sub humid agro-climatic zone of Himachal Pradesh which receives average annual rainfall of about 2750 mm. These soils belong to the order Alfisol and subgroup Typic Hapludalf and owe their origin to different kind of rocks such as slates, phyllites, quartzites, schists and gneisses. The weekly maximum and minimum temperature ranged between 26.0 to 30.5 and 13.0 to 20.1°C, respectively during crop growth period. The maize crop received a total rainfall of about 1449.0 mm and the weekly relative humidity varied from 57.95 to 92.05 per cent.

Experimental details: The experiment comprised of a randomized block design with three replications consisting eleven treatments (Table 1). Recommended dose of fertilizers for maize is 120:60:40 kg ha^{-1} of N:P_2O_5:K_2O. Urea, single super phosphate, and muriate of potash were applied as source of nitrogen, phosphorus and potassium, respectively. At the time of sowing, half dose of N and full doses of P and K were applied in treatments comprising inorganic fertilizers. The remaining half dose of N was given as top dressing in two equal splits at knee high and pre-tasseling stage of maize. Before sowing, whole quantity of FYM was given as per the treatments of the experiment. NPK content of FYM was 0.98, 0.47 and 0.85 %, respectively. Lime ($CaCO_3$) was thoroughly incorporated in the specified plots (@ 3.2 t ha^{-1}) about four weeks prior to sowing of the maize. In organic farming plots, 60 kg N ha^{-1} (50 per cent of RDF) was provided through FYM and another 60 kg N ha^{-1} was supplemented through vermicompost. In NFS plots, before sowing, the seeds were treated with *beejamrit* for 30 minutes. *Ghan-jeevamrit* was applied along with sieved FYM, followed by application of jeevamrit at sowing, and sprays of *jeevamrit* were given 5 times at 21days interval during crop growth. *Ghan-jeevamrit* was applied @ 250 kg ha^{-1} along with sieved FYM @ 250 kg ha^{-1}, jeevamrit @ 500 l ha^{-1} at sowing, and sprays of 10% *jeevamrit* were given 5 times at 21days interval during crop growth. Soybean was intercropped in the ratio of 2:1 in between the rows of maize plants. Mulching with locally available organic residues was also done. In addition to this, fermented butter milk was sprayed at 60 days after sowing (DAS) and at grain filling stage of maize (@ 12.5 l ha^{-1}). Microbial count present in *beejamrit, jeevamrit, and ghanjeevamrit* formulations prepared using products of different cattle (Table 2).

Sample analysis: After the harvest of maize, soil samples

Table 1. Effect of conventional, organic and natural farming treatments on bulk density, water holding capacity and mean weight diameter

Treatments details	BD (g cm^{-3})	WHC (%)	MWD (mm)
T_1- 100% NPK	1.36	45.14	2.15
T_2- 100% NPK+FYM	1.34	46.34	2.18
T_3-100% NPK+Lime	1.35	46.15	2.17
T_4- Organic farming	1.26	48.92	2.24
T_5-NFS-*Desi* cow	1.27	48.89	2.22
T_6-NFS-Crossbred cow	1.27	48.78	2.21
T_7-NFS-Buffalo	1.32	48.65	2.19
T_8-T_4+25% NPK	1.24	49.07	2.26
T_9-T_5+25% NPK	1.26	48.92	2.24
T_{10}-T_6+25% NPK	1.26	48.90	2.23
T_{11}-T_7+25% NPK	1.31	48.69	2.20
LSD (P=0.05)	0.02	0.91	0.03
Initial	1.36	44.42	2.12

BD: Bulk density, WHC: Water holding capacity, MWD: Mean weight diameter

Table 2. Microbial count in natural farming formulations prepared from products of different cattle

Excreta used	Bacteria (10^6cfu ml^{-1})	Fungi (10^4 cfu ml^{-1})	Actinomycetes (10^2cfu ml^{-1})
Beejamrit			
Desi Cow	25.1	10.3	22.6
Crossbred Cow	23.3	7.4	21.3
Buffalo	19.8	4.8	18.2
Jeevamrit			
Desi Cow	19.6	15.4	21.3
Crossbred Cow	16.4	12.3	18.6
Buffalo	14.5	10.5	16.2
Ghanjeevamrit			
Desi Cow	28.6	12.5	21.3
Crossbred Cow	26.6	11.4	19.6
Buffalo	22.4	9.8	17.4

Table 3. Method used for analysis of soil samples

Soil property	Method employed	Reference
Bulk density	Core sampler	Singh (1980)
Water holding capacity	Keen's moisture box	Richard (1954)
Mean weight diameter	Wet sieving	Yoder (1936)
Microbial biomass N	Incubation	Brookes et al (1985)
Urease activity	Colorimetry	Tabatabai and Bremner (1972)
Viable microbial count	Serial dilution pour plate	Alef and Nannipieri (1995)

were collected from 0-0.15 m depth from each plot. For determining the bulk density, soil samples from each replication were taken by using a core sampler having cores of 5 cm height and 5 cm diameter. For aggregate analysis, surface soil samples were collected in cores at field capacity moisture content. After drying, they were broken by giving gentle strokes in a wooden hammer and only aggregates of 4-8 mm size were used for analysis. The aggregate size distribution of soil was determined by wet sieving method using Yoder's apparatus. For microbiological properties, about 500 g of fresh soil samples from each plot were immediately preserved under refrigerated conditions for further analysis. Physical (bulk density, water holding capacity and aggregate stability) and biological properties (MBN, soil urease activity, viable bacterial, fungal and actinomycetes count) of soil samples were estimated (Table 3).

Data analysis: The data recorded was analyzed using MS-Excel, OPSTAT and SPSS 16.0 package as per design of the experiment.

RESULTS AND DISCUSSION

Physical Properties

Bulk density: The lowest bulk density (1.24 g cm^{-3}) was in organic farming + 25% NPK which was statistically at par with NFS-*Desi* cow + 25% NPK , NFS-Crossbred cow + 25% NPK and organic farming treatments (Table 1). Moreover, no change in bulk density was observed where 100% NPK was applied which might be due to low soil organic matter content and soil structure degradation. Bulk density reduced under organic farming + 25 per cent NPK treatment that might be due to higher soil organic carbon content, better aggregation, increased root growth, and biopores in the combined fertilizer and manure treated plots Sharma et al (2016).

Water holding capacity: Highest water holding capacity (49.07%) of soil was observed under organic farming + 25% NPK with non-significant differences with T_5, T_6, T_7, T_4, T_9, T_{10} and T_{11}, while the lowest (45.14%) was in T_1 (Table 1). Application of small dose of chemical fertilizers in combination with organic manures significantly increased the water holding capacity of soil over sole application of chemical fertilizers which could be attributed to enhanced soil structure and stable aggregates, as well as an increased number of storage pores which in turn, increased the moisture retention capacity. This clearly showed that the amount of organic matter in soil greatly affects ability to retain moisture, and organic matter not only increased the soil's water holding capacity but also increased the amount of water accessible for plant growth. Similar findings were reported by Amipara and Jadhav (2017). Water holding capacity was also improved under integrated nutrient management treatments *viz.,* 100% NPK + FYM and,100 % NPK + lime from the initial value which might be attributed to improved soil structure as well as aggregation with the application of FYM and lime). Water holding capacity was also recorded high in all the NFS treatments which could be due to increased build-up of soil carbon as a result of increased microbial activities.

Aggregate stability: MWD of soil ranged from 2.15 mm under 100% NPK treatment to 2.26 mm under organic farming + 25% NPK treatment. Initially mean weight diameter recorded was 2.12 mm which increased to 2.26 mm after the application of organic manures in combination with fertilizers (organic farming + 25 per cent NPK) which was statistically at par with NFS-*Desi* cow + 25% NPK, NFS-Crossbred cow + 25% NPK and organic farming treatments (Table 1). The highest mean weight diameter in organic farming + 25 per cent NPK might be due to release of various organic acids after decomposition of organic residues which led to greater binding of soil particles, increased soil organic matter content and greater earthworms activity thereby, resulting in significant increase in MWD (Shepherd et al 2006). In manure amended soil, the formation of macro-aggregates was favoured if soil organic matter content increased which resisted slaking, and earthworms present might also improve aggregate stability.

Biological Properties

Microbial biomass nitrogen (MBN): The maximum MBN (26.4 µg g^{-1}) was in T_8 which was statistically at par with T_9 and T_4. Lowest MBN (19.9 µg g^{-1}) was in conventional treatment i.e., 100% NPK (Fig. 1). Farm yard manure and lime amended treatments exhibited an increase of 11.1 and 5.5% in MBN over 100% NPK treatment, respectively. The significantly highest soil MBN found under organic farming practices combined with inorganic fertilizers which might be attributed to the balanced fertilization resulted in improved plant growth, root biomass and rhizosphere activity. Moreover, increased inputs to the soil from above and below ground residues as well as rhizo-deposition might be another reason for significant increase in MBN as these materials are the main carbon sources for soil microorganisms (Chahal et al 2019). .

Soil urease activity: The significantly highest soil urease activity (42.12 µg NH_4^+-N g^{-1} soil hr^{-1}) was in organic farming + 25% NPK , while the lowest (37.21 µg NH_4^+-N g^{-1} soil hr^{-1}) was in NFS-Buffalo treatment. Organic farming + 25% NPK treatment produced non-significant differences with NFS-*Desi* cow + 25% NPK, ,NFS-Crossbred cow + 25% NPK and NFS-Buffalo+25% NPK treatments (Fig. 1). Among organic treatments, organic farming treatment recorded higher soil urease activity (39.09 µg NH_4^+-N g^{-1} soil hr^{-1}) which was found statistically at par with , NFS-*Desi* cow treatments (38.32 µg NH_4^+-N g^{-1} soil hr^{-1}). The highest urease activity recorded under organic farming practices supplemented with small amount of chemical fertilizers might be due to increasing population of microorganisms like bacteria and increased availability of substrate through organic manures (Sireesha et al 2017). It was also likely that ureolytic heterotrophic microbes were encouraged by the addition of organic materials, consequently resulted in an increased urease activity.

Viable bacterial count: The highest viable bacterial count (24.5 x 10^6 cfu g^{-1} soil) was in T_8 which produced non-significant differences with NFS-*Desi* cow + 25% NPK treatment. The lowest viable bacterial count (13.5 x 10^6 cfu g^{-1} soil) was in T_1 followed by T_3 (Fig. 2). Among organic treatments, T_4 recorded higher viable bacterial count (19.5x 10^6 cfu g^{-1} soil) which was statistically at par with T_5. The superiority of organic farming combined inorganic fertilizers practices in microbial population (bacteria and fungi) could be due to the fact that addition of FYM and vermicompost might have served as a source of carbon and energy for microorganisms. Application of organic matter provided proper aeration, moisture content and nutrients which might resulted in proliferation of microorganisms (Sharma and Banik, 2016). NFS-*Desi* cow treated plots also recorded higher viable bacterial count than other NFS treatments which might be due to high microbial count present in the NF products prepared from dung and urine of *Desi* cow.

Viable fungal count: The highest viable fungal count (14.3 x 10^4 cfu g^{-1} soil**)** was in T_8 which was statistically at par with T_9. The lowest viable fungal count (3.5 x 10^4 cfu g^{-1} soil) was in 100% NPK treatment (Fig. 2). Organic farming treatment recorded higher viable fungal count (10.0 x 10^4 cfu g^{-1} soil) with NFS-*Desi* cow treatment. Organic farming supplemented with 25 per cent of chemical fertilizers showed the highest viable fungal count which might be attributed to increased organic carbon and mineral nitrogen content providing energy and conducive environment for microbial proliferation thereby leading to increase in total fungal count. Low microbial population count in inorganic treatment might be due to poor availability of substrate to sustain microbial biomass (Sudhanshu et al 2015).

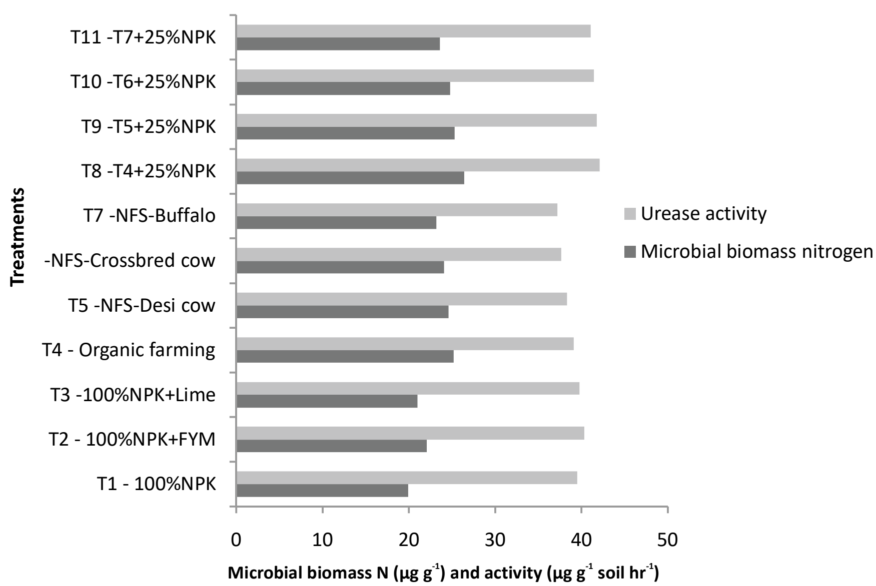

Fig. 1. Soil microbial biomass nitrogen and urease activity under different treatments

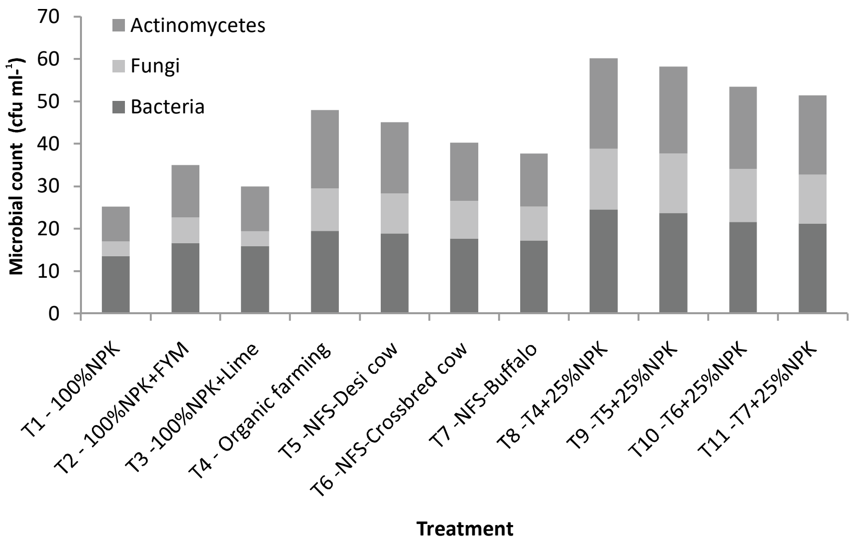

Fig. 2. Soil microbial count under different treatments

Viable actinomycetes count: The highest viable actinomycetes count (21.4 x 10^2 cfu g^{-1} soil) was in organic farming + 25% NPK treatment and was found statistically at par (20.5 x 10^2 cfu g^{-1} soil) with NFS-*Desi* cow + 25% NPK treatment (Fig. 2). Among organic treatments, higher viable actinomycetes count (18.5 x 10^2 cfu g^{-1} soil) was in organic farming, followed by T_5. The lowest viable actinomycetes count (8.2 x 10^2 cfu g^{-1} soil) was recorded under 100% NPK treatment. The highest viable actinomycetes count recorded under organic farming + 25 per cent NPK treatment could be due to the fact that majority of soil microorganisms are chemoheterotrophs, which require an organic source of carbon as food and obtain energy through the oxidation of organic substances. The result corroborates the findings of Watts et al (2010). Moreover, organic manures provided with mineral fertilizer increased crop residue and root exudates, which provide organic matter for microorganisms resulting in higher culturable microbial counts in organic manure treatments.

CONCLUSIONS

The integration of organic farming packages with inorganic fertilizers exerted a beneficial impact on soil physical properties as well as on microbial properties over sole application of organic manures or chemical fertilizers. Natural farming system had also significant positive impact on studied soil properties over conventional farming.

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Effect of Vermicompost and Fertilizer on Carbohydrate and Functional Indicator Microbes

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Abstract: An incubation experiment was carried out at Dr. RPCAU, Pusa, department of soil science consisting of four levels of vermicompost (0, 1.25, 2.5 and 3.7 t ha^{-1}) and three levels of fertilizer doses (0, 100, 50 % RDF) in different combinations along with calcareous sandy loam soil in the incubation boxes to find out the effect of vermicompost and fertilizer on soil microbiological growth and to study about effect on acid hydrolysable carbohydrate content in incubated soil. For analysis of acid hydrolysable carbohydrate content 24N H_2SO_4 and 0.2% anthrone solution was used. The microbiological analysis was conducted using different media solution and after that serial plate dilution technique. Acid hydrolysable carbohydrate and all functional indicator microbes (*Bacillus, Pseudomonas, Azotobacter, Azospirillum, Phosphate Solubilizing Bacteria, Starch hydrolyzing bacteria and Cellulose hydrolyzing bacteria*etc.) increased from 0^{th} DAI to 65^{th} DAI and then decreased from 65^{th} DAI to 115^{th} DAI. The combination of 3.75t ha^{-1} of vermicompost and full dose 100% RDF was found superior among all other treatments. Combined application of vermicompost and fertilizer always provide better results in case of soil health and microbial studies.

Keywords: Vermicompost, Fertilizer, Microbes, Carbohydrate, Incubation

Soil microbes are the important key components of soil ecosystem and their diversified activities regulates the different biochemical cycles. Soil microbial community is considered as sensitive indicator of soil quality and also acts as bio-indicator of ecosystem sustainability and very much affected by nutrient management systems; its diversity is also affected by environmental factors as well as human activities related to agriculture. Continuous fertilization especially organic material applications increase soil fertility, organic carbon thus led to enhanced soil functional microbial population (Tamilselvi et al 2015) and finally elevated yield is seen. Heavy use of nitrogenous fertilizer reduces soil base saturation, increase acidity and also reduce soil microbial population.

Organic sources are very much important in case of improving overall soil health and soil ecosystems. Sustainable microbial growth can only be possible by the application of safe, nontoxic and nutrient enriched vermicompost application in soil. The fine granular peat-like end product, vermicompost that is produced is reported to contain elevated levels of nitrogen, phosphorus, and potassium (NPK) in available form, micronutrients, microflora, enzymes, and growth regulators. Because of this, the vermicompost when applied or supplemented in soil improves crop growth and yield (Alshehrei and Ameen 2021). The INM practices with biofertilizer an organic sources enhanced the microbial biodiversity in rhizosphere and rhizospheric microbial population variation were seen (Arunjith and Isaac, 2021). The earthworms, the drivers of many processes in soil, apart from the known vermicomposting, are also found to enhance phyto-extraction of metals from contaminated soils. In addition, vermicompost, produced by the joint action of earthworms and microbes, contains nutrients in available form with increased microbial activity (Prakash 2021). To analyze the effect of vermicompost and fertilizer doses on microbial biodiversity and acid hydrolysable carbohydrate content in soil the following study was taken out.

MATERIAL AND METHODS

An incubation experiment was carried out at Dr. RPCAU Soil Science laboratory during *Kharif*, 2018 consisting of four levels of vermicompost (0, 1.25, 2.5 and 3.7 t ha^{-1}) and three levels of fertilizer doses (0, 100 and 50 % RDF) with recommended RDF dose N: P_2O_5:K_2O:: 120:60:40 kg per hector with twelve treatments replicated thrice with factorial completely randomized design. The days of incubation are indicated as S_1(0^{th} Days after incubation), S_2(30^{th} Days after incubation), S_3(65^{th} Days after incubation), S_4(115^{th} Days after incubation).

T_1: $V_0 F_0$:- No manure + No Fertilizer- Control, T_2:$V_0 F_{100}$:- No manure + 100% RDF,

T_3: $V_0 F_{50}$:- No manure + 50% RDF, T_4:$V_{1.25}F_0$:- Vermicompost (1.25tha^{-1}) + No Fertilizer,

T_5: $V_{1.25}F_{100}$:- Vermicompost (1.25t ha^{-1}) +100% RDF, T_6:$V_{1.25}F_{50}$:-Vermicompost (1.25t ha^{-1}) + 50% RDF , T_7:$V_{2.50}F_0$:- Vermicompost (2.5t ha^{-1}) + No Fertilizar, T_8:$V_{2.50}F_{100}$:- Vermicompost (2.5t ha^{-1}) + 100%RDF , T_9:$V_{2.50}F_{50}$:- Vermicompost (2.5t ha^{-1}) + 50% RDF, T_{10}:$V_{3.75}F_0$:- Vermicompost (3.75t ha^{-1}) + No Fertilizar, T_{11}:$V_{3.75}F_{100}$:- Vermicompost (3.75t ha^{-1}) + 100%RDF, T_{12}: $V_{3.75}F_{50}$:- Vermicompost (3.75t ha^{-1}) +50% RDF

Acid hydrolysable carbohydrate: Acid hydrolysable carbohydrate estimation was done by following the methods of Chesire and Mundie (1966).

Functional indicator microbes: Ten gram of soil sample was taken in 90 ml water in 500ml flask and serial dilution was made up to 10^{-5}. 0.5 ml of sample was taken from 10^{-5} dilution was spread in petri plate along with different standard media for different microbes studies like *Bacillus, Pseudomonas, Azotobacter, Azospirillum, PSB, Cellulose hydrolyzing Bacteria, starch hydrolyzing microbes* as described further by Schmidt and Coldwell (1967).

Statistical analysis: All the data obtained in the experiment will be analyzed using OPSTAT (http://14.139.232.166/opstat/default.asp) analysis software.

RESULTS AND DISCUSSION

Acid hydrolysable carbohydrate (mg kg^{-1}): The acid hydrolysable carbohydrate content in soil irrespective of incubation stages, fertilizer and vermicompost doses increased from 0^{th} DAI to 65^{th} DAI and then decreased up to 115^{th} DAI (Table 1). Irrespective of all incubation periods, the increasing

Table 1. Effect of vermicompost and fertilizer on acid hydrolysable carbohydrate (AHC) in incubation study (mg kg^{-1})

Vermicompost/Fertilizer	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
S_1						
F_0	22.07	34.51	33.33	35.92	31.46	
F_{100}	30.75	37.32	40.14	44.13	38.09	
F_{50}	28.40	34.74	36.62	42.25	35.50	
Mean	27.07	35.52	36.70	40.77		
S_2						
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	32.63	36.62	35.68	40.85	36.45	
F_{100}	35.92	39.20	45.31	50.94	42.84	
F_{50}	31.69	37.56	42.96	46.48	39.67	
Mean	33.41	37.79	41.32	46.09		
S_3						
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	61.27	111.74	107.98	131.22	103.05	
F_{100}	80.75	130.28	148.59	176.06	133.92	
F_{50}	73.94	115.26	137.32	165.73	123.06	
Mean	71.99	120.09	131.30	157.67		
S_4						Grand mean (Fertilizer)
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	55.63	97.65	88.50	100.70	85.62	64.15
F_{100}	72.30	108.45	114.55	130.28	106.40	80.32
F_{50}	67.14	103.99	105.16	125.59	100.47	74.68
Mean	65.02	103.36	102.74	118.86		
Grand mean (Vermicompost)	49.37	74.19	78.02	90.85		

Factors	CD (5%)
Factor (S)	1.33
Factor (V)	1.33
Interaction S x V	1.15
Factor (F)	2.65
Interaction S x F	2.30
Interaction V x F	2.30
Interaction S x V x F	4.60

Grand Mean is the average mean of respective values for fertilizer and vermicompost in each respective stage.
V_o= Vermicompost (0 t ha^{-1}), $V_{1.25}$= 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 (0 t ha^{-1} vermicompost +no fertilizer)

levels or doses of vermicompost increased the mean acid hydrolysable carbohydrate from 49.37 to 90.85 mg kg^{-1} soil and along with increasing fertilizers levels increased the from 64.15 to 80.32 mg kg^{-1} soil. The combination of vermicompost dose of 3.75 t ha^{-1} with 100 % RDF always showed 44.13-130.28 mg kg^{-1} at 0thto 115th DAI respectively followed by vermicompost dose of 2.5 t ha^{-1} with 100% RDF and both these two treatment combinations were always significant over other treatment combinations.The highest dose of vermicompost along with highest dose of fertilizer showed significant increase in the acid hydrolysable carbohydrate, which was superior to other vermicompost +NPK treatments but significantly superior to sole doses of vermicompost, NPK and control. Manna et al(2015) observed highest microbial activity at initial stages and then decreased further which could be due to used carbohydrate source by microbes.

Bacillus (x 10 c.f.u. g^{-1} dry soil): Irrespective of incubation stages, vermicompost and fertilizer doses the *Bacillus* count increased from 0th DAI to 65th DAI and then decreased up to 115th DAI (Table 2). Irrespective of all incubation periods, the increasing levels or doses of vermicompost increased the mean *Bacillus* population from 11.11 to 15.87 (x 10^{7} c.f.u.g^{-1}

Table 2. Effect of vermicompost and fertilizer on *Bacillus* population in incubation study10 ^{7}c.f.u. g^{-1})

Vermicompost/Fertilizer	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
S_1						
F_0	6.43	8.23	7.73	8.53	7.73	
F_{100}	7.47	8.87	10.30	11.67	9.58	
F_{50}	6.40	8.17	9.40	10.30	8.58	
Mean	6.77	8.42	9.14	10.17		
S_2						
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	7.85	9.90	8.99	9.77	9.13	
F_{100}	9.01	11.00	14.17	15.20	12.35	
F_{50}	7.92	9.87	12.80	13.93	11.13	
Mean	8.26	10.26	11.99	12.97		
S_3						
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	14.00	17.00	15.67	17.67	16.09	
F_{100}	16.33	20.33	21.33	22.00	20.00	
F_{50}	14.17	17.21	18.33	21.33	17.76	
Mean	14.83	18.18	18.44	20.33		
S_4						Grand mean (Fertilizer)
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	13.48	17.48	15.67	17.00	15.91	12.22
F_{100}	15.74	19.33	20.67	21.67	19.35	15.32
F_{50}	14.45	17.33	18.00	21.33	17.78	13.81
Mean	14.56	18.05	18.11	20.00		
Grand mean (Vermicompost)	11.11	13.73	14.42	15.87		

Factors	CD (5%)
Factor (S)	0.30
Factor (V)	0.30
Interaction S x V	0.26
Factor (F)	0.61
Interaction S x F	0.53
Interaction V x F	0.53
Interaction (s x v x f)	1.05

See Table 1 for details

) and along with increasing fertilizers levels increased from 12.22 to 15.32 (x 10 ^{7} c.f.u.g^{-1}) .The application of vermicompost dose of 3.75 t ha^{-1} with 100 % RDF 11.67 - 21.67 (10 ^{7} c.f.u.g^{-1}) 0thto 115th DAI respectively. The other combination of 2.5 t ha^{-1} vermicompost with 100% RDF showed second highest results and these two treatment combinations were always superior over other treatment combinations. Application of organic nutrient management resulted in higher microbial count (Pradhanet al 2016). Organic matter being the food of microorganisms provides the substrate for growth and activities of microorganism. The *Bacillus* population increased and then slightly decreased in later stages could be due to the more amount of depletion of organic sources at later days.

Pseudomonas (x 10 ^{7}c.f.u. g^{-1} dry soil): The*Pseudomonas* population increased significantly with increasing levels of vermicompost and fertilizers irrespective of different incubation periods. Irrespective of all incubation periods, the increasing levels or doses of vermicompost increased the mean pseudomonas population from 10.86 to 15.12 (x 10 ^{7}c.f.u. g^{-1}) and along with increasing fertilizers levels increased the from 11.98 to 14.49 (x 10 ^{7}c.f.u. g^{-1}) (Table 3).

Table 3. Effect of vermicompost and fertilizer on pseudomonas population in incubation study (x 10 ^{7}c.f.u. g^{-1})

Vermicompost/Fertilizer	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
S_1						
F_0	5.77	7.98	8.03	8.63	7.60	
F_{100}	6.97	8.52	9.36	10.48	8.83	
F_{50}	6.27	8.27	8.95	9.60	8.27	
Mean	6.34	8.26	8.78	9.57		
S_2						
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	7.94	9.51	8.82	9.66	8.98	
F_{100}	9.07	10.33	11.33	12.37	10.78	
F_{50}	8.30	9.77	10.80	11.87	10.19	
Mean	8.44	9.87	10.32	11.30		
S_3						
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	13.87	16.97	15.47	16.87	15.80	
F_{100}	15.63	18.63	20.47	21.47	19.05	
F_{50}	14.20	17.36	18.20	20.87	18.41	
Mean	14.57	17.65	18.05	19.74		
S_4						Grand mean (Fertilizer)
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	13.34	16.48	15.71	16.51	15.54	11.98
F_{100}	15.60	19.52	20.11	21.91	19.29	14.49
F_{50}	13.29	17.31	17.65	21.17	17.36	13.56
Mean	14.08	17.77	17.82	19.86		
Grand mean (Vermicompost)	10.86	13.39	13.74	15.12		

Factors	CD (5%)
Factor (S)	0.28
Factor (V)	0.28
Interaction S x V	0.24
Factor (F)	0.58
Interaction S x F	0.48
Interaction V x F	0.48
Interaction S x V x F	0.96

See Table 1 for details

The *Pseudomonas* population content increased in initial and then slightly decreased that might be due to the more availability of organic sources increased microbes populations at initial stages but in later stages depletion of more number of organic sources decreased microbial population. Similartrend was observed by Pradhanet al *(*2016) and Richard and Ogunjobi (2016).

Azotobacter (x 10 ^{7}c.f.u. g^{-1} dry soil): The *Azotobacter* count in soil irrespective of incubation stages, fertilizer and vermicompost doses increased from 0th DAI to 65th DAI and then decreased up to 115th DAI (Table 4). The depletion or exhaustion of available organic matter for microbial activities might have decreased the population from initial days of incubation which further decreased and similar results were observed by Deubel et al (2002).

Azospirillum (x 10 ^{7}c.f.u. g^{-1} dry soil): Irrespective of incubation stages, vermicompost and fertilizer doses the *Azospirillum* count increased from 0th DAI to 65th DAI and then decreased up to 115th DAI (Table 5). The application of Vermicompost dose of 3.75 t ha-1 with 100 % RDF gave excellent results i.e.,8.87, 13.07, 20.27 and 17.57 (x 10 ^{7}c.f.u. g^{-1} dry soil) at 0th, 30th, 65th and 115th DAI respectively. The

Table 4. Effect of vermicompost and fertilizer on Azotobacter population in incubation study (x 10 ^{7}c.f.u. g^{-1})

Vermicompost/Fertilizer	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
S_1						
F_0	5.20	6.23	6.60	7.83	6.47	
F_{100}	6.60	7.83	8.70	10.10	8.31	
F_{50}	5.33	7.23	8.37	9.20	7.53	
Mean	5.71	7.10	7.89	9.04		
S_2						
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	6.33	6.53	7.43	8.37	7.17	
F_{100}	7.10	9.00	11.03	12.40	9.88	
F_{50}	6.63	8.10	9.23	11.37	8.83	
Mean	6.69	7.88	9.23	10.71		
S_3						
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	9.77	10.80	12.13	13.83	11.63	
F_{100}	11.27	15.97	17.50	18.97	15.92	
F_{50}	10.57	13.60	14.57	17.70	14.11	
Mean	10.54	13.46	14.73	16.83		
S_4						Grand mean (Fertilizer)
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	8.37	11.30	11.50	12.97	11.04	9.08
F_{100}	11.87	14.70	15.90	16.70	14.79	12.23
F_{50}	9.67	12.97	13.87	16.03	13.14	10.90
Mean	9.97	12.99	10.32	15.23		
Grand mean (Vermicompost)	8.23	10.36	10.54	12.95		

Factors	CD (5%)
Factor (S)	0.29
Factor (V)	0.29
Interaction S x V	0.25
Factor (F)	0.58
Interaction S x F	0.50
Interaction V x F	0.50
Interaction S x V x F	1.00

See Table 1 for details

Azospirillum population content increased in all incubation stages and then slightly decreased and that might be due to the more availability of organic sources increased microbes populations at initial stages but in later stages exhaustion of organic matter decreased microbial populations and was supported by Deubel et al (2002).

Phosphate solubilizing bacteria (x 10 ^{7}c.f.u. g^{-1} dry soil): The phosphate Solubilizing bacteria population increased significantly with increasing levels of vermicompost and fertilizers irrespective of different incubation periods (Table 6). Irrespective of all incubation periods, the increasing levels or doses of vermicompost increased the mean phosphate solubilizing bacteria population from 10.92 to 14.80 (x 10 ^{7}c.f.u. g^{-1}) and along with increasing fertilizers levels increased the from 15.57 to 19.30 (x 10 ^{7}c.f.u. g^{-1}). The phosphate Solubilizing bacteria population content increased in all incubation stages and then slightly decreased which might be due to the more availability of organic sources increased microbes populations at initial stages but in later depletion of organic matter declined the microbial growth and similar results were supported by Richard and Ogunjobi (2016).

Table 5. Effect of vermicompost and fertilizer on Azospirillum population in incubation study (x 10 ^{7}c.f.u. g^{-1})

Vermicompost/Fertilizer	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
S_1						
F_0	6.95	7.61	7.77	7.86	7.55	
F_{100}	7.85	8.35	8.61	8.87	8.42	
F_{50}	7.31	8.07	8.27	8.46	8.03	
Mean	7.37	8.01	8.22	8.40		
S_2						
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	7.67	9.11	9.35	9.83	8.99	
F_{100}	8.75	10.19	12.35	13.07	11.09	
F_{50}	7.49	9.57	11.45	12.53	10.26	
Mean	7.97	9.62	11.05	11.81		
S_3						
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	11.27	15.57	15.07	16.27	14.55	
F_{100}	12.87	18.87	18.87	20.27	17.72	
F_{50}	13.97	16.49	17.37	19.17	16.75	
Mean	12.70	16.98	17.10	18.57		
S_4						Grand mean (Fertilizer)
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	10.37	13.07	12.57	13.47	12.37	10.87
F_{100}	11.47	13.97	15.77	17.57	14.70	12.98
F_{50}	11.07	13.27	14.87	16.47	13.92	12.24
Mean	10.97	13.44	14.40	15.84		
Grand mean (Vermicompost)	9.75	12.01	12.69	13.66		

Factors	CD (5%)
Factor (S)	0.20
Factor (V)	0.20
Interaction S x V	0.17
Factor (F)	0.40
Interaction S x F	0.35
Interaction V x F	0.35
Interaction S x V x F	0.69

See Table 1 for details

Starch hydrolyzing microbes (x 10 ^{7}c.f.u. g^{-1} dry soil): Irrespective of incubation stages, vermicompost and fertilizer doses the Starch hydrolyzing microbes count increased from 0th DAI to 65th DAI and then decreased up to 115th DAI (Table 7). The application of Vermicompost dose of 3.75 t ha-1 with 100 % RDF gave excellent results i.e., 10.74, 12.28, 21.37 and 18.82 (x 10 ^{7}c.f.u. g^{-1} dry soil) at 0th, 30th, 65th and 115th DAI, respectively. The starch hydrolyzing microbes population content increased in all incubation stages from 0th DAI to 65th DAI and then decreased up to 115th DAI might be due to the more availability of organic sources increased microbes populations at initial stages but in later stages depletion of organic matter content declined the microbial activities and similar studies were with pseudomonas population and was supported by Kumar et al (2012).

Cellulose hydrolyzing bacteria (x 10 ^{7}c.f.u. g^{-1} dry soil): The Azotobacter count in soil irrespective of incubation stages, fertilizer and vermicompost doses increased from 0th DAI to 65th DAI and then decreased up to 115th DAI (Table 8). The combination of Vermicompost dose of 3.75 t ha^{-1} with 100

Table 6. Effect of vermicompost and fertilizer on phosphate solubilizing bacteria population (PSB) in incubation study (x 10 ^{7}c.f.u. g^{-1})

Vermicompost/Fertilizer	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
S_1						
F_0	6.30	8.23	7.13	8.53	7.55	
F_{100}	7.63	8.63	10.07	11.36	9.42	
F_{50}	6.77	7.67	9.40	9.77	8.40	
Mean	6.90	8.18	8.87	9.89		
S_2						
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	7.63	9.53	8.34	9.19	8.67	
F_{100}	8.73	10.23	13.40	13.63	11.50	
F_{50}	7.03	9.01	10.97	12.94	9.99	
Mean	7.80	9.59	10.90	11.92		
S_3						
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	13.80	16.70	15.73	17.23	15.87	
F_{100}	16.20	19.27	21.23	22.07	19.69	
F_{50}	14.10	17.10	17.93	19.97	17.28	
Mean	14.70	17.52	18.30	17.76		
S_4						Grand mean (Fertilizer)
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	13.37	16.60	15.60	16.70	15.57	11.92
F_{100}	16.10	19.43	20.17	21.50	19.30	14.98
F_{50}	13.40	16.93	17.90	20.63	17.22	13.22
Mean	14.29	17.65	17.89	19.61		
Grand mean (Vermicompost)	10.92	13.24	13.99	14.80		

Factors	CD (5%)
Factor (S)	0.28
Factor (V)	0.28
Interaction S x V	0.25
Factor (F)	0.57
Interaction S x F	0.49
Interaction V x F	0.49
Interaction S x V x F	0.98

See Table 1 for details

% RDF always showed excellent results i.e., 10.81- 18.41 (x 10^7c.f.u. g^{-1} dry soil) at 0^{th} - 115^{th} DAI respectively. The cellulose hydrolyzing bacteria population content increased in all experiment stages and then decreased which might be due to the more availability of organic sources increased microbes populations at initial stages but in later stages exhaustion of available organic matter decreased microbial growth. The complex organic matter in vermicompost provides substrate for proliferation of microbial colonies in soil. The similar results were found by Bahadur et al (2012) and also by Lakshmi et al (2014).

CONCLUSION

The application of highest doses of vermicompost (3.75 t ha^{-1}) and inorganic (N-P-K) fertilizer at the recommended dose of 100% showed superiority in all observations such as acid hydrolysable carbohydrate, functional indicator microbes over all other treatments and also over control. The higher dose of vermicompost along with fertilizer provided prolonged substrate for the growth of microorganisms and other rhizospheric microorganism thus enhanced the soil nutrients availability and soil health. The buildup of organic matter in soil improved the fertility status of soil with ensuring

Table 7. Effect of vermicompost and fertilizer on starch hydrolyzing microbes population in incubation study (x 10^7c.f.u. g^{-1})

Vermicompost/Fertilizer	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
S_1						
F_0	5.87	8.07	7.77	8.63	7.59	
F_{100}	7.40	8.70	9.60	10.74	9.11	
F_{50}	6.23	8.30	9.20	9.87	8.40	
Mean	6.50	8.36	8.86	9.75		
S_2						
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	6.58	8.50	8.84	9.59	8.38	
F_{100}	7.91	9.51	11.27	12.28	10.24	
F_{50}	6.81	8.83	10.67	11.60	9.48	
Mean	7.10	8.95	10.26	11.16		
S_3						
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	11.97	12.54	15.87	17.23	14.40	
F_{100}	13.07	17.78	20.00	21.37	18.06	
F_{50}	12.36	17.10	17.93	20.93	14.58	
Mean	12.47	15.81	17.93	19.84		
S_4						Grand mean (Fertilizer)
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	12.80	15.07	15.63	16.53	15.01	11.35
F_{100}	13.78	16.80	17.50	18.82	16.73	13.54
F_{50}	13.24	16.05	17.04	17.91	16.06	12.13
Mean	13.27	15.97	16.72	17.75		
Grand mean (Vermicompost)	9.84	12.27	13.44	14.63		

Factors	CD (5%)
Factor (S)	0.23
Factor (V)	0.23
Interaction S x V	0.20
Factor (F)	0.46
Interaction S x F	0.40
Interaction V x F	0.40
Interaction S x V x F	0.80

See Table 1 for details

Table 8. Effect of vermicompost and fertilizer on cellulose hydrolyzing bacteria population in incubation study cellulose hydrolyzing bacteria (x 10^7 c.f.u. g^{-1})

Vermicompost/Fertilizer	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
S_1						
F_0	5.80	8.20	7.13	8.47	7.40	
F_{100}	7.57	8.60	9.70	10.81	9.17	
F_{50}	6.17	7.80	9.10	9.87	8.24	
Mean	6.51	8.20	8.64	9.72		
S_2						
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	7.13	9.00	7.92	8.83	8.22	
F_{100}	7.98	9.84	11.35	12.13	10.33	
F_{50}	6.63	8.89	10.27	11.38	9.29	
Mean	7.25	9.24	9.85	10.78		
S_3						
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	11.06	14.18	15.08	14.18	13.63	
F_{100}	12.15	16.12	18.95	19.83	16.76	
F_{50}	11.58	15.26	16.21	19.31	15.59	
Mean	11.60	15.19	16.75	17.77		
S_4						Grand mean (Fertilizer)
	V_0	$V_{1.25}$	$V_{2.5}$	$V_{3.75}$	Mean	
F_0	10.56	13.00	12.90	14.06	12.63	10.47
F_{100}	11.15	14.01	16.71	18.41	15.07	12.83
F_{50}	10.50	13.86	15.03	17.50	14.22	11.84
Mean	10.74	13.62	14.88	16.66		
Grand mean (Vermicompost)	9.03	11.56	12.53	13.73		

Factors	CD (5%)
Factor (S)	0.33
Factor (V)	0.33
Interaction S x V	0.29
Factor (F)	0.67
Interaction S x F	0.58
Interaction V x F	0.58
Interaction S x V x F	1.16

See Table 1 for details

highest productivity of crops for future sustainable agriculture with broad scope. The vermicompost as a source of organic is cost effective, eco-friendly and might replace some amount of chemical fertilizer on long term application purposes.

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Overlooked Influence of Indian Hemp (*Cannabis sativa*) Cultivation on Soil Physicochemical Fertility of Humid Tropical Agroecosystems: Upland Soils

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Abstract: The extensive root growth of Indian hemp (*Cannabis sativa*) or *Marijuana* may imply greater depletion of soil nutrients. Because of the high demand for various parts of this plant especially the leaves that have smoking value, such nutrients may also not be recycled, impoverishing the soil the more. To empirically support or invalidate this hypothesis, this study compared soil physicochemical properties among uplands differing in *Cannabis* cultivation status; land not used for *Cannabis* cultivation (NUC), land currently under *Cannabis* cultivation (CCC), farmlands converted from *Cannabis* to alternative use (CAU), and *Cannabis* farmlands abandoned or seized (ABS) at a representative location in southwestern Nigeria. Particle size distribution of the soils differed under the land-use options. Soil bulk density exceeded the critical limit (1.30 Mg/m^3) for crop production in these upland soils under CCC but not NUC, both for which values were lower compared to CAU and ABS. Soil pH, soil organic C, total N, available P, and cation exchange capacity were highest under NUC which had the lowest base saturation. The Ca^{2+} was lowest in CCC, but soil contents of K^+, Mg^{2+} and Na^+ were unaffected by *Cannabis* cultivation. Also, copper and manganese were highest in NUC and lowest in CAU, while iron was higher in ABS than the rest. *Cannabis* cultivation could compact the soil and decrease plant nutrient levels leading to a reduction in legitimate agricultural production below the soil potential.

Keywords: *Cannabis* fields, Illicit cultivation, Alternative land uses, Soil drain, Food security

Indian hemp (*Cannabis sativa*), also called *Marijuana*, is a narcotic vegetable plant grown in Nigeria primarily for the premium on its leaves with a smoking value. The prohibition of the cultivation, processing, storage, trafficking, distribution and use of any part of *Cannabis* plant was adopted in Nigeria from the International Conventions on narcotics. Yet, significant amounts of *Cannabis* are illicitly cultivated in Nigeria for trafficking into the black markets of the United States of America, Europe and some Asian countries (Kolo 2019). In many of these industrialized nations, the roots, stems, leaves, flowers and the seeds of licit *Cannabis* are competitively used for recreational, medicinal and industrial purposes (Ester 2020). In tropical agro-ecosystems, vegetation directly influences soil physical and chemical properties (Watanabe et al 2010, Obalum et al 2012, 2013a, Uzoh et al 2020 and Oguike et al 2022). Vegetation could facilitate nutrient cycling by extracting nutrients from lower soil horizons and redistributing them to the topsoil through leaf falls and residue decomposition. Because most tropical soils are highly leached (Igwe 2011, Igwe and Obalum 2013), plants with extensive lateral roots in these soils scavenge nutrients from beneath the root zone and redistribute them to the immediate soil environment (Rhoades 2006).

Continuous cultivation of plants grown essentially for their leaves could therefore drain the soil of its nutrients reserve as nutrients taken up by such plants may not be recycled. Removal of crop residue accentuates nutrient depletion (Blanco-Conqui and Lal 2009, Smith et al 2012). This situation could be worse with *Cannabis* because its roots mine water and nutrients extensively in the soil (Bergman 2011). The nutrients so mined provide the building blocks for *delta-9-tetrahydrocannabinol*, the most active psychoactive chemical ingredient in *Cannabis* smoke which, though promotes appetite and relieve pains, could cause hallucinations, disconnected thoughts and panic reactions (NIDA, 2021). Some upland soils in Ondo State in southwestern Nigeria are used for illicit growing of *Cannabis*, often with poor soil and water management practices (Kolo 2019). Though the Nigerian *Cannabis* cultivation prohibition law provides for the confiscation of lands under *Cannabis* among other penalties, a sizeable hectarage is still under *Cannabis* mostly in Southwestern Nigeria (Giade 2014, NDLEA 2015). *Cannabis* farmers are thus not deterred due to the enormous financial gains (Kolo 2019). Considering the pressure on agricultural lands in Nigeria, losing more lands to *Cannabis* may put the country in danger of food crisis (Fagge 2014). *Cannabis* cultivation thus threatens not only human health but also food security (Fagge 2014, NDLEA 2015). The possible adverse effects of this *Cannabis'* displacement of arable crops on soil nutrients status which would aggravate food insecurity are thinly supported with empirical data. The need to check the scourge has to be established starting from any such effects for inference on sustainability or otherwise of this illicit enterprise, hence this study which assessed the effects of *Cannabis* on physicochemical fertility of upland soils in tropical agro-ecosystems.

MATERIAL AND METHODS

The study was conducted near Akure town in Southwestern Nigeria in 2016 (Fig. 1). The climate is humid tropical, with mean annual rainfall, temperature and relative humidity of 2000 mm, 24.8°C, 75.4%, respectively; while the soils, formed on the Precambrian basement complex rock, are mostly sandy-loam Alfisols (Kolo 2019). Rainfed root/tuber, cereal and vegetable crops are commonly grown, with some used as perimeter crops for illicit *Cannabis* plants on these well-drained uplands that are usually abandoned when the soil fertility status 'seemingly' drops.

Using satellite imagery, four land-use options were identified in communities known for illicit cultivation of *Cannabis*; land not used for *Cannabis* cultivation (NUC), land currently under *Cannabis* cultivation (CCC), farmlands previously under *Cannabis* but now converted from *Cannabis* to alternative use (CAU), and farmlands previously under *Cannabis* but now abandoned or seized (ABS).Those on elevations of 340-380 m asl were selected using free survey technique and sampled (Figure 2). Before the study, NUC had been under legitimate crops for 15-20 years, and CCC under *Cannabis* for 4-5 years. Both CAU and ABS previously were under *Cannabis* for 3-6 years before being changed to their current status; CAU had been under this alternative use for 3-5 years; while ABS had been abandoned for at least 3 years. To get the above vital information on the land use history of the sampled farmlands, the National Drug Law Enforcement Agency (NDLEA) hired native agents who were familiar with the agricultural practices in the local environment. Local communities and farmlands were accessed through subterfuge. Studies at CCC farmland locations were achieved by ensuring the presence of armed guards during *Cannabis* plant destruction by the NDLEA

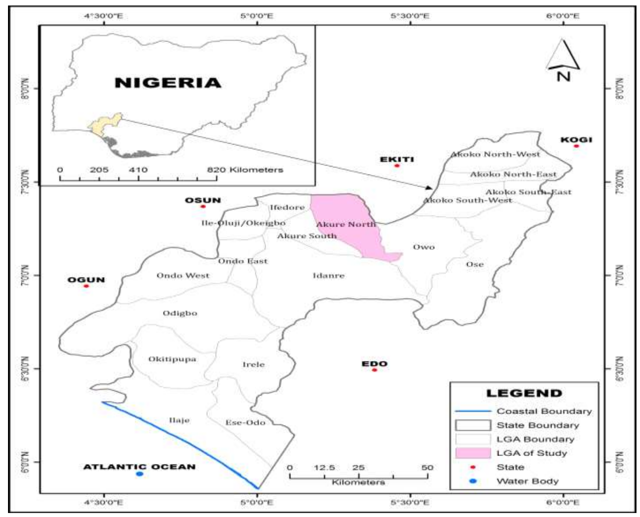
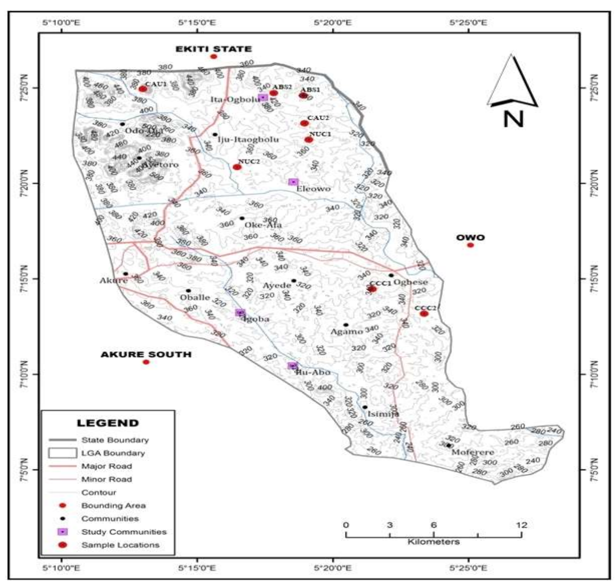


Fig. 1. Ondo State showing the local government area of study (Inset: Map of Nigeria)



NUC - land not used for *Cannabis* cultivation,
CCC - land currently under *Cannabis* cultivation,
CAU - farmlands converted from *Cannabis* to alternative use,
ABS - *Cannabis* farmlands abandoned or seized

Fig. 2. Contour/topographic map of Akure North Local Government Area, Ondo State of Nigeria showing local communities and sample locations at the upland topographic setting (340-380 m asl)

Operatives. The entire soil profile was considered in the assessment of *Cannabis* effects in the soil, using two strategically sited profile pits per land-use option (Kolo et al 2022). The pedons were 52-170 m deep, with 3-5 pedogenetic horizons. Undisturbed and disturbed soil samples were collected in triplicates from these horizons and used to determine some soil hydraulic and physicochemical fertility indices, respectively, following exactly the same standard procedures already detailed as for the lowland soils of the bigger project (Kolo et al 2022). Data were analysed by subjecting the average values of the soil parameters across the pedogenetic horizons (giving six means for two pedons per land-use option) to appropriate ANOVA procedure using the software GenStat (Discovery Edition 4). The least significant difference was used to make inference on differences between means at $P < 0.05$ (LSD 0.05).

RESULTS AND DISCUSSION

Particle size distribution of the upland soils under the four land-use options: The particle size distribution of the upland soils shows higher clay in the NUC and CCC than the CAU and ABS soils (Table 1). Silt contents were higher in the NUC and CAU than the ABS and CCC soils. Significant decreases in coarse sand content of the soils followed the order CCC> NUC>CAU>ABS. These variations in soil texture were not likely caused by the differences in feature and management among the land-use options. Similar observation and remark were made for the lowland soils of this study, with plausible reasons proffered (Kolo et al 2022, Oguike et al 2022).

Influence of land-use option on some hydraulic properties of the upland soils: Soil bulk density was lower in the NUC and CCC than the CAU and ABS. Specifically, the lower values in the NUC compared with those that were previously used to grow *Cannabis* (CAU and ABS) were likely due to the high soil organic associated with conservation practices typified by the NUC farmlands (Bitew and Abera 2019). Generally, these variations were attributed to the interaction between soil texture, soil organic C (Ezeaku et al 2020), and traffic load across the fields. Apart from the NUC, soil bulk density was generally above the critical limit of 1.30 Mg/m^3 for cultivation of certain crops in these soils (Oritsejafor et al 2022). High soil bulk density implies reduced permeability. Soil total porosity mirrored the bulk density, since the two are inverse derivatives of each other. The high values in the fallowed ABS suggests enhanced soil structural stability compared to others (Obalum and Obi 2014). The saturated hydraulic conductivity (K_s) was higher in the NUC and CCC than the CAU and ABS soils, implying faster transmission of water through the soils in the former than the latter. The high bulk density values and the corresponding low K_s values in the duo of CAU and ABS are attributed to the removal of *Cannabis* plant residue exposing the soil to greater raindrop impact, soil loss and compaction (Blanco-Conqui and Lal 2009, Ramteke et al 2018). Being that bulk density data were used to compute total porosity here, the similar trend for bulk density and K_s also suggests a good correlation between total porosity and the proportion of water-conducting macropores in the soils (Obalum et al 2011).

Influence of land-use option on some chemical properties of the lowland soils: Soil pH, soil organic C, total N, available P and CEC were higher in the NUC than the rest (Table 2). These results reflect nutrient drain by *Cannabis* with scavenging roots, crop residue removal and losses to the expected increased runoff and leaching (Bergman 2011, Kolo 2019). Intense litter recycling increases soil nutrient levels (Rhoades 2006, Watanabe et al 2010 and Krause and Rotter 2018). The higher values of these soil fertility indices in the NUC are attributed to plant residue return to this farmland, as opposed to the present or previous removal of the entire *Cannabis* plants from the other farmlands. The higher available P in the NUC than the CCC contrasts with the results attained at the poorly drained lowlands, reported elsewhere (Kolo et al 2022).The $Ca^{2+}$and ECEC varied alike,

Table 1. Influence of land-use option on some physical properties of the soils at the upland topographic positions in Akure area of southwestern Nigeria

Land-use option	Clay	Silt	Fine sand (g/kg)	Coarse sand	BD (Mg/m^3)	% TP	K_s (cm/h)
NUC	91	154	281	474	1.31	50.28	31.06
CCC	73	92	275	560	1.41	46.62	23.22
CAU	173	153	256	418	1.64	38.13	15.55
ABS	206	134	304	356	1.62	38.84	22.63
LSD (0.05)	60	40	NS	60	0.15	5.35	7.57

NUC - Land not used for *Cannabis* cultivation, CCC - land currently under *Cannabis* cultivation,
ABS - *Cannabis* farmlands abandoned or seized;
CAU - Farmlands converted from *Cannabis* to alternative use,
BD - Bulk density, TP - total porosity, K_s - saturated hydraulic conductivity,
LSD (0.05) - Least significant difference at P< 0.05, NS -not significant

Table 2. Influence of land-use option on chemical properties of the soils at the upland topographic positions in Akure area of southwestern Nigeria

Land-use option	Soil pH		SOC	TN	AvP	CEC	K^+	Ca^{2+}	Mg^{2+}	Na^+	EA	ECEC	% BS
	H_2O	KCl	(g/kg)		(mg/kg)	(cmol/kg)							
NUC	6.6	5.7	18.4	2.3	13.62	18.38	0.22	6.01	1.94	0.19	1.10	9.13	45.49
CCC	6.1	5.2	9.3	1.7	7.46	9.87	0.27	5.81	1.71	0.19	0.78	8.87	80.84
CAU	5.5	4.6	10.1	1.7	4.51	12.87	0.22	6.73	2.06	0.17	0.96	10.15	71.40
ABS	4.9	4.1	6.2	1.9	4.66	13.90	0.20	7.26	2.06	0.18	1.21	10.82	69.77
LSD (0.05)	0.4	0.7	3.6	0.4	4.93	6.06	NS	1.08	NS	NS	0.23	1.22	11.56

NUC - land not used for *Cannabis* cultivation, CCC - land currently under *Cannabis* cultivation, CAU - farmlands converted from *Cannabis* to alternative use, ABS - *Cannabis* farmlands abandoned or seized; SOC – soil organic carbon, TN - total nitrogen, AvP - available phosphorus, CEC - cation exchange capacity, K^+ - exchangeable potassium, Ca^{2+} - exchangeable calcium, Mg^{2+} - exchangeable magnesium, Na^+ - exchangeable sodium, EA - exchangeable acidity, ECEC - effective CEC, BS - base saturation, $LSD_{(0.05)}$ - least significant difference at *P*< 0.05, NS - not significant

Table 3. Influence of land-use option on the soils' contents of some micronutrients (mg/kg) at the upland topographic positions in Akure area of southwestern Nigeria

Land-use option	Iron	Manganese	Zinc	Copper
NUC	2.24	5.41	3.95	2.96
CCC	3.11	3.76	3.50	2.56
CAU	2.37	3.10	3.16	1.74
ABS	4.93	4.47	3.92	1.89
LSD (0.05)	1.41	1.05	NS	1.01

NUC - Land not used for *Cannabis* cultivation
CCC - land currently under *Cannabis* cultivation,
CAU - Farmlands converted from *Cannabis* to alternative use,
ABS - *Cannabis* farmlands abandoned or seized,
LSD (0.05)- Least significant difference at *P*< 0.05, NS - not significant

being higher in the ABS than the CCC and NUC, while K^+, Mg^{2+} and Na^+ were unaffected. These data implicate Ca^{2+} as the basic cation most depleted by *Cannabis*, the effect of which reflects in the ECEC, the all-important index of soil fertility. Exchangeable acidity was increased under the ABS due likely to the low soil pH linked to the previous removal of *Cannabis* plant from this farmland (Randall et al 2006). The NUC with about the lowest clay content but highest soil organic C showed the highest CEC, suggesting that these upland soils are fairly structurally stable (Obalum et al 2013b). However, the NUC showed lower base saturation than the rest. With the four exchangeable bases being similar in the NUC and CCC, the low base saturation in the former was due to its high CEC and not any sparing effects of *Cannabis* on these plant nutrients.

The Cu and Mn contents were highest in the NUC and lowest in the CAU (Table 3).The highest Cu and Mn in the NUC may be by the highest soil organic C in this farmland. The Fe was lowest in the NUC and highest in the ABS with lowest soil pH and soil organic C, indicating that acid tropical soils with depleted organic C could potentially accumulate high amounts of Fe (Aweto and Enaruvbe 2010). This situation may increase the risk of plinthite formation, reducing soil permeability and restricting root growth (Eze et al 2014). Overall, the ABS approximating fallowing in this study was not always better than the CCC farmlands. This highlights the enormity of soil degradation due to *Cannabis* cultivation. Fallowing is an effective way of restoring soils degraded by continuous cultivation in the humid tropics (Alarima et al 2020, Onah et al 2021 and Oguike et al 2022). The data show that rejuvenation may not occur unaided in confiscated *Cannabis* farmlands. But for soil pH and organic C, the ABS and CAU with crop residue were similar. Granted that residue-retaining cropping systems may not always fully restore degraded tropical soils (Uzoh et al 2017, Adama et al 2022), the CAU being rather ineffectual is yet additional pointer to the enormous soil degradation due to cultivation of *Cannabis*.

CONCLUSION

The former *Cannabis* farmlands were too compacted for root growth and less permeable compared with those that never grew the crop; current ones assumed central values. *Cannabis* also decreased soil fertility and the supply of plant nutrients. Considering soil quality holistically, *Cannabis* cultivation led to its decline, with fallowing being generally less effective than changing to arable crops. These findings have implications for food production and the environment, and so call for immediate intervention. Beyond retrieving uplands from *Cannabis*, therefore, is the need for research on effective agricultural practices, especially those that favour rapid accretion and stabilization of soil organic C.

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Planning of Suitable Soil and Water Conservation Structures Using Remote Sensing and GIS Approach: A Case Study of Madar Watershed

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Abstract: The present study was conducted for Madar watershed of Udaipur Rajasthan, India to plan appropriate water conservation structures on the basis of Drainage map and Land Capability Classification (LCC) using remote sensing and GIS. The objective of the study was to decide the optimal locations for conservation measures and water harvesting structures. In this study, location of puerto-rico terraces, contour trenches, staggered trenches and contour bund were planned for soil conservation measures, while check dams, gully plugs, and farm ponds for water harvesting structures. SRTM data has been used for extracting necessary geomorphologic parameters, while Lands at 8 data has been used for LCC. The results revealed that the Madar watershed must be treated with appropriate conservation measures and water harvesting structures. The results suggest for twelve check dams, fifteen gully plugs, and three farm ponds can be constructed for Madar watershed.

Keywords: Land capability classification, Water harvesting, Drainage map, Madar watershed

Due to anthropogenic causes, now agricultural land and natural water resources on earth are no more in plenty of amount. Almost every country of Asia has water scarcity problems. Besides having twenty river basins in India (http://nca.gov.in/nb_basin.htm), India has very uneven distributions of water in every state. States with low monsoonal rainfall, such as Rajasthan has severe water scarcity problems. Water harvesting planning and management can help a region to use water resources in a more judicial way. Countries like Israel have adopted many methods for water harvesting such as roof water harvesting on every governmental and school building. For India, water harvesting technology is important, since about 60 percent of total arable land (142 million ha) in the country is rain-fed (Panhalkar et al 2014). Water harvesting planning should be properly done to avoid unnecessary economic loss due to the failure of structures. Thus, while planning Water harvesting structures, the soil, the slope of land, the land cover, etc. should be optimally studied. Through Land capability classifications, the lands could be demarked and classified on the basis of soil erosion and slope of that area (Amir et al 2010, Atalay 2016). According to the USDA (1973) guidelines, land capability class range from a I to VIII. Land capability class from I to IV is suitable for agriculture. The susceptibility of the land to erosion and limitation in use, however, become progressively greater from Class I to Class IV Capability classes ranging from V to VIII are generally not suitable for agriculture but can be used for controlled grazing, pasture, forest, woodland and wildlife purposes (Oluwatosin et al 2006, Maryati 2013, Gad 2015, Abdel Rahman et al 2016, Atalay 2016, Saranaathan and Vaishaly 2021, Jeelani et al 2022). For locating water harvesting and conservation techniques surveying techniques will require time-consuming fieldwork and also it requires a large number of technical experts working in the field. These watershed action plans were adopted in the past because at that time, remote sensing data was not available. But, nowadays, remote sensing coupled with GIS is used for natural resources management (Agarwal 2003). In the present study, water harvesting and conservation measures planning have been done on the basis of LCC.

MATERIAL AND METHODS

Study area: The study area (Madar) is a village situated in Badgaon Tehsil in Udaipur District of Rajasthan, India and lies between $73^{0}35'$ to $73^{0}36'$ E longitude and$24^{0}40'$ to $24^{0}42'$ N latitude having a total geographical area of 35.239 km^2. It falls under agro-climatic zone-IV A of Rajasthan i.e. "Sub-humid Southern Plains of the Aravalli hills" (Fig. 1). Average annual rainfall of 607 mm received mostly during the monsoon months of July to September.. Distribution of the rainfall in monsoon season is uneven and erratic marked by prolonging rainless days. The temperature of the study area varies from 19-48 ºC during summer while during the winter season, the temperature varies between 3.2 to 28.90 ºC. The study area comprises of undulating uplands fields and hills. The general slope of the area is north-east to south-west direction and slope ranges are even more than 30 percent. The main rock formations of the area under study are phyllites, schist, and quartzite. maize, urd, moong, are the commonly grown crops in Kharif season whereas, wheat, mustard, gram, linseed, are grown in Rabi season.

Data acquisition: Topographic and drainage features were extracted from Digital Elevation Model (DEM) from Shuttle Radar Topographic Mission (srtm) data, and Geographical Toposheet at 1:50,000 scale which was procured from Survey of India (SOI) obtained from Panchayat Samiti Office, Udaipur, Rajasthan, India. The soil information and soil map of the area at 1:250,000 scale was gathered from the Regional Centre of the National Bureau of Soil Survey and Land Use Planning (NBSS & LUP), Udaipur, Rajasthan (Jain et al 2005). Landsat satellites are considered a valuable source of observation and monitoring of global changes because of the medium spatial resolution and the availability of long-term data (Wulder et al 2008). The remote sensing data of the area was used from satellite imagery IRS-IC-LISS-III dated 3 March 2015 at Regional Centre of the National Bureau of Soil Survey and Land Use Planning (NBSS & LUP), Udaipur, Rajasthan. The catchment boundary was delineated in GIS by using Universal Transverse Mercator (UTM) projection system. Annual rainfall data from 1986-2017 were obtained from the portal of Water Resource Department, Rajasthan. The thematic maps were prepared using ArcGIS 10.1 software.

Methodology: The present study was taken to use the puissance of the remote sensing and GIS technique in locating the site suitability for conservation measures and water harvesting structures. Due to hilly topography, the rainfall is not managed properly which leads to severe erosion in the site selected for the study and hence needs action for suitable conservation measures. The study was planned to make use of the freely available satellite data processed in a GIS environment. The flowchart for the methodology of the present study is given in Figure 2. The first stage includes collections of data from different sources and all the data have been converted into digital format, if it was not so, such as toposheet map for Madar area. The second stage comprises the development of a thematic layer of information from distinct sources. It includes digital image processing of satellite data, processed maps, and field data for the extraction of the necessary information. The third stages involve the integration of data and implementing the objectives of the study. For generating an LCC map of watershed, georeferencing, digitizing the drainage map using toposheet, FCC generation, ground-truthing, unsupervised and supervised classification has been done. DEM file, obtained from SRTM, was used for delineation of the watershed in the ArcGIS environment and further used for the preparation of the slope and drainage map (Minakshi and Verma 2014). The georeferenced toposheet was used for validating this delineated watershed and in the generation of streams map of the watershed. The satellite Landsat 8 data were used for classification of land use land cover using the shapefile of the watershed which was further validated by site visiting and using toposheet and google earth imagery. The soil map of the study area was obtained from NBSS & LUP, Udaipur, Rajasthan were used for textural class, depth and erosion hazard of the study area. The other thematic maps, including slope map, land use and land cover map, depth classes, and erosion hazards were also prepared in ArcGIS 10.1 for further analysis. The Integrated Mission for Sustainable Development (IMSD) given by National Remote Sensing Agency (NRSA) (currently NRSC), India, guidelines were used for suitable sites for water harvesting structures.

Data availability: For Digital Elevation Model (DEM) and Landsat 8 data were downloaded from site https://earthexplorer.usgs.gov/. Geographical Toposheet at 1:50,000 scale which was procured from Survey of India (SOI) obtained from Panchayat Samiti Office, Udaipur, Rajasthan, India. The soil information and soil map of the area at 1:250,000 scale was gathered from the Regional Centre of the National Bureau of Soil Survey and Land Use Planning (NBSS & LUP), Udaipur, Rajasthan. "Data that support the findings of this study are available from the corresponding author upon reasonable request."

RESULTS AND DISCUSSION

Drainage: In the present study, the watershed was observed 5^{th} order type and number of streams present in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, and 5^{th} order streams were 93, 50, 23, 2, and 1 respectively for the study area. A check dam is suitable for 1-3^{rd} order type (Sinha et al 2015). The 2nd, 3rd or 4th order streams are suitable for Storage Tank and Percolation Tank (Prasad et al 2014 (Fig. 4).

DEM and slope: The slope of the watershed was varied accordingly and classified into six categories i.e. 0-1 t, 1-3 t, 3-8 t, 8-15 and 15-30, greater than 30 percent. The area that comes under these categories was 918.137, 241.377, 336.645, 606.628, 426.3 and 994.11 ha respectively. Thus, the maximum area falls under the slope greater than 30 percent depict 28 percent of the total watershed area. The second highest area falls in the categories of the slope varies from 0-1 percent depicts 26 percent of the total area. Seven percent of the total area falls under 1-3 percent which accounts minimum area under this category (Fig. 5).

Soil: The depth of soil for study area classified into d_1(<25 cm soil depth), d_3 (50-75 cm), d_4 (75-100 cm)) having area of 3079.03, 99.2529, 261.237 and 84.4115 ha land respectively. Therefore, 87% area of the total area was dominated by the soil having a depth of less than 25 cm. The textural class of the study area was classified into loamy skeletal soils (lsk) and fine loamy soils (fl) (Fig. 6) having a contributing area were 3079.028 and 444.902 ha respectively. Therefore, dominated texture class in the study area was loamy skeletal soils having a contributing area was 87% of the total area. The other component is erosion hazards which were classified into severe (e_3), moderate (e_2) and slight erosion (e_1) (Fig. 7).

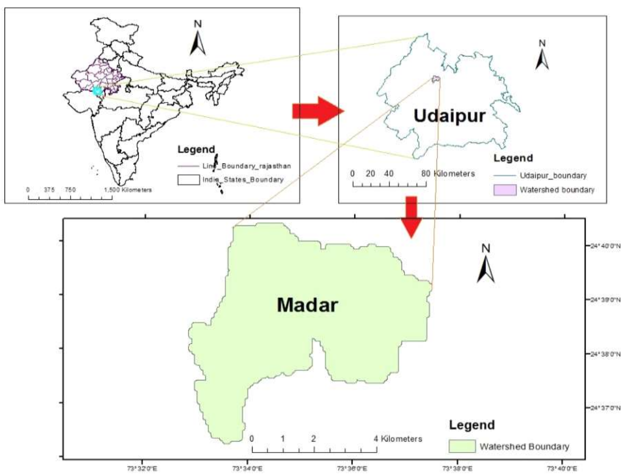

Fig. 1. Study area

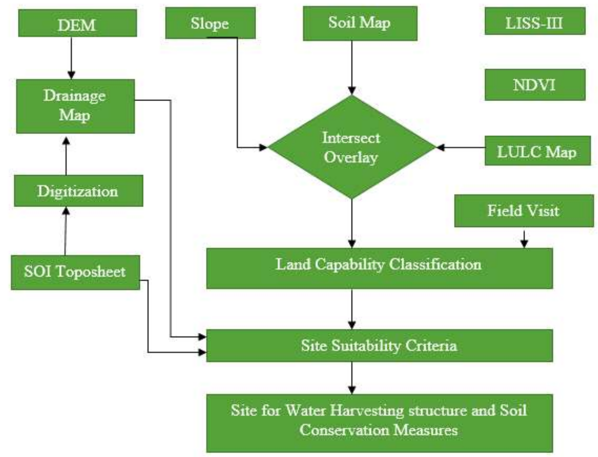

Fig. 2. Flow chart of the methodology of research work

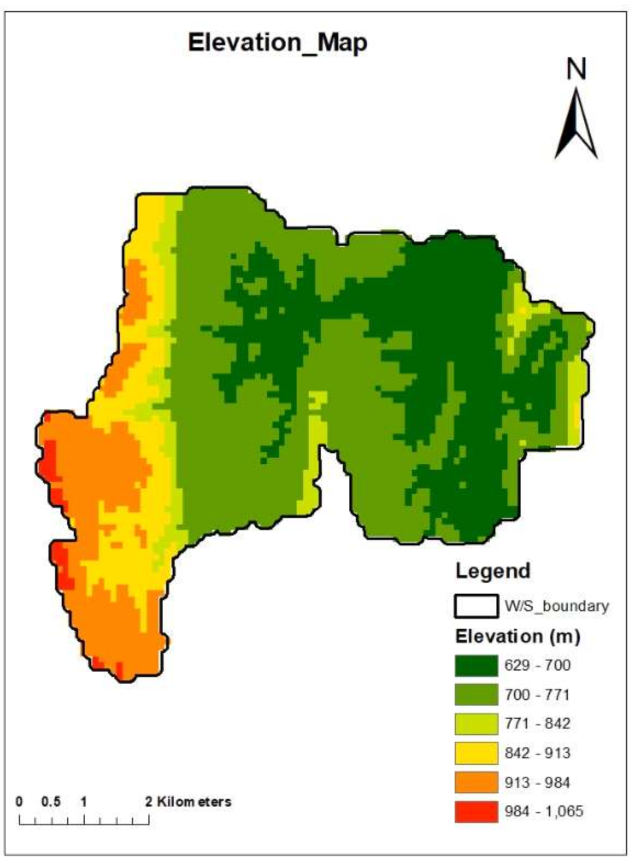

Fig. 3. DEM of the study area

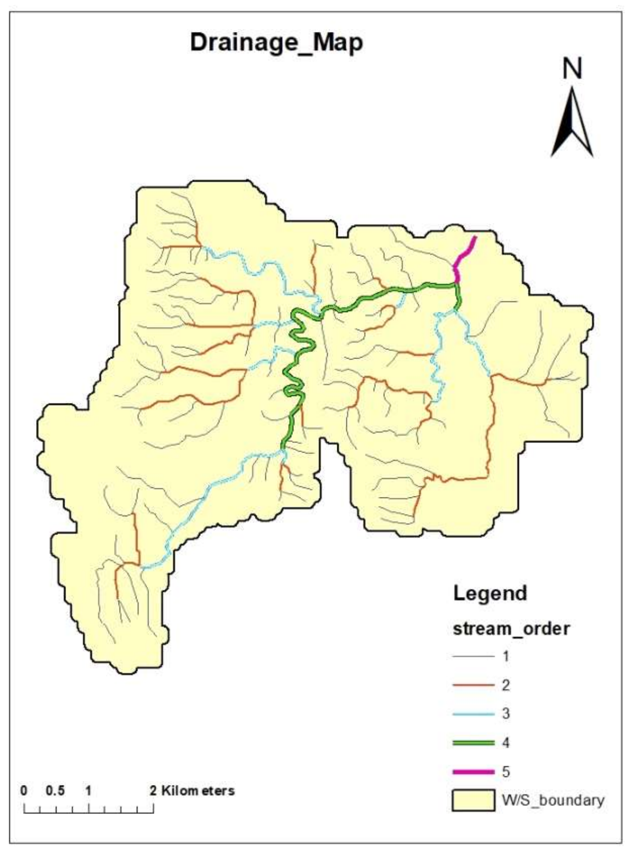

Fig. 4. Drainage map of the study area

Land use/land cover: First, unsupervised classification and then supervised classification was carried (Khalaf and Younis 2021; Fig. 8). The validation of these features, the site visiting with the help of GPS recorder (pixel-based) at several points and also used google earth imagery of dated 8 March 2015 to verify the accuracy of the results. the majority of land dominated by barren land, forest cover, cultivate the land, and water bodies. The area covered by cultivated land, forest cover, barren land, and water bodies were 1035.908, 1637.453, 689. 422, and 160.416 ha, respectively (Table 2). Therefore, most of the area was unused due to barren land and forest cover. Therefore, need to be a proper mechanism of conservation structures.

Land capability classification (LCC) : The study area was

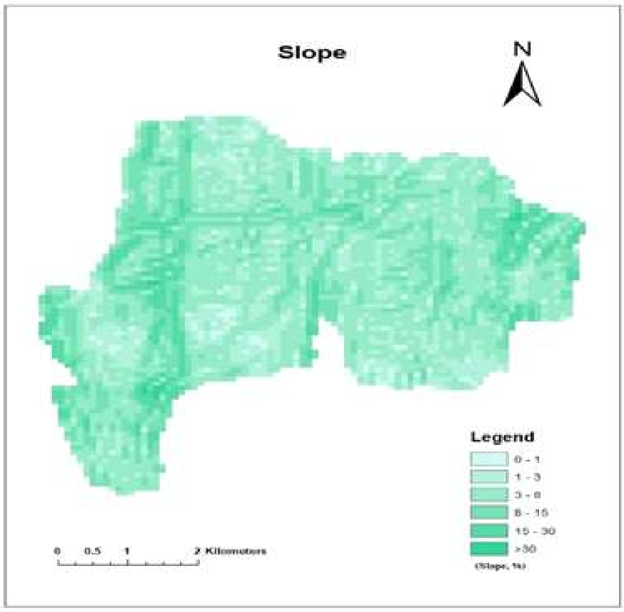

Fig. 5. Slope map of the study area

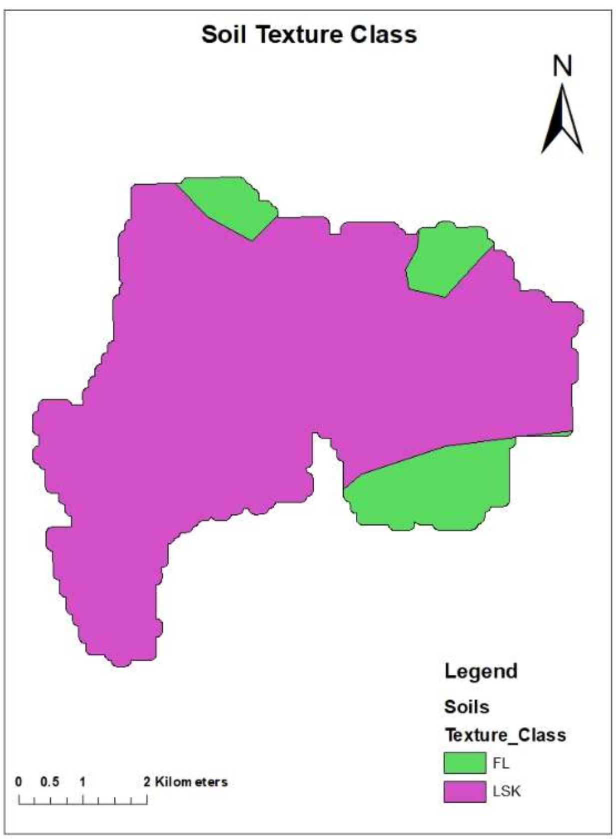

Fig. 6. Soil texture map of the study area

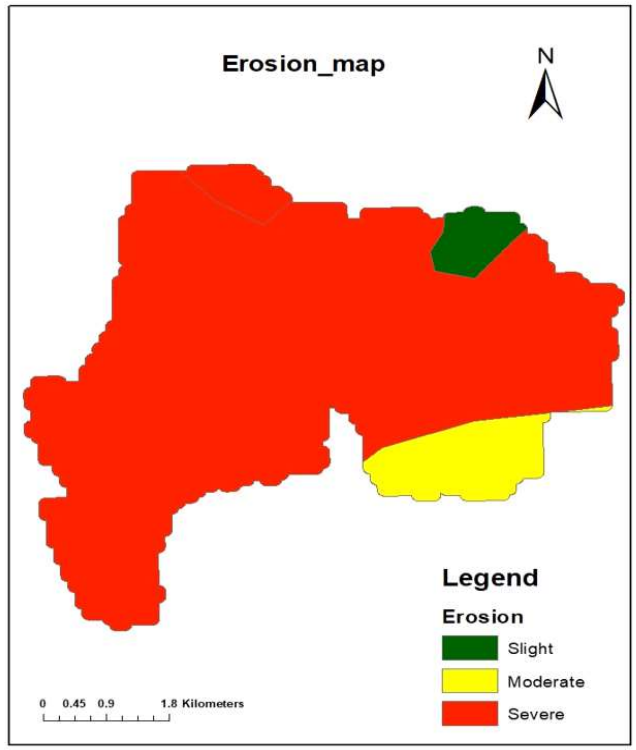

Fig. 7. Soil erosion hazard map

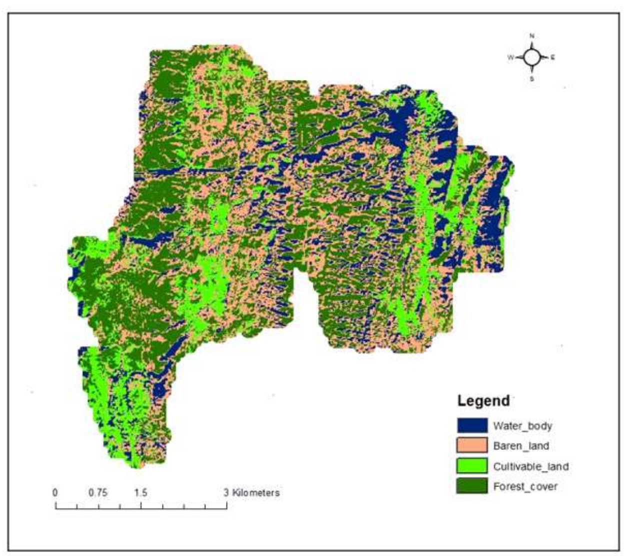

Fig. 8. Land use land cover map of the study area

classified into the class-II, class-III, class-IV, class-VI, class-VIII/ rock, and rest of areas into water bodies. The contributing areas to these classes are obtained in the range of 142.974 to 1235.885 ha (Table 3). In percentage of the total area of watershed, minimum contributing area of LCC is class-II 4 percent). This shows that the study area comes under less farming activities and there is a need for more mechanization to rejuvenate area into cultivated land. The highest contributing area is class-IV which comprises 35 percent of the total area. This shows this area may be treated with suitable conservation measures to having cultivation on them according to neediness. The next highest contributing area is 31 percent of the total study area falls in class-III categories. This also shows that the area was treated with

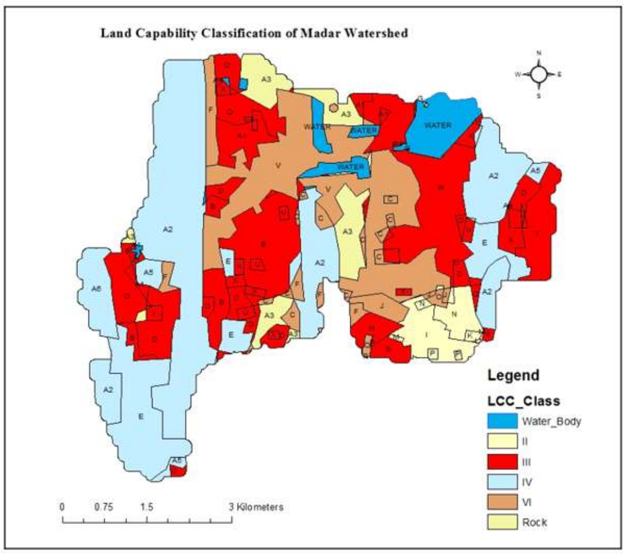

Fig. 9. Land use capability classification map of the study area

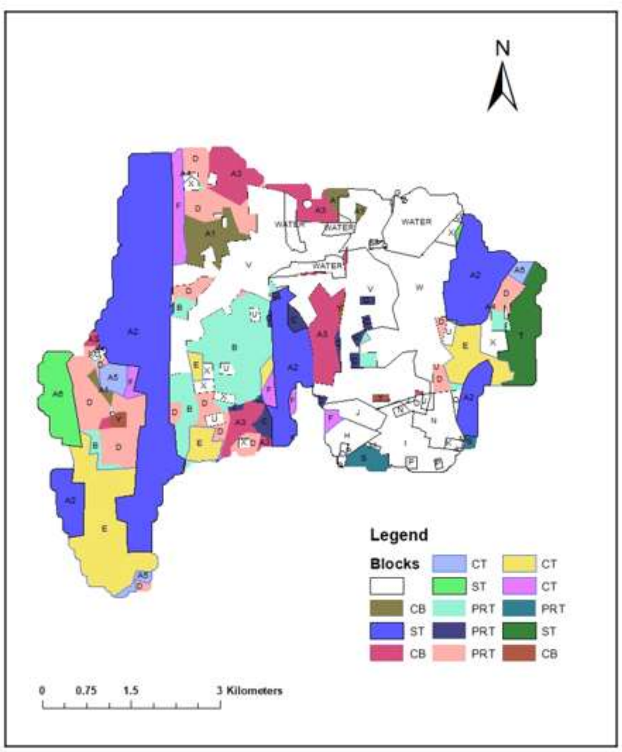

Fig. 10. Proposed area with conservation structures

Table 1. Components of soil map obtained from NBSS, Udaipur

Erosion hazard	Texture class	Depth (cm)	Area (ha)
Severe	LSK	<25	3079.027794
Moderate	FL	75-100	261.23728
Slight	FL	50-75	99.252946
Severe	FL	Rock	84.411543

Table 2. Land use/land cover classification area

Type	Area (ha)	Area (%)
Cultivated land	1035.908	29.40
Barren land	689.4222	19.57
Waterbody	160.4161	4.55
Forest cover	1637.453	46.48

Table 3. Land capability classification area

Class	Area (ha)	Area (%)
II	142.974	4
III	1108.091	31
IV	1235.885	35
VI	677.112	19
VIII/Rock	197.999	6
Water bodies	160.416	5

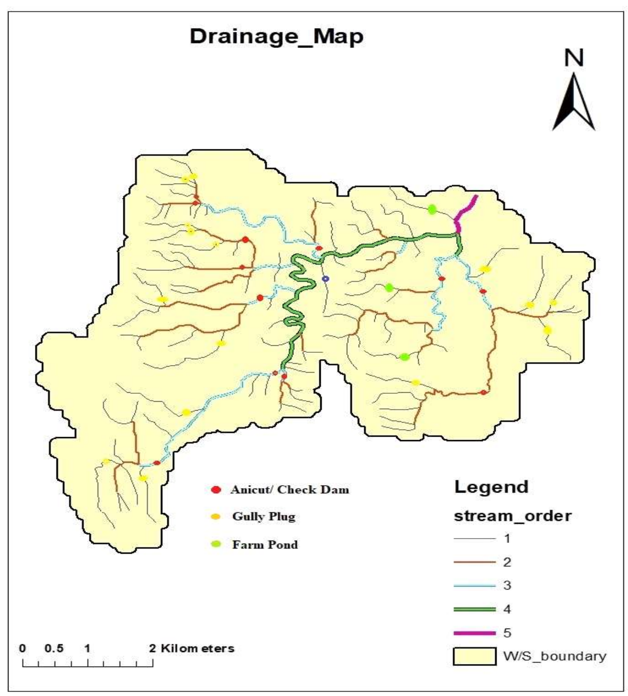

Fig. 11. Proposed site suitable for water harvesting structures

Table 4. Land use capability classification

Block	Area (ha)	Soil mapping unit	LCC	Limiting factor
A1	74.374	$\frac{lsk-d_1}{c-e_3}$	III	Land has been severe eroded (e_3)
A2	828.343	$\frac{lsk-d_1}{f-e_3}$	IV	Land has been severely eroded (e_3) and higher slope (e)
A3	197.999	Rock	VIII	Rock land
A4	2.089	$\frac{lsk-d_1}{b-e_3}$	III	Land has been severely eroded (e_3)
A5	37.391	$\frac{lsk-d_1}{e-e_3}$	IV	Land has been severely eroded (e_3) and highly slope (E)
A6	79.517	$\frac{lsk-d_1}{f-e_3}$	IV	Land has been severely eroded (e_3) and higher slope (E)
B	244.012	$\frac{lsk-d_1}{d-e_3}$	III	Land has been severely eroded (e_3) and moderately slope (D)
C	45.435	$\frac{lsk-d_1}{d-e_3}$	VI	Land has been severely eroded (e_3) and moderately slope (D)
D	285.598	$\frac{lsk-d_1}{d-e_3}$	III	Land has been severely eroded (e_3) and moderately slope (D)
E	290.634	$\frac{lsk-d_1}{e-e_3}$	IV	Land has been severely eroded (e_3) and highly slope (E)
F	67.736	$\frac{lsk-d_1}{e-e_3}$	VI	Land has been moderately eroded (e_2) and higher slope (E)
G	0.419	$\frac{fl-d_3}{b-e_1}$	II	Less erosive land
H	30.539	$\frac{fl-d_4}{e-e_2}$	III	Land has been moderately eroded (e_2) and moderately slope (D)
I	81.174	$\frac{fl-d_4}{a-e_2}$	II	Land has been moderately eroded (e_2)
J	32.821	$\frac{fl-d_4}{a-e_2}$	VI	Land has been moderately eroded (e_2)
K	4.475	$\frac{fl-d_4}{b-e_2}$	II	Land has been moderately eroded (e_2)
L	0.535	$\frac{fl-d_4}{b-e_2}$	II	Land has been moderately eroded (e_2)
M	2.077	$\frac{fl-d_4}{b-e_2}$	II	Land has been moderately eroded (e_2)
N	48.615	$\frac{fl-d_4}{c-e_2}$	II	Land has been moderately eroded (e_2)
O	10.367	$\frac{fl-d_4}{c-e_2}$	VI	Land has been moderately eroded (e_2)
P	5.290	$\frac{fl-d_4}{c-e_2}$	II	Land has been moderately eroded (e_2)
Q	4.561	$\frac{fl-d_4}{d-e_2}$	III	Land has been moderately eroded (e_2) and moderately slope (D)
R	0.148	$\frac{fl-d_4}{d-e_2}$	VI	Land has been moderately eroded (e_2) and moderately slope (D)
S	26.876	$\frac{fl-d_4}{d-e_2}$	III	Land has been moderately eroded (e_2) and moderately slope (D)
T	86.250	$\frac{fl-d_4}{f-e_2}$	III	Land has been moderately eroded (e_2) and highly slope (F)
U	25.779	$\frac{lsk-d_1}{a-e_3}$	III	Land has been severely eroded (e_3)
V	520.605	$\frac{lsk-d_1}{a-e_3}$	VI	Land has been severely eroded (e_3)
W	257.369	$\frac{lsk-d_1}{a-e_3}$	III	Land has been severely eroded (e_3)
X	58.333	$\frac{lsk-d_1}{b-e_3}$	III	Land has been severely eroded (e_3)
Y	12.311	$\frac{lsk-d_1}{b-e_3}$	III	Land has been severely eroded (e_3)
Z	0.389	$\frac{fl-d_3}{a-e_1}$	II	Less erosive land
Water	160.416		-	-

appropriate conservation measures before cultivation starts. The study area has 25 percent of the land in which cultivation cannot be possible according to the U.S. Soil conservation service (1958, 1959, 1963 and 1992). They have 19 percent class-VI, 6 percent class-VIII or rocky land. This area needs to reclaim with water harvesting structures to protect this land from wastage due to erosion. The study area has water bodies into patches at different places contributing 5 percent of the total land. Therefore, it is clear that there is an absence of class-I land which requires less or no treatment to land for cultivation. The study area has 70 percent of total cultivable land which needs to be treated with suitable soil and water conservation measures (Table 4 and shown in Fig. 9).

The criterion for their use is given in Table 5 (Singh 1998). With the help of information extracted from land capability classification, various thematic map, toposheet, google earth imagery and rainfall characteristics of the study area, the suitable conservation measures proposed for study area were puerto-rico terraces, contour trenches, staggered trenches, and contour bund in benefits for the conserving soil moisture, reducing soil erosion of the study area. The suitable site is shown in Figure 10.

Puerto-rico terrace (PRT): PRT is proposed along the contours with dry stone which further develops into level bench terraces because of ploughing. It is applicable for the area having more than 6 percent slope and also having shallow soil depth in arable land. e. Generally, PRT is constructed on the boundary of field because it is difficult to follow absolute contour in the real field conditions. The site for PRT and their contributing area is given in Table 6. The area planned to treated with PRT comes under the block-B, block-C, block-D, and block-S. The area proposed under these blocks is 231.92, 43.18, 271.46, and 25.54 ha respectively.

Contour trenches: Generally, contour trenches are suggested to be constructed for the area having slope up to 30 percent only, because of it is not stable above this slope and also, vertical interval between two contour trenches becomes less hence loss of area due to it is more. The parts of the study area planned to treat with contour trenches are block-A5, block-E, and block-F having proposed area 35.53 ha, 276.20 ha, and 64.38 ha respectively (Table 6).

Staggered trenches: Construction for staggered trenches are suggested in the area having slope greater than 30 per

Table 6. Proposed area for soil and water conservation structures

Block/conservation structures	Proposed area (Ha)
P.R.T.	
B	231.92
C	43.18
D	271.46
S	25.54
Contour Trenches	
A5	35.53
E	276.20
F	64.38
Staggered Trench	
A2	787.34
A6	75.57
T	81.97
Contour Bund	
Y	11.70
A1	70.70
A3	188.21

Table 5. Criterion for site selection of different soil and water conservation measures

Structure	Rainfall/ runoff (mm)	Slope (%)	Infiltration rate/seepage	Soil depth	Desired use
P.R.T.	400-800	> 6	Moderate	Shallow soil depth	Protection of valley slopes
Contour trench	400-1200	10-30	Good	-	In-situ moisture conservation
Staggered trenches	400-1200	> 20	Moderate	Shallow soil depth	In-situ moisture conservation
Contour Bund	< 600	< 6	Moderate	Must not be Black cotton soil	In-situ moisture conservation

Table 7. Criterion for site selection of different water harvesting structures

Structure	Runoff potential	Porosity & permeability	Stream order	Slope (%)
Anicut	Medium/High	Low	1-4	<15
Gully Plug	High	Low	1	15-20
Farm Pond	Medium/High	Low	1	0-5

The site selected for farm pond is such that there is less excavation and more runoff extracted can be possible. There are three sites proposed to be suitable for farm pond cent. The parts of study area planned to propose under this category are block-A2, block-A6, and block-T (Table 6).

Contour bund: Contour bunds are proposed in Block-Y, Block-A1 and Block-A3 having land capability class II with the slope range of 1.75 to 5.60 percent. The total area under contour bund is 270.61 ha (Table 6).

Water harvesting structures: The proposed site for different water harvesting is shown in Figure 11. The IMSD guidelines are used to identify the site for water anicut/ check dam, gully plug, and farm pond (Table 7). Check dams applied mostly in the Central India region are one of the popular water harvesting structures (Khonkaen et al 2011, Arnab et al 2018, Khattab and Basman 2021). It works as both for water-conserving structures as well as soil erosion.

CONCLUSIONS

The study was conducted for planning and designing of Madar micro-watershed using remote sensing and GIS. The project area lies between 73^{0}35' to 73^{0}36' E longitude and24^{0}40' to 24^{0}42' N latitude. Madar is a Village in Bargaon Tehsil in Udaipur District of Rajasthan State, India. The total geographical area of the delineated watershed was determined to be 3000 ha and divided into thirty-one different blocks on the basis of slope groups. The appropriate soil and water conservation measures for the watershed were planned on the basis of rainfall, land use capability classification and topography of the area. Contour bunds, Puerto rico terrace, staggered trench, and contour trench were proposed for soil and water conservation measures. Water harvesting structure such as anicut/ check dam, gully plug, and farm pond has been proposed in the watershed area on the basis of site condition and its functional utility.

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Consequences of Soil Quality on Crop Yield: An Update

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Abstract: The soil is the medium that can fulfill the basic requirement of food, shelter, and clothes for human beings. It is an essential part of our agriculture system. Therefore the crop yield depends on the nutrients intake of soil via plants in the form of cation or anion. It is the mixture of macro (needed in large amounts) and micro (required in significantly less quantity) nutrients essential for a better crop yield. A proper understanding of the physicochemical and nutrient dynamics of any soil is the key to knowing the productivity of crops. Various parameters such as soil temperature, pH, electrical conductivity, moisture content, bulk density, texture, soil organic matter, organic carbon, and sodium adsorption ratio, are essential sources to understand the properties of soil. This information will helpful to the farmers for producing better quality crops. In today's scenario, due to the shortage of water, people are using anthropogenic water as a source of irrigation. The irrigation by sewage water may change the chemical properties of the soil, affecting the equilibrium between acidity and alkalinity. Likewise, it may affect agricultural growth and farming quality. Besides this, excessive use of chemical fertilizers adversely alters the quality of the agriculture field. In this review, we have focused on various soil parameters that may affect the growth and nutrient quality of yield. This information will also develop the awareness among farmers for economic productivity.

Keywords: Physico-chemical parameters, Nutrients dynamics, Soil, Agriculture field

Soil is the part of the earth's crust serving as a significant resource for the socio-economic development of the living being. For livelihood, a total of 70% of the population depends upon agriculture and related allied activities; therefore, agriculture proves to be the predominant part of the world economy. The formation of soil took in both constructive and destructive manner and constituted of broken rock material, which has been reformed by chemical and mechanical processes either through weathering or erosion (Pujar et al 2012, Sumithra et al 2013). Soil is not just a mixture of mineral particles but also the biotic system of living beings as well as other related constituents. Besides this, soil formation is also affected by various meteorological parameters such as rainfall, humidity, air temperature, etc. (Pietraszko et al 2018). Soil is a combination of organic and inorganic constituents with minerals, water, and a small portion of air. The plants themselves do not synthesize the essential nutrients, so they are extracted from the soil. Therefore fertility of soil plays an important role in controlling the yield of crops. The water type used for irrigation may also affect the quality of the soil to some extent. Besides increasing the nutrient value of soil through irrigation, wastewater from various anthropogenic activities is constantly contaminating the soils and crop products (Kumar et al 2019a). Likewise, it is loaded on plant portions via a root system and transported to the food chain. Similarly, soil fertility may also be responsible for controlling the production of the crop (Kumar et al 2019a). The properties of any soil may vary spatially and temporally from a field to a larger regional scale. It gets influenced by both intrinsic and extrinsic factors. The intrinsic factor includes the soil formation process like soil parent material. The extrinsic factors may include soil management practices such as fertilization and crop rotation (Cambardella and Karlen 1999). Besides all the above problems, dumping of solid waste is one of the major issues that make soil infertile, and barren, while mixing with dumping materials. Solid waste with water contact forms leachate that contaminates soil as well as groundwater (Kamboj and Choudhary 2013). The disposal site must be located far away from the water sources like rivers and lakes, residing areas, areas in and around the forest, and agricultural land (Kamboj and Pandey 2017). This review article emphasizes is on the effects of soil physicochemical and nutrient quality on the growth and production of crops.

Role of Physicochemical Properties

Soil temperature: Soil temperature has a role in heat exchange between the soil and the atmosphere, known as heat flux (Elias et al 2004). It can also define the function of the internal energy of the loam. The heat transport in the ground (Zhao et al 2007) and the exchange of energy at the surface (Nwankwo and Ogugurue 2012) are the fundamental sources of the change in soil temperature. Solar radiation is an essential source of soil temperature. Temperature is the major factor that affects the physical, chemical, and biological properties of soil and plant growth. Therefore, the soil is the key reservoir of energy during the day and a source of heat to the surface at night and stores energy during the summer season and releases it into the atmosphere during the cold climatic condition (Gieger et al 2003). The amount of absorbed energy interacts with the lost energy from the soil, which decides the temperature of the soil. However, the fluctuation in soil temperature on daily basis depends on the changes in the air temperature and solar radiation. The rate of absorption of dark-colored soil is greater than that of light-colored soil (Sandor and Fodor 2012, Nwankwo and Ogugurue 2012) and the dark-colored soil have high temperature than the lighter one. (Lehnert 2013, Probert 2000). The metabolic activity of microbes can maintain the proper nutrient cycle in soils. The microbial optimum temperature in the soil ranges between 10°C and 35.6°C, for performing their activities. There will be a decrease in the soil microbial activities when the temperature is low, and at the freezing point, most of the activities cease (Allison 2005). During the soil microbial interaction, the breakdown of complex organic matter to a simpler form and nutrient mineralization is affected by the climate. Even the water retention capacity of the loam, transmission, and availability to the plants get influenced by the weather. Due to the elevation in metabolic activity, soil temperature increases that stimulate the availability of nutrients for plants because of changing soil water viscosity and root nutrient transport (Grossnickle 2000). Lower temperature induces the reduction of nutrient uptake. Many factors affect the soil temperature

Soil mulch: The moisture content of the soil gets increases as the material inhibits the rate of evaporation (Horton et al 1996). Thus, more mulch materials reduce the temperature of the soil surface (Shinner et al 1994). Usually, more heat will flow into bare loam as compared to the mulched soil. Consequently, mulching of the earth's surface insulated the energy in the form of radiation (heat), lowered the temperature, and kept the loam cool (Dahiya et al 2007).

Slope of the land: The land slope is important in expressing the amount of radiant energy scattered through the land surface. This energy strike at a right angle dispersed to the minimum surface area compared to the left angle strike. So that, if the land slope increases, the amount of radiation per unit area of the land surface decreases (Elisabarashivilli et al 2010).

Vegetative cover: Vegetative cover of any land act as an insulator, affecting the soil temperature. The exposed soil absorbs more heat during the hot season, while in the cold season, the absorption rate is less, so it remains cool. Due to the presence of a dense amount of vegetative cover, the soil of the area neither becomes hot during the dry season nor too cold during the rainy season (Nwankwo et al 2012).

Organic matter content: A high amount of organic matter in the soil can increase water holding capacity that changes the color of the soil. Likewise, the increased amount of radiant energy absorption may increase the soil temperature (Fang et al 2005).

Evaporation: Considerable amount of energy is required in the process of evaporation of water. The water present in the soil utilizes the solar energy radiation to evaporate, thus exposing it engaged for heating the loam. Greater is the rate of evaporation, more speedily, the earth gets cooled, and its temperature gets decreased (Geiger et al 2003).

Water uptake: The lower the temperature, the lesser will be the water uptake. It is usually due to the decreased rate of absorption and increased rate of viscosity. Due to the decrease in uptake of water, the rate of photosynthesis gets reduced (Toselli et al 1999).

pH: pH is one of the essential physical parameters that play a significant role in maintaining the hydrogen ion concentration in soil (Pandesswari et al 2012). It is also the measurement of the intensity of acidity and alkalinity (Kamboj et al 2013). An increase in acidity and alkalinity harms water chemistry, thus resulting in corrosive behavior. When these types of water are used for irrigation in the agricultural fields, alter the biochemical reactions that occur in the soil (Fig. 1, Chitragar et al 2016). Due to the buffering function, the soil's pH value usually remains relatively stable (Masto et al 2009). Once there is a change in the pH, it may result in changes in the chemical properties. This change dramatically affects the existing form, transformation, and availability of soil nutrients directly (Ma and Zhao 2010). The changes in the pH value are generally related to the type of water for irrigation and the category of soil in taking it (Wan et al 2015). It was observed that there was no apparent effect on the pH of the agriculture fields when the sewage came from the breeding of livestock (Zhang et al 2011).

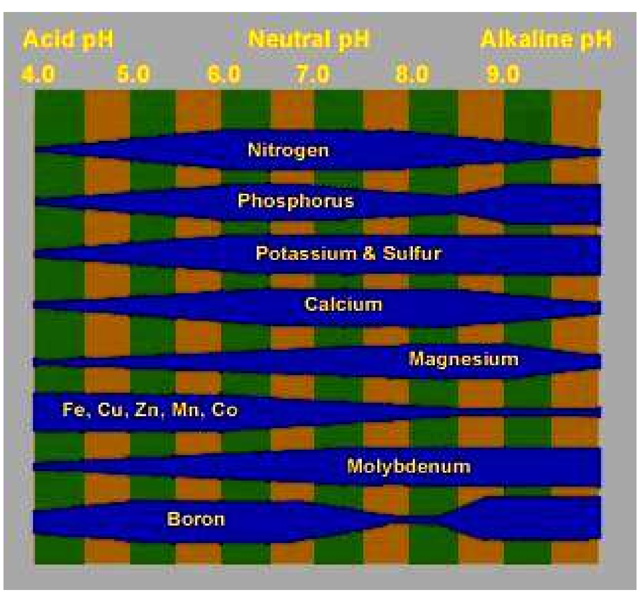
Electrical conductivity: Electrical conductivity is a measurement that has a relationship with soil properties that dramatically affects the texture, cation exchange capacity, level of organic matter, drainage pattern and condition, salinity, sub-surface characteristics, etc. (Solanki and Chavda 2012). Conductivity indicates the presence of dissolved solids and another major ionic form in the water bodies (Deshpande and Aher 2012). The increasing concentration of ions indicates a high electric conductive effect. High conductivity lowers the availability of water to the plants, thus affecting the rate of growth (Bauder et al 2013). Soil health is rapidly measured by Electric Conductivity. With the change in depth, conductivity differs, and variation is comparatively less in the unplanned soil profile. Likewise, the changes in the unplanned soil profile are due to land-surface slope, elevated permeability, and high rainfall, responsible for leaching out of alkali and alkaline bases (Dutta and Ram 1993). In the measurement of salinity and estimation of soluble salt concentration in soil, electrical conductivity plays a crucial role. Salinity is a leading water characteristic that affects crop yield (Wagh et al 2013).

Moisture content: Soil moisture is one of the most vital physical parameters of soil. It impacts crop development by influencing supplement accessibility (Behera 2014). The nutrient absorption of soil depends on the moisture content. Texture and the structure of any soil decide its water content. Void ratio, size of the soil particles, minerals in the clay, organic matter, and condition of groundwater also play an essential role in the moisture content of the soil (Yennawar et al 2013). Clay has a larger moisture content, due to its high quality of porosity, while the sandy soil has a lesser amount of moisture due to a lower porosity level. The porosity is the deciding factor in the wetness of soil (William 2005). If the water holding capacity of any soil is adequate, it shows the physical activeness of the soil (Soffe 1995). However, the type of red and black soil has maximum water holding capacity (Thakre et al 2012, Vanderlinden et al 2005).

Texture: The retention of nutrients and capability of drainage, both are directly related to the texture. Soil texture is considered to be a persistent feature and is not readily getting changed in the field (Brady and Weil 2007). Based on the proportion of various sized particles, the soil could be classified into different textural groups. Besides this, Soil-water retention, aeration, and root penetration get influenced by soil texture directly. The texture of the soil plays a vital role in carbon storage and strongly influences the retention of nutrients and their availability (Hamarashid et al 2010). Soil with a tremendous amount of clay content will be more conducive than the earth with a higher amount of sand. Black soil has a loamy and clay texture, yellow soil, earthy clay, and red soil silt clay and loamy (Jain and Singh 2014). The surface of the loam influences the diversity of the microbial communities together with pH, organic matter content, and cation exchange capacity. The structure of the microbial community provides an appropriate home for the growth of the specific microorganism, making the degradation process efficient (Girvan et al 2003).

Bulk density: There are water-filled air pores in an "ideal soil" that fulfill the plants' needs with easy root penetration. The mineral particles provide the strength that helps in providing physical support and essential plant nutrients. Bulk density is also influenced by soil's physical and chemical properties (Chaudhari et al 2013). The amount of organic matter present in the soil, its texture, mineral constituent, and porosity get influenced by its bulk density. It is essential for soil management, soil compaction, and the planning of modern farming techniques (Sharma and Bhattacharya 2017). Aubertin and Kardos (1965) reported that the bulk density of clay ranges between 1.0 to 1.6 mg/m3. For sand, the typical thickness varies between 1.2 to 1.8 mg/m3 with probable root dispersion occurring at ≥ 1.4 mg/m3 for clay and ≥ 1.6 mg/m3 for sand.

Sodium adsorption ratio: Sodium adsorption proportion (SAR) is a proportion of the measure of sodium (Na+) comparative with calcium (Ca2+) and magnesium (Mg2+) in the water extracted from an immersed soil paste. It is the ratio of the Na concentration divided by the square root of one-half



Source: Chitragar et al 2016

Fig. 1. Values for pH of different nutrients in the soil

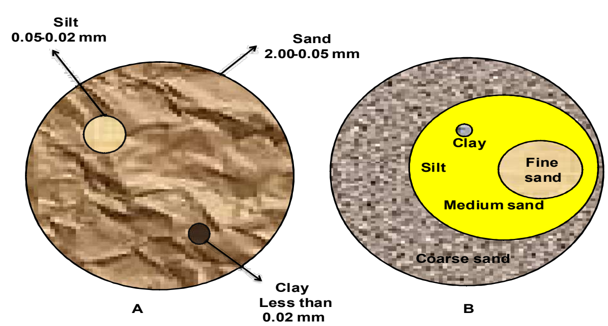


Fig. 2. 2A-Relative size of sand, silt, and particles; 2B-Identification of soil separates

of the Ca + Mg concentration. The SAR can measure the suitability of water used in agricultural irrigation. The water will be less suitable for irrigation if the SAR is high. El-Morsy et al (1991) reported that the water transmission properties of the soil get significantly affected by the SAR of irrigation water. Characterization of soil sodicity can be done by using the SAR index. The high value of SAR in the soil represents high content of sodium which has the potential to result in poor physical situations owing to crusting, logging of water, and poor permeability. The high concentration of sodium is responsible for clay swelling, dispersion, or disaggregation of soil. Irrigational water is greatly responsible for clay swelling. If the concentration of sodium and salinity in the irrigational water is high greater will be the rate of swelling (Hanson et al 1999).

Soil organic matter and organic carbon: Supply of nutrients, contributing to cation exchange capacity, and soil texture all are controlled by soil organic matter (It can define as the non-living extract of the plant and animal alterations). Besides, it includes total biomass, micro, and macrofauna, and entire decomposition products (Roscoe et al 2004).The non-living biomass composition has 95% of soil organic matter. It is divided into two categories: (i) organic matter in numerous phases of disintegration (non-humic substrate in which the morphological aspects of original biomass can be recognized) and (ii) material that is altered with no morphological characters from original biomass (active fractions and stabilizes organic matter) (Fig. 3).

The storage of soil organic matter depends on the primary addition of carbon from the plant detritus (residue) and the loss of carbon through the soil organic matter decomposition. (Machado et al 2006, Awale et al 2013). The more considerable amount of carbon sink in the biosphere is mainly due to the change in the soil organic matter. It acts as a building block of the soil organization and plays a vital role in balancing CO_2. It drives out the climatic variation in the environment (Lal 2004, Smith 2012). Soil organic matter and organic carbon both are interlinked. Other metabolic reactions, such as the exchange of ions absorption, or dissolution reaction, result in constituting dissolved organic carbon (Guggenberger and Kaiser 2003). The increase of organic content in the soil may also increase the water-holding capacity of the loam (Bharti and Kamboj 2018).

Soil nutrients dynamics: Nutrients are the compounds required for performing different metabolic activities that are essential for fertility, growth, development, and production. If the soil is deficient in nutrients or its amount is excess, it will affect the proper functioning and growth of a plant, resulting in various diseases. Nutrients in the soils determine the genetic expression of the crop plants' physiological and morphological traits (Kumar et al 2019b). The nutrients are further divided into two categories viz. micronutrients and macronutrients (Table 1).

Macro-nutrients: The nutrients are required in a large amount for the growth of the crops and are significant elements due to their presence in a large amount. The

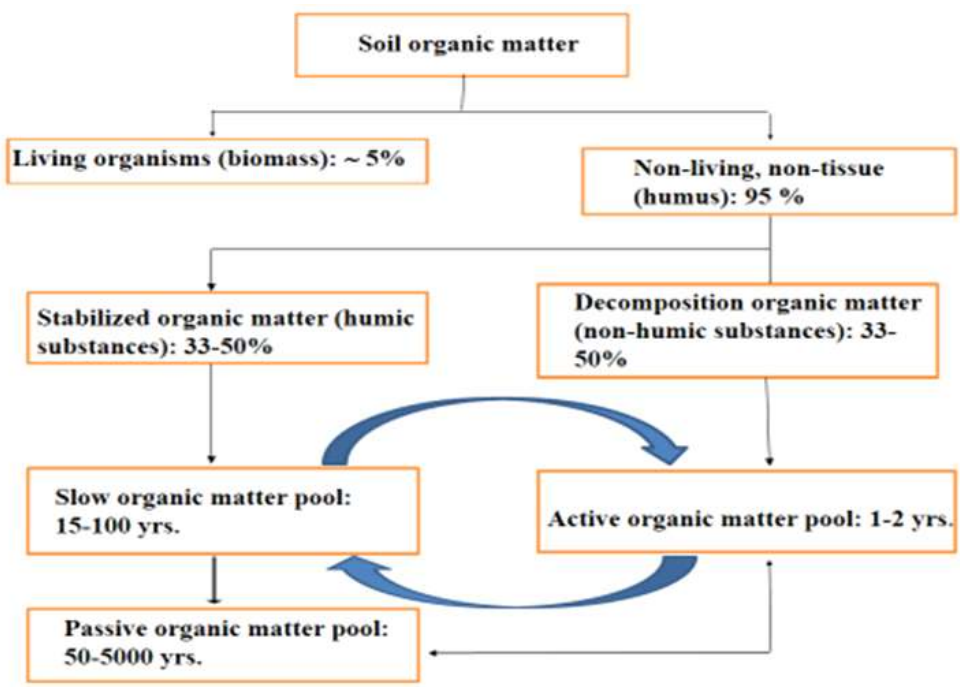


Fig. 3. Classification of soil organic components and a conceptual model of the various pools of soil organic matter

majority of the macro-nutrients are minerals except a few viz., C, H, and O. They are easily get absorbed by the roots of the plants (Chitragar et al 2016). The toxic level of macro-nutrients is very high if their concentration level exceeds the optimum level it may cause stunted growth and poor yields for plants. Testing of the soil gives enlightening information about the accessibility of supplements as a result, compost is given to amplify the harvest yield. Evaluating the fertility status of soils helps us to determine the nutritional disorders and factors that affect crop productivity. The source of irrigational water plays an important role in it. The different types of water sources used for irrigation purposes in the agricultural field affect the nutrient balance of the soil (Kamboj et al 2021; Fig. 4). Either soil becomes rich in nutrients or gets deficit, due to which fertility, productivity, or yield of the crop gets severely affected. For the proper growth of the crops, nutrient equilibrium should be present in the water, soil, and plants. In case of the irrigation through wastewater, the soil gets highly enriched with macro and micronutrients that play a crucial role in the productivity and yield of crops. (Kumar et al 2018).

Micro-nutrients: Micronutrients or trace elements are required in very little quantity but their lack may result in serious concern for the plant, which simultaneously affects crop production. All the micro-nutrients are absorbed through roots. Through weathering of mineral and organic matter decomposition, nutrients become available to the soil, which plants intake in the form of ions. Threatening to agricultural

Table 1. Different types of micro and macronutrients and their role in plant metabolism

Micronutrients	Macronutrients
Boron (B) • It plays an important role in the metabolism and movement of sugar compounds in plants. • Synthesis of plant harmonics and nucleic acids and formation of lignin of cell wall takes place with the help of Boron.	Nitrogen (N) • Amino acids are the building blocks of protein including mostly all the enzyme which controls the biological processes and nitrogen is the major part of all the amino acids. • If the supply of nitrogen is good then the growth and development of roots will be enhanced. Consecutively, uptake of other nutrients also gets increased.
Chlorine (Cl) • Plays an important role in ionic balance and photosynthesis and is essential for osmosis.	Phosphorus (P) • It enhances many elementary processes counting photosynthesis, flowering, maturation, fruiting, maturation, and fixation of nitrogen. • DNA and RNA moleculesare made up of phosphate groups. • Present in both the form i.e., organic and inorganic, that can be easily translocated in the plants. • Most of the energy transfer in the plants is dependent on phosphorus. • The availability of phosphorus to the plants is in a very limited quantity in most of the soils since it is released very slowly.
Copper (Cu) • Formation of the cell wall, photosynthesis, respiration, and other such processes involving nitrogen.	Potassium (K) • Mostly it occurs in all the parts of the plant in significant amounts. • Through the potassium ion pump, it regulates stomatal closing and opening. • As a nutrient, the rate of its mobility solubility is outstanding.
Iron (Fe) • The main constituent of chlorophyll formation and many reactions involving enzymes. • It also plays an important role in the process of photosynthesis and respiration.	Calcium (Ca) • The majority of the amount is present in the leaves of the plant. A small concentration of it is also present in roots, seeds, and fruits. • A major constituent of the cell wall. • It is also involved in photosynthesis and plant structure.
Manganese (Mn) • Many of the enzymes have manganese content in them. • It is involved in photosynthesis and the growth of roots also takes place in its presence. • It is also involved in N_2 fixation.	Magnesium (Mg) • Chlorophyll contains magnesium as a constituent material. When the plant is deficient in magnesium molecules, its translocation starts from the older tissues to the younger side, showing the deficiency of nutrients to the older part first and then to the younger area.
Molybdenum(Mo) • It is involved in N_2 fixation.	Sulfur (S) • It is important in the development of chloroplast and is a structural part of some amino acids and vitamins.
Zinc (Zn) • It is part of DNA proteins and many organic complexes. • It also acts as an important enzyme in protein synthesis. It is also responsible for harmonic production and seed development.	

potentials is due to the soil nutrients because their availability depends on soil organic matter content, soil pH, adsorptive surface, soil texture, and interaction of nutrients in the soil. Throughout the world, the soil's micro-nutrients are available in fewer amounts, due to which the plants growing in the soil suffer from nutrient deficiencies, affecting their growth and showing various disorders (Rengel 2007, Alloway 2008). Strong leaching and high precipitation result in humid tropical regions' micro-nutrient deficiencies and humid temperate regions. The symptoms of micronutrient deficiencies appear on younger leaves at the apical part of the plant, while the toxicity symptoms appear on the older leaves of the plants (Chitragar et al 2016). Various factors viz., clay content, pH, soil organic matter, cation exchange capacity, level of phosphorus in the soil, and tillage practices affect the solubility and availability of micronutrients in the soil.

Nutrients Essential for Growth of Plants

Nitrogen: Nitrogen is the main element and gets quickly absorbed by the roots of the higher plants in the form of nitrate and ammonium ion (Sumithra et al 2013, Masto et al. 2009). Sewage irrigation shows a lesser effect on ammonium ions present in the deep inside soil and groundwater, but long-term irrigation results in a higher concentration of nitrate, causing groundwater pollution inthe deep soil layer (Liu and Lu 2002). Nitrogen (N) is an integral part of all the proteins, enzymes, and metabolic processes involved in the formation of energy and it transfers to the cell. (Singh et al 2014). The nitrogen cycle is greatly influenced by biological processes and act a significant role in the soil system (Fig. 5). Roots of some higher plants havea symbiotic relationship

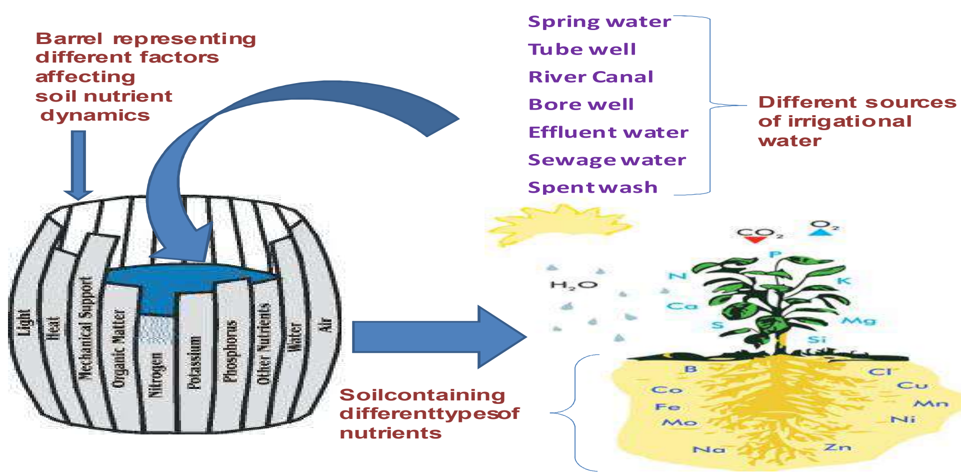


Fig. 4. Representation of different types of nutrients present in the soil

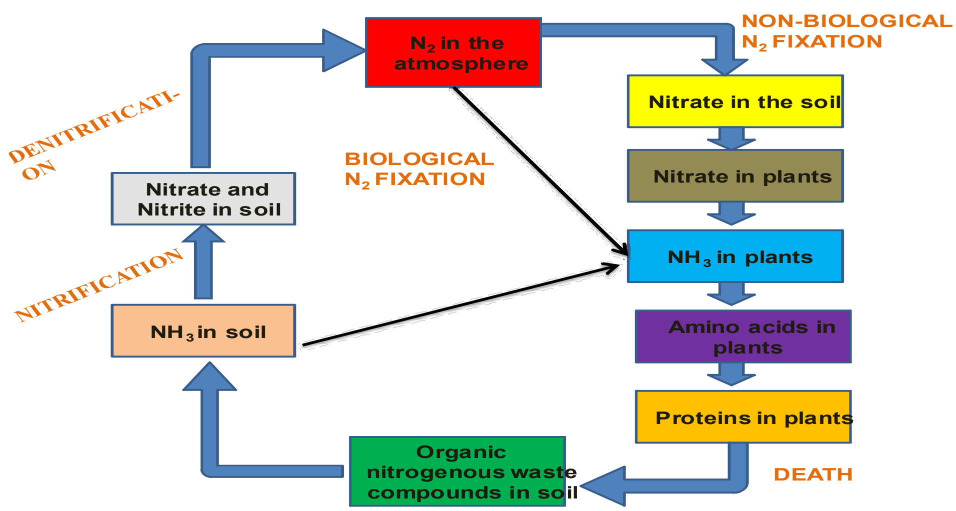


Fig. 5. Nitrogen cycle representation representing biological and non-biological N_2 fixation pathway

with certain bacteria like Nostoc and Anabaena that fixes atmospheric nitrogen in the form of ammonium ion (e.g., Corolloid root of Cycas).

Phosphorus: Every plant cell contains phosphorus as one of its constituents. It is also counted as a key reservoir responsible for better plant growth. Therefore it acts as limiting nutrients that remain in the plant nuclei as an energy storage packet (Jain et al 2014). For the growth of the plant, a large amount of phosphorus is required, which plays a vital role in photosynthesis and is concerned with the creation of oils, sugars, and starches (Singh et al 2014). There is an abundant amount of phosphorus present in the fruits and seeds of plants. For the germination of the seed, flowering, and fruit formation, phosphorus plays an essential role, and its deficiency results in the purple stem of leaves and poor yield of fruits in crop fields (Wagh et al 2013). Phosphorus is the vital micro-nutrient required for the metabolic activity and proper growth of the plant. Processes like growth, respiration, and reproduction depend upon the availability of phosphorus in the soil in which the plant grows (Wagh et al 2013). In the topographic region, the soil's available phosphorus content is higher than the soil in the lower topographic level (Singh et al 2014). The soil with an ample amount of phosphorus content results in the early growth of the plants and hastens ripeness (Solanki and Chavda 2012). The soil with the maximum leaching rate has a high phosphorus ratio as compared to the soil with the minimum leaching rate (Ashraf et al 2012).

Potassium: Potassium (K) is considered to be one of the essential elements found abundantly in soil. Potassium in the soil is found in four forms viz. solution, exchangeable, fixed or non-exchangeable, and structure or mineral (Fig. 6 & 7). The interchangeable level of K and Non-exchangeable level of K contain a lesser portion of the whole K. The bulk of the entire soil K is concentrated in the mineral portion of the soil (Sparks and Huang 1985). The availability of K to the plants and microbes is in the order: solution> exchangeable> fixed (non-exchangeable)> mineral (Spark and Huang 1985, Sparks

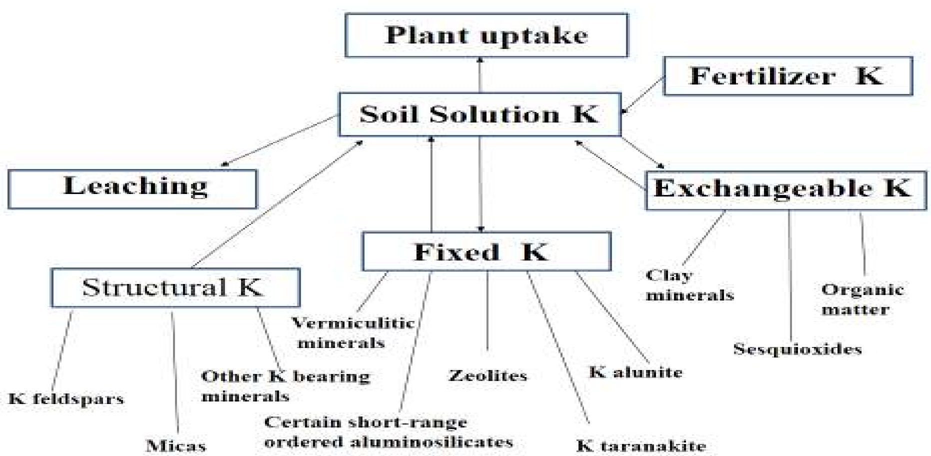


Fig. 6. Various forms of K found in the soil, their inter-relationship (Sparks and Huang 1985)

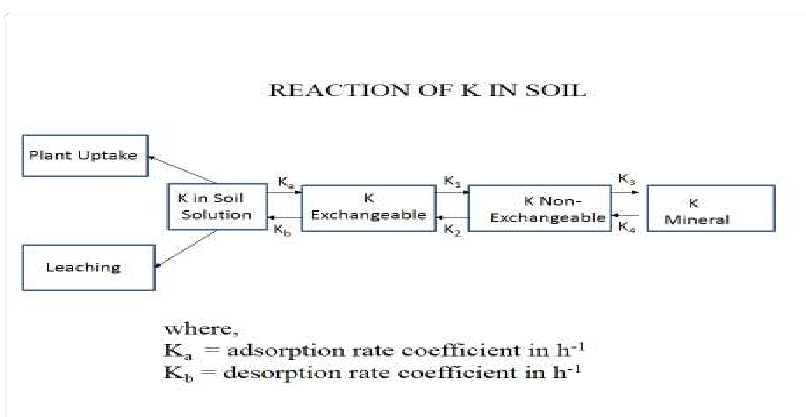


Fig. 7. Exchangeable K dynamics in soil

1987, Sparks 2000). There is a kinetic and equilibrium reaction exists between the four forms of soil potassium, which affects the level of soil solution K at a particular time, and thus, the amount available for plants. The frequency and a way of reaction between the solution and exchangeable forms of K govern whether the applied K will be leached into the lower distances, taken up by the plants, converted into unavailable forms, or released into the available forms. The rate of reaction between the soil solution and exchangeable phases is strongly dependent on the kind of clay mineral present and the methods implied to measure the kinetics of K interchange (Amacher 1991, Sparks et al 1996). Though it is not an essential element for plants still it plays a key role in plant growth, protein synthesis, maintenance of plant water stability, etc. (Sumithra et al 2013). Many of the metabolic reactions ranging from lignin and cellulose used for the formation of cellular structures, for the regulation of photosynthesis, and manufacture of plant sugars used for several plant metabolic needs, are dependent on the concentration of potassium (Solanki et al 2012).

If the soil is deficient in nutrient content, organic or chemical fertilizers containing nitrogen, phosphorus, and potassium are added up. The chemical fertilizers bring up a quick reaction with the soil and promote plants to grow up at a faster rate. They are available to the plants for a shorter period and get diminished. On the other hand, organic fertilizers are sustained in the soil for a longer period and are available to the plants constantly, promoting their growth and yield. So, the better option will be to use a mixture of chemical and organic fertilizers in a specific ratio. This will help the plant to grow properly without affecting the quality of the soil (Raj and Joshi 2019).

Impact of soil nutrients on crop yield: The fast growth in the anthropogenic activities in the agricultural field to increase the crop yield, in some way or the other affects the productivity of the soil to a greater extent. The quality and fertility of soil directly influence the nutritive content of the food crops and the overall nutrient output of the agricultural system (Bruulsema et al 2012). Soil nutrients after increasing upto the saturation level, become intolerant to the further intake of external supplements and with time start degrading the soil properties thus making it sodic. The micronutrient deficiency in soil has become one of the major concerns about plant growth and yield. In the last few decades, it was observed that deficiency of micronutrients viz., zinc, boron, and molybdenum is ascertained. Soil with the deficiency of zinc was mainly seen in Asia including the countries viz., India, Turkey, China, and Indonesia. The deficiency was also observed in the north-western part of South America and sub-Saharan Africa as reported by Udo de Haes et al (2011) emphasized that the soil with maximum calcium content dominant in the Middle East has high pH with lesser organic content (i.e., <1%), therefore the crops obtained from these regions have low micronutrient content. Similarly, a lesser amount of rainfall and variance in the fertilization rate also adds to micronutrient deficiency in these regions. Kiran (2017) assessed the soil quality of the Gurdaspur region and found out that some of the micro-nutrients viz., zinc and copper were present in a very lesser amount. Even boron and iron were also present in fewer quantities. Due to the nutrient deficiency, the productivity of soil and yield of wheat gets lessened. Similarly, the Rupnagar region had high to very severe nutrient deficiency in zinc, copper, and boron resulting in lesser yield. The districts such as Nawanshahr, Jalandhar, and Sangrur had moderate to high yields in respective nutrients such as zinc, boron, manganese, etc. In the overall context, the areas with low wheat yields have a greater micro-nutrient deficiency compared to the area with high yields.

Methods and techniques to be adopted to manage the soil fertility and crop yield: In order to improve the variety of crops and yield, different methods and techniques are to be adopted in a way to cope with the nutrient loss or hyper accumulative conditions. Some of the techniques are well illustrated below:

Nuclear or isotopic techniques: In this technique, an isotopic form and nitrogen and phosphorus i.e., N-5 and P-32 are used. This isotopic form helps in tracing out the labeled fertilizers containing nitrogen and phosphorus into the soil, water, and crops. It gives overall quantitative data on the efficiency of the usage, their translocation, their residual effects, and also their transformation from one form to another. N-15 helps in quantifying the nitrogen fixed from the atmosphere via nitrogen fixation by the crops from the leguminous family. The isotopic carbon i.e., C-13 helps to quantify the residues of crop that is incorporated for the stabilization and fertility enhancement as reported by Kirda et al (1999).

Zai technology: In sub-Saharan Africa (SSA) due to the low or unreliable rainfall and less moisture content, the fertility of the soil is deteriorating at a greater pace. This has resulted in a low crop yield. Zai technology is one of the trending ways to promote soil moisture enhancement by retaining the water, thus increasing the fertility of the soil. In this technique, the organic method gets buried beneath the soil in small pits that helps in restoring fertility and also conserves water in the soil. Though adoption of this technique is not that high, it is one of the eco-friendly techniques as reported by Danso-Abbeamet al (2019).

Machine learning technique: For the prediction of the yield of a crop, the machine learning technique proves to be one of the most effective tools. Machine learning helps to predict the yield based on machine learning as reported by Pandith et al (2020). Machine Learning is a technology that allows computers to learn and improve automatically over time by regularly training them. It consists of a series of well-defined models that gather certain data and use precise algorithms to obtain the desired outcomes. In order to improve the productivity and quality of the crops grown, machine learning techniques have been used in the agriculture area. Machine Learning algorithms are used to evaluate which conditions will generate the best yield for a specific crop.

CONCLUSION

Soil degradation is taking place at a greater rate. It is due to the conventional agricultural practices that are mostly dependent on concentrated input of chemicals. The physicochemical and nutrient quality of soil is essential in improving the productivity and yield of crops that may fulfill the increasing human demands. Looking towards a few years back, we can observe that the farmers are using excessive amounts of fertilizers and pesticides to improve productivity without knowing much about their negative roles. On one hand, yield is increasing, and on the other hand, soil quality is decreasing. The crops with the shorter height get wilted out, thus suppressing the expected growth. So, analysis of the soil parameters becomes an essential part of any research work. The use of intensive agriculture practices may have a large number of disadvantages in the long run. Therefore, modern techniques are adopted for sustainable agriculture by applying bio-fertilizer and bio-pesticides instead of chemical ones. For a better tomorrow, a new hope has confronted the economic and environmental viewpoints. Same as bio-fertilizers, bio-pesticides used for pest control appears to be an essential tool in recent years. Due to the intrinsic and extrinsic factors, soil fertility variation is also very high in many of the nutrients. Before starting any experiment, one should keep the field un-planted for one season to reduce the error resulting due to the soil fertility variation. Therefore, it has become an important parameter to maintain soil health for the security of food for the future generation and increasing agricultural yield. The matter discussed in this article may help the farmers to utilize the information in managing the condition of nutrient equilibrium that is an essential part of plant metabolism and growth.

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Macronutrient Status of Soils under Different Plantation Cycles of Poplar (*Populus deltoides* Bartr.) Based Agroforestry System in Punjab

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***Abstract*:** The study was conducted to determine depthwise (0-15, 15-30, 30-60 and 60-90 cm) available primary (N, P and K) and secondary (Ca, Mg and S) macronutrients in five land use systems viz., sites having continuous poplar (*Populus deltoides*)-based agroforestry system (AFS) for 10, 20 and 30 years (Y), fodder (pearlmillet/sorghum)-fodder (oats/berseem) rotation (F-F) and fallow land. Available N, P and K in different soil layers were generally lowest in fallow land (62.7, 16.78 and 120.5 kg/ha, respectively in surface depth) and increased significantly with adoption of AFS and were highest in 30 Y plantation site of AFS (183.1, 43.53 and 195.2 kg ha^{-1}, respectively in surface depth). These were highest in surface and declined with increase in depth. In 0-15 cm soil depth, an increase of 13, 84.3 and 29.6% in exchangeable Ca, exchangeable Mg and available sulphur was observed in mature (30 Y) plantation sites over young (10 Y) plantation sites of AFS. The macronutrient index value was low for N, medium to high for P and low to high for K in different treatments. Therefore, the long-term adoption of poplar based agroforestry helps in improving the soil health, productivity and sustainable agriculture.

***Keywords*:** Agroforestry system, Fodder-fodder rotation, Macronutrient index value, Primary macronutrients, Secondary macronutrients

Paddy-wheat rotation is the main cropping system in Punjab and other parts of north western India. The continuous paddy straw burning results in annual nutrient losses at an estimated amount of 3.85 million tons of organic carbon (OC), 59 thousand tons of nitrogen (N), 20 thousand tons of phosphorus (P) and 34 thousand tons of potassium (K) and it has a detrimental impact on nutrient budget of soil (Kumar et al 2015). When crop residue is burnt, the existing minerals present in the soil are ruined, which subsequently hamper the next crop's cultivation. Moreover, straw carbon, nitrogen and sulphur are fully burnt in the cycle of burning and ultimately lost in the environment. According to Singh et al (2008), around 17 million tons of paddy straw is produced every year in Punjab, of which 90% is burnt in open fields. A solution to address such nutrient declines is the adoption of agroforestry systems, because the trees have a profound impact on soil nutrient pools as deep and extensive tree root systems enable them to accumulate significant quantities of nutrients below the crop root zone and move them to the surface soil through litterfall and root turnover (Allen et al 2004, Singh et al 2016). However, the return of nutrients through litterfall differs widely depending on parameters such as tree species, spacing, age of plantation, intercrops and management strategies (Singh 2009, Sirohi and Bangarwa 2017, Kaur et al 2020).

Among numerous tree species, thinly crowned poplar (*Populus deltoides* Bartr.) has emerged as a prominent tree of short rotation for agroforestry in north-western India due to its economic viability and more sustainability. In addition, its rapid growth, pruning-tolerant nature, low level of competition with associated crops and generation of supplementary income from its wood has resulted into its considerable acceptance among farmers (Kumar et al 2019). Besides these, many studies have reported higher content of available and total nutrients in poplar based agroforestry systems in comparison to sole cereal crops rotation such as rice-wheat, maize-wheat, cotton-wheat and soyabean-wheat (Sharma et al 2015, Prakash et al 2018, Di bene et al 2011). Poplar plantation accretes approximately 20.1 Mg ha^{-1} litterfall over 6 year period which upon mineralization adds macronutrients such as N (176 kg ha^{-1}), P (21.7 kg ha^{-1}), K (133 kg ha^{-1}), Ca (368 kg ha^{-1}) and S (55.4 kg ha^{-1}), respectively which improves soil health (Singh 2009). This signifies that poplar incorporation in existing cereals rotation should be encouraged in order to maintain soil nutrient status for ecological sustainability and this will ultimately reduce the use of chemical fertilizers in agricultural systems. In many studies, positive impact of chronosequence of agroforestry systems on soil fertility has been reported; for instance by Nath et al (2015) in bamboo-based agroforestry systems and Sharma et al (2009) in alder-cardamom agroforestry system.

However, the studies on effect of different rotations or planting cycles of poplar based agroforestry on soil nutrient build up are lacking. Therefore, the present study was conducted in order to appraise the impact of different plantation cycles of poplar based agroforestry system (AFS) and other land uses (fodder-fodder rotation and fallow land) on content of major nutrients in the soil profile.

MATERIAL AND METHODS

Selection of study sites: Soil samples were taken from Khehra Bet and other surrounding villages in Ludhiana district (latitude 30°91'N and longitude 75°85'E) of Punjab, India. The area receives an annual rainfall of 700-800 mm per annum, more than 75 percent of which is recorded over the three monsoon months (July-September). Maximum and minimum mean monthly temperatures vary from 17.4°C (January) - 39°C (May) and 6.0°C (January) -26.8°C (July), respectively.

Sites were chosen where poplar-based agroforestry system (AFS) had been adopted for the past 10, 20 and 30 years in addition to fodder - fodder cropping system (pearlmillet/sorghum-oats/berseem) and control (no poplar/crop). Five replications of each system were selected. The selected sites were similar in soil texture of the surface layer (0-15 cm). First, 2nd and 3rd plantation cycles represented about 10, 20 and 30 years of continuous poplar stands, respectively. Along with these poplar plantations, five sites having fodder-fodder cropping system and five control sites (without crops or trees), were also selected for comparison of soil characteristics. Pearlmillet or sorghum in the *kharif* (summer) season was grown by farmers as intercrops in the inter-row area for the first 3 years of plantation age, while wheat in the *rabi* (winter) season was grown throughout poplar rotation due to deciduous nature of trees in winters. No crop was grown in *kharif* season after 3 years of tree age. The fertilizers were applied to the intercrops as per the recommended practices. For pearlmillet, sorghum and wheat; 100 and 60, 12.5 and 40, and 125 and 62.5 kg of N and P_2O_5 per hectare respectively, were applied.

Description of soil sampling: Five replications were chosen for each plantation cycle of poplar and other agro-ecosystems (treatments). Six spots from these chosen sites were randomly selected for soil sampling under each treatment. Soil samples were taken from each treatment and replication, using an auger from 0-15, 15-30, 30-60 and 60-90 cm soil depths. A composite soil sample was obtained by accurately mixing the soil from these six random spots. Soil samples were air dried, ground and passed through a 2 mm sieve to assess different soil characteristics. Acronyms for the treatments are: Sites having poplar plantation for 10 years (10 Y), 20 years (20 Y), 30 years (30 Y), fodder-fodder cropping system (F-F) and fallow (no poplar or crop) (FL).

Laboratory analysis: Particle size distribution (mechanical analysis) was assessed by using the International pipette method (Jackson 1973). Furthermore, by using obtained percentages of sand, silt and clay, the texture was determined using TAL 4.2 software programme (Table 1). Water holding capacity was examined by using the standard procedures (Jackson 1973).

Available Nitrogen (N) was assayed by the Alkaline-Permanganate method described by Subbiah and Asija (1956). Available phosphorus (P) was scrutinized calorimetrically in 0.5 M $NaHCO_3$ extract on Elico spectrophotometer as outlined by Olsen et al (1954) and available potassium (K) was determined by flame photometer after extraction with neutral normal ammonium acetate (1 M CH_3COONH_4)as described in Jackson (1973). Exchangeable calcium (Ca) and magnesium (Mg) in the soil were evaluated by versenate titration method as suggested by Cheng and Bray (1951). Available sulphur (S) was estimated colorimetrically by turbidimetric method with 0.15% calcium chloride ($CaCl_2.2H_2O$) extractant (Jackson 1973).

Macro-nutrients index value (MNIV) was calculated to compare the levels of soil fertility of one treatment with those of another by obtaining a single value for each macronutrient. Therefore, nutrients index values of primary macronutrients were calculated by using methodology of Parker et al (1957).

From the proportion of soils under low, medium and high available nutrient categories, MNIV is represented by the following expression:

$$\text{Macro-nutrient index value (MNIV)} = \frac{(NL*1) + (NM*2) + (NH*3)}{NT}$$

Table 1. Depth-wise proportion of primary particles (%) and soil texture in different land use systems

	Soil depths (cm)			
	0-15	15-30	30-60	60-90
Sand	68.09-78.77	66.57-77.37	68.03-83.83	66.90-79.27
Silt	8.47-13.31	12.07-15.87	6.73-18.50	7.50-17.51
Clay	12.07-18.6	9.73-21.37	9.43-14.43	9.43-15.60
Soil texture	Sandy loam	Loamy sand-Sandy clay loam	Loamy sand-Sandy loam	Loamy and-Sandy loam

Where, NT stands for total number of samples analyzed in a given area.

NL, NM and NH represent the proportion of total soil samples falling in the low, medium and high nutrient categories and are assigned weightage of 1, 2 and 3, respectively.

Correspondingly, areas with macro-nutrient index value below 1.5 could be considered low category, those with MNIV in between 1.5 to 2.5 could be considered medium category, and those with values above 2.5 could be grouped as high category in native supply of that nutrient.

Statistical analysis: The data thus obtained were contrasted for significance of the differences by Least Significant Difference (LSD) with Tukey's Honestly Significant Difference (HSD) post hoc test at 5 percent level of significance by Analysis of Variance (ANOVA) using completely randomized design in SAS software version 9.4.

RESULTS AND DISCUSSION

Physical properties: The soil texture in 0-15 cm soil layer was sandy loam in all land use systems, with sand ranging from 68.09-78.77%, silt from 8.47-13.31% and clay from 12.07-18.6% (Table 1). The lowest water holding capacity (WHC) in 0-15 cm soil depth was observed in fallow land (31.79%), which was significantly lesser than all the treatments (Fig. 1). Meanwhile, it increased considerably with increase in number of years under poplar from 39.7% in young plantation site (10 Y) to 44.2% in mature AFS (30 Y) in the surface soil layer. Similar trend was reported in subsurface layers where it increased from 10 year old to 30 year old AFS.

The higher WHC of AFS than F-F and FL may be attributed to addition of litter biomass and extensive root systems which might have altered the distribution of micro and macropores and thus helped the soil to retain more water (Ramya et al 2021). Ramesh et al (2008) stated that water retention capacity of soil was directly proportional to organic matter and clay content. Temporal positive effect can also be seen in AFS as WHC increased linearly in all depths in mature AFS (30Y) than young AFS (10 Y) and it can be ascribed to higher accumulation of organic matter through litter and root mass in older plantation cycles in contrast to younger AFS. It has been made clearer by the findings of Nath et al (2015) who reported a strong positive empirical relation ($r^2 = 0.95$) between WHC and age of bamboo based AFS plantation cycles.

Available primary macronutrients: The content of available N, P and K in all the treatments were found to be diminishing with the depth as the plough layer (0-15 cm) had higher content in comparison to subsurface layers (Table 2). The order of macronutrients in the soil profile (0-90 cm) of every treatment followed an order K>N>P and all were higher in the mature plantation cycles of agroforestry system in comparison to other land uses (Fig. 2). Box plot representation of available K (Fig. 3) signifies that adoption of vegetation on fallow land and chronosequence of AFS resulted in noticeable increase in the range as well as mean value. For instance, 75% of the soils under 10 Y agroforestry had available K ranging between 148 to 160 kg ha^{-1} as

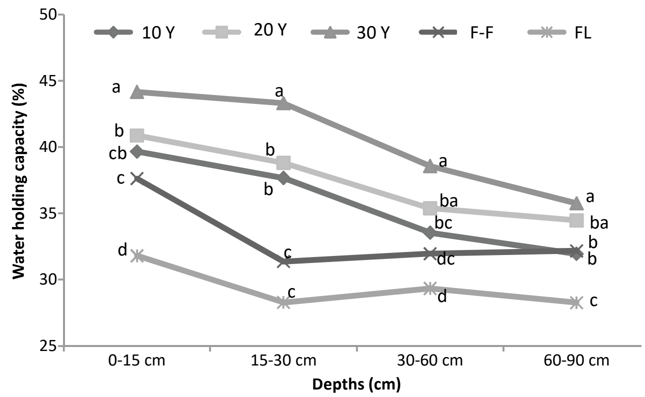

Fig. 1. Depthwise distribution of water holding capacity under different poplar plantation cycles and other land uses. Values in the same row within same soil layer followed by same small letters are not significantly different at P<0.05 [LSD: 3.12 (0-15 cm); 3.41 (15-30 cm); 3.30 (30-60 cm); 3.36 (60-90 cm)]

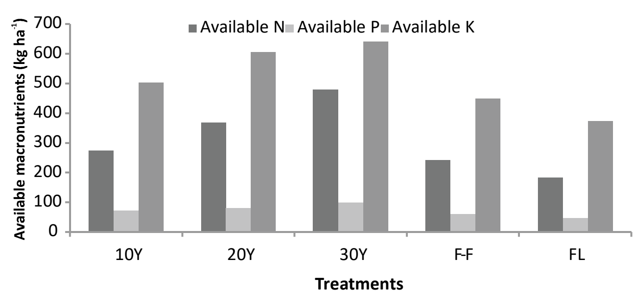

Fig. 2. Distribution of available nutrients in 0-60 cm soil profile under different poplar plantation cycles and other land uses

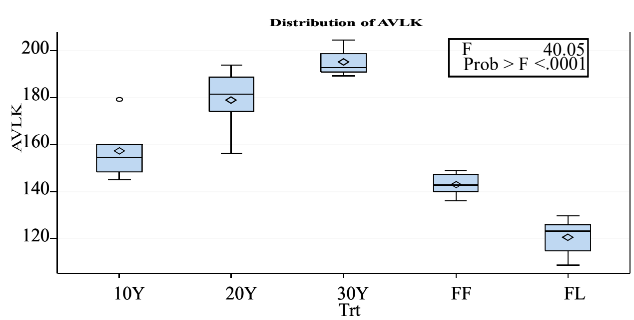

Fig. 3. Box plot representation of available K in surface soil with chronosequence of poplar based agroforestry system and other land use systems

opposed to 191 to 199 kg ha^{-1} under 30 Y in 0-15 cm depth of soil. Available K in the soil profile (0-90 cm) was highest in mature plantation cycles (641 and 605 kg ha^{-1}) in 30 and 20 year old agroforestry followed by younger plantation cycle (504 kg ha^{-1}) than F-F sequence (449 kg ha^{-1}) and least 372 kg ha^{-1} in fallow land (Fig. 2). In comparison to plough layer, the available K declined drastically in FL by 54.8% compared to 42.3% and 33.8% decline in F-F and mature plantation cycle of AFS (Table 2).

In comparison to FL, adoption of AFS or F-F led to significant increase in primary macronutrients (P and K) but significant increase in available N in surface soil layer was only reported under AFS. In plough layer (0-15 cm), both major primary macronutrients (available N and P) increased considerably with advancement of AFS i.e. up to 37.8% and 17%, respectively in 20 Y plantation cycle over 10 Y and further inclined to 43.2% and 33.9%, respectively in 30 Y plantation cycle. Available N, P and K contents were conspicuously higher in the upper layer (0-15 cm) in comparison to lower layers. However, sub-surface layers also followed the same order i.e. agroforestry > fodder-fodder > fallow land in all the depths. Higher content of nutrients in AFS and F-F system is due to the regular addition of organic matter through leaf litter and rhizodeposition in the agroforestry system and a finer but dense network of root mass of fodder crops that has remained in the soil year after year, both of which increase the overall turnout of N through biological processes (Konthoujam et al 2021). In addition, released organic decomposition products such as organic anions regulate the adsorption-desorption reactions of P and K by forming complexes or chelates with Fe, Al, Mg and Ca, which would otherwise restrict the availability of P and K. Singh (2009) reported that a total of 20.1 Mg ha^{-1} litterfall was added during 6 year rotation of poplar plantation which added approximately 176, 21.7 and 133 kg ha^{-1} of N, P and K, respectively through litterfall. Numerous studies assessed the variability of primary macronutrients under agroforestry following different agronomic management practices such as different tree species (Singh et al 2010), soil texture (Mao et al 2012), different tree spacing (Sirohi and Bangarwa 2017) and tree canopy positions (Pandey et al 2000). Apart from these, plantation cycles of agroforestry also had a considerable effect on nutrient pools throughout the soil profile as the present study recorded relatively 97.2% N, 56.7% P and 23.9% K higher in mature plantation cycle (30 year) over 10 year old agroforestry system in 0-15 cm soil depth. Lower available macronutrients in uncultivated soil are presumably attributed to lack of vegetative cover, more soil degradation and higher losses by leaching (Moges et al 2013).

Secondary macronutrients: The positive impact of the age continuum of poplar-based agroforestry can also be witnessed in case of secondary macronutrients (calcium, magnesium and sulphur), which increased dramatically from the early successional stage (10 Y) to the mid-successional stage (20 Y) and added substantial quantities of soil nutrients after 30 years of agroforestry establishment. In 0-15 and 15-30 cm soil depth, an increase of 13% and 6.79% exchangeable Ca (Exch-Ca), respectively and 84.3% and 61.3% exchangeable Mg (Exch-Mg), respectively was observed in mature (30 Y) plantation sites over young (10 Y) plantation sites of agroforestry system (Table 3). Among land uses, Exch-Ca and Exch-Mg followed the order: agroforestry system [8.08-9.13 Ca; 2.93-5.40 Mg cmol (+) kg^{-1}] followed by fodder-fodder [7.89 Ca; 2.60 Mg cmol (+) kg^{-1}] and fallow land [5.90 Ca; 1.76 Mg cmol (+) kg^{-1}] in surface soil (0-15 cm). Irrespective of land uses, decrease in exchangeable bases (Ca and Mg) was observed in all the treatments with increase in depth from 0-15 to 15-30, 30-60 and 60-90 cm (Table 3). However, the diminution of both Exch-Ca and Mg in case of land uses was highest in FL (51% Ca and 40% Mg) followed by F-F sequence (49% Ca and 27% Mg) and10Y AFS (40.8% Ca and 23% Mg). Available S in the soil profile (0-90 cm) was highest in mature plantation cycles (128.3 and 117 kg ha^{-1}) in 30 and 20 Y old agroforestry followed by younger plantation cycle (102 kg ha^{-1}) than F-F sequence (95 kg ha^{-1}) and least 80.9 kg ha^{-1} in fallow land (Table 3). Integration of trees and fodder crops in fallow land built considerable amount of available S in plough layer (0-15 cm) by 19.4% and 12.1% in 10 Y AFS and F-F. Subsequently, advancement of AFS further accrues available S by 40.2% and 54.7% after 20 and 30 Y of AFS establishment on fallow land.

Higher content of Exch-Ca and Mg under tree based systems may be ascribed by leaf litter composition of poplar as it exhibited higher level of Ca and Mg in the leaves as a constituent of cell wall. Singh (2009) reported that 368 kg ha^{-1} and 55.4 kg ha^{-1} of Ca and S were added to the soil through litterfall by 6 year old poplar plantation throughout its rotation age. Similarly, Singh et al (2018) also asserted that exchangeable bases were well maintained in agroforestry systems in comparison to agriculture land use as they reported higher concentration of Exch-Ca and Mg in all agroforestry systems. Higher level of available S under tree based system than F-F and FL can be owed to higher availability of organic matter with low C:S ratio, which upon mineralization enhances the availability of sulphate S in the soil (Wainwright et al 1986). In the effect of soil layer, secondary macronutrients were found to be higher in the top layer of all treatments, which was probably due to pumping of bases (Ca and Mg) and S element from the subsoil by the profuse root system of vegetation and returning them in to topsoil (Yimer et al 2008). In comparison to surface layer, the curtailment of secondary macronutrients in lowers layers was maximum in case of FL followed by F-F and poplar based AFS which may be ascribed to lack of vegetation in case of FL, shallow root system of F-F whose most of the roots are concentrated up to 30 cm layer and minimum in poplar as its roots can penetrate up to depth of 1 m.

Macro-nutrient index value (MNIV): The major macronutrients (N, P and K) were assigned macro-nutrient index values (Table 4) as a better way of nutrient management on sustainable basis. Nitrogen (N) had low critical value (1.0) in all the treatments. Phosphorus (P) was medium (1.6 and 2.4) in fallow and F-F sequence, whereas

Table 2. Effect of different plantation cycles of poplar based agroforestry in comparison to other land uses on depth-wise distribution of available primary macronutrients

Depths	Indicators (kg ha^{-1})	10 Y	20 Y	30 Y	F-F	FL	LSD (P=0.05)
0-15 cm	Available N	92.83^{c}	127.9^{b}	183.1^{a}	77.8^{dc}	62.7^{d}	18.87
	Available P	27.77^{c}	32.49^{b}	43.53^{a}	22.73^{d}	16.78^{e}	3.57
	Available K	157.5^{c}	178.9^{b}	195.2^{a}	143.0^{d}	120.5^{e}	13.68
15-30 cm	Available N	70.25^{c}	107.9^{b}	153.0^{a}	72.76^{c}	50.18^{d}	18.87
	Available P	17.95^{c}	20.31^{b}	25.20^{a}	14.80^{d}	12.74^{d}	2.13
	Available K	141.5^{b}	165.5^{a}	176.1^{a}	129.4^{b}	112.2^{c}	15.93
30-60 cm	Available N	67.74^{b}	85.30^{a}	90.32^{a}	50.18^{c}	42.65^{c}	13.85
	Available P	13.97^{b}	15.17^{b}	17.13^{a}	13.74^{b}	9.83^{c}	1.76
	Available K	132.3^{b}	156.9^{a}	140.5^{b}	93.97^{c}	85.12^{c}	13.77
60-90 cm	Available N	42.65^{a}	47.67^{a}	52.68^{a}	40.14^{a}	27.60^{a}	NS
	Available P	10.85^{b}	11.88^{b}	13.37^{a}	7.84^{c}	7.53^{c}	1.48
	Available K	72.35^{c}	103.9^{b}	129.3^{a}	82.54^{c}	54.43^{d}	14.36

Values in the same row within same soil layer and parameter followed by same small letters are not significantly different (P = 0.05) according to Tukey's honestly significant difference (HSD) post hoc test

Table 3. Effect of different plantation cycles of poplar based agroforestry in comparison to other land uses on depth-wise distribution of secondary macronutrients - exchangeable Ca [cmol (+) kg^{-1}], exchangeable Mg [cmol (+) kg^{-1}] and available sulphur (kg ha^{-1})

Secondary macronutrients	10 Y	20 Y	30 Y	F-F	FL	LSD (P=0.05)
0-15 cm						
Exch- Ca	8.08^{b}	8.96^{a}	9.13^{a}	7.89^{b}	5.90^{c}	0.63
Exch- Mg	2.93^{c}	3.64^{b}	5.40^{a}	2.60^{c}	1.76^{d}	0.54
Available S	27.70^{c}	32.53^{b}	35.90^{a}	26.01^{c}	23.20^{d}	2.75
15-30 cm						
Exch- Ca	7.95^{b}	8.05^{ba}	8.49^{a}	7.91^{b}	5.58^{c}	0.47
Exch- Mg	2.87^{cb}	3.36^{b}	4.63^{a}	2.74^{c}	1.22^{d}	0.50
Available S	27.52^{c}	31.04^{b}	34.83^{a}	26.18^{c}	22.38^{d}	2.23
30-60 cm						
Exch- Ca	6.55^{a}	6.74^{a}	6.91^{a}	5.75^{b}	4.19^{c}	0.36
Exch- Mg	2.36^{b}	2.85^{a}	2.92^{a}	2.11^{b}	0.93^{c}	0.38
Available S	25.41^{bc}	27.81^{ba}	30.32^{a}	22.47^{dc}	19.73^{d}	3.16
60-90 cm						
Exch- Ca	4.78^{b}	5.13^{b}	5.74^{a}	4.03^{c}	2.89^{d}	0.41
Exch- Mg	2.25^{b}	2.88^{a}	2.86^{a}	1.90^{c}	1.06^{d}	0.26
Available S	22.07^{b}	25.59^{a}	27.27^{a}	20.24^{b}	15.68^{c}	2.78

Values in the same row within same soil layer and parameter followed by same small letters are not significantly different (p = 0.05) according to Tukey honestly significant difference (HSD) post hoc test

Table 4. Macro-nutrients index value (MNIV) of different plantation cycles of poplar based agroforestry system and other land use soils

Chronosequence	Nutrients	Range (kg ha^{-1})	MNIV	Category
10 Y	N	87.8 - 100	1	Low
	P	21.8 - 33.9	2.8	High
	K	145 - 179	2	Medium
20 Y	N	100 - 150	1	Low
	P	29.0 - 36.1	3	High
	K	156 -193	2	Medium
30 Y	N	163 - 213	1	Low
	P	39.1 - 46.7	3	High
	K	189 - 204	2	Medium
F-F	N	62.7 - 87.8	1	Low
	P	19.8 - 25.4	2.4	Medium
	K	136 - 148	2	Medium
FL	N	50.1 - 75.2	1	Low
	P	11.7 - 18.5	1.8	Medium
	K	104 - 133	1.6	Low

* Macro-nutrients index categories are assigned on the basis of nutrient critical values: <1.50-low, 1.50-2.50-medium, >2.50-high

all plantation cycles of AFS were rated as high [2.8 (10 Y), 3 (20 Y) and 3 (30 Y)]. Potassium (K) was categorized low in no vegetation land use (FL) and highest (2.0) in F-F and all plantation cycles of AFS.

In order to compare the soil fertility levels of one region with those of another, it was obligatory to obtain a single value for each nutrient. Nutrient index (NI) value is a measure of nutrient supplying capacity of soil to plants. Higher P and K index value in mature plantation sites over rest can be attributed to higher addition of organic anions which increase their availability through solubilization or chelation mechanisms. Moreover, lesser consumption of available P and K by intercrops in comparison to available N could be the reason behind variability. Similarly, Nath et al (2015) also reported significant positive impact of chronosequence of bamboo based agroforestry system in improving soil fertility through profuse root system and on site nutrient conservation as they found positive correlation of increasing clump age with total N (R^2 = 0.95*), available P (R^2 = 0.94*) and exchangeable K (R^2 = 0.91*).

CONCLUSIONS

The introduction of poplar-based agroforestry system leads to a considerable accumulation of primary as well as secondary macronutrients in the soil and this content increased tremendously with advancement of AFS from young plantation cycle (10 Y) to mature sites (30 Y). This change is not restricted to plough layer but increment occur throughout the soil profile (0-90 cm). The order of macronutrients in the soil profile (0-90 cm) in poplar tree based system followed an order K>N>S>P. Apart from these, physical property such as water retention capacity also improved significantly with establishment of AFS on fallow land. Overall, buildup of macronutrients in mature sites over young sites suggests that a long-term adoption of agroforestry may help in mitigating the ill effects of continuous paddy straw burning and also a better alternate to improve soil health and productivity.

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Morphological and Molecular Identification of New species of *Coprinopsis iraqicus* sp. nov. from Iraq

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Abstract: The wild macro fungi, commonly named mushroom, have been rarely studied in Iraq for many reasons. In this study macrofungal specimens were collected from a site in the Tikrit province, Salah ad-Din, that located in the mid-north of Iraq from December to January 2019-2020 for describing a cryptic species of *Coprinopsis* depending on morphological and molecular analyses. Description of fruiting bodies on their microhabitats was recorded and photographed in their natural microhabitats. Molecular and phylogenetic analyses based on the nuclear ribosomal internal transcribed spacer (ITS) region were performed. In this study, the results of Bayesian and Maximum Likelihood phylogenetic analysis based on ITS nucleotide sequences and morphological description revealed that *Coprinopsis iraqicus* sp. nov. was a cryptic species and formed distinct morphological features and a clade among its related species belonging to the genus *Coprinopsis.* More studies are needed to determine the distribution of this genus generally and the presented species specifically.

***Keywords*:** Basidiomycota, Bioinformatic analysis, *Coprinopsis*, Iraqi mushrooms, Phylogenetic tree, ITS region

Coprinoid species are an interested group of macro fungi in the field due to undergoing autolysis to their deliquescent lamellae and basidiocarps with age. However, this group is highly divers phenotypically and poly-phyletically. Based on phenotype descriptions, this group stared by taxonomic classification into one broadly defined genus *Coprinus* Pers. (1797). Later based upon molecular phylogeny, the genus has been evident for cryptic speciation, and split into four main lineages appointed to four genera each of which has own sections (Nagy et al 2013a). These genera are *Coprinus* in Agaricaceae, *Coprinellus* P. Karst., 1879, *Coprinopsis* P. Karst., 1881, and *Parasola* Redhead, Vilgalys & Hopple, 2001 in the new family Psathyrellaceae (Hopple & Vilgalys 1999, Redhead et al 2001). The reclassification of coprinoid fungi was mainly depending on macro-micro morphological characters such as the cap texture, the presence or absence of the universal veil. The genus *Coprinopsis* contain species that have been mostly described based on the classical methods, morphological features as being grayish, deliquescent, and having a cuticular pileipellis with diverse structures of veil (Nagy et al 2012). However, this method shows slight discriminatory differentiation and/or significant overlap between several species (Nagy et al 2013a). The genus *Coprinopsis* has approximately 200 species around the world (Kirk et al 2008). Due to significant genetic diversity among the collections of its species, taxa are divided into twenty sections according to index fungorium (www.indexfungorum.org) based on veil anatomy and the presence or absence of cap pileocystidia. Functionally, basidiocarps of coprinoid species are terrestrial habitat and saprotrophs collected from distinctive microhabitats such as leafy and woody litter materials on the forest floor (Al Anbagi et al 2019), and woody chip and herbivore dung (Schafer 2010). The propagules of *Coprinopsis* or and Coprinoid species have also been dispersed widely across 10 plots (each 10 m x 10 m) on the leaf litter of the forest floor (Al Anbagi et al 2021).

The wild macrofungi, commonly named mushroom, have been rarely studied in Iraq due to several reasons. Scientifically, there are few specialists in this research area. That type of research needs researchers who not only have experience in field works but also experts in morphological descriptions with all advantages and disadvantages of traditional survey methods. In Iraq, the climate is another crucial cause. The weather is mainly a hot desert climate. It is externally hot and dry in summer with mild in winter. The temperature in the Baghdad could reach 50 C, making Baghdad one of the hottest capitals in the world with receiving only 150 mm of rainfall throughout the year. In the north-eastern part of Iraq, occupied by the mountains, it is the only part that receives substantial rainfall between October and April with annual precipitation 700 -1000 mm with temperature 8-25°C. However, the temperatures and precipitations with cooler nights due to the high altitude may be appropriate for fungal growing and producing some fruiting bodies through the year in different parts of Iraq (https://www.climatestotravel.com/climate/iraq). These factors may lead to insufficient studies on macrofungi, but higher fungal diversity that needs to be investigated is also expected. From 2014 until today, many studies have been recorded various macrofungi using morphological descriptions and later molecular techniques. These were first-time recording and describing a wild macrofungal species of the discomycetous fungus, *Cheilymina theleboloides* from Babylon province, in the middle south of Iraq (Al anbagi 2014). There have been many efforts for first-time documentations and morphological classifications of macrofungi from Kurdistan region - Northern Iraq, Salah ad-Din province (north- central Iraq); Anbar province (western Iraq) (AL- Qaissi 2014, Muslat & Owaid, 2015, Al-Khesraji 2016, Suliaman et al 2017, AL-Khesraji 2018, AL-Khesraji et al 2018, Suliaman, 2019, Al-Khesraji & Suliaman, 2019). During these efforts, molecular approaches have been applied to confirm species identifications for some species (Suliaman, 2017, AL-Khesraji et al 2019, Aish et al 2020, Al-Khesraji et al 2021).

Nevertheless, the central and southern regions have been poorly studies. Due to geographic and environmental influences of Iraq, many genera may be expected to have new species. These have been evident by recording new species reported by molecular identification. In this study, we described a distinct species of *Coprinopsis* depending on morphological and molecular aanalyses. These were different from all other known species of the subsection Nivei of *Coprinus* (Citerin 1992, Ulje´ 2005) and from remaining recognized *Coprinopsis* species.

MATERIAL AND METHODS

Specimen sampling, processing, and morphological identifications : The specimens were collected from site in the Tikrit province that located in the mid-north of Iraq about 140 Km northwest of Baghdad (34° 43´ 51.1" N 43° 38´48.0" elevation 137m). The climate of Tikrit system is characterized by a hot desert with a mean annual temperature of 14.9-28.95°C (July, 25.8-43.6°C; January, 4-14.3°C) and precipitation of 15 mm (Jun – September 0 mm and December 37 mm that has the highest rainfall; https://www.weather-atlas.com). After rainy days, a series of visits were conducted for fruiting body inventories from December to January 2019- 2020. The site was dominated by native trees including *Populus* sp. and *Salix* sp. and fruit trees. The floor site was covered by mixed litter microhabitats. Fruiting bodies were collected directly from the carpeting mixed materials on the forest floor. Description of mycelia and fruiting bodies on their microhabitats were recorded and photographed in their natural microhabitats. Small pieces of fresh fruit bodies were put in paper bags and transfer to the lab. Samples were divided in two groups: one group for morphological and molecular analyses and another group for preserving. The preserved samples were oven-dried at 45–50 °C for 24 hours and conserved in paper bags based on relevant literature and field (Al Anbagi et al 2019). Later, specimens are deposited in Biology Department, College of Sciences, Tikrit University. For species identifications and classification, the macromorphological and micromorphological features were performed based on fresh samples. Light microscopy was used for describing microcharacters. Slides were prepared from fresh specimens in an aqueous solution. Observations and measurements were taken directly through the light microscope under an 40x. The gill tissue was described. Then, spore features were described and measured by random selecting of 20 well-formed spores. The morphological characters of basidia and cystidia were described, and their dimensions were presented after measuring 25 elements.

DNA extraction, PCR amplification and Sanger sequencing techniques : Genomic DNA was extracted from fresh materials, amplified, visualized and sequenced in the same manner in detail elsewhere (Al Anbagi et al 2019). The small portion of fruiting body was used to extract the genomic DNA using the Wizard Genomic DNA purification Kit (Promega, Madison, Wisconsin following the manufacturer's instructions. Later, the extracted DNA was amplified targeting the nuclear ribosomal DNA (rDNA) genes and using the universal primers ITS1(5´-TCCGTAGGTGAACCTTGCGG-3´) and ITS4 (5`-TCCTCCGCTTATTGATATGC-3`) (White et al 1990).

The PCR products were sized and visualized on 1% agarose gel via electrophoresis stained with Red Safe. The amplified DNA was sent for Sanger sequencing (Microgen, Seoul, Korea). The represented sequencing was manually edited and then spontaneously assembled in the Geneious program version 9.1.8 (Biomatters Ltd., Newark, New Jersey). Similar to Al Anbagi et al (2019) the sequence identity was verified using the Basic Local Alignment Search Tool (BLAST) available at the National Center for Biotechnology Information (NCBI) GenBank (www.ncbi.nlm.nih.gov/genbank/). The species sequence was compared with fungal nucleotide sequences using query coverage of > 80% and > 97–100% sequence similarity. The current taxonomic level of the investigated species was based on Index Fungorum (www.indexfungorum.org). Later, the sequences were deposited in the NCBI's GenBank database under the accession number MZ265188 and MZ265189. The interesting species was putatively assigned to a particular ecological functional group based on the information available in the relevant literature (Rinaldi et al 2008, Tedersoo et al 2014).

Bioinformatic and phylogenetic analyses: The ITS forwarded and reversed sequences of two fungal isolates were trimmed, and low-quality edges were removed. Then, individual sequences of each isolate were assembled to contig with the Geneious program (Kearse et al 2012). The sequences of these isolates were blasted against the ITS database. Later, ninety-nine close related sequences to the investigated sequences were obtained from the GenBank. All sequences were aligned using ClustalW (Thompson et al 1994). Preliminary trees were created using Neighhor-joining. For the final tree, sequences of isolates were aligned with covering all available GenBank sequences of *Coprinopsis* species (81 species), reduced to one sequence per species, and included sequences generated by experts of *Coprinopsis* taxonomy (Keirle et al 2004, Navarro-Gonza´lez et al 2006, Nagy et al 2010a,b, 2011, 2013a, b Geml et al 2012, Osmundson et al 2013, Ruiz et al 2013, O¨ rstadius et al 2015, Elwess et al 2016)and some unpublished sequences available in GenBank. Some species from close related genera (5 species) were chosen as the outgroups (Table 1) for the preliminary hypothesis on the phylogenetic placement of *Coprinopsis*. These trees were used default settings of MAFFT v7.309 (Katoh & Standley 2013), and the alignment was manually adjusted as necessary (data not shown). The outgroup (*Parasola*) was chosen based on related references (Nagy et al 2013b). Bayesian analyses were completed using Metropolis Coupled Markov Chain Monte Carlo (MCMC) methods as fulfilled in MrBayes V3.2.6 to infer phylogenetic trees (Huelsenbeck & Ronquist 2001) in Geneious version 9.1.8 under a general time- reversible (GTR) model of sequence evolution (Darriba et al 2012). These analyses run for 1,100,000 generations and begun with random starting trees. Those trees were sampled every 200 generations with the heating chain temperature set at 0.2. The first 100,000 trees were discarded, and the remaining trees were used to calculate posterior probabilities (PP) of the individual clades in the majority rule consensus tree. The unconstrained branch lengths and uninformative topology priors were set based on default settings of MrBayes. For maximum likelihood (ML) analyses, RAxML V7.2.8 were performed (Stamatakis 2014) and run with rapid bootstrap for 1000 replicates in Geneious version 9.1.8. The general time- reversible GTR model of nucleotide substitution with the additional options of modeling gamma rate heterogeneity (G) and proportion invariable sites (I) was accommodated. The resulting trees were visualized in Geneious version 9.1.8.

RESULTS AND DISCUSSION

Classification Position of *Coprinopsis iraqicus* sp. nov.

Kingdom: Fungi

Phylum: Basidiomycota.

Class: Agaricomycetes

Order: Agaricales.

Family: Psathyrellaceae (the family formerly treated under the name Coprinaceae).

Ecology: Terrestrial habitat, Saprotrophic nutrition (lignocellulosic substrates).

Habit and Habitats: Gregarious often fruiting in dense clusters within various substrates including wood chips, plant litter materials, and dung manured soil. It has been also found in both mixed straw- dung, and in the burned plant straw on wet, muddy soils.

Phenology: Specimens found from November to January in 2019 -2020.

Edibility: Unknown.

Distribution: The species was observed in the village in Tikrit province, Salah ad-Din in mid-north of Iraq. It could be a local species till now. However, few studies have been investigated macrofungi generally in Iraq, thus, it could be suggested to be collected from other areas in Iraq. The current species is the fourth species related to the genus that has been published. The previous three species mentioned were also collected from Erbil, Salah ad-Din, Dukan and Sulaymaniyah areas.

Morphological Characters

Macromorphological characters : *Basidiocarps* thin and soft when fresh, small-sized, fragile and short-lived. *Pileus* 4-25 × 5-10 mm; first sub-globular nearly conical and mostly bill-shaped then expanding to become broadly convex; margin softly striated, splitting in age; white when young, then change to white to pale cream, tan and becoming grayish at old specimens, the changing of pileus color starting from the end towards the top of the pileus. The surface dry radially lined and splitting in old specimens; covered with a massive white powdery granular to scaly leading to snowy pileus initially; the centers of surface pilus whiteish turned to yellowish tan and eventually light brown, sometimes fading to tan, with flattened-scaly. The fruiting body types non-deliquescent when being seen in the field and after being transported to the lab. *Lamellae* attached to the stipe, white, then change to pale cream, dark brown, and grayish becoming black with age; one series lamellae, distant to mostly sub-distant spaced. Spore print dark brown. Odor and test not distinctive. *Stipes* 5-10 ×18-60 mm; white to cream

Table 1. Species sequences obtained from Genbank used for construction of phylogentic tree

Taxa of phylogentic trees	Accession number
Coprinellussclerocystidiosus CBS 195.52 from TYPE material	NR_164277.1
Coprinopsis acuminata voucher SZMC-NL-0958	JX118698.1
C.aesontiensis voucher LZ P-7614	KY554753.1
C.afrocinerea MJ-2017a voucher CNF 1/5838	MG662162.1
Coprinopsisafronivea PB-2016a	KX017208.1
Coprinopsis ammophilae strain WAT 24982	HQ847008.1
Coprinopsis annulopora strain Enderle 30.71987	HQ847017.1
Coprinopsis argentea strain SZMC-NL-1678 i	HQ847040.1
Coprinopsis atramentaria voucher Seq_MP15	MW553148.1
Coprinopsis babosiae voucher SZMC-NL-0871	JX118685.1
Coprinopsis bicornis voucher Ulje 1216	JX118690.1
Coprinopsis brunneistragulata voucher SZMC-NL-FVBD 3821	JX118724.1
Coprinopsis brunneofibrillosa voucher Pegler 2704	JX118664.1
Coprinopsis calospora strain CBS 612.91	MH862284.1
Coprinopsis candidolanata voucher 794	JF907837.1
Coprinopsis canoceps voucher MushroomObserver.org/352292	MK346221.1
Coprinopsis cerkezii voucher CNF 1/7253	KX869912.1
Coprinopsis cf.*atramentaria* voucher JLF7063	MK874613.1
Coprinopsis cf. *cinerea* voucher Mushroom Observer # 316138	MH497221.1
Coprinopsis cf. *uliginicola*voucher JLFNSCFS51	MK874610.1
Coprinopsis clastophylla CBS 473.70	NR_154756.1
Coprinopsis cothurnata strain CBS 174.49	MH856479.1
Coprinopsis depressiceps voucher WTU-F-018322	MK169334.1
Coprinopsis episcopalis voucher ASIS25879	KP004959.1
Coprinopsis filamentifera voucher HB20171117A	MK069600.1
Coprinopsis fluvialis strain SZMC-NL-0840	HQ847011.1
Coprinopsis friesii voucher AM954	MK072829.1
Coprinopsis geesterani voucher HB20100810A	MK063784.1
Coprinopsis gonophylla strain CBS 144.47	MH856190.1
Coprinopsis insignis voucher HMAS 281305	MK966570.1
Coprinopsis jonesii voucher SZMC-NL-0154	JX118726.1
Coprinopsis krieglsteineri voucher SZMC-NL-3413	JX118701.1
Coprinopsis kubickae voucher CNF 1/6614	MH422562.1
Coprinopsis laanii strain CBS 476.70	MH859802.1
Coprinopsis lagopides voucher S.D. Russell iNaturalist #8536159	MN892574.1
Coprinopsis luteocephala strain SZMC-NL-2754	HQ847012.1
Coprinopsis macrocephala strain VKT-1	EU591956.1
Coprinopsis marcescibilis strain CBS 165.47	MH856199.1
Coprinopsis marcida voucher WTU-F-018311	MK169335.1
Coprinopsis martinii strain O50524	GU234126.1
Coprinopsis melanthina voucher WU19918	KC992961.1
Coprinopsis musae C J. Vesterholt 06-179	NR_148070.1
Coprinopsis narcotica strain CBS 171.39	MH855976.1

Cont...

Table 1. Species sequences obtained from Genbank used for construction of phylogentic tree

Taxa of phylogentic trees	Accession number
*Coprinopsis neolagopus*AB097564.1	AB097564.1
Coprinopsis neophlyctidospora CBM FB-37998	NR_137526.1
Coprinopsis nevillei GG08090401	HM126488.1
Coprinopsis nivea voucher 4585	JF907848.1
*Coprinopsis novorugosobispora*AB978534.1	AB978534.1
Coprinopsis ochraceolanata voucher SZMC-NL-0192	JX118697.1
Coprinopsis pachyderma voucher FVDB 3237	JX118731.1
Coprinopsis pannucioides voucher LO143-03	DQ389727.1
Coprinopsis phlyctidospora voucher 15575	JF907842.1
Coprinopsis picacea strain SZMC-NL-0174	JN943110.1
Coprinopsis pinguispora voucher UBC:F33455	MN954725.1
Coprinopsis poliomalla voucher HB20151219A	MK072612.1
Coprinopsis psammophila voucher CNF 1/6401	MK491274.1
Coprinopsis pseudofriesii strain SZMC-NL-2631	HQ847016.1
Coprinopsis pseudomarcescibilis AH 33711	NR_158341.1
Coprinopsis pseudonivea specimen voucher SZMC:NL:2340	FM163181.1
Coprinopsis pseudoradiata voucher SZMC-NL-0956	JX118687.1 1
*Coprinopsis rugosobispora*specimen_voucher: BR-44338-09	AB983245.1
Coprinopsis rugosomacrospora KRAM F-58717	NR_148112.1 1
Coprinopsis sclerotiger strain CBS596.80	GQ249277.1
Coprinopsis sclerotiorum strain SZMC-NL-0564	HQ847039.1
Coprinopsis scobicola strain Orton964	HQ847021.1
Coprinopsis semitalis strain CBS291.77	GQ249278.1
Coprinopsis spelaiophila voucher WU 14574	JX118674.1
Coprinopsis spilospora voucher JLF8953	MW555596.1
Coprinopsis stangliana strain SZMC-NL-2153	FM878027.1
Coprinopsis striata voucher HMAS 290163	MK966572.1
Coprinopsis strossmayeri voucher JU16585	MG981027.1
*Coprinopsis sylvicola*MN809536.1	MN809536.1
Coprinopsis tectispora voucher Schafer20090720001	JX118666.1
Coprinopsis trispora voucher MR180722	MN227299.1
Coprinopsis udicola GB A. Melzer 1240	NR_148071.1
Coprinopsis undulata voucher WTU-F-041708	MK169349.1
Coprinopsis urticicola strain ZMGR16	MT446068.1
Coprinopsis utrifer strain SZMC-NL-0591	FN396140.1
Coprinopsis variegata voucher SDR-MM5698	MG748581.1
Coprinopsis vermiculifer strain CBS132.46	GQ249279.1
Coprinopsis verticillata strain CBS 254.	MH873439.1
Coprinopsis xenobia voucher G. Mu oz	KF178383.1
Parasolaochracea BP NL-3621	NR_158793.1

color, central, smooth, lined, hollow, surface dull, dry; slender shape without seeing volva or annual, subglobose base when young and clavate to slightly enlarged at mature, sometimes equal (Fig. 1).

Micromorphological characters : *Pileus* cuticle, lamella tissue composed of interwoven or parallel, thin-walled, hyaline hyphae, *Basidiospores* 5-7.5 × 7.5-12.5 micrometer (µm), smooth, thick-walled, light brown and dark reddish-brown, ellipsoid to ovoid and narrowly amygdaloid to limoniform with some rounded base with prominent apiculus and with central, papillate germ pore, but few eccentric pores, mostly 1 µm wide germ-pore (Fig. 2). *Basidia* hyaline; clavate, 7.5-10 × 27.5-37.5 µm (measured without sterigmata)**,** come out from the terminal cells of hyphal tissues; developing 4 Sterigmata 1 µm long on which 4-spored generated. *Cheilocystidia* found on the edge of the lamella**,** scattered, broadly ellipsoid to clavate 10-12.5 × 27-35 µm, thin-walled, hyaline. *Pleurocystidia* found on the face of the lamella, broadly ellipsoid to clavate shape, thin-walled, hyaline, similar to cheilocystidia, 10-12.5 × 25-37.5 µm.

The wild macrofungi, commonly named mushroom, have been rarely studied in Iraq. The *Coprinellus disseminates*, *C. radians*, *C. flocculosus*, *C. comatus*, *Coprinopsis atramentaria*, *C. strossmayrei*, *C. romagnesiana*, *Parasola plicatilis*, *Psathyrella candolleana*, and *P. spadiceogrisea* were the first coprinoid fungi described from Salah ad-Din governorate in north central Iraq (Al-Khesraji et al 2017, Suliaman 2017, Al-Khesraji 2018, AL-Khesraji et al 2018). All the previous taxa have been characterized mainly based on the morphological techniques, except *C. strossmayrei*. and *P*. *candollena*, that were conformed to their classification using a molecular technique (Suliaman 2017, AL-Khesraji et al 2019). However, several studies confirmed that coprinoid fungi are complex taxa and have cryptic species with polyphyletic groups as being recognized for species of *Coprinus* and *Coprinopsis*. Furthermore, taxonomic changes happened for some species of both genera before they gained their current name. Therefore, it is difficult to accurately identify species of the current *Coprinopsis* with only morphological features and limited studies. In the current study, the new species of *Coprinopsis* is the first new record and described based on morphological and molecular characterizations from Iraq. That diverges from all known species of *Coprinopsis* species as well as the subsection Nivei of *Coprinus* s.l. (Cite´rin 1992, Ulje´ 2005) later included in the genus *Coprinopsis* (Hopple & Vilgalys 1999, Moncalvo et al, 2002). Its macromorphological descriptions are similar to species related to the subsection Nivei by having small fruiting bodies, pilus white to grey, fragile snowy white caps when young with pale yellow-brown in the center of pileus and expended pileus, and radially grooved and splitting in old specimens with limoniform, dark pigmented spores (Uljé & Noordeloos 1993). However, this species has a unique and distinct pattern of morphological characters (Figs. 1& 2). According to the taxa key, the species would be classified into *C. utrifer* depending on the expended pileus and the average length of spores or species with spores shorter than 9 µm (Uljé & Noordeloos1993, Nagy et al 2012). However, a phylogenetic tree based on rDNA in the present study provided another suggestion. The species has small dimensions of basidiocarps and average lengths of basidiospores that overlapped between the *Coprinopsis cerkezii* with very small pileus 3-11mm diameter and with an average length of spores 3-3.4 µm and *C. utrifer* with expended pileus up to 25 mm and average spore lengths more than 9 µm (Ulje´& Noordeloos 1993, Jayasiri et al

Fig. 1. Basidiocarps of *Coprinopsis* sp. from Iraq include different ages of basidiocarps (A & B); old specimen and lamellae color (C); young basidiocarps in cluster on natural habitats in burned plant materials (D & E); specimens covered with white snowy mass (F)

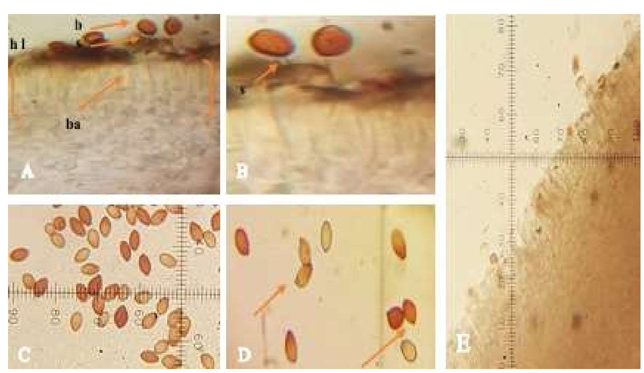

Fig. 2. Micromorphological characters of *Coprinopsis* sp. (A), illustrated hymenium layer (hl) with short clavate basidia (ba) bearing spores (b) on sterigmata (s), basidia with four sterigmata and 2 attached basidiospores (B); centric and eccentric germ pore of basidios-pores (C & D); lamellae tissue with basidia and cheilocystidia in the hymenium layer(E)

2015). The investigated species has larger pileus with smaller Cheilocystidia compared to *C. afronivea,* 19-18 mm and 12–17 µm. On the other hand, its pileus and Cheilocystidia are smaller than *C. pseudonivea* 28-57 mm and 28–57 µm and *C. nivea* 20-54 mm 29–71 m respectively. The phylogenetic tree has presented these differences clearly, as shown below.

Phylogenetic analyses: The results of blast and preliminary trees (neighbor joining tree built with 99 close related sequences) revealed that isolates of a species collected in this study have low identical similarity to close related sequences (<91.2%) and clustered in a distinct clade belong to the genus *Coprinopsis* (data not shown). Furthermore, Bayesian and Maximum phylogenetic analysis based on ITS nucleotide sequences revealed that *Coprinopsis iraqicus* nov. in this study was a cryptic species and formed a distinct clade among its related species belong to the genus *Coprinopsis* (Fig. 3). The new Iraqi *Coprinopsis* with other members of the genus *Coprinopsis* in the present tree split into several smaller clades. Its position nested in an isolated position as a sister clade and basal to the assemblage of *Coprinopsis* species belong to subsection known as pseudoneviea such as the *C. marcibilies, C. utrifer, C. pseudoneviea*, *C. afronivea*, and *C. cerkezii* some of them known as pseudoneviea. The topology of Bayesian and Maximum Likelihood phylogenetic trees were similar and strongly supported by Maximum Likelihood bootstrap value (100) and the Bayesian posterior probability (1.00) for the clade of this cryptic species, indicating that *Coprinopsis iraqicus* sp. nov. is distinct taxon (Fig. 3). Although *Coprinopsis iraqicus* sp. nov. had morphologically some similarity with other coprinoid species, a phylogenetic tree based on rDNA successfully discriminated against the present species of *Coprinopsis* (Fig. 3). The position of new Iraqi *Coprinopsis* in the present phylogenetic tree takes an isolated position. Its position with other *Coprinopsis* members split into several smaller clades (Fig. 3). In the present clade, the *Coprinopsis* sp. is placement close to

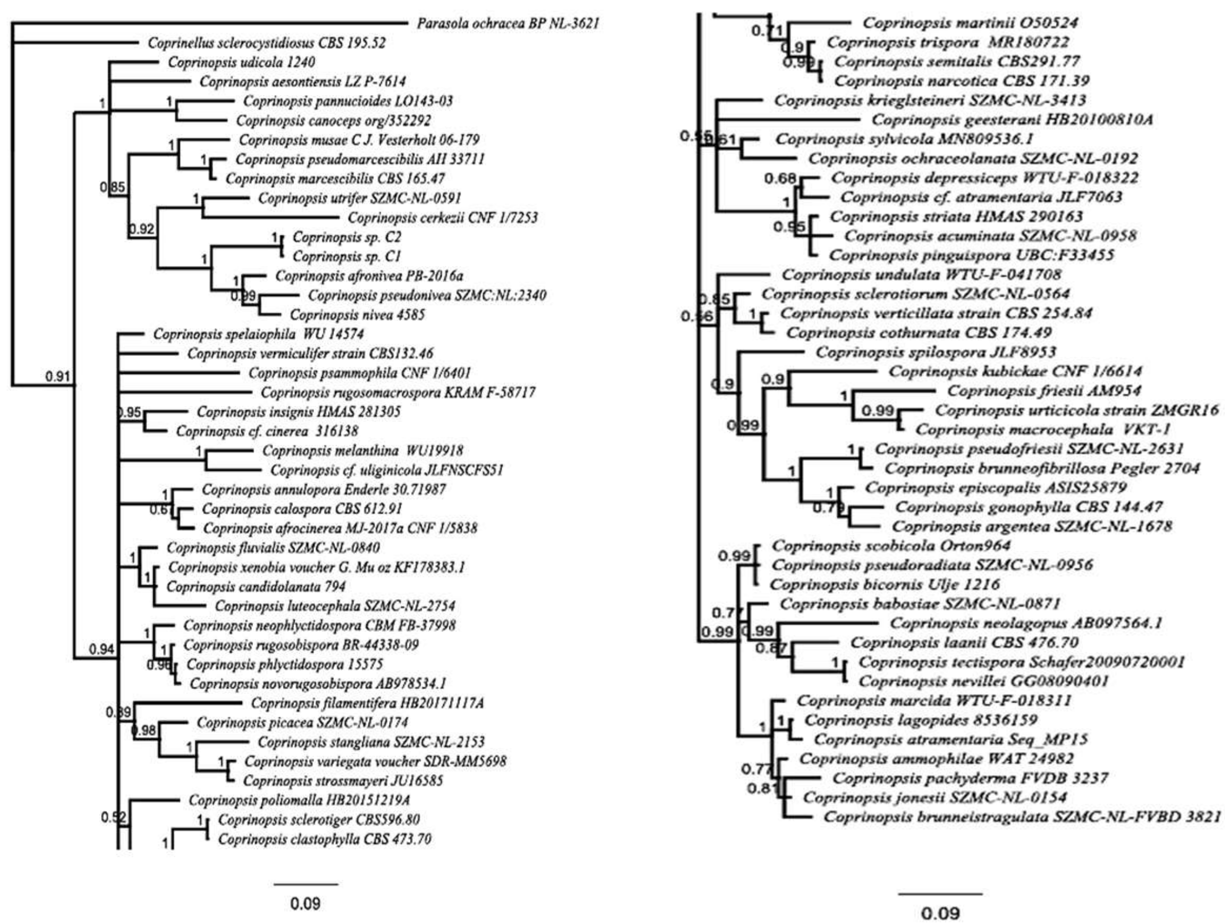

Fig. 3. Bayesian phylogenetic tree inferred from the dataset of ITS region's sequences from *Coprinopsis iraqicus* sp. nov. and related species. The isolates of new species are shown in bolt. Bayesian posterior probability values are indicated at the nodes. The tree is rooted with two outgroup species, *Coprinellus sclera cystidiosus,* and *Parasola ochracea*. The number of nucleotide substitutions per site is indicated as the bar

species known as pseudoneviea. *Pseudoneviea* species as defined by Nagy et al (2012) are characteristic by usually having pure white or whitish basidiomes with cuticular pileipellis when the fruiting body is deliquescent. With adding more sequencing from identified species and new species into databases, and with uncertainties in the placement of some clades. However, it may be difficult to certain the current species clad. The morphological features are no longer evidence for describing new species or recording the identified species because difficulties in recognizing homologous traits and molecular techniques have evident there are complex species within a species. These could be even spilt into new genera as being suggested for coprinoid fungi (Nagy et al 2013b), although the larger clades of phylogenetic analyses can be distinguished by morphological traits clearly.

CONCLUSION

The study revealed the presence of new *Coprinopsis* sp. and fourth recorded species in Iraq after those isolated from the north part of the country. The current species record indicated that genus distribution extends to Iraq. More studies are needed to determine the distribution of this genus generally and the presented species specifically. The high temperature and few precipitations are important factors preventing macrofungi from producing the sexual form, fruiting bodies in Iraq. Therefore, more efforts are needed to inventory macrofungi under suitable environmental conditions in other regions of the country.

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Distribution of Butterflies in Karanthamalai Reserve Forest, Tamil Nadu, Southern India

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Abstract: Butterflies are monopolizing insects of the Order Lepidoptera, form an important part of the food chain and they are crucial for pollination. The present study was carried out to record the distribution and abundance of butterfly species at Karanthamalai Reserve Forest, Natham, Dindigul District, Tamil Nadu in Southern India from August 2016 to July 2018 using transect count method. The study revealed the occurrence of 79 individual species around 80.166 Km^2. Amid the five families Nymphalidae was dominant in respective to species, genus and abundance. Specific distribution was observed for *Papilio clytia* at Karrupusamy Temple and *Graphium cloanthus* at Thekkal Reserve Forest. Amid the Lycaenidae family *Zizina otis* dominated all study areas. Irrespective to the distribution and abundance Thekkal Reserve Forest was rich in the diversity of butterflies. These results suggest that the Karanthamalai Reserve forest harbours a rich diversity of butterfly species. The present study can be used as reference for the establishment of national or regional forest biodiversity evaluation indicator systems in India in future.

Keywords: Butterfly diversity, Karanthamalai, Lepidoptera, Natham, Reserve forest

Ecosystem has been evaluated based on the diversity of organisms. Biodiversity has rather an important key indicator of the healthy ecosystem. The class Insecta is the one of the most important among the phylum Arthropod due to its species richness. The winged wonders the butterflies (Order Lepidoptera) were most attracted by the scientist as the richest resources of all terrestrial ecosystem (Ghazoul 2002). Nearly, 20,000 species has been documented (Tiple 2012) worldwide, among which1504 species were recorded in Indian subcontinent (Kunte 2009).In terrestrial ecosystems, butterflies play a major role in pollination and herbivory (Tiple et al 2006). Butterflies as a pollinator, reflect the overall plant diversity of an ecosystem in a given area. Hence, they act as good indicators of a healthy ecosystem (Padhye et al 2006). Butterflies were rather very sensitive to habitat destruction and climatic fluctuation (Kunte 2000). Hence, butterflies are used as model organisms to study the threats posed to the environment (Kunte 2008). United Nations Convention on Biological Diversity (CBD) and The United Nations Environment Programme (UNEP) emphasizes the importance of biodiversity and insisted all nations to take part in monitoring and assessing the diversity of flora and fauna to eliminate the root cause of biodiversity loss. The average conservation value of butterflies in the evergreen forests was more than any other habitat, followed by riparian patches, deciduous forests, grasslands, and shrubs. In the Western Ghats, deforestation, as well as plantation activities are taking place on a large scale (Jha et al 2000, Tiple 2011). Monitoring of the butterflies is essential for formulating conservation priorities and the management of its diversity. With this backdrop the present work has been carried out to study the distribution of butterflies at Karanthamalai Reserve Forest.

MATERIAL AND METHODS

Study area: The present study is intended to enumerate the diversity of butterfly at Karanthamalai reserve forest, as there is no published data of butterfly diversity. Karanthamalai is a scenic village in the Perumalai hill ranges of Dindigul District, Tamil Nadu, India (Fig. 1, Table 1). Perumalai hills are part of the Sirumalai range of Eastern Ghats, and the natural vegetation comprises of dry forests. It is located in the reserved forest area with numerous wild streams, waterfalls and dense forest.

Methodology

The field survey of butterflies was conducted from August 2016 to July 2018 approximately 900m long and 5 m wide line transect was set up in each sampling unit and marked in the field along with GPS data for repeated observations. Transect in each sampling unit was observed from 9.00 forenoon to 12.00 noon. Collected butterfly individuals were identifies either in the field or after reaching the laboratory following the standard field guide proposed by Gunathilagaraj et al (1998) and Kunte (2000). All the species were photographed with Canon EOS 1500D DSLR Camera, identified (Varshney 1983, Ackery 1984). For species level identification the butterflies were caught using sweep net and released back to the environment.

RESULTS AND DISCUSSION

India has 1,800 species and subspecies of butterflies (Kunte et al 2017) of which peninsular India hosts 350 species, while 331 species are found in the Western Ghats (Kunte 2001). Butterflies in all habitats showed a highly seasonal trend.

In the present study 79 species of butterflies were observed which comes under five families (Fig. 2; Table 2 to

Table 1. Sampling sites at Karanthamalai Reserve Forest

Sampling site	Latitude	Longitude
Karrupusamy Temple	78°14'38.835"E	10°17'51.344"N
Irranjan Medu	78°13'35.06"E	10°17'51.578"N
Malaiyur	78°13'32.343"E	10°18'32.099"N
Manikayan Kada Water Falls	78°14'18.522"E	10°19'3.683"N
Ayyanar Temple	78°12'55.7"E	10°20'49.788"N
Ayyanar Aruvi	78°12'14.687"E	10°19'28.346"N
Thekkal Reserve Forest	78°9'33.388"E	10°19'16.022"N
Punnapatti	78°11'10.061"E	10°16'37.816"N

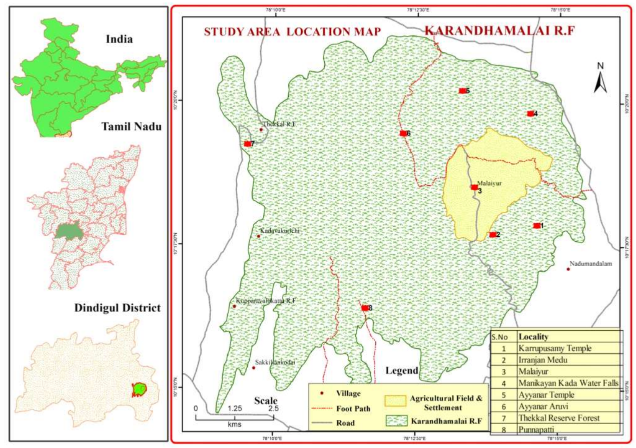

Fig. 1. Map showing the eight different sampling locations (indicated in red squares) in Karanthamalai Reserve Forest

Table 2. Butterflies of Nymphalidae family in Karanthamalai Reserve Forest

Genus	Scientific name	Common ame
Acraea	*Acraea violae* (F. 1775)	Tawny Coster
Ariadne	*Ariadne ariadne* (L. 1763)	Angled Castor
Charaxes	*Charaxes athamas* (Drury 1770)	Common Nawab
	C.solon (F. 1793)	Black Rajah
Chirrochora	*Chirrochora thais* (F. 1787)	Tamil Yeoman[2&3]
Danaus	*Danaus chrysippus* (L. 1758)	Plain Tiger
	D. genutia (Cramer 1779)	Striped Tiger
Euthalia	*Euthalia aconthea* (Cramer 1779)	Common Baron**
	E. nais (Forster1771)	Baronet
Euploea	*Euploea core* (Cramer 1780)	Indian Common Crow***
Hypolimnas	*Hypolimnas bolina* (L. 1758)	Great Eggfly[1&2]
	H. misippus (L. 1764)	Danaid Eggfly***[1]
Junonia	*Junonia almana* (L. 1758)	Peacock Pansy
	J. atlites (L. 1763)	Grey Pansy
	J. hierta (F.1798)	Yellow Pansy
	J. iphita (Cramer 1779)	Chocolate Pansy
	J. lemonias (L. 1758)	Lemon Pansy
	J. orithya (L. 1764)	Blue Pansy
Melanitis	*Melanitis leda* (L. 1758)	Common Evening Brown
Mycalesis	*Mycalesis mineus* (L. 1758)	Dark Brand Bush Brown
Neptis	*Neptis hylas* (L. 1758)	Common Sailer
Phalanta	*Phalanta phalantha* (Drury 1773)	Common Leopard
Tirumala	*Tirumala septentrionis* (Butler 1874)	Dark Blue Tiger
Thaumantis	*Thaumantis diores* (Sparrman 1768)	Jungle Glory
Ypthima	*Ypthima baldus* (F. 1775)	Common Five Ring
	Y. ceylonica (Hewitson 1865)	White Four Ring
	Y. huebneri (Kirby 1871)	Common Four Ring

Indian Wildlife Protection Act:*-Schedule I; **-Schedule II; ***-Schedule I,II& IV; [1] Endemic to Peninsular India;[2]Srilanka;[3]Southern India ; WG-Western Ghats

Fig. 2. Butterflies observed at Karanthamalai Reserve Forest

6). Nymphalidae is the dominant family in terms of species composition and its abundance irrespective of the sampling sites at Karanthamalai Reserve Forest. *Euploea core* species is abundant in site 1 followed by *Tirumala septentrionis* and *Junonia lemonias* at the same time *Neptis hylas* species is observed to be dominant followed by *Euploea core* and *Junonia iphita* at Irranjan Medu. Meanwhile the dominance varies among the study sites such

Table 3. Butterflies of Pieridae family in Karanthamalai Reserve Forest

Genus	Scientific name	Common name
Appias	*Appias albino* (C&R Felder 1865)	Common Albatross**
	A. libythea (F. 1775)	Striped Albatross
	A. lyncida (Cramer 1779)	Chocolate Albatross
Belenois	*Belenois aurota* (F. 1793)	Pioneer
Catopsilia	*Catopsilia pomona* (F. 1775)	Common Emigrant
	C. pyranthe (L. 1758)	Mottled Emigrant
Cepora	*Ceporanadina* (Lucas 1852)	Lesser Gull
	C. nerissa (F. 1775)	Common Gull**
Colotis	*Colotis amata* (Butler 1876)	Small Salmon Arab
	C. danae (F. 1775)	Crimson Tip
	C. fausta (Olivier 1804)	Large Salmon Arab
	C. vestalis (Butler 1876)	White Arab
Delias	*Delias eucharis* (Drury 1773)	Common Jezebel[1]
Eurema	*Eurema andersoni* (Moore 1886)	One Spot Grass Yellow
	E. brigitta (Stoll 1780)	Small Grass Yellow
	E. blanda (Boisduval 1836)	Three Spotted Grass Yellow
	E. hecabe (L. 1758)	Common Grass Yellow
Hebomoia	*Hebomoia glaucippe* (L. 1758)	Great Orange Tip
Ixias	*Ixias Marianne* (Cramer 1779)	White Orange Tip
	I. pyrene (L. 1764)	Yellow Orange Tip
Leptosia	*Leptosia nina* (F. 1793)	Psyche
Pareronia	*Pareronia valeria* (Cramer1776)	Common Wanderer
Pieris	*Pieris canidia* (Sparrman 1768)	Indian Cabbage White

Indian Wildlife Protection Act:*-Schedule I; **-Schedule II; ***-Schedule I,II& IV; [1] Endemic to Peninsular India; [2]Srilanka; [3]Southern India ; WG -Western Ghats

Table 4. Butterflies of Papilionidae family in Karanthamalai Reserve Forest

Genus	Scientific name	Common name
Atrophaneura	*Atrophaneura aristolochiae (L. 1758)*	Common Rose
	A. hector (L. 1758)	Crimson Rose*[2&3]
Graphium	*Graphium Agamemnon* (L. 1758)	Tailed Jay
	G. cloanthus (Westwood 1841)	Glassy Blue Bottle
	G. doson (Felder & Felder 1864)	Common Jay
	G. nomius (Esper 1798)	Spot Swordtail
Papilio	*Papilio crino* (F. 1792)	Common Banded Peacock
	P. clytia (L. 1758)	Common Mime
	P. demoleus (L. 1758)	Limeyellow Butterfly
	P. polymnestor (Cramer 1775)	Blue Mormon
	P. polytes (L. 1758)	Common Mormon
Troides	*Troides minos* (Cramer 1779)	Southern Birdwing*[3&WG]

Indian Wildlife Protection Act:*-Schedule I; **-Schedule II; ***-Schedule I,II& IV; [1] Endemic to Peninsular India; [2]Srilanka; [3]Southern India; WG -Western Ghats as *Tirumala septentrionis* at Malaiyur; Among the 27 species of Nymphalide family *Euploea core* was noted to be dominant along with genus *Junonia* and *Tirumala* and *Danaus chrysippus* was observed specific at Punnapatti. Among the sampling site Thekkal Reserve Forest was noted to be dominant (16.27 %) in distribution of Nymphalidae and Pieridae family. *Appias* sp. was dominant among the Pieridae family. *Atrophaneura aristolochiae* and *Atrophaneura hector* of Papilionidae family was observed in all sampling sites. Malaiyur has richness in diversity of Papilionidae family. In the present study species uniqueness was recorded for *Papilio clytia* at Karrupusamy Temple and *Graphium cloanthus* at Thekkal Reserve Forest. Amid the Lycaenidae family *Zizina otis* dominated all study areas. Irrespective of the distribution and abundance Thekkal Reserve Forest was richness in the diversity of butterflies.

The nymphalids are a large group of robust bodied butterflies that come in almost every shape and colour. The highest numbers of butterfly species, belong to this family among the total reported in the study area. Totally 16 genera and 27 species of nymphalids were recorded during the study period (Fig. 3). The species of this family are distributed throughout the area. This may due to the fact that the monsoon in this region gradually diminishes in the month of September. Because of heat the thin layer of soil loses moisture very quickly and causes the grass to turn yellow at the end of October. The current work is in accordance with Afaq et al (2021), where highest number of Nymphalids were with 16 genera and 23 species. The next groups of butterflies were the family Pieridae with 23 species under 12 genera and the family Papilionidae with 12 species under 4 genera. In Assam University campus, Cachar district, Bora and Meitei (2014) observed 4 genera and 13 species of the family Papilionidae representing the swallow tails. Loss of suitable

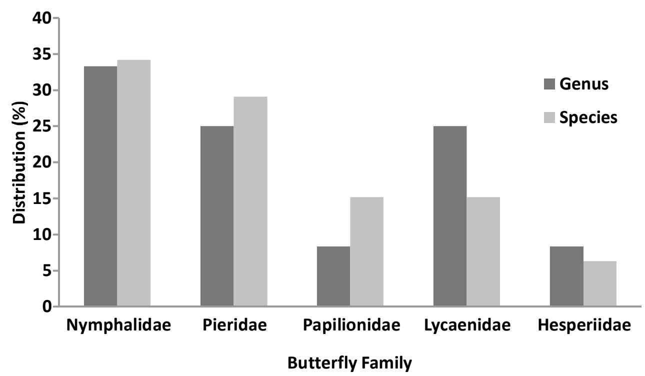

Fig. 3. Family level distribution of the existing butterflies in Karanthamalai Reserve Forest

Table 5. Butterflies of Lycaenidae family in Karanthamalai Reserve Forest

Genus	Scientific name	Common name
Abisara	*Abisara echerius* (Stoll 1790)	Plum Judy Brown
Arhopala	*Arhopala centaurus* (Moore 1884)	Centaur Oak Blue
Azanus	*Azanus jesous* (Guerin & Meneville 1849)	African Babul Blue
Caleta	*Caleta decidia* (Hewitson 1876)	Angled Pierrot
Castolius	*Castolius rosimon* (F. 1775)	Common Pierrot*
Catochrysops	*Catochrysops strabo* (F. 1793)	Forget Me Not
Chilades	*Chilades lajus* (Stoll 1780)	Lime Blue
Curetis	*Curetis thetis* (Drury 1773)	Indian Sun Beam
Jamides	*Jamides celeno* (Cramer 1775)	Common Cerulean
Prosotas	*Prosotas dubiosa* (Evans1925)	Tailless Lineblue
Spindasis	*Spindasis lohita* (Horsfield 1829)	Long Banded Silverline
Zizia	*Zizina otis* (Murray 1874)	Lesser Grass Blue

Indian Wildlife Protection Act :*-Schedule I; **-Schedule II; ***-Schedule I,II & IV; [1] Endemic to Peninsular India; [2]Srilanka; [3]Southern India and WG -Western Ghats

Table 6. Butterflies of Hesperiidae family in Karanthamalai Reserve Forest

Genus	Scientific name	Common name
Caprona	*Caprona ransonnetti* (R Felder 1868)	Golden Angle
Hasora	*Hasora chromus* (Cramer 1780)	Common Banded Owl
	H. taminatus (Hubner 1818)	White Banded Awl
Tagiades	*Tagiades japetus* (Stoll 1781)	Common Snow Flat
Thoressa	*Thoressa astigmata* (Swinhoe1890)	Southern Spotted Ace

habitat was considered to be a reason for the decline in population. Saravanan and Venkatramalingam (2021) have also reported a total of 22 butterfly species and among them the most dominant family was Nymphalidae (45.45%) followed by Pieridae (31.82%), Papilionidae (13.64%), and Lycaenidae (9.1%). The next family of dominating butterflies were represented by the members of Lycaenidae with 12 species under 12 genera (Table 5) followed by Hesperidae with 5 species under 4 genera (Table 6). Lycaenidae is the most abundant family of the Western Ghats, compared to all other families (Kunte 2000). Most browns are normal and frequently sighted, yet are less observed because of their resigning propensities. The members of Lycaenidae are seen in moderate numbers with jerky flight near the ground. Hesperiids fly vigorously and have greyish colour pattern on their wings so that their appearance is not easily noted. Occasionally these rapid fliers are visualized on flowers and near mud puddles. The small size, mysterious colouration and swift mobility of the butterflies belonging to the families Hesperiidae and Lycaenidae, make them very difficult to be identified (Arun and Azee 2003). Hence a very few members of this family were spotted in the study area. Deepak et al (2016) reported a total of 172 species, belonging to 117 genera and 18 subfamilies under six families in Dakshina Kannada District of Karnataka. Nymphalidae with 57 species (33.13%) was the dominant family followed by Hesperiidae with 37 species (21.51%), Lycaenidae with 45species (26.16%), Papilionidae with 17species (9.88%), Pieridae with 15 species (8.72%) and Riodinidae with one species (0.58%). Sushmita et al(2021) during month of June 2020 to November 2020, in Butterfly park of Nawab Wajid Ali Shah Zoological Garden, Lucknow, India recorded the highest population of family Nymphalidae with 20 species, followed by Lycaenidae comprising of 16 species, Hesperiidae with 2 species, Pieridae with 13 species and Papilionidae with 7 species. Similarly the present study also illuminates the abundance of butterfly species in different families with 79 species belonging to 48 genera under five families.

Butterflies are categorized under different schedules according to Wild Life Protection Act 1972. Several authors have attempted to find the status of the butterflies present in their study area. Gowda et al(2011) have reported 54 species in Lakkavalli range of Bhadra Wildlife Sanctuary in Karnataka. Among them Crimson Rose and Danaid Eggfly were in Schedule - I and Common Baron and Gray Count were in Schedule -II. Sharmila and Thatheyus (2013) recorded 101 species in Alagar Hills, Madurai, Tamil Nadu. Among them the species namely, Crimson Rose and Danaid Eggfly were classified under Schedule -I and Schedule -II contained Common Baron. Kumar and Murugesan (2014) recorded 64 species of Butterflies around 30km radius of Kudankulam Nuclear Power plant area of Tamil Nadu, India. Out of 64 species, Danaid Eggfly, Common Pierrot, Southern Bird wing and Crimson Rose were listed under Schedule –I; Common Albatross, Common Gull, Danaid Eggfly, and Gram Blue under Schedule -II and Common Indian Crow in Schedule -IV. In the present study also 79 species are recorded, in which Common Pierrot, Crimson Rose and Southern Birdwing come under schedule-I of Indian Wildlife Protection Act, 1972 and other species like Common Baron, Common Albatross and Common Gull are noted under the schedule –II. Butterflies such as Danaid Eggfly and Indian Common Crow are noted under schedule -I, II, & IV.

There is need to investigate endemic status of butterflies. Some previous works have been proposed by researchers in which endemism has been highlighted. Rajagopal et al (2011) suggested the presence of Blue Mormon and Common Jezebel that are endemic to Penninsular India and Sri Lanka and well as species like Crimson Rose, and Blue Mormon are endemic to Western Ghats and Sri Lanka. The Southern Birdwing the largest Indian butterfly which is endemic to Peninsular India. The Common Jeszebel, Danaid Eggfly, Baronet and Great Eggfly are endemic species which are found in Peninsular India and Sri Lanka (Sharmila and Thatheyus 2013, Gowda et al 2011). Tamil Yeoman that has been declared as state butterfly of Tamil Nadu (Shrikumar 2019) was spotted in the study area. This species is endemic to Southern India and Sri Lanka (Gaonkar 1996). The current research work also recorded the above mentioned endemic species in the study area. Species like Dark Blue Tiger (Krishna and Swamy 2014), and Indian Common Crow belonging to the family Nymphalidae; Common Mormon, Common Rose and Lime Yellow Butterfly belonging to the family Papilionidae; Common Emigrant, Common Grass Yellow, Large Salmon Arab and Mottled Emigrant belonging to the family Pieridae; Common Pierret of the family Lycanidae were more abundant in the study area and are observed throughout the year.

CONCLUSION

The present study reveals that the study area provides favourable ecological conditions and habitat for the survival of butterflies. The uncharted areas such as the population dynamics and seasonal patterns of butterflies in this particular geographical area may be thrown light to understand the species change and fluctuations in abundance over time. Moreover, the presence of eight threatened species and eight endemic species in this reserve forest makes it ideal butterfly habitat in terms of future restoration and conservation projects.

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Genetic Divergence and Phylogeny of North-Western Indian Himalayan Population of Honey Bee (Hymenoptera: Apidae) Inferred from Mitochondrial DNA Sequences

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***Abstract*:** The utilization of molecular techniques to predict the phylogenetic similarity within the honey bee topologically divided these into three groups *viz*., giant bees, dwarf bees and cavity-nesting bees. Four species of honey bee, i.e., Asian honey bee (*Apis cerana*), dwarf bee (*Apis florea*), the rock or giant honey bee *(Apis dorsata*) and European honey bee (*Apis mellifera*) collected from North Western Himalaya region were molecularly characterized using partial mitochondrial Cytochrome oxidase I (COI). Nucleotide frequency analysis revealed that partial COI gene sequences were A+T biased (>73%), while the amino acid frequency analysis showed higher frequency of leucine (13.71%) and serine (12.27%). Among the tested species of honey bees *A. florea* showed exceptionally higher concentration of Cystine Amino acid. The overall variation in 72 SNPs was revealed by multiple sequence alignment of COI sequences from four species. The results reveal more transversion (56.5%) compared to transition (43.5%). Additionally, the pairwise genetic distance analysis described that *A. cerana* and *A. dorsata* had least genetic distance (0.102). The Ts/Tv value of 0.61 suggests that there is insignificant neutral selection in honeybees of north western Himalayan region of Uttarakhand. Such researches are significantly important to analyze the biodiversity of area, distinguish cryptic species and develop distinct taxonomic tools for integrative taxonomy of Honey bees.

***Keywords*:** Honey bees, Molecular characterization, Cytochrome oxidase I, Phylogenetic analysis, Genetic divergence, Indian Himalayas

Pollinators are fundamental vehicles for plant reproduction, ecosystem health, and agricultural production (Ollerton 2017 and Klein et al 2007). The most essential and diverse group of pollinators are the insects, with honey bees specifically regarded the most essential group due to their significant numbers and expertize on floral resources (Ollerton 2017). They contribute up to 73% pollination services provided by animals in total (Michener 2007, Abrol 2009 and Ollerton et al 2011). Worldwide the services of bee pollinators to the agriculture are predicted around $235- $577 billion per year (FAO 2018). Honey bees of genus *Apis* (Hymenoptera: Apidae: Apini), comprise around seven species and are ranked as best pollinators due to their eusocial nature (Alqarni et al 2011, Oleksa and Tofilski 2015, Haddad et al 2016, Cridland et al 2017, Eimanifar et al 2018). Generally, all the honey bee species have same biological characters viz., i) the occurrence of one queen in the colony (for egg laying), ii) thousands of workers are responsible for all activities such as foraging, cleaning of the hive, defending the enemy (Abou-Shaara et al 2017), and iii) drones with main role to mate virgin queen (Heidinger et al 2014 and Abou-Shaara et al 2021), iv) the similar thermoregulation capacity (Abou-Shaara et al 2017). These resemblances direct the occurrence of the common genetic characters between them. These characteristics are based mainly on the coding regions in the nuclear and the mitochondrial DNA (mtDNA) (Abou-Shaara 2019a).

During the last few decades, numerous identification approaches like developing the database of prototypes and comparing the unknown specimen with the well identified specimen within the database are practiced but, with only probable accuracy has been achieved (Ratnasingham and Hebert 2007). Distinguishing among the honey bee sub-species is intricate and necessitates specific knowledge. It is normally based on morphometric characteristics of bees, like head width in frontal view (including eyes), antennal scape length, and hind tibia length (Gruber et al 2013). Angles in the forewing have been used to distinguish within bee groups (Nedic et al 2011 and Kulici et al 2014). Besides these wing morphometrics are also utilized (Silva et al 2015). Automated Bee Identification (Schröder et al 2002) and Digital Automated Identification System (DAISY) (Weeks et al 1999) are two such systems, although they are rarely effective because a new specimen whose prototype does not exist in the library cannot be identified with 100% accuracy. Using molecular techniques, researchers were able to better understand the genetic diversity pattern, phylogenetics, biogeography, evolution, and population genetics of insects (Susanta 2006 and Andrade- Souza et al 2017). Molecular characterization methods such as DNA barcoding using mitochondrial cytochrome oxidase I genes have shown promise in identifying the bee biota. (Magnacca and Brown 2010, Magnacca and Brown 2012 and Wang et al 2014). The molecular techniques provide numerous other information's like identification of the sympatric and cryptic species within a habitat (Eriksson et al 2017 and Nneji et al 2020), classification of dimorphic sex species (Gibbs 2009 and Sheffield et al 2009), characterizing of species which are otherwise difficult to identify on morphological basic (Gibbs 2009, Rehan and Sheffield 2011 and Williams et al 2012) and determination of the evolutionary relationship and genetic distances within the targeted insect species (Will and Rubinoff 2004 and Kekkonen and Hebert 2014). Use of COI gene, has been utilized successfully to understand the population genetic structure and variations among the insects (Pramual et al 2005 and Patel and Jadhav 2019). Therefore, utilizing this method in combination with other mitochondrial and nuclear markers has unlocked new perspectives for the identification of species and studies related to genetic diversification of insects (Low et al 2016 and Pramual and Nanork 2012). In this study, we investigated the population genetic diversity of *Apis* species of Northwestern Himalayas. We sequenced the cytochrome c oxidase subunit 1 gene (COI) of individuals of *Apis* species collected from different eco-geographic regions in Northwestern Himalayas. The objectives present study was to use the DNA (COI) barcode data to reliably identify and characterize *Apis* species and evaluate the mitochondrial genetic diversity pattern and population structure.

MATERIAL AND METHODS

Test insects: The specimens of four *Apis* species (*A. cerana, A. mellifera, A. florea and A. dorsata*) were collected from crops grown in the Experimental Farm, Hawalbagh of ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora situated at 29°37′N and 79°40′E with an altitude of 1250 m in Uttarakhand state of North Western (NW) Himalayan Region, India. The *Apis* species are dominant pollinators of the crops in the Northwestern Himalayas.

DNA extraction: The standard methodology of CTAB as described by Subbanna et al (2016) was followed for extraction of genomic DNA from the honey bee specimens. The three pairs of legs of honey bee species were crushed in liquid nitrogen utilizing autoclaved pestle and mortar, the crushed material was transferred to a micro-centrifuge tube. 600 µl of CTAB solution (20 parts of 1M Tris-HCl, 8 parts of 0.5M EDTA, 56 parts of 5M NaCl and 4 parts of CTAB and the final volume makeup 1000ml and the pH adjusted to 8. After this 0.4 parts of ß-mercaptaethanol were added along with 1 mg/ml of proteinase K was transferred to the tube and incubated in a water bath at 57^0C for 3-5 hours. The contents were vertexed manually after every 20 minutes for thorough degradation of the tissues. The degraded material was treated twice with phenol-chloroform-isoamyl solution (25:24:1) to extract the genomic DNA and ice-cold isopropyl alcohol was used to precipitate the DNA at -20^0C for 30 minutes. The DNA pellet obtained after centrifugation at 10000 rpm for 7 minutes was washed with 70% chilled ethyl alcohol to remove the excess salts and was suspended in 40 µl of TE buffer. DNase-free RNase A treatment was followed for 1 h at 37^0C to remove the RNA residues. Electrophoresis with 0.8% agarose gel was carried out to visualize intact genomic DNA and the DNA samples were diluted if required to obtain a working solution of 20-25 ng/µl.

PCR protocol: The insect specific universal mitochondrial cytochrome oxidase I primers (JM 76 (5-GAGCT GAATTAGG(G/A)ACTCCAGG-3) and JM 77 (5-ATCACCTCC(A/T) CCTGCAGG ATC-3)) were used for amplification of the target region from four *Apis* species. The PCR reaction mix utilized for the study was as follows, 50 ng of DNA template, 200nM of dNTPs, 1mM of each primer, 2.5 units of Taq DNA polymerase and 5µl of PCR reaction buffer was added to make the make a final volume of 50 µl. The PCR reactions were performed in a thermal cycler (Biorad) with an initial 3 min denaturation step at 95^0C, followed by 35 amplification cycles consisting of 1 min denaturation at 95^0C, 45 seconds annealing at 52^0C and 1 min extension at 72^0C with an additional final step of extension for 10 min at 72^0C. The presence of amplified PCR product was visualized and confirmed in the gel documentation system (Alpha Image Analyzer, Alpha Innotech Corporation) by 1.2% agarose-EtBr 10 mg/ml gel electrophoresis with 2.5 µl PCR product.

Sequencing and data analysis: Gel elution columns (Sigma) were used for purification of the amplified products of the target gene. The purified products were sequenced directly by an automated DNA sequencer (ABI 377) following manufacturers guidelines for the Big Dye terminator kit (Applied Biosystems). The sequence thus obtained were aligned with Clustal Omega (1.2.2) multiple sequence alignment (Sievers and Higgins 2018). Further analysis on phylogenetic and molecular evolutionary analyses, pairwise genetic distance among *Apis* species, variation in nucleotide sequences as well as transition/transversion rate ratios were calculated by comparing with the CO1 sequences of other closely related *Apis* spp. in the NCBI GenBank database by

BLASTN. The MEGA X 10.0.5 software (Molecular Evolutionary Genetic Analysis version X) (Kumar et al 2018) was used for the construction of Maximum likelihood evolution tree (Saitou and Nei 1987) utilizing the distance matrix from the alignment. The confidence level of each branch was tested by bootstrapping 1000 replicates generated with random seed. The nucleotide sequences were translated into amino acid sequences with the help of invertebrate mitochondrial genetic code through the ExPASy translate: SIB bioinformatics resource portal (Artimo et al 2012) and were aligned using Clustal omega software (Sievers and Higgins 2018). The variation in the amino acid concentration within and among the species was estimated by computing the amino acid composition by MEGA X 10.0.5 software. The generated sequences of partial mitochondrial COI region were further submitted to NCBI GenBank database through Bankit submission tool (https://www.ncbi.nlm.nih.gov/WebSub/?tool=genbank) to acquire the individual accession number (*Apis dorsata* COI-Almora 1-ON506691, *Apis florea* COI-Almora 1 - ON506690, *Apis mellifera* COI-Almora 1 - ON506267, *Apis cerana indica* COI-Almora 1 - ON506013).

RESULTS AND DISCUSSION

Four species of honey bees were collected from the premises of experimental form hawalbagh of VPKAS-ICAR, Almora while pollinating various crops. During the present investigation, the partial mitochondrial COI regions of (683-696) bp of *A. cerana*, *A. mellifera*, *A. florea* and *A. dorsata* were amplified. These species were morphologically characterized but to clear the ambiguity at molecular level, the molecular characterization with partial mitochondrial Cox 1 gene were carried out. The sequences of honey bee obtained were BLASTn to determine the resemblance index and recognition of every isolate from North-western Himalayas of India with closely related sequences are displayed in the phylogenetic tree. For mitochondrial COI gene fragment, there was 97-100, 98.53-99.25, 85.28-95.23 and 91.29-99.84 percent nucleotide identity for *A. cerana*, *A. mellifera*, *A. florea* and *A. dorsata* sequences respectively, when these sequences were analyzed by BLASTn separately in NCBI database. The evolutionary relationship of honey bees based on mitochondrial COI gene categorized the whole assemblage into four main groups (Fig. 1). *Apis cerana indica* voucher specimen Almora form a sub-cluster with 100% similarity with the *Apis cerana* isolate CL24 mitochondrion species form Bangalore, India, collected by Sudhagar et al (2014), *Apis cerana* isolate KYY-41, and *Apis cerana* isolate KYY-42 from China isolated by Wang (2021), *Apis cerana* mitochondrion, a species from USA (having NCBI accession number KU963188.1, MZ191825.1, MZ191824 and NC_014295.1 respectively). All these species form separate sub-clade in the maximum likelihood tree. *Apis florea* voucher specimen Almora represents 99% similarity in its sequence with *Apis florea* cytochrome oxidase subunit I specimen collected from Bangalore, Karnataka (NCBI accession number KU666428.1), both forming a separate cluster in maximum likelihood tree (Sangeetha et al 2016). *A. dorsata* voucher specimen Almora shows 96% sequence identity with *Apis dorsata* specimen cytochrome oxidase subunit I (NCBI accession number KJ755328) recorded from Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bangalore, Karnataka (Sudhagar et al 2014). Both these form a separate sub-cluster in maximum likelihood tree.

A. mellifera form two sub-cluster, in which our specimen i.e., *Apis mellifera*-COI-Almora 1 represent a separate sub-cluster. Although *A. mellifera show* cent percent identity with *Apis mellifera* voucher PHEL: 4132 from USA (KU601503.1), *Apis mellifera* voucher HY37(NC001566.1) from USA, *Apis mellifera* anatoliaca mitochondrion (MT188686.1) from USA, *Apis mellifera caucasica* mitochondrial DNA (AP018404.1) from Japan, *Apis mellifera* voucher HY37 from Bangladesh and *Apis mellifera carpatica* isolate 6-27 (MF100917.1) from Russia (Dhami et al 2016, Aslam et al 2016, Aslam et al 2018, Boardman et al 2020 and Ilyasov et al 2019) it did not show close evolutionary relationship with any of these sequences. As the entire mitochondrial DNA sequence was used for the construction of phylogeny, the Cox I region proves to be highly conserved region, through which

Table 1. Pair-wise genetic distance analysis between four species of *Apis* spp

Cox1 nucleotide sequences	*Apis_cerana_Indica*_COI-Almora_1	*Apis_mellifera*-COI-Almora_1	*Apis_florea*-COI-Almora_1	*Apis_dorsata*-COI-Almora_1*
*Apis_cerana_Indica*_COI-Almora_1*				
Apis_mellifera-COI-Almora_1	0.127			
Apis_florea-COI-Almora_1	0.255	0.263		
Apis_dorsata-COI-Almora_1	0.102	0.114	0.277	

*The nucleotide sequences of *Apis_dorsata*-COI-Almora_1 and *Apis_cerana_Indica*_COI-Almora_1 showed no pairwise genetic relationship, thus yielding the Pair-wise genetic distance analysis value of 0.000

molecular characterization of insects can be taken up with higher degrees of specificity.

When the phylogenetic tree was constructed with the MEGA X 10.0.5 software (Fig. 1), it was observed that the four species of *Apis* native to Indian Himalayas formed four separate groups in the maximum likelihood evolution tree. The node support estimated using 1000 bootstrap pseudoreplicates, showed that *A. dorsata, A.florea* and *A. mellifera* species evolved together as they showed 100% node value. The results showed that the *A. cerana* species showed 98% node value using 1000 bootstrap pseudoreplicates in the evolutionary analysis.

The pairwise genetic distance analysis carried out between the Four tested *Apis* species, recorded that *A. cerana indica* and *A. dorsata* had least genetic distance of 0.102 followed by *A. dorsata* and A. *mellifera* with genetics distance of 0.114 while the genetic distance between *A. florea* and *A. mellifera* was the highest with the value 0.263. Further to understand the inter-specific diversity among the four experimented species on the basis of 684 bp CO1 sequence, the variation in single nucleotide polymorphism (SNPs) was estimated by aligning the four sequences in CLUSTAL Omega (1.2.4) multiple sequence alignment

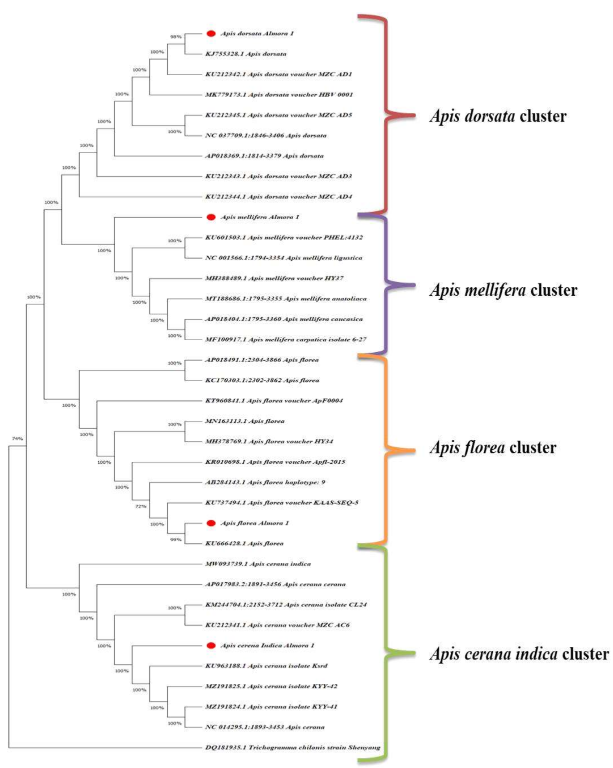

Fig. 1. Phylogenetic analysis through maximum likelihood method of four species of *Apis* along with 32 closely associated *Apis* species sequences (*Trichogramma chilonis* strain shenyang was taken as out group)

software (Fig. 2). It was observed that a total variation in 72 SNP's was recorded, which was large enough to differentiate the species at inter-specific level. The mean number nucleotide frequency amongst the four species of *Apis* was also examined (Fig. 3) and COI sequences were usually A+T biased with the concentration of A+T exceeding 73.49%, while the concentration of G+C was well below 26.51%. The highest concentration was found to be of T/U i.e., 40.57 %. Along with nucleotide frequencies, the transition/transversion rate ratios were also calculated (Table 2) for the 36 species of four *Apis* species and it was reported that the ratios were *k1* = 1.294 (purines) and *k2* = 1.738 (pyrimidines) and the overall transition/transversion bias was R=0.627, where R = [A*G*k1 +T*C*k2]/[(A+G)*(T+C)]. The tested honey bee specimen show more transversion (56.5%) compared to transition (43.5). Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution showed maximum base substitution between C to T and vice versa with a maximum of 19.91. To further analyze the variation in amino acid composition among the four sequences of *Apis* species, the sequences were translated by ExPASy translate software and the nucleotide compositions were estimated by MEGA X software (Fig. 4). Majority of deviations in polypeptides were associated with substitution of small amino acids with other. The per cent amino acid composition among the four *Apis* species showed that Leucine and Serine were found at the highest frequencies with an average of 13.71% and 12.27% respectively. Among all the tested specimen Cysteine amino acid was found with higher frequency in *A. florea* only. Moreover, it was found that the variation in amino acid frequency indicates huge variation among the four *Apis* species native to Indian Himalayas. Throughout the world, 20,355 species of bee pollinators has been documented (Ascher and Pickering 2019), their taxonomic identification is still in dearth stages due to lack of distinguishing morphological characters in bee taxonomy (Packer et al 2009), identification of bees become more herculean task for taxonomists due to huge biological diversity and presence of cryptic species (Hines and Williams 2012 and Vamosi et al

Table 2. Maximum composite likelihood estimate of the pattern of nucleotide substitution of 34 species of *Apis*

Nucleotides	A	T	C	G
A	-	11.46	4.23	4.22
T	9.3	-	7.35	3.26
C	9.3	19.91	-	3.26
G	12.03	11.46	4.23	-

2017). Morphological identification is no longer enough to obtain accurate data on the position of a species. Molecular identification using CO1 gene as a genetic barcode has been accepted universally (Herbert et al 2003). Saini and Chandra (2019) observed that , the Indian subcontinent is estimated to have a diverse bee fauna, including over 766 species divided into 71 genera and six families, although the actual figure may be much higher. Pakrashi et al (2020) conducted a survey of Himalayan bee fauna and molecular characterization showed huge diversity of bees in the Indian Himalayas. Out of the 156 bee species collected through extensive surveys, the bees belonging to the four major families were reported viz., Apidae (40 species), Halticidae (five species), Megachilidae (nine species), and Melittidae

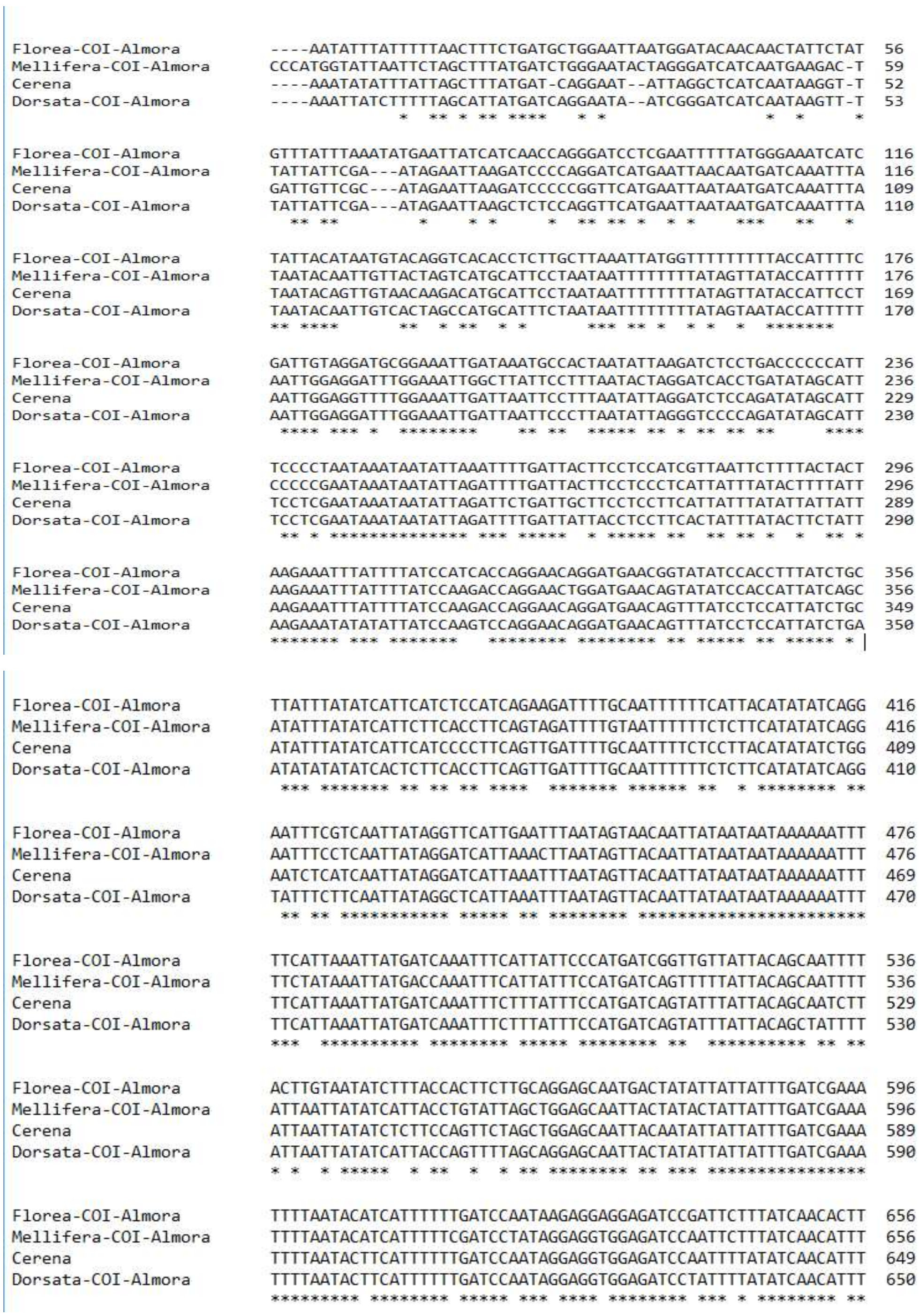

Fig. 2. Multiple sequence alignment of four species of *Apis* for analyzing the variation in single nucleotide polymorphism (SNPs)

(one species) which are widely distributed all over the Himalayas.

Saini et al (2019) discovered a new species of wild bee (*Melitta indica*) (Hymenoptera: Melittidae) from North Western Himalayan region of Uttarakhand through molecular characterization and DNA barcoding methods that has sparked the importance of molecular techniques in understanding the bees species delimitation in several areas of the world (Smith et al 2008; Butcher et al 2012). Through morphological Characterization it is difficult to identify a specimen (Gibbs 2009, Rehan and Sheffield 2011 and Williams et al 2012), appropriate identification of the specimens up to species level (Schmidt et al 2015), clarifying level of speciation of various species associated with various taxonomic levels (Trunz et al 2016 and Oh et al. 2013), association of sexes in dimorphic species classification (Gibbs 2009 and Sheffield et al 2009), cryptic species detection (Packer and Taylor 1997) and determining the evolutionary relationship and genetic distances among the targeted insect species (Kekkonen and Hebert 2014). Besides the above-highlighted applications molecular techniques, these support taxonomists in integrative taxonomy to decode the biological diversity of the given region (Dayrat 2005). Globally, nine species have been commonly recognized under the genus *Apis*, including A. *andreniformis*, *A. cerana*, *A. dorsata*, *A. florea*, *A. koschevnikovi*, *A. laboriosa*, *A. mellifera*, *A. nigrocincta* and *A. nuluensis* (Koeniger et al 2011). Based on recent studies, the origin of *Apis* spp. has been predicted from tropical regions, and of all the honey bee species including *A. mellifera* and *A. cerana* have spread northward into the temperate zone, explaining their large scale role in agriculture (Ji 2021). Using molecular techniques to predict the phylogenetic similarity with the help of mitochondrial genes, strongly supported the basic topology that these groups of honeybee species belong to three main clusters: giant bees (*A. dorsata* and *A. laboriosa*), dwarf bees (*A. andreniformis* and *A. florea*) and cavity-nesting bees (*A. mellifera*, *A. cerana*, *A. koschevnikovi*, *A. nuluensis* and *A. nigrocincta*) (Koeniger et al., 2011 and Ji 2021).

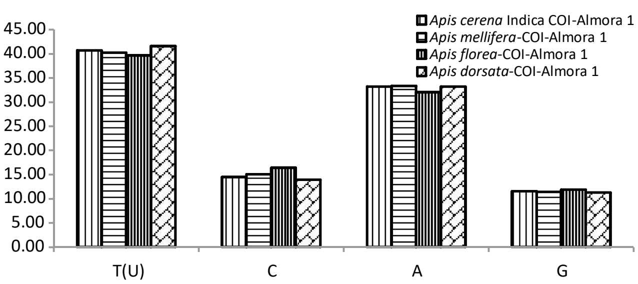

Fig. 3. Average nucleotide frequency among the four species of *Apis*

In present study investigated the genetic diversity and population structure among the honeybee populations in North western Himalayan region. In our study, characterization of four *Apis* sp., three native to India viz., *A. cerana*, *A. florea* and *A. dorsata* and one introduced i.e., *A. mellifera* utilizing molecular techniques like DNA barcoding and phylogenetic analysis with the closely related *Apis* species obtained from NCBI data base were carried out. The phylogenetic hierarchy of the *Apis* species from Northwestern Himalayas and closely related sequences obtained form NCBI form four separate cluster in the maximum likelihood tree. All the sequences fall into respective groups, which are in agreement with Arias and Sheppard (2005). The result highlighted that *A. cerana* voucher Almora show close resemblance with *Apis cerana* species from India, China and USA. Similarly *A. mellifera*

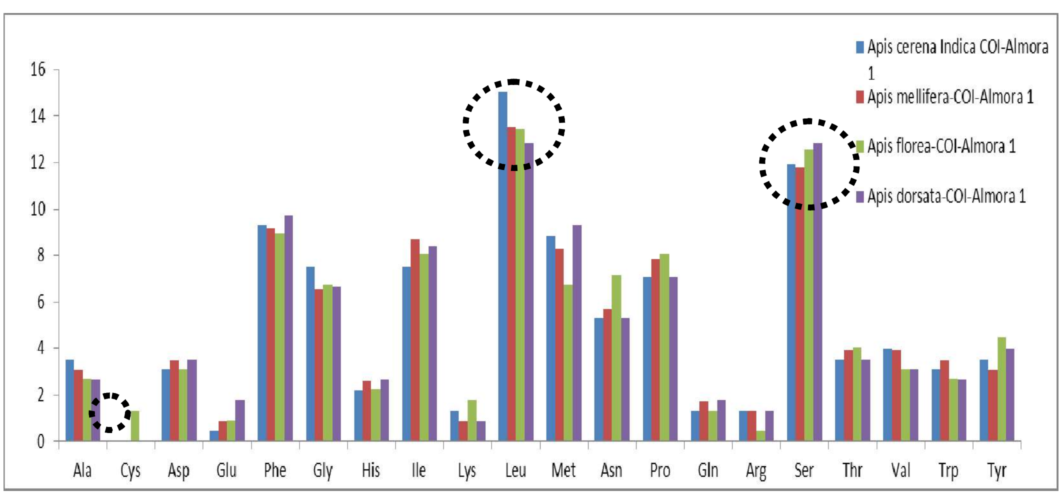

Fig. 4. Variation in amino acid composition of four *Apis* species native to North Western Himalayas

show resemblance with species from USA, Bangladesh, China, Japan and Russia. This genetic variation in mitochondrial COI represent that this gene has high average genetic diversity. Similar results were obtained by Ballard and Whitlock (2004). Oldroyd et al (2006) suggested that mitochondrial COI genes are well suited for the characterization of phylogenetic divergence within species. Meixner et al (2013) revealed that this method is effective in inferring bee identification and evolution of subspecies. Packer and Ruz (2017) and Kevan and Packer (2009) suggested that COX 1 genes are the commonly used universal genes for the identification of honey bee species worldwide. Ozdil and Ilhan (2012) also followed DNA barcoding technique for characterizing honey bee subspecies in Turkey. The transition/transversion (Ts/Tv) ratio is instrumental in inferring the direction and magnitude of natural selection. The Ts/Tv value of 0.61 suggests that there is insignificant neutral selection in honeybees of north western Himalayan region of Uttarakhand. The ratio of more than 1, implies a positive or Darwinian selection; whereas the ratio less than 1, implies purifying selection and a ratio of one indicates neutral (i.e., no) selection (Chalapathy et al 2014). However the positive and purifying selection at different points within the gene or at different times along its evolution may cancel each other out giving an average value that may be lower, equal or higher. Thus, it could be inferred that the honeybees of north western Himalayan region of Uttarakhand might be in the verge of diversification. Moreover, the exact transition rate (43.5%) and transversion rate (56.5%) suggests no significant alteration representing frequent migration, mutations, gene flow amongst these populations. Transitions do not contribute heavily to genetic divergence, whereas transversion generate significant impact on the evolution of species. The comparable values of transitions and transversions in the current study suggest the possible occurrence of genetic divergence over evolutionary time scales. Chalapathy et al (2014) showed similar result in the honey bees from Karnataka, India. Like Willis et al (1992), suggested honeybees are AT biased and the mean number of A+ T and C+G satisfied of the findings is in the ratio of approximately (73.49:26.51) 3:1 respectively. Willis et al (1992) additionally discussed that AT rich sequence may be the result of numerous factors including small effective population size, selection drift, and mitochondrial polymerase inefficiency. Historical sign suggest rapid changes in the morphology of *Apis* and these rapid changes are predicted between upper Eocene and Oligocene approximately 10 million years ago (Culliney 1983). The A+T bias pointed out for various codons has been elucidated either as a mutational practice favouring the buildup of those nucleotides or the result of an ineffective repair system (Chalapathy et al 2014).

CONCLUSION

The comparative analysis of honey bee populations was carried out, taking into account the residual phylogenetic relationships. As the Indian Himalayas are undisturbed as well as unexplored ecosystems on the earth where biodiversity has not been studied comprehensively due the hilly location where taking up survey is very much difficult. Our investigation not only forms the point of commencement for study of bee fauna in Indian Himalayas, but also opens the doors of opportunities to discover the faunal diversity of the Himalayas.

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Diversity and Abundance of Insect Pollinator Fauna in Coriander (*Coriandrum sativum* L.) and Ajwain (*Trachyspermum ammi* L.) in North Karnataka

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Abstract: Coriander (*Coriandrum sativum* L.) and ajwain (*Trachyspermum ammi* L.) are the two important seed spices being domesticated and marketed in India. Understanding the abundance and diversity of insect fauna which are actively involved in cross pollination of them is crucial in production of higher quality seed yield. During the study period, coriander flowers were visited by 17 species of pollinators (5 Hymenopterans, 6 Dipterans, 2 Coleopterans and 4 Lepidopterans) and 23 species of pollinators (6 Hymenopterans, 10 Dipterans, 2 Coleopterans and 5 Lepidopteran) were documented on ajwain flowers from the districts surveyed (Gadag, Haveri and Dharwad districts for coriander; Vijayapura, Bagalkot and Dharwad districts for ajwain). Diversity (Simpson index of diversity, Shannon-Wiener index, Evenness and Similarity index) of pollinators in coriander was higher in Gadag followed by Haveri and Dharwad districts. In ajwain, the diversity (Simpson index of diversity, Shannon-Wiener index, Evenness and Similarity index) of pollinators was higher in Vijayapura followed by Bagalkot and Dharwad districts. Meanwhile, in both coriander and ajwain, *Apis florea* was the most predominant pollinator followed by *A. cerana indica* which need to be conserved through potential conservative agronomical practices to enhance the seed yield.

Keywords: Abundance, Ajwain, Coriander, Diversity, Insect pollinators

Since ancient times, Indian spices and spicy cuisine have been popular all across the world. Traders from all over the globe have come to our subcontinent in search of spices. Out of 109 spices found in the world, 75 are grown in India, which includes fourteen seed spices (cumin, coriander, fennel, fenugreek, ajwain, anise, dill, nigella, pepper, cardamom, clove, cinnamon, nutmeg and bay leaf) commercially grown across the country. India is one of the biggest producer, consumer and exporter of seed spices because of its diverse soil and climatic regions, ideal to grow many of the seed spices. These crops are mostly grown in the country's semi-arid and arid zones, which have dry or rainy chilly weather conditions. Together, the states of Rajasthan, Gujarat, and parts of Madhya Pradesh are known as the "bowl of seed spices," accounting for more than 80 per cent of the country's yearly production. Coriander (*Coriandrum sativum* L.) also known as cilantro or Chinese parsley, belongs to the family Apiaceae (2n=22). It is a smooth, erect annual medium height herb, grown for its seeds and tender green feathery leaves. Green leaves are used in the preparation of chutney and also used as seasonings in curries, soups and sauces. All plant parts are used due to the presence of essential oils and pleasant aromatic character. Coriander produces a large amount of nectar, which attracts a variety of insects for pollination; an external effect that is both ecological and economic in value; as a result, it is also a good melliferous plant, producing 500 kg of honey from a single hectare of coriander. Ajwain (*Trachyspermum ammi* L.), commonly known as carom seed or bishop's weed, is a member of the Apiaceae family and a major seed spice crop in India. It is an annual herbaceous shrub with little greyish brown egg-shaped fruits. Because of the inclusion of an essential oil, it has a distinct odor and flavor (2- 4%). Thymol is mostly found in ajwain oil. Its distinctive fragrant scent and strong flavor make it popular as a spice in curries. It can be used alone or in combination with other spices and condiments. Ajwain's most significant usage is medical, and it is a common home cure for indigestion. Its seed and oil are highly sought after for its antispasmodic, stimulant, tonic, and fragrant carminative effects (Rathore et al 2014). The pollination constraints have raised serious threats to crop production and plant diversity. Approaches for integrated pollination services using well-managed pollinators for crop species and to make the habitats favourable for the survival of insect pollinators is much needed (Shivanna et al 2020). Over the major pollinators, coriander and ajwain crops are cross-fertilized by insects to the extent of 60-70 per cent and play a vital role in increasing crop productivity. Hence, the present investigation was undertaken to document the abundance and diversity of insect pollinators through random survey in major cropping area of northern Karnataka.

MATERIAL AND METHODS

Survey: Roving survey was carried out during *rabi* 2020-21 in coriander and ajwain growing districts of Karnataka covering Gadag (15.4315° N, 75.6355° E) and Gajendragad (15.7361° N, 75.9710° E) talukas of Gadag district, Haveri (14.7951° N, 75.3991° E) and Ranebennur (14.6154° N, 75.6288° E) talukas of Haveri district to record pollinators diversity in coriander ecosystem. Similarly, Basavana bagevadi (16.5681° N, 75.9754° E) and Muddebihal (16.3396° N, 76.1291° E) talukas of Vijayapura district, Hungunda (16.0576° N, 76.0609° E) and Guledgudda (16.0496° N, 75.7895° E) talukas of Bagalkot district were surveyed to record pollinators diversity in ajwain ecosystem. From each taluka, two villages were selected and in each village one field was surveyed. In Main Agricultural Research Station (MARS) Dharwad, the experimental plots of coriander (Variety: DWD-3) and ajwain (Variety: Ajmer ajwain-1) were raised as per the package of practices except plant protection measures under the black soil condition.

Observations: Randomly 20 net sweeps were taken by transect walk under open pollination conditions during peak flowering period on clear sunny days (Devi et al 2015). During the period of survey, pollinators visiting the crops were collected, preserved and identified with the help of available taxonomic keys and by experts to know the diversity of pollinators. Village wise collected data were pooled and the relative abundance of pollinators was expressed in percentage taluka wise.

Diversity indices of pollinators

a. Shannon-Wiener diversity index (H): The Shannon-Wiener diversity index is calculated by using the following equation: $H = -\Sigma\, pi \ln pi$

Where, pi is the proportion of the i^{th} species of pollinator and ln is the natural log with base e=2.718.

b. Simpson index of diversity (1-D): It was calculated by $D = 1 - \Sigma pi$

c. Evenness (J): Relative abundance of each species in a particular habitat was calculated using the following formula: $J = H' / \ln S$

Where, H' is the Shannon-Wiener diversity index or Simpson index of diversity and 'S' is the total number of species present.

d. Sorenson's similarity index:

$$S = \frac{2C}{A+B}$$

Where, A= number of species in sample A, B= number of species in sample B and C= number of species common to both samples

e. Relative abundance of insect pollinators: It was calculated by using formula to know the dominance of species in coriander and ajwain ecosystem.

$$\text{Relative abundance} = \frac{\text{Abundance of the species}}{\text{Total abundance of all species}} \times 100$$

RESULTS AND DISCUSSION

Pollinator fauna of coriander: Most of the insect pollinators encountered during the present study have been reported earlier. However, the occurrence of *Nyctemera coleta* (Stoll), *Prosotas dubiosa* (Semper) and *Eristalinus obliquus* Wiedemann as pollinators of coriander is the first record from Karnataka. Gadag district (Gajendragad and Gadag talukas), recorded 13 species of pollinators from 6 families under 4 orders (Table 1). Among the 4 orders of insect pollinators recorded, Hymenopterans were the most dominant which constituted 58.85 per cent followed by Dipterans (27.78 %), Coleopterans (9.35 %) and Lepidopterans (3.61 %). Among the order Hymenoptera, Apidae accounted for 4 species of honey bees and a lone species belonged to Halictidae. Order Diptera was represented by 4 species belonging to a Syrphidae family. Similarly, the order Coleoptera was represented by two species belonging to Coccinellidae while, Lepidoptera was represented 2 species belonging to the family Lycaenidae. Haveri district (Haveri and Ranebennur talukas), accounted for 12 species belonging to 4 families under 4 orders (Table 1). Among the 4 orders of insect pollinators recorded, Hymenopterans were the most predominant which constituted 60.14 per cent followed by Dipterans, Coleopterans and Lepidopterans. Among the order Hymenoptera, Apidae accounted for 4 species of honey bees. Order Diptera was represented by 4 species belonging to a Syrphidae family and Coleoptera was represented by two species belonging to Coccinellidae. Lepidoptera was represented by 2 species belonging to the family Lycaenidae. During fixed plot survey conducted in Dharwad, 13 species belonging to 6 families under 4 orders were recorded (Table 1). Among the 4 orders of insect pollinators recorded, Hymenopterans were the most prominent pollinators (73.47 %) followed by Dipterans, Coleopterans and Lepidopterans. Among the order Hymenoptera, Apidae accounted for 4 species of honey bees and a lone species belonged to Halictidae. Order Diptera was represented by 5 species of which 4 species belonged to Syrphidae and a lone species from Muscidae. Similarly, order Coleoptera was represented by two species belonging to Coccinellidae while, Lepidoptera was represented by a lone species belonged to Lycaenidae.

Between the location there is no variation in diversity of pollinators at taxonomic levels in coriander ecosystem.

The present results are partially in agreement with the findings of Prasanna (2003) who reported 14 species of pollinators belonging to 9 families (Apidae, Halictidae, Syrphidae, Asilidae, Pieridae, Pyraustidae, Danaidae, Coccinellidae and Crysomelidae) included in 4 different orders. Further *A. florea* was the most prominent pollinator (45.52 %) followed by *A. cerana indica* and *A. dorsata* in Dharwad. However, during the present study reports of the family Asilidae, Pieridae, Pyraustidae, Crysomelidae and Danaidae were not recorded. Sharma and Meena (2019) reported 28 insect species belonging to 18 families of 6 orders were visited coriander flowers. Among the pollinators, *A. florea* was the most dominating species, followed by *A. mellifera* and *E. balteatus*. Shivashankara *et al*. (2016)

Table 1. Diversity of pollinator fauna of coriander

Common name	Scientific name	Family	Species abundance (%)						
			Gadag district (December, 2020)			Haveri district (December, 2020)			Dharwad (December 2020 to January 2021)
			Gajendragad	Gadag	Mean	Haveri	Ranebennur	Mean	
Hymenoptera									
Little bee	*Apis florea* Fabricius	Apidae	30.18	28.27	29.22	25.27	22.72	23.99	31.63
Indian bee	*A. cerana indica* Fabricius		12.5	15.20	13.85	20.44	30.00	25.22	26.17
Rock bee	*A. dorsata* Fabricius		6.77	10.90	8.83	9.29	7.72	8.50	5.86
Stingless bee	*Trigona iridipennis* Smith		4.33	3.63	3.98	2.60	2.27	2.43	5.53
Halictid bee	*Lasioglosum* sp.	Halictidae	3.49	2.45	2.97	-	-	-	4.28
Relative abundance (Order)			58.85 %			60.14 %			73.47 %
Diptera									
Common hover fly	*Ischiodon scutellaris* Fabricius	Syrphidae	11.23	11.20	11.21	5.94	5.18	5.56	4.07
Long hover fly	*Sphaerophoria indiana* Bigot		8.66	8.18	8.42	6.69	3.63	5.16	4.05
Syrphid	*Sphaerophoria macrogaster* Thomson		6.13	6.81	6.47	8.55	5.20	6.87	4.26
Marmalade hoverfly	*Episyrphus balteatus* De Geer		2.16	1.21	1.68	-	-	-	3.80
Syrphid	*Episyrphus obliquus*		-	-	-	0.37	-	0.18	-
Musca sp.	*Musca* sp.	Muscidae	-	-	-	-	-	-	1.76
Relative abundance (Order)			27.78 %			17.77 %			17.94 %
Coleoptera									
Transverse ladybird	*Coccinella transversalis* Fabricius	Coccinellidae	7.22	5.45	6.33	8.92	10.1	9.51	4.07
Indian wave stiped ladybug	*Cheilomenes sexmaculata* Fabricius		3.24	2.81	3.02	5.94	7.72	6.83	3.59
Relative abundance (Order)			9.35 %			16.34 %			7.66 %
Lepidoptera									
Tailless lineblue	*Prosotas dubiosa* (Semper)	Lycaenidae	2.88	3.63	3.25	1.85	1.81	1.83	-
Pea blue	*Lampides boeticus*		-	-	-	4.08	3.63	3.85	-
Plain cupid	*Luthrodes pandava* (Horsfield)		-	-	-	-	-	-	0.88
Marbled moth	*Nyctemera coleta* (Stoll)	Erebidae	0.72	-	0.36	-	-	-	-
Relative abundance (Order)			3.61 %			5.68 %			0.88 %

reported that coriander flowers were visited by insect species belonging to Hymenoptera, Diptera, Lepidoptera and Coleoptera. Among these, *A. cerana indica*, *A. mellifera*, *A. florea*, *E. balteatus*, *E. tenax*, *Syrphus* spp. and *Musca* sp. were the dominant pollinators in Pantnagar (Uttarakhand). Thus, various studies have highlighted the dominance of

Table 2. Diversity of pollinator fauna of ajwain

Common name	Scientific name	Family	Species abundance (%)						
			Vijayapura (January, 2021)			Bagalkot (January, 2021)			Dharwad (January to February, 2021)
			Basavan bagevadi	Muddeb hihal	Mean	Hungunda	Guledagudda	Mean	
Hymenoptera									
Little bee	*Apis florea* Fabricius	Apidae	18.51	22.54	20.52	26.66	23.78	25.22	27.67
Indian bee	*A. cerana indica* Fabricius		12.50	15.16	13.83	15.71	14.07	14.89	20.68
Rock bee	*A. dorsata* Fabricius		4.62	9.42	7.02	7.61	5.33	6.47	3.82
Stingless bee	*Trigona iridipennis* Smith		5.09	5.32	5.20	6.19	4.90	5.54	3.94
Halictid bee	*Lasioglosum* sp.	Halictidae	-	-	-	2.38	1.40	1.89	2.96
Scoliid wasp	*Scolia affinis* Guerin	Scoliidae	-	-	-	-	-	-	0.15
Relative abundance (Order)				46.57 %			54.07 %		59.22 %
Diptera									
Common hover fly	*Ischiodon scutellaris* Fabricius	Syrphidae	7.94	4.91	6.42	3.33	7.76	5.54	4.45
Long hover fly	*Sphaerophoria indiana* Bigot		5.82	4.09	4.95	-	-	-	4.02
Syrphid	*Sphaerophoria macrogaster* Thomson		6.94	6.55	6.74	7.14	5.82	6.48	3.83
Marmalade hoverfly	*Episyrphus balteatus* De Geer		5.59	3.70	4.64	-	-	-	4.27
Syrphid	*Phytomia errans* Fabricius		9.25	6.96	8.10	8.57	9.70	9.13	5.14
Syrphid	*Eristalinus arvorum* Fabricius		-	-	-	-	-	-	5.01
Syrphid	*Serratoparagus serratus* Fabricius		-	-	-	-	-	-	4.25
House fly	*Musca domestica*	Muscidae	3.20	3.25	3.23	-	-	-	0.84
Winged fly	*Physiphora anea* Fabricius	Ulidiidae	-	-	-	-	-	-	0.94
Relative abundance (Order)				34.08 %			21.14 %		32.75 %
Coleoptera									
Transverse ladybird	*Coccinella transversalis* Fabricius	Coccinellidae	11.10	8.19	9.65	9.04	10.67	9.85	3.73
Indian wave stiped ladybug	*Cheilomenes sexmaculata* Fabricius		6.01	6.55	6.29	7.60	8.25	7.93	3.25
Relative abundance (Order)				15.94 %			17.83 %		6.98 %
Lepidoptera									
Tailless lineblue	*Prosotas dubiosa* (Semper)	Lycaenidae	-	-	-	-	-	-	0.25
Pea blue	*Lampides boeticus*		3.24	3.27	3.26	3.33	3.88	3.60	0.56
Plain cupid	*Luthrodes pandava* (Horsfield)		-	-	-	1.42	2.91	2.16	-
Common shot silverline	*Cigaritis ictis* (Hewitson)		-	-	-	0.95	1.45	1.20	0.20
Karwar swift skipper	*Caltoris canaraica* (Moore)	Hesperiidae	-	-	-	-	-	-	0.12
Relative abundance (Order)				3.26 %			6.96 %		1.13 %

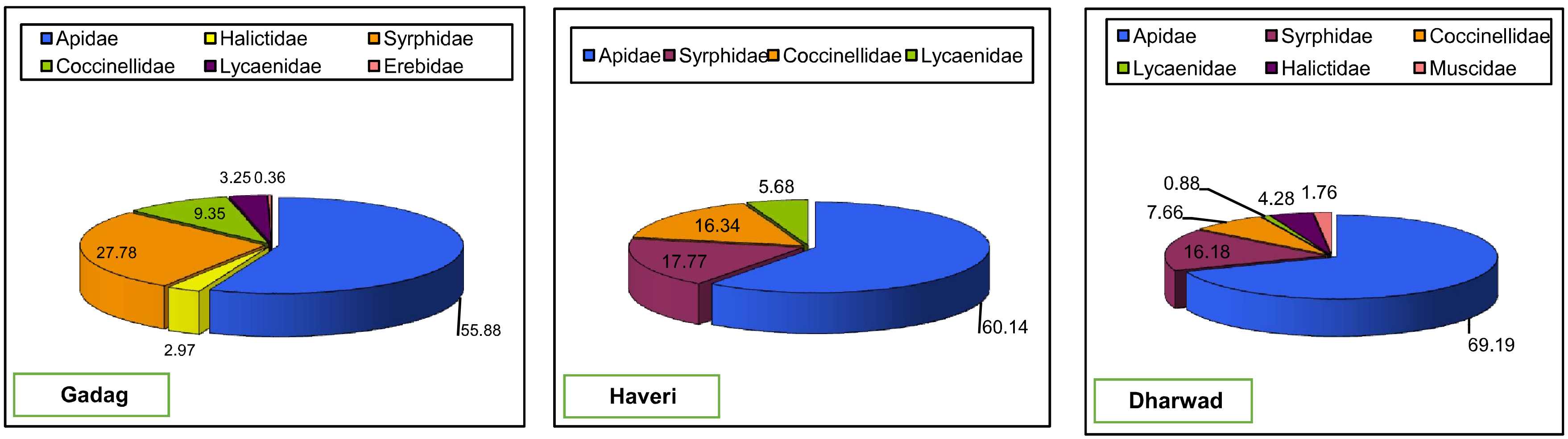

Fig. 1. Relative abundance (%) of insect pollinators in coriander from different surveyed districts (Family)

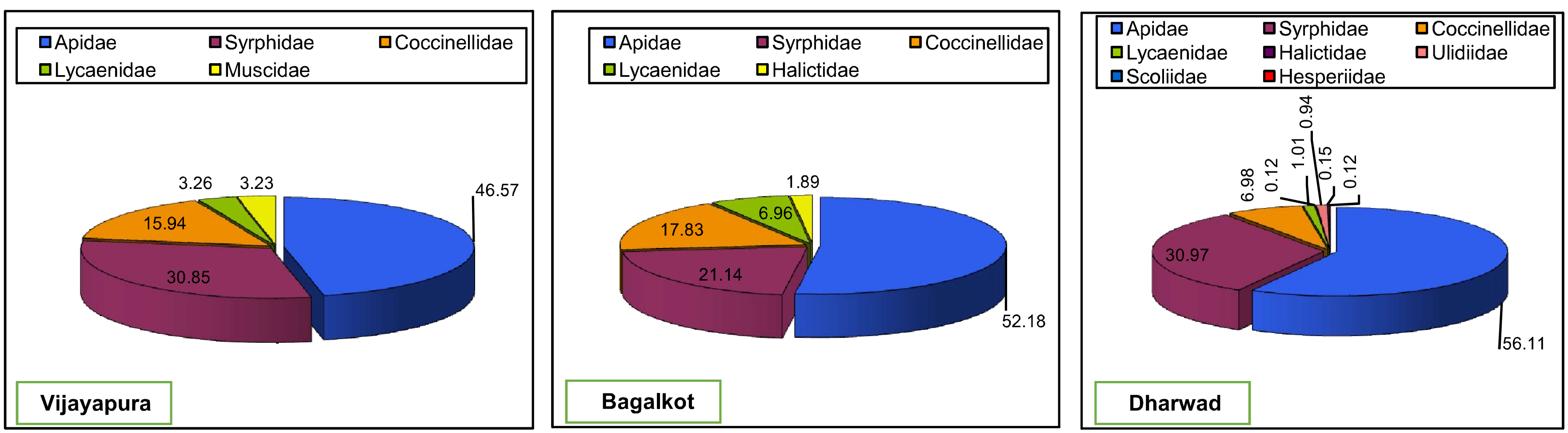

Fig. 2. Relative abundance (%) of insect pollinators in ajwain from different surveyed districts (Family)

Table 3. Diversity indices of pollinators in coriander

Diversity indices	Coriander			Ajwain		
	Gadag	Haveri	Dharwad	Bagalkot	Vijayapura	Dharwad
Simpson index of diversity (1-D)	0.87	0.85	0.83	0.85	0.89	0.83
Shannon-Wiener index (H)	2.21	2.16	2.09	2.29	2.41	2.27
Evenness (J)	0.76	0.67	0.62	0.74	0.87	0.48
Similarity index (%)	96	95	82	100	100	68

honey bees as the most important pollinators not only in coriander also in other crops.

Pollinator fauna of ajwain: The majority of the pollinators observed during this investigation have previously been documented. However, the occurrence of *Phytomia errans* Fabricius, *Eristalinus arvorum* Fabricius *Physiphora anea* Fabricius, *Sphaerophoria macrogaster* Thomson, *Serratoparagus serratus* Fabricius, *Lampides boeticus* Linnaeus, *Cigaritis ictis* (Hewitson) and *Scolia affinis* Guerin as pollinators of ajwain is the first record from Karnataka. Vijayapura district (Basavan bagevadi and Muddebhihal talukas), recorded 13 species of pollinators belonging to 5 families under 4 orders (Table 2). Among the 4 orders of insect pollinators recorded, Hymenopterans were the most prominent pollinators (46.57 %) followed by Dipterans and Coleopterans. Among the order Hymenoptera, Apidae accounted for 4 species of honey bees. Order Diptera was represented by 6 species of which 5 species belonged to Syrphidae and a lone species from Muscidae. Similarly, the order Coleoptera was represented by two species belonging to Coccinellidae while, Lepidoptera was represented by a lone species belonging to the family Lycaenidae. Bagalkot district (Hungunda and Guledagudda talukas), recorded 13 species of pollinators belonging to 5 families under 4 orders (Table 2). Among the 4 orders of insect pollinators recorded, Hymenopterans were the most prominent pollinators (54.07 %) followed by Dipterans, Coleopterans and Lepidopterans. Among the order Hymenoptera, Apidae accounted for 4 species of honey bees and a lone species belonged to Halictidae. Order Diptera was represented by 3 species belonging to a Syrphidae family. Similarly, the order Coleoptera was represented by two species belonging to Coccinellidae while, Lepidoptera was represented by 3 species belonging to the family Lycaenidae. 21 species belonging to 9 families under 4 orders were documented during fixed plot survey, conducted in Dharwad (Table 2). Among the 4 orders of insect pollinators recorded, Hymenopterans were the most predominant (59.22 %) followed by Dipterans and Coleopterans. Among the order Hymenoptera, Apidae accounted for 4 species of honey bees and a lone species from Halictidae and Scoliidae. Order Diptera was represented by 9 species of which 7 species belonged to Syrphidae and one each from Muscidae and Ulidiidae families. Similarly, order Coleoptera was represented by two species belonging to Coccinellidae while, the order Lepidoptera was represented by 4 species of which 3 species belonged Lycaenidae and a lone species to belonged to the family Hesperiidae. Among the districts surveyed in ajwain crop ecosystems, the diversity of pollinators was more in Dharwad compared to Vijayapura and Bagalkot districts. Several authors have reported honey bees as dominant pollinators in related coriander crop (Khalid et al 2008, Kant et al 2013, Roopashree 2011, Ranjitha et al 2019). Ajwain being a member of same family as that of coriander, it is possible that the diversity and abundance of pollinators is similar to coriander.

In both coriander and ajwain crops, 70 per cent of pollinators are similar from the above obtained results. Among the pollinators recorded, *A. florea* is the most prominent pollinator followed by *A. cerana indica* and Syrphids. Coriander and ajwain flowers are rich in pollen and nectar content which attracted the pollinators. Coccinellid species might act as fortuitous pollinators while moving from one plant to another plant in search of their prey. Similarly, in the process of visiting flowers, nectar feeding lepidopterans also frequently act as pollinators.

Diversity indices of pollinators in coriander and ajwain: In both coriander and ajwain crops, the Simpson index of diversity and Shannon-Wiener index did not vary much among the districts surveyed (Gadag, Haveri and Dharwad districts for coriander, Vijayapura, Bagalkot and Dharwad districts for ajwain) (Table 3). The Evenness of species differed much between the districts. However, similarity was noticed in Gadag and Haveri, Vijayapura and Bagalkot than Dharwad district in coriander and ajwain ecosystem respectively. Diversity and species richness of pollinators varies from region to region. The density of insects on blossom depends on several factors like flower color, availability of floral rewards, shape, size, variety, crop protection measures and weather conditions. Since, it was the first kind of attempt, supported literatures on diversity indices of pollinators are not available to make any meaningful comparison.

CONCLUSIONS

The ajwain flowers are visited by a greater number of pollinators than the coriander due to floral scents, floral rewards and duration of flowering. Meanwhile, in both coriander and ajwain, *A. florea* was the most predominant pollinator followed by *A. cerana indica* which need to be conserved through potential conservative agronomical practices to enhance the seed yield. The information on the type of pollinators and peak activities is expected to conserve the pollinators naturally or may be pollinators rearing through artificially and to schedule crop protection measures.

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Population Dynamics of Major Insect Pests of Castor, *Ricinus communis* L. as Influenced by Weather Variables in South-west Haryana

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Abstract: The population dynamics of major insect pests of castor was studied during 2018-19 at CCSHAU RRS, Bawal on castor hybrid DCH 177. Among the major insect pests, castor semilooper, *Achaea janata* was the first to infest the crop reaching its peak population of 6 larvae per plant in 35th SMW. The peak population of *Spodoptera litura* was 3.4 larvae/plant during 41st SMW. Peak nymphal population of leafhopper was in 44th SMW. Maximum capsule damage by castor shoot and capsule borer, *Conogethes punctiferalis* was during 50th SMW of 2018. The rainfall (r= 0.688) and relative humidity were positively (r= 0.836) related with *A. janata* larval population. Maximum temperature (r= 0.900) and bright sunshine hours (r= 0.737) exhibited a positively significant correlation with the larval population of *S. litura*. Leafhopper nymphal population and capsule damage by *C. punctiferalis* were negatively influenced by evening relative humidity (r= -0.775) and rainfall (r= -0.505) and maximum (r= -0.698) and minimum temperature (r= -0.828), respectively. Regression analysis revealed that the weather variables exhibited a significant effect on the population dynamics of the pests with adjusted coefficients of determination to the tune of 0.67, 0.90, 0.58 and 0.67 for *A. janata*, *S. litura*, *E. flavescens* and *C. punctiferalis*, respectively.

Keywords: Castor, DCH 177, *Achaea janata*, *Spodoptera litura*, *Empoasca flavescens*, *Conogethes punctiferalis*

In India, major oilseed crops of *kharif* season are soybean, groundnut, sesamum and castor. Castor (*Ricinus communis* L.) is a non-edible oilseed crop which belongs to the family Euphorbiaceae. Main use of castor crop is to extract non-edible oil from its seeds. Well drained sandy loam soils are suitable for its cultivation. In India, castor was cultivated on 1.05 million ha area producing about 1.84 million tonnes during 2019-20 with a productivity of 1.76 tonnes/ha (Anonymous 2022). It plays vital role in Indian economy and fetched Rs. 6802 crores in terms of valuable foreign exchange by exports of about 0.73 million tonnes of castor oil during 2020-21 (APEDA 2022). There are many constraints in the cultivation of castor but insect pests are of prime importance especially in the rainfed areas. More than 107 species of insects and six species of mites are recorded on castor at different stages of crop growth (Lakshminarayana and Raoof 2005) and most of the insect pests infesting castor crop are either defoliators or sucking pests (Sarma et al 2005). The economically important insect-pest species associated with castor in south-west Haryana are castor shoot and capsule borer (*Conogethes punctiferalis*), castor semilooper (*Achaea janata*), leafhopper (*Empoasca flavescens*), castor hairy caterpillar (*Euproctis lunata*) and tobacco caterpillar (Gaur 2014, Puneet et al 2020, Ranga et al 2021). Various insect pests account for 17.2-63.3 per cent seed yield losses in castor (Lakshminarayana and Duraimurugan 2014). Insects are poikilothermic organisms and are largely influenced by the ambient conditions as far as their growth, development, life cycle, population dynamics and other life history traits are concerned. The various weather variables are known to cause variability in population dynamics of castor major insect pests to the tune of 74.2- 97.5 per cent (Manjunatha et al 2019). Studies on incidence of insect pests in relation to weather parameters give better understanding of plant insect relationship, especially in recognizing the time of economic management.

MATERIAL AND METHODS

The present investigations were carried out in field experiment during 2018-19 at CCS Haryana Agricultural University Regional Research Station, Bawal located at latitudes 28°10' N, longitudes 76° 50' E and 266 m above mean sea level (Rao et al 2005). The area of 250 m^2 (r hybrid DCH 177) was sown during 2nd fortnight of July, 2018 as per recommendations of package of practices of *Kharif* crops by CCS HAU, Hisar (Anonymous 2018). The experiment area was not sprayed with any insecticide.

Observations recorded: Thirty plants in each experimental plot were randomly selected and tagged for recording observations for incidence of major insect pests of castor *viz*., castor semilooper, *Achaea janata*; tobacco caterpillar, *Spodoptera litura*; leafhopper, *Empoasca flavescens* and castor shoot and capsule borer, *Conogethes punctiferalis*. Infestation levels of castor semilooper and tobacco caterpillar were recorded by counting the number of larvae per plant. The nymphal population of leafhopper was recorded from 3 leaves from randomly selected thirty plants starting from germination at weekly intervals. The leaves were selected on the main shoot - one from top (excluding two top-most leaves), middle (medium matured leaves) and bottom (leaving two bottom-most leaves). The incidence of castor shoot and capsule borer was observed by calculating the number of infested capsules as compared to total number of capsules from 3 branches per plant at weekly intervals.

Weather data: Meteorological data on various weather parameters like temperature (maximum and minimum), relative humidity (morning and evening), wind velocity, evaporation, rainfall and bright sunshine hours was collected from the Meteorological observatory, RRS, Bawal.

Statistical analysis: All the observations recorded in the due course of investigations were subjected to statistical analysis by using SPSSv23 (IBM Corp 2015). Karl Pearson coefficient was calculated to elucidate the impact of weather variables on population dynamics and fluctuations of insect pests. Graphs and correlograms were prepared using OriginPro® (2022). Correlation analysis was conducted with weather data of lag weeks also as the previous weather conditions ultimately determines the current pest population. Step-wise linear regression models were formulated to quantify the impact of weather variables on insect pests.

RESULTS AND DISCUSSIONS

The mean larval populations of *A. janata* and *S. litura* are presented in Table 1 along with respective SMW and weather data. Similarly, mean nymphal population and mean per cent capsule damage is tabulated as Table 2.

Castor semilooper, *Achaea janata* Linn.: The castor semilooper (*A. janata*) first appeared during 32nd standard meteorological week (SMW) with an average larval population of 3.5 per plant. It started increasing and reached to a peak population of 6 larvae per plant during 35th SMW (Fig. 1). The larval population reduced to 1.2 larvae per plant during 41st SMW (second week of October). The larval population of castor semilooper was active from 30th SMW to 47th SMW (Singh et al 2016b) and *A. janata* larval population from 28th to 44th SMW on castor hybrid DCH 177 (Ranga et al 2021).

During current week, rainfall (r= 0.688) and evening R.H. (r= 0.836) were positively and significantly correlated with *A. janata* larval population, whereas maximum temperature (r= -0.799) and evaporation (r= -0.836) exhibited a negatively significant (Fig. 2). During lag week I and II both, minimum temperature negatively influenced the larval population. During lag week I, the evening R.H. (r= -0.757), minimum temperature (r= 0.620) and maximum temperature (r= -0.677) were major influencers of larval populations of *A. janata*. Umbarkar and Patel (2016) who reported that *A. janata* had negative correlation with maximum temperature. The results are also in agreement with Singh et al (2016a) where evening relative humidity had positive significant correlation with larval population of *A. janata*. Rainfall was

Table 1. Mean larval populations of *S. litura* and *A. janata* on castor hybrid DCH 177 during *kharif*, 2018

SMW	Temperature (ºC)		Relative humidity (%)		Wind velocity (km/hr)	Sun shine (hrs)	Evaporation (mm)	Rainfall (mm)	Mean larval population per plant*	
	Maximum	Minimum	Morning	Evening					*S. litura*	*A. janata*
31	34.9	24.2	72	51	6.1	3.6	6.6	0	-	-
32	33.5	25.9	88	60	3.8	3.3	3.2	11.9	-	3.5
33	34.9	26.4	83	54	3.6	6	5	1.5	-	1.2
34	33.7	25.8	86	60	5.4	4.3	4.4	6	-	2.2
35	30.9	24.9	91	76	3.9	2.2	3	134.5	1.2	6.0
36	30	24.4	93	76	4.2	2.1	2.6	41.2	1.2	5.2
37	32.5	23.5	87	62	4.4	7.2	3.6	3.4	1.6	4.8
38	32.4	22.7	88	59	5.9	6.5	4.9	20	1.9	3.2
39	32.2	21.1	94	57	3.7	8.3	3.5	60.3	2.1	3
40	35.4	20.1	83	39	2.8	8.8	4.7	0	3.3	2.2
41	38.6	16.7	89	36	2.1	7.2	4.1	0	3.4	1.2
42	33.8	15.8	83	34	1.9	7.4	4.3	0	2.8	-
43	32.2	13.6	82	31	1.6	6.8	3.7	0	2.4	-
44	31.4	14.2	92	38	1.8	6.2	3.1	0	1.8	-

***Each larval population is the mean of 30 observations**

reported to be positively correlated with larval population of *A. janata* (Manjunatha et al 2019, Ranga et al 2021). Linear step-wise regression models of *A. janata* larval population with weather parameters are presented. Among the various weather variables, maximum temperature was significant and accounted for 67 per cent variability in the population (Table 3). Manjunatha et al (2019) reported 95.2 per cent variation in semilooper larval population on castor due to weather conditions

Tobacco caterpillar, *Spodoptera litura* Fabricius : The larval population of *S. litura* was first observed during 35th SMW with population of 1.2 larvae per plant and started increasing gradually and reached to a peak population of 3.4 larvae per plant during 41st SMW (Fig. 3). The population started to decrease and reached up to 1.8 larvae per plant during 44th SMW. More or less similar appearance pattern of this pest was observed by Duraimurugan (2018) where the peak incidence was during 33rd to 43rd SMW with a gradual decline in population till 44th SMW. Ranga et al (2021) observed *S. litura* to be active from 37th to 46th SMW. . During current study, bright sunshine hours (BSS) (r= 0.737), maximum temperature (r= 0.900) and evaporation (r= 0.695) positively influenced *S. litura* larval population (Fig. 4). BSS (r= 0.610) was positively and significantly correlated with larval population of *S. litura* during Lag week I. Kanani (2013) stated that evening relative humidity was highly negatively significant correlation with leaf damage. Ahir et al (2017) recorded negative and non-significant correlation of *S. litura*

Table 2. Mean incidence of *E. flavescens* and *C. punctiferalis* on castor during *kharif,* 2018

SMW	Temperature (ºC)		Relative humidity (%)		Wind velocity (km/hr)	Sun Shine (hrs)	Evaporation (mm)	Rainfall (mm)	Leafhopper nymphs/3 leaves Per plant*	Per cent larval infestation of *C. Punctiferalis**
	Maximum	Minimum	Morning	Evening						
37	32.5	23.5	87	62	4.4	7.2	3.6	3.4	5.6	-
38	32.4	22.7	88	59	5.9	6.5	4.9	20	2.4	-
39	32.2	21.1	94	57	3.7	8.3	3.5	60.3	2.2	-
40	35.4	20.1	83	39	2.8	8.8	4.7	-	7.6	-
41	38.6	16.7	89	36	2.1	7.2	4.1	-	12.4	-
42	33.8	15.8	83	34	1.9	7.4	4.3	-	13.2	3.20
43	32.2	13.6	82	31	1.6	6.8	3.7	-	13.8	4.20
44	31.4	14.2	92	38	1.8	6.2	3.1	-	16.2	5.20
45	28.3	9.3	82	33	2.6	6	2.9	-	14.2	5.80
46	28	12.5	92	47	3.4	6.2	2.7	-	14.4	5.90
47	27.4	10.7	84	29	2.8	7	3	-	13.8	6.20
48	26.1	9	86	34	1.8	6.4	2	-	13.2	6.80
49	24.3	6.7	91	35	1	6.4	1.7	-	12.6	7.40
50	20.6	7	93	45	2.7	4.8	1.2	1	9.5	12.20
51	20.5	2.5	93	32	1.5	6.6	1.6	-	9.2	12.00
52	20.2	2.3	91	35	2	6.8	1.6	-	9	11.80
1	19.6	5	91	52	2.8	4	2.8	1.5	6.8	11.20
2	19.7	5.2	92	38	3.3	5.3	1.8	-	7.6	11.40
3	21.2	4.8	90	41	2.1	5	1.6	-	6.2	8.80
4	16.6	7.5	93	59	3.8	5.1	1.6	11	5.8	9.40
5	18.4	5.6	89	52	3	4.4	1.9	-	6	7.80
6	20.4	7.2	94	52	3.2	5.3	1.7	10.6	4.8	6.40
7	20.8	9.5	94	58	3.7	4.3	1.6	1.7	4.4	6.20
8	23.1	10.5	88	49	3.5	5.4	2.5	0.7	3.2	5.80
9	20.2	8.8	88	55	3.1	4.2	1.6	1		5.60
10	25.7	9	83	29	3.4	7.6	3.5	0.4		4.80
11	25.2	9.9	87	33	3.1	5.5	3.3	-		3.40

***Mean of 30 observations**

with temperature and negatively significant with relative humidity. Dry and hot weather was reported to favor *S. litura* larval population (Akashe et al 2015). Ranga et al (2021) revealed a positive correlation of *S. litura* larval population with temperature (maximum and minimum), evening relative humidity, wind velocity, bright sunshine hours and evaporation. Maximum temperature and evening relative humidity together caused 90 per cent variability in *S. litura* larval population. Maximum temperature alone accounted for 79 per cent variability. Manjunatha et al (2019) observed 95.6 per cent variability by weather parameters.

Leafhopper, *Empoasca flavescens* Fabricius: The population of leafhopper was first observed during 37th SMW with 5.6 nymphs per 3 leaves per plant. The peak population of leafhopper 16.2 nymphs per 3 leaves per plant was observed during 44th SMW (Fig. 5). Similar appearance of

Table 3. Step-wise linear regression models for weather parameters influencing the incidence of major insect pests of castor (2018-19)

Insect pest	Equation	Adjusted R^2
Achaea janata	Y= -0.56 T_{max} + 22.07	0.67
Spodoptera litura	Y= 0.21 T_{max} - 0.20 RH_e – 3.67	0.90
	Y= 0.29 T_{max} - 7.29	0.79
Empoasca flavescens	Y= -0.32 RH_e+ 22.69	0.58
Conogethes punctiferalis	Y= -0.66 T_{min} + 12.94	0.67

Y= incidence of corresponding insect pest, T_{max}- maximum temperature, T_{min}- minimum temperature, RH_e- evening relative humidity, R^2- coefficient of determination

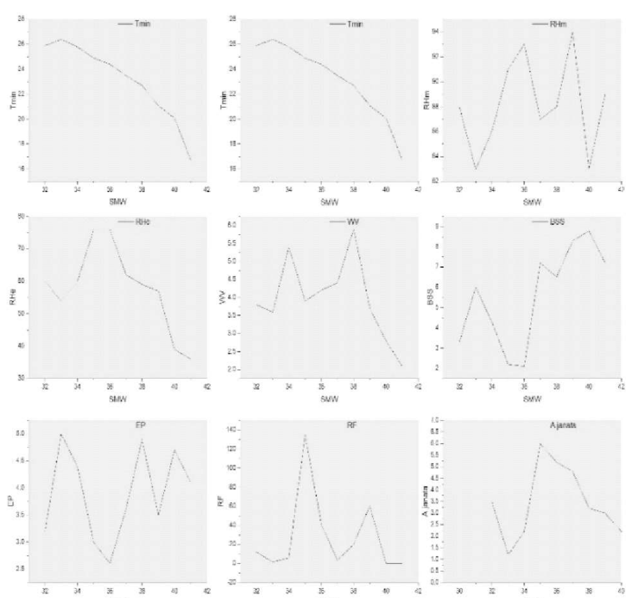
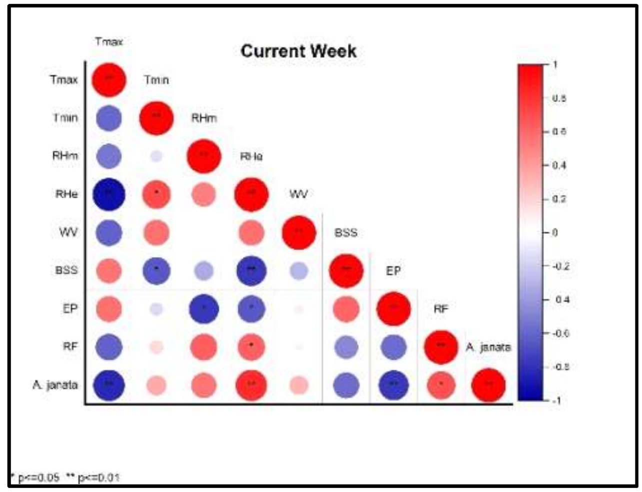
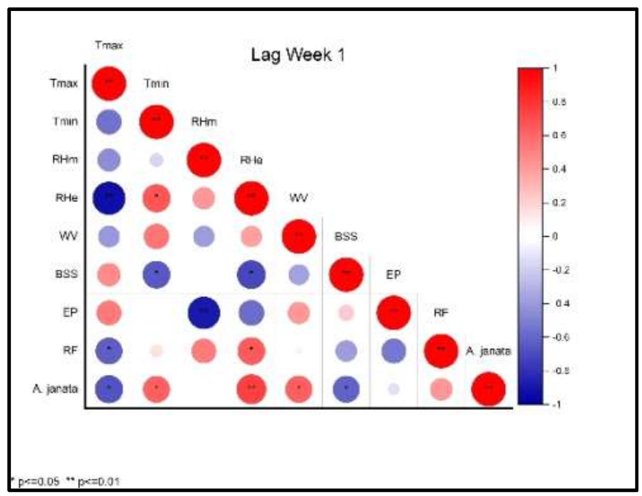


Fig. 1. *A. janata* larval population on castor in relation to weather parameters during 2018



a



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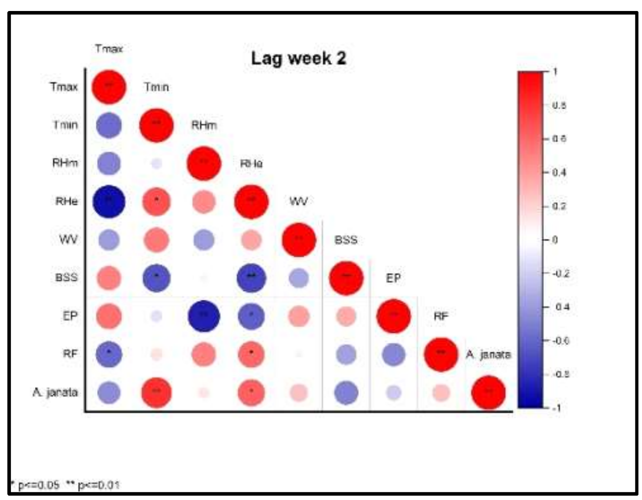


c

Fig. 2. Correlograms with coefficients of correlation (r) between weather variables and larval population of *A. janata* on castor during 2018-19 for (a) Current week (b) Lag week 1 (c) Lag week 2

pest was observed by Patel et al (2015). Leafhopper population was recorded from mid-September to mid-February by Ranga et al (2021).

Correlation with weather variables: The evening R.H. (r= -0775), wind velocity (r= -0.677) and rainfall (r= -0.505) significantly influenced the leafhopper nymphal population Figure 6. During lag week I, maximum temperature (r= 0.455), morning R.H. (r= -0.473), evening R.H. (r= -0.687) and wind velocity (r= -0.423) exhibited significant effects on nymphal population. All the weather variables except minimum temperature (r= 0.128) and rainfall (r= -0.142) were reported to exert significant effects on *E. flavescens* nymphal population during lag week II.

Mounica et al (2018) observed negative correlation of leafhopper nymphal population with rainfall, maximum temperature, minimum temperature, number of rainy days and evaporation while sunshine hours and relative humidity were found to be positively correlated. Anjani et al (2018) observed high and significant correlation with all the abiotic factors *viz*. maximum temperature, minimum temperature, evening relative humidity, morning relative humidity and rainfall. Relative humidity was correlated in a negatively significant manner with leafhopper nymphal population by Ranga et al (2021).

Minimum temperature caused significant variability in leafhopper nymphal population (58 per cent) (Table 3). Ranga et al (2021) observed that evening relative humidity and maximum temperature account for 40 to 69 per cent variability in leafhopper nymphal population. Variability to the tune of 74.2 per cent in leafhopper population on castor was recorded by Mounica et al (2018). Narayanamma et al (2017) reported 33 to 52 per cent variability due to weather variables.

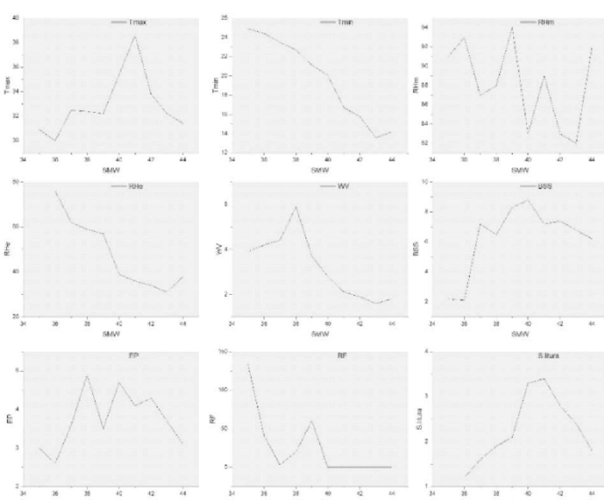
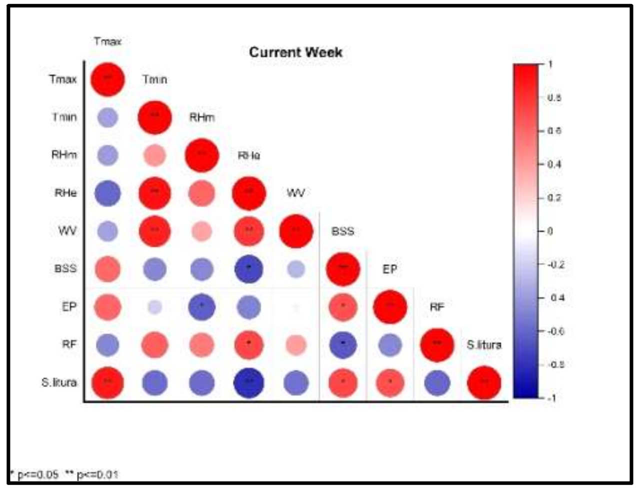


Fig. 3. *S. litura* larval population on castor in relation to weather parameters during 2018



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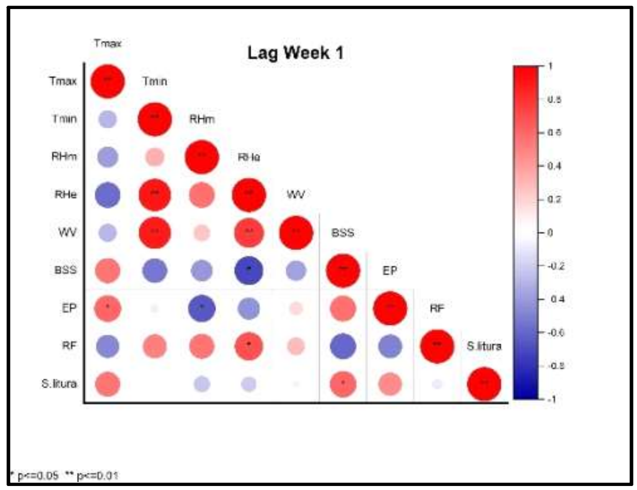

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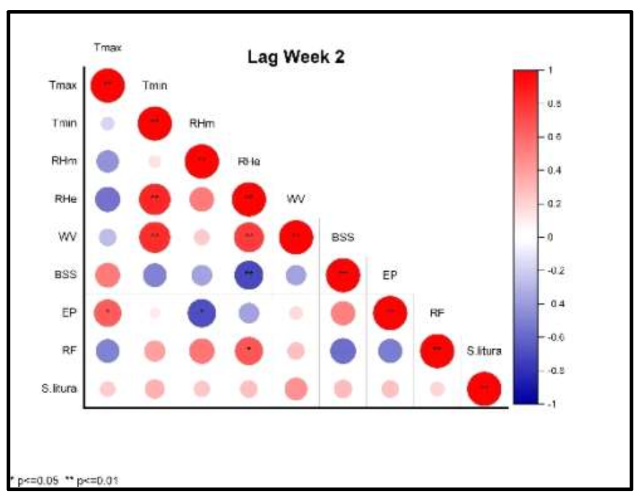

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Fig. 4. Correlograms with coefficients of correlation (r) between weather variables and larval population of *S. litura* on castor during 2018-19 for (a) Current week (b) Lag week 1 (c) Lag week 2

Castor shoot and capsule borer, *Conogethes punctiferalis* Guenée: The infestation of castor capsule borer, *C. punctiferalis* was first observed during 42nd SMW with 3.20 per cent capsule damage. Capsule damage increased gradually and reached to a peak level of 12.20 per cent during 50th SMW (Fig. 7). The incidence of *C. punctiferalis* started decreasing after 50th SMW of 2018 and reduced to 3.40 per cent by 11th SMW of 2019 (Table 2). Similarly, Akashe et al (2015) reported that the castor capsule borer remains active during October to January. Minimum temperature and evaporation exhibited highly negative and significant correlation with capsule damage during current, lag I and lag II weeks Figure 8. Maximum temperature (r= -0.698, -0.504) and morning R.H. (r= 0.603, r= 0.496)

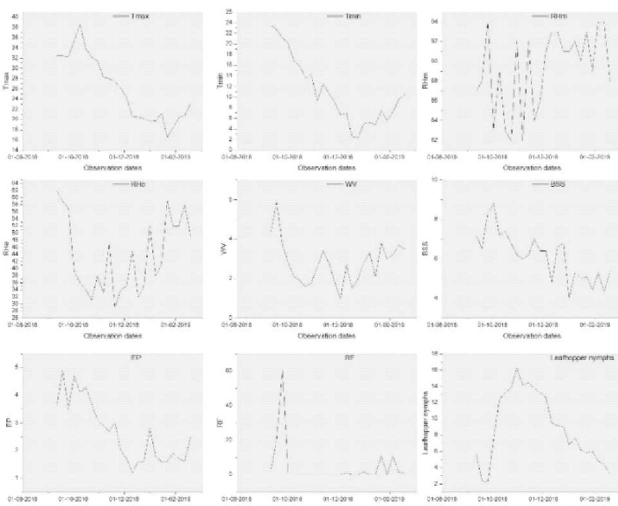


Fig. 5. Nymphal population of *E. flavescens* on castor in relation to weather parameters during 2018-19

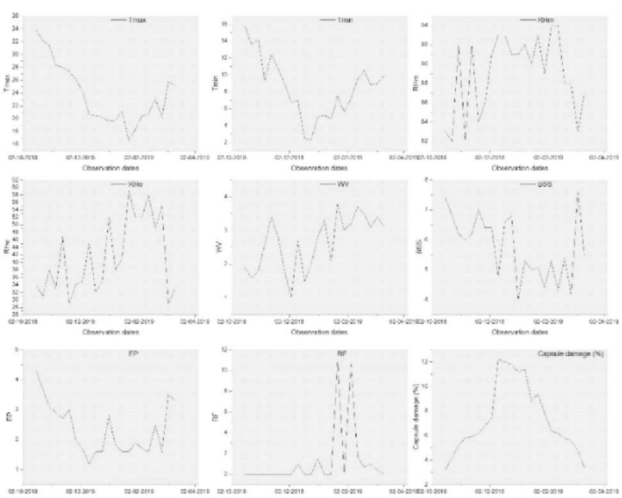
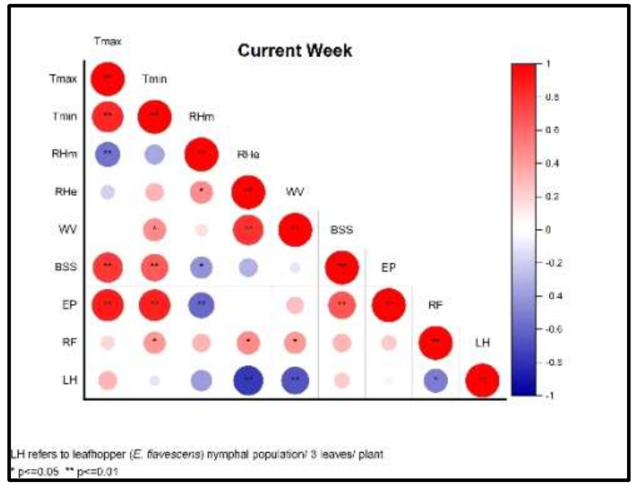


Fig. 7. Per cent capsule damage by *C. punctiferalis*on castor in relation to weather parameters during 2018-19



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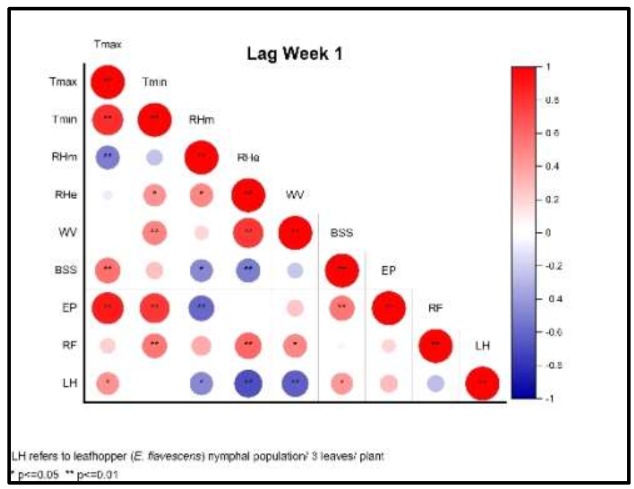

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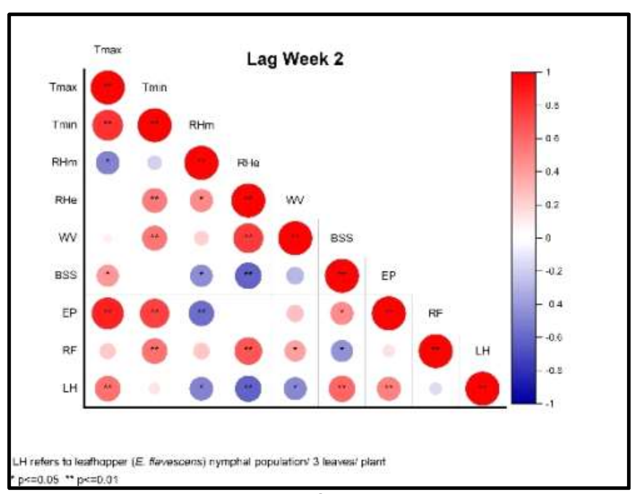

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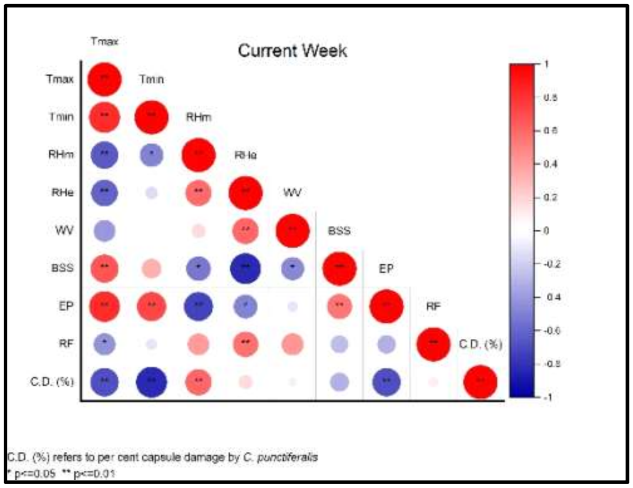
Fig. 6. Correlograms with coefficients of correlation (r) between weather variables and nymphal population of *E. flavescens* on castor during 2018-19 for (a) Current week (b) Lag week 1 (c) Lag week 2



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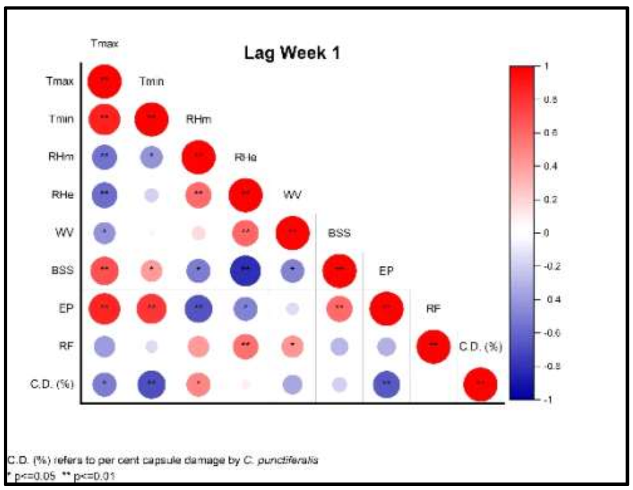

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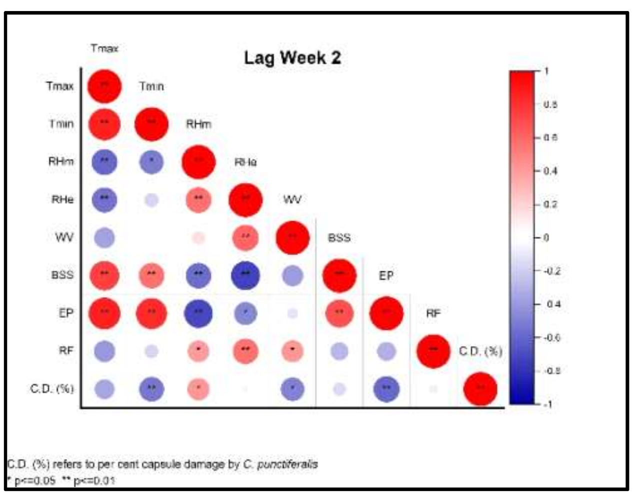

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Fig. 8. Correlograms with coefficients of correlation (r) between weather variables and capsule damage (%) by *C. punctiferalis* on castor during 2018-19 for (a) Current week (b) Lag week 1 (c) Lag week 2

significantly influenced capsule damage during both current and lag I week. Goel and Kumar (1990) who stated that minimum and maximum temperature were positively and significantly correlated with infestation of *C. punctiferalis* (=*D. punctiferalis*). A negative correlation of weather variables with capsule damage was reported with maximum temperature, minimum temperature and rainfall (Shivakumar 2016, Manjunatha et al 2019, Ranga et al 2021).Minimum temperature accounted for 67 per cent variability in capsule damage (Table 3). Weather variables accounted for 97.5 per cent variability in capsule damage by *C. punctiferalis* (Manjunatha et al 2019). Ranga et al (2021) reported weather variables causing 45 to 96 per cent variability in capsule damage by *C. punctiferalis*.

CONCLUSION

Castor semilooper was first observed during second week of August and continued to infest castor crop up to mid of October. The incidence of *S. litura* started during first week of September and continued up to first week of November and reached to a peak during second week of October. The infestation of *C. punctiferalis* initiated from third week of October and lasted till second week of March with peak capsule infestation during second week of December. The attack of *E. flavescens* started from second week of October and continued to third week of February with a peak population during first week of November. The abiotic factors i.e., weather variables undoubtedly exhibited significant effects on population dynamics of the insect pests of castor under consideration as reflected by correlation and regression analysis.

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Efficacy of Different Insecticide Modules on Tomato Pin Worm, *Phthorimaea absoluta*

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***Abstract*:** Tomato pin worm, *Phthorimaea absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is an invasive pest on tomato and was first reported in India during 2014 and has spread to several tomato growing states. An experiment was conducted to know the efficacy of different modules. The three modules include Integrated module: Installation of pheromone traps (25/ha), collection and destruction of infested parts, spraying of Azadirachtin @3-5ml/L after 30 Days after transplanting, spraying of emamectin benzoate @0.4g/L 10 days after first spray, spraying of chlorantraniliprole @0.3ml/L 10 days after second spray; Bio intensive model: installation of pheromone traps (25/ha), spraying of NSKE5% after appearance of pest, spraying of Pongamia soap @10g/L 10 Days after first spray, spraying of Neem soap @10g/L 10 days after second spray, Spinosad 45 SC @0.3ml/L 10days after third spray, Chemical Module: Spraying of Lamdacyhalothrin @2.5ml/L at 3days after transplanting, followed by spraying of indoxacarb 14.5 SC @ 1ml/L at 10 days after first spray followed by Chlorantraniliprole @ 0.3 ml/L 10 days after second spray and Novaluron 10EC @ 1.5 ml/L at 10 days after third spray and Untreated control. All the modules were significantly superior over control. Among the modules tested chemical module and integrated modules were superior in the management of *P.absoluta* followed by biointensive module. Live mines in integrated module reduced from 5.08 to1.97 per plant as against increase in live mines from 3.25 to 8.06 per plant in untreated control.

keywords: Tomato, Pinworm, *P. absoluta*, Insecticide, IPM Module

The South American tomato pinworm, *Phthorimaea absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is an exterminatory pest presently undermining the worldwide tomato industry. Over the recent 10 years, it has spread and extended to a large portion of Europe, Africa and Asia, making broad harm the actual harvest and to the global tomato market (Campos et al 2017). *Phthorimaea absoluta* is an oligophagous pest related with solanaceous harvests, *viz*. tomato, brinjal, pepper, potato, tobacco and hardly any weeds (Ferracini et al 2012). It causes 50-100% decreases in yield and affects product quality in nurseries and fields (EPPO 2005). This pest was first detected infesting tomato fields in some districts of Maharashtra, India in 2014 (Shashank et al 2015). Subsequently was reported from Karnataka (Sridhar et al 2014, Kalleshwaraswamy et al 2015), Andhra Pradesh, Telangana (Kumari et al 2015), Tamil Nadu (Shanmugam et al 2016) and Gujarat (Ballal et al 2016). It has a huge biotic potential, as multivoltine nature, short generation period and expanded protection from chemical insecticides, and it achieved key pest status even in new environments (Biondi et al 2018). In general, around 12 classes of insecticides are used to control tomato pests (IRAC2009). Organophosphates and pyrethroids have been used since 1970 while during 1990s, abamectin, spinosad, tebufonzide and chlorfenpyr have been attempted (Lietti et al 2005). In any case, continuous failure to control the insect with organophosphates and pyrethroids in South America (Lietti et al 2005), has led to the appraisal of new more secure chemical insecticides for successful insect control (IRAC 2009). Indoxacarb, spinosad, imidacloprid, deltamethrin and *Bacillus thuringiensis* var. *kurstaki* have effectively been utilized against *P. absoluta* hatchlings in Spain (Russell 2009). Chlorpyrifos and pyrethrins are often utilized in Italy (Garzia et al 2009). The main control technique against *P. absoluta* is the utilization of synthetic insecticides which can give up to 95% control in the Mediterranean basin (Urbaneja et al 2012, Derbalah et al 2012). Hanafy and El-Sayed (2013) observed spinetoram displayed better impact in decreasing population of *P. absoluta* followed by spinosad. Other workers reported that various pesticides were successful against *P. absoluta* like spinosad (Bratu et al 2015, Abdelgaleil et al 2015), azadirachtin, emamectin benzoate, spinosad, chlorantraniliprole (Eleonora and Vili 2014) chlorantraniliprole + abamectin (Ali et al 2014), cyantraniliprole (Patricia et al 2014), indoxacarb and chlorantraniliprole (Roditakis et al 2013).

Sridhar et al (2019) considered entomopathogens (*Bacillus thuringiensis, Metarhizium anisopliae, Beauveria bassiana* and *M. rileyi*), egg parasitoids (*Trichogramma chilonis, T. pretiosum* and *Trichogrammatoidea bactrae*), light snares, pheromone traps, pesticides for their efficacy. Among the egg parasitoids *T. pretiosum* and among chemical insecticides spinetoram were observed to be exceptionally useful. Yellow light traps, Azadirachtin 5% EC showed good efficacy along with *M. anisopliae* demonstrating their combined use can be adequately used in the eco-accommodating management of *T. absoluta*. Consequently, utilization of synthetic compounds has debilitated the stability of crop ecosystems. Under such circumstances, the colonization and spread of this destroying pest might be the main challenge for conventional and organic farming. So, there is a pressing need to embrace information on its degree of harm, conservation of natural enemies and host plant resistance for shaping Integrated Pest Management (IPM) module for containment of this pest. It is necessary to create awareness among farmers about its *T. absoluta* and continuous monitoring to prevent its spread.

MATERIAL AND METHODS

The experiment was conducted in Telangana (17° 7' 23.4624'' N and 79° 12' 31.7664'' E) between 2017-2020. The experiment was laid out in 3 module T_1-Integrated module, T_2-Bio intensive module, T_3-Chemical Module and untreated control. Three replications were laid out for each module and untreated control. Observations on live mines of *P. absoluta* (hatchlings) were recorded on selected plants, representatively leaves each from top, center and lower part of the plant per replication. Fruits damaged by *P.absoluta* were recorded from each replication along with healthy fruits.

The components of T_1-Integrated module were : Installation of pheromone traps (10/acre), collection and destruction of infested parts, spraying of Azadirachtin @ 3-5ml/L at 30 Days after transplanting, spraying of Emamectin Benzoate @0.4g/L 10 days after first spray, spraying of chlorantraniliprole @ 0.3ml/L 10 days after second spray. Components of T_2-Bio intensive model were : Installation of pheromone traps (10/acre), spraying of NSKE5% after appearance of pest, spraying of Pongamia soap @10g/L 10 Days after first spray, spraying of Neem soap @10g/L 10 days after second spray, spraying of Spinosad 45 SC @0.3ml/L 10days after third spray. T_3-Chemical Module consisted of spraying of Lamdacyhalothrin @2.5ml/L at 3days after transplanting, Spraying of indoxacarb 14.5 SC @ 1ml/L at 10 days after first spray, spraying of chlorantraniliprole @ 0.3 ml/L 10 days after second spray, spraying of Novaluron 10EC @ 1.5 ml/L at 10 days after third spray and T_4 was Untreated control.

RESULTS AND DISCUSSION

All the three modules recorded higher damage control compared to the untreated control in all the three years of study (Table 1). In the first year in the vegetative stage, chemical module recorded highest control of larvae (77.24%) followed by integrated module and biointensive module. In the second year also, the same trend followed where all the modules performed better than the untreated control. Chemical module recorded highest damage control

Table 1. Evaluation of IPM modules for tomato pin worm (*Tuta absoluta*) (2017-18 to 2019-20)

Treatment	Vegetative precount (Number of larvae per plant)				Damage control (%)				Reproductive precount (Number of larvae per plant)				Damage control (%)			
	2017-18	2018-19	2019-20	Pooled	2017-18	2018-19	2019-20	Pooled	2017-18	2018-19	2019-20	Pooled	2017-18	2018-19	2019-20	Pooled
T_1	6.70	8.20	9.28	8.06	74.20	72.54	71.34	72.69	8.56	10.88	13.18	10.87	82.26	79.20	76.74	79.40
	(2.77)	(3.02)	(3.20)	(3.00)	(59.57)	(58.43)	(57.68)	(58.56)	(3.09)	(3.44)	(3.75)	(3.43)	(65.25)	(62.98)	(61.17)	(63.13)
T_2	7.97	9.12	10.54	9.21	62.76	60.64	51.56	58.32	7.83	9.36	11.52	9.57	67.93	63.14	61.48	64.18
	(2.99)	(3.18)	(3.39)	(3.19)	(52.44)	(51.16)	(45.88)	(49.83)	(2.96)	(3.21)	(3.52)	(3.23)	(55.57)	(52.62)	(51.64)	(53.28)
T_3	8.23	10.08	11.10	9.80	77.24	74.32	74.56	75.37	9.57	11.00	14.96	11.84	79.16	75.88	72.74	75.93
	(3.03)	(3.33)	(3.47)	(3.28)	(61.55)	(59.57)	(59.76)	(60.29)	(3.25)	(3.45)	(3.98)	(3.56)	(62.95)	(60.59)	(58.54)	(60.69)
T_4	8.11	9.60	12.12	9.94	15.50	17.56	18.36	17.14	10.32	12.15	16.26	12.91	16.76	16.82	18.96	17.51
	(3.01)	(3.25)	(3.62)	(3.29)	(23.10)	(24.75)	(25.35)	(24.40)	(3.36)	(3.62)	(4.15)	(3.71)	(24.09)	(24.16)	(25.80)	(24.68)
CD (5%)	NS	NS	0.98	0.85	7.32	5.55	6.09	6.42	1.81	NS	NS	1.24	6.62	6.14	3.57	4.34
CV	13.83	10.91	6.51	4.48	9.15	7.08	8.10	5.64	14.34	13.73	17.77	5.37	7.19	7.49	4.45	3.60
SeM±	0.48	0.45	0.31	0.24	2.35	1.78	1.96	1.82	0.58	0.67	1.11	0.35	2.12	1.97	1.15	1.23

(74.32%) followed by integrated module ,biointensive module. Untreated module recorded least damage control (17.56 %). In the third year the chemical module fared best again (74.56%) followed by integrated module and biointensive module (Table 1). Pooled data also confirmed the same findings. Chemical module recorded maximum damage control (75.37%) followed by integrated module and biointensive module. Untreated control recorded least damage control (17.14%) proving the efficacy of the chemical module over the integrated module and biointensive modules.

In the first year in the reproductive stage integrated module recorded highest damage control of larvae (82.26%) followed by chemical module and Bio intensive model. Untreated module recorded least damage control (16.76%). In the second year of reproductive stage Integrated module recorded highest damage control of larvae followed by chemical module and Bio intensive module Untreated module recorded least damage control in 2018. In the second year also, the same trend followed where all the modules performed better than the untreated control. In the third year of reproductive stage integrated module recorded highest damage control of larvae followed by chemical module and Bio intensive module. Untreated module recorded least damage control (18.96%) in 2019. In the third year again, the same trend followed where all the modules performed better than the untreated control. Pooled data also confirmed the same findings reproductive stage Integrated module recorded highest damage control of larvae (79.40%) followed by chemical module (and Bio intensive module. Untreated control recorded least damage control (17.51%) proving the efficacy of the treated module over the untreated module. Lamdacyhalothrin, indoxacarb, chlorantraniliprole in T_3-Chemical Module and biopesticides (T_1-Integrated module and T_2-Bio intensive module) were found effective in reducing the infestation of *P. absoluta*.

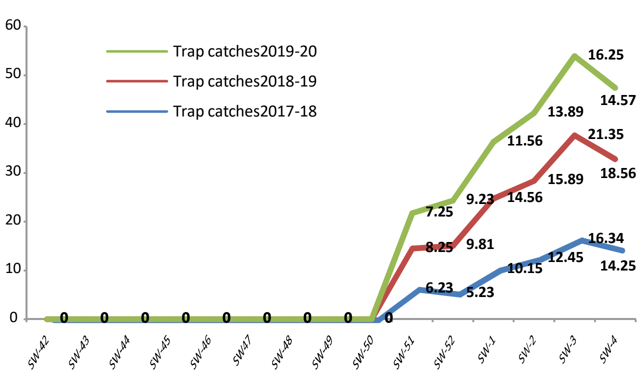

Fig. 1. Pheromone trap catch during the study period (2017-18 to 2019-20)

Pheromone trap catches during the study period indicated that *P. absoluta* catches started to rise from the 50th Standard week (6.23 to 8.25/trap/week) in all the years of study and increased at an increasing rate with a peak between the 3rd and 4th Standard meteorological week (14.25-21.35/trap/week). Trap catch came down after that as the crop sown in November was harvested by January second fortnight (5th standard week) (Fig. 1). Sridhar et al (2016) o reported that Spinetoram 12 SC @ 1.25ml/L, cyantraniliprole 10 OD @1. 8 ml/L, flubendiamide 480 SC@ 0.3ml/L and spinosad 45 SC@0.3ml/L were found more effective against *P.absoluta.* Halder et al (2019) reported that among the biopesticides, *Bacillus thuringiensis* var *kurstaki* gave encouraging results causing 66.7 and 73.37% mortality at 48 and 72 h after the treatment followed by *B. subtilis*-2

CONCLUSION

The integrated module along with the chemical module recorded lesser pest damage in both vegetative and reproductive stages of crop growth, but since keeping in view the importance of ecofriendly management techniques, the integrated module would help the farmer and ecosystem in the long run.

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Current Status of Avifauna Diversity in Lahaul and Spiti, Himachal Pradesh: Synthesis from Current and Past Surveys

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Abstract: Birds are the most prominent wildlife species in most forests and ecosystems. We attempted to enlist the existing avifaunal species in trans-Himalayan Lahaul and Spiti, Himachal Pradesh based on our observations, 22 previously published checklists/observations and eBird data. A total of 278 species belonging to 20 orders and 59 families of birds have been reliably recorded in this region. Species that belongs to the Muscicapidae family were the most sighted during the study period. We also report six new species for the region: Short-eared owl (*Asio flammeu*), Lesser Cuckoo(*Cuculus poliocephalus*), Large-spotted Nutcracker (*Nucifraga multipunctate*), Scarlet Minivet (*Pericrocotus flammeus*), Blyth's Leaf-warbler(*Phylloscopus reguloides*) and Red-tailed Wheatear (*Oenanthe chrysopygia*). Among these, Large-spotted Nutcracker, Scarlet Minivet andBlyth's Leaf-warbler were sighted frequently during the study period but never been reported previously for the region.

Keywords: Avifauna, New records, Trans-Himalaya, Lahaul-Spiti

The Himalaya is a fragile but biodiversity rich region with a vast repository of floral and faunal diversity (Jodha 2000, Pun and Mares 2000, Rautela and Lakhera 2000, Mishra et al 2006). The Himalaya harbours around 970 (~80%) of the bird species of the Indian Subcontinent (Naithani and Bhatt 2010, Praveen et al 2016). Out of 970 species, 15 endemic species, 11 were reported only from the western Himalaya including Himalayan Quail *Ophrysia superciliosa* (Critically Endangered: CR), Cheer Pheasant *Catreus wallichii* (Vulnerable: VU), and Western Tragopan *Tragopan melanocephalus* (VU) (Stattersfield et al 1998, Naithani and Bhatt 2010). The region is also well known for the presence of globally threatened species like White-rumped Vulture *Gyps bengalensis* (CR), Slender-billed Vulture *Gyps tenuirostris* (CR), Red-headed Vulture *Sarcogyps calvus* (CR) Himalayan Griffon*Gyps himalayensis* (NT) etc, (Chandra et al 2018). Anthropogenic pressure, habitat loss and extensive tourism is a serious concern in the region resulting in depletion of native Himalayan habitat (Samal et al 2003, Pandit et al 2007). Continuous monitoring and long-term datasets on the population status of birds provides an indication for ecological change (Amat and Green 2010). Therefore, this article is an attempt to highlight the avifaunal composition since 1923 to till date in the Lahaul and Spiti Landscape. Lahaul and Spiti has attracted a number of ornithologist's and naturalists over the last eight decades. Over the past several decades numerous locality-specific avifaunal checklists of Lahaul and Spiti, have been published and contributed significantly to the enhancement of knowledge on birds of the area. Due to large landscape and locality-specific checklists the exact number of bird species present in the entire landscape (defined as only site specific) remained unclear. Most part of the landscape have been explored and baseline information generated through 23 observational records from different localities, however, none of the study provides a complete information on bird assemblage of the whole district. Although, the avifauna of various locations in the region is well known, but we surveyed and complied all previous checklists since 1923 to make a comprehensive list of birds and compare the past and present scenario in Lahaul, Udaipur and Spiti. As per Bird life International a total of 354 species should be present in the region. In this article an attempt is made to determine the present status of the birds in three localities (Lahaul, Udaipur and Spiti), also present, six new sighting records from Lahaul and Spiti district.

MATERIAL AND METHODS

Study area: Lahaul and Spiti in the Trans-Himalayan region of Himachal Pradesh (13,833 sq. km.) consists of the two formerly separate districts and shares international boundary with Tibet. It lies between 31.74917° to 32.99917° N Latitude and 76.77472° to 78.69278° E Longitude with an altitude ranging from 2240 m to 6400 m above sea level (Fig. 1). The physical features of these two valleys differs from each other, where Spiti is more barren and rugged with semi-desertic to desertic conditions, on the other hand Lahaul and Udaipur is much greener and has dry temperate to dry alpine conditions with willows and other coniferous forests (Fig. 2). The district have three sub divisions i.e. Keylong in Lahaul valley, Kaza in Spiti valley, Udaipur is the west most part of Lahaul valley. Rainfall occurs mainly in May to mid-September and ranges between 10 mm to 300 mm. From late November to April, winter colds blow here and there is heavy snowfall (120-400 cm),the temperature drops to -19°C (Kumar et al 2014). The extreme environmental conditions of the landscape such as low temperature and rainfall, high wind speed and rough ground topography result in a floral and faunal diversity different from most of India (Wagnon et al 2007).

Methods

The survey was conducted between July and September 2018 and June to Augest 2019 in three different administrative blocks (Fig. 2, Table 1). During the survey birds were observed using Olympus 10X42 DPSI binoculars, photographed by Canon SX60HS camera and co-ordinates were taken by Garmin eTrex 30. Birds were identified using Ali and Ripley (1983), Rasmussen and Anderton (2012), Grimmett et al (2015). Three methods (*Ad libitum* observations, vantage point watch and call play-back) were used for surveying the birds. In addition, compiled 22 previously published checklists as well as we check the submitted checklists from eBird for the region (http://www.ebird.org), because nowdays the emerging field of citizen science useful for knowning the biodiversity.

Ad libitum observations: Observer recorded whatever they saw during the trail walks in the forest as an opportunistic method.

Vantage point watch: This protocol was adopted mainly for observing raptor species. A prominent vantage point (usually on hill tops) were chosen from where all birds of prey were counted. This protocol was only considered when weather was clear and surveys were conducted only during the first half of the day (from 0730 to 1030 hrs).

Call play-back: Recorded songs/calls of a select bird species were played back using a portable loudspeaker-digital recorder combination to elicit response, mainly from nocturnal birds such as Owls to confirm the presence of species.

RESULTS AND DISCUSSION

The checklist presented here compiles 23 previously published bird lists from the year 1923 (Baker; Whistler) up to 2017 (Rawal et al), observation records submitted in eBird and the birds sighted during the current study period (2018). This revealed 278 bird species (Spiti-236, Lahaul-178 and Udaipur-133) belonging to 20 orders and 59 families (03-EN, 01-VU and 07-NT species) from the three administrative blocks of Lahaul and Spiti district (Table 2). The family Muscicapidae (Old world Flycatchers and Chats) had the most species followed by Fringillidae and Accipitridae (27, 24 and 17 bird species respectively). Six new records of birds for this region are reported. The six birds were identified either with their diagnostic features through photographic evidence or through there vocalization (Figure 3). Details of these first records are given below.

***Short-eared Owl* (*Asio flammeus* Pontoppidan, 1763):** Three individuals of the species were sighted on 29 July 2018 around 17:00 h on the rocks of mountainous terrain near village Kibber (32.35106˚N, 77.99989˚ E; 4012 m a.s.l.). Individuals were difficult to notice because of camouflage plumage with rocks. Birds were identified from the large and round head with very small tufts and round facial discs. It is a wide spread resident however the species has not been reported from the North-west Himalaya.

Large-spotted Nutcracker (*Nucifraga multipunctata* Gould, 1849): A single individual of the species was encountered in Udaipur (32.71128˚ N, 76.65772˚ E; 3387 m a.s.l.) on 02nd September 2018 on *Cedrus deodara ,* but later several individuals were sighted and heard in nearby areas repeatedly. The individuals while making harsh grating calls, often perched on the highest branches of pine trees in Lahaul forests. We identified this bird from the conspicuous white spots throughout its body, heavily spotted under parts reaching the vent. It is different from *N. caryocatactes* which has a non-spotted chocolate brown vent.

Table 1. Lahaul and Spiti district, (A) Lahaul, (B) Udaipur and (C) Spiti where bird survey was carried out during the study period

Lahaul (2200-4,270m) (A)	Udaipur (2,400-3,500m) (B)	Spiti (3,048-4,880m) (C)
Keylong, Tandi,Jispa, Gramphu, Batal, Rarig, Chhatru, Sissu, Yoche, Khoksar	Arat, Bardang, Barur, Bhujund, Chachagoat, Chaling, Changut, Chamrat, Chokhang, Ghari, Karpat, Koraki, Kurched, Nain Gahar, Nalda, Salgaraon, Salpat, Shakoli, Sheiling, Sukto, Miyar Valley, Tindi, Trilokinath, Thanwani, Udaipur, Urgosh	Chandra Taal, Chicham, Demul, Kibber, Hansa, Hikkim, Kaza, Kee, Hinsa, Khanamo, Langza, Mud, Kunzum La,Komic, Lari, Siachin Nala, Sagnam, Losar

Table 2. Checklist (observed during the survey and previous records) and direct sighting from Lahaul and Spiti district of Himachal Pradesh

Species name	Location in 2019	Reference from	Residential Status	WPA (1972)
Order: Galliformes; Family: Phasianidae				
Chukar Partridge * *Alectoris chukar*	A, B, C	1,3,5,7,11,12,13, 14,16,19,23	R	
Common Quail *Coturnix coturnix*	A, C	3, 5, 19	W/PM	IV
Snow Partridge * *Lerwa lerwa*	A, B, C	1, 3, 7, 12, 16, 19, 23	R	IV
Himalayan Monal *Lophophorus impejanus*	A	23	R	I
Koklass Pheasant * *Pucrasia macrolopha*	A, B	3,23	R	
Himalayan Snowcock * *Tetraogallus himalayensis*	A, B, C	1,3,5,12,13,16,19,23	R	I
Tibetan Snowcock *Tetraogallus tibetanus*	C	16	R	I
Order: Anseriformes; Family: Anatidae				
Northern Pintail *Anas acuta*	A, C	3,5,19,23	W	IV
Eurasian Teal *Anas crecca*	A, C	3, 4, 13,19	W	IV
Mallard *Anas platyrhynchos*	A, B, C	3,4,23	Br/W	IV
Indian Spot-billed Duck *Anas poecilorhyncha*	C	16	R	IV
Bar-headed Goose *Anser indicus*	B, C	19,23	W	IV
Tufted Duck *Aythya fuligula*	B, C	16,23	W	IV
# Ferruginous Duck *Aythya nyroca*	A	3	W	IV
Gadwall *Mareca strepera*	A, B, C	23	W	IV
Common merganser *Mergus merganser*	C	3	W	
### White-headed Duck *Oxyura leucocephala*	B	23	W	IV
Northern Shoveler *Spatula clypeata*	A, B, C	16,23	W	IV
Garganey *Spatula querquedula*	A, C	5,6,19,23	W	IV
Ruddy Shelduck * *Tadorna ferruginea*	A, C	1,3,5,13,16,19,23	Br/W	IV
Order: Podicipediformes; Family: Podicipedidae				
Little Grebe *Tachybaptus ruficollis*	C	19	WR	IV
Order: Ciconiiformes; Family: Ciconiidae				
Black Stork *Ciconia nigra*	A	23	W/PM	
Order: Pelecaniformes; Family: Ardeidae				
Grey Heron * *Ardea cinerea*	C	13,19	R/PM/W	IV
Indian Pond-Heron * *Ardeola grayii*	A, C	9,13,19,23	WR	IV
Eurasian Bittern *Botaurus stellaris*	C	19	W	IV
Little Egret *Egretta garzetta*	B	23	WR	IV
Order: Suliformes; Family: Phalacrocoracidae				
Great Cormorant * *Phalacrocorax carbo*	B	23	R	IV
Order: Falconiformes; Family: Falconidae				
Merlin *Falco columbarius*	C	19	W	
Peregrine Falcon *Falco peregrinus*	C	23	R/W	I
Eurasian Hobby * *Falco subbuteo*	A, C	3,19,23	Br/W	
Common Kestrel * *Falco tinnunculus*	A, B, C	1,3,5,12,13,14,16,19,23	R	
Order: Accipitriformes; Family: Accipitridae				
Shikra * *Accipiter badius*	B, C	16	WR	I
Eurasian Sparrowhawk * *Accipiter nisus*	A, B, C	3,5,6,19,23	R/W	I
# Cinereous Vulture *Aegypius monachus*	A, B, C	12	W	

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Table 2. Checklist (observed during the survey and previous records) and direct sighting from Lahaul and Spiti district of Himachal Pradesh

Species name	Location in 2019	Reference from	Residential Status	WPA (1972)
Golden Eagle * *Aquila chrysaetos*	A, B, C	3,5,11,13,14,16,19,23	R	I
## Eastern imperial eagle *Aquila heliaca*	A, B, C	3	W	
### Steppe Eagle *Aquila nipalensis*	A, C	12,15	W	
Upland Buzzard *Buteo hemilasius*	C	19,23	W	I
Himalayan Buzzard *Buteo refectus*	A, B	23	R/W	I
Western Marsh Harrier * *Circus aeruginosus*	C	5,19	W	I
# Pallid Harrier * *Circus macrourus*	C	5	W	I
Montagu's Harrier * *Circus pygargus*	C	5,19	W	I
# Bearded Vulture * *Gypaetus barbatus*	A, B, C	1,3,5,7,11,12,13,14,16,19,23	R	I
Griffon Vulture * *Gyps fulvus*	C	1,23	R	I
# Himalayan Vulture * *Gyps himalayensis*	A, B, C	3,5,7,12,13,14,16,19,23	R	I
### Pallas's Fish Eagle *Haliaeetus leucoryphus*	C	19,20	R	I
Booted Eagle *Hieraaetus pennatus*	B	23	Br	I
Black Kite * *Milvus migrans*	A, B, C	3,5,7,12,14,19,23	R/W	I
Order: Gruiformes; Family: Rallidae				
Corn Crake *Crex crex*	C	19	V	
Eurasian Coot *Fulica atra*	B	23	R/W	IV
Eurasian Moorhen *Gallinula chloropus*	C	5,6,13,19,23	R/W	
Water Rail *Rallus aquaticus*	C	19	W	IV
Ruddy-breasted Crake *Zapornia fusca*	C	19	W	
Baillon's Crake *Zapornia pusilla*	C	5,19	Br	
Order: Charadriiformes; Family: Charadriidae				
Kentish Plover * *Charadrius alexandrinus*	A, B, C	23	W	I
Lesser Sand-Plover **Charadrius mongolus*	A, B, C	1,2,3,5,12,16,19,23	Br	I
Red wattled lapwing *Vanellus indicus*	C	16	R	
# Northern Lapwing *Vanellus vanellus*	C	13	W	
Family: Ibidorhynchidae				
Ibisbill *Ibidorhyncha struthersii*	A, C	3,5,19	R	I
Family: Recurvirostridae				
Black-winged Stilt * *Himantopus himantopus*	A, C	6,16,19,23	W	IV
Family: Jacanidae				
Pheasant-tailed jacana *Hydrophasianus chirurgus*	A	3	R	
Family: Scolopacidae				
Common Sandpiper * *Actitis hypoleucos*	A, B, C	3,5,19,23	Br	IV
# Curlew Sandpiper *Calidris ferruginea*	C	19	W	IV
Little stint *Calidris minuta*	C	16,19	W	IV
Ruff *Calidris pugnax*	C	5,19	W/PM	
Long-toed Stint *Calidris subminuta*	C	19	W	IV
Temminck's Stint *Calidris temminckii*	A, C	5,6,19	W	IV
Common Snipe *Gallinago gallinago*	C	19	Br	IV
Solitary Snipe * *Gallinago solitaria*	A, C	3,5,12,14,19,23	R/W	IV
Pin-tailed Snipe *Gallinago stenura*	C	19	W	IV

Cont...

Table 2. Checklist (observed during the survey and previous records) and direct sighting from Lahaul and Spiti district of Himachal Pradesh

Species name	Location in 2019	Reference from	Residential Status	WPA (1972)
Red-necked Phalarope *Phalaropus lobatus*	A, C	19,23	W	
Wood Sandpiper *Tringa glareola*	C	3,5,19	W	
Common Greenshank *Tringa nebularia*	C	5,19	W	
Green Sandpiper * *Tringa ochropus*	A, B, C	1,3,5,13,19,23	W	
Common Redshank *Tringa totanus*	A,C	3,19	Br	
Family: Laridae				
Whiskered Tern *Chlidonias hybrida*	A,B,C	19,23	W	
Slender-billed Gull *Larus genei*	C	19	W	
Black-headed Gull *Larus ridibundus*	B	23	W/PM	
Order: Pterocliformes; Family: Pteroclidae				
Tibetan Sandgrouse *Syrrhaptes tibetanus*	A,C	3,5,12, 19,23	R	IV
Order: Columbiformes; Family: Columbidae				
Snow Pigeon * *Columba leuconota*	A,B,C	1,3,5,7,12,13,14,16,19,23	R	IV
Rock Dove * *Columba livia*	A,B,C	1,3,5,7,12,13,14,16,19,23	R	IV
Hill Pigeon * *Columba rupestris*	A,B,C	1,3,4,5,7,11,12,13,14,16,19,23	R	IV
Spotted Dove * *Spilopelia chinensis*	A,B,C	7,12,13,14,23	R	IV
Laughing Dove *Spilopelia senegalensis*	A,B,C	5,19,23	R	IV
Eurasian Collared Dove *Streptopelia decaocto*	A,C	3,13	R	IV
Oriental Turtle-Dove * *Streptopelia orientalis*	A,B.C	3,5,7,14,13,19,23	R/W	IV
Order: Psittaciformes; Family: Psittaculidae				
Rose-ringed Parakeet *Psittacula krameri*	C	13	R	IV
Order: Cuculiformes; Family: Cuculidae				
Jacobin cuckoo *Clamator jacobinus*	A,B,C	12	R/ Pr M	
Common Cuckoo * *Cuculus canorus*	A,B,C	3,5,14,19,23	Br	IV
Lesser Cuckoo * *Cuculus poliocephalus*	A,B		Br	IV
Himalayan Cuckoo **Cuculus saturatus*	B	23	Br	IV
Order: Strigiformes; Family: Strigidae				
Boreal Owl *Aegolius funereus*	A,B,C	12,23	RR	IV
Short- eared Owl * *Asio flammeus*	C		R	IV
Northern Long-eared Owl *Asio otus*	A,C	3,12	W	IV
Little Owl *Athene noctua*	C	19	R	IV
Eurasian Eagle-owl *Bubo bubo*	A,B,C	3,12,19	R	IV
Tawny Owl *Strix aluco*	C	13	R	IV
Himalayan Owl * *Strix nivicolum*	A,B	23	R	IV
Order: Apodiformes; Family: Apodidae				
Himalayan Swiftlet * *Aerodramus brevirostris*	C	13,14,23	R	
Little Swift *Apus affinis*	C	13	WR	
Common Swift * *Apus apus*	A,C	1,5,13,14,19,23	S	
Pacific Swift *Apus pacificus*	A	3	Br	
White-throated Needletail *Hirundapus caudacutus*	C	13	S	
Alpine Swift *Tachymarptis melba*	C	13	R?	

Cont...

Table 2. Checklist (observed during the survey and previous records) and direct sighting from Lahaul and Spiti district of Himachal Pradesh

Species name	Location in 2019	Reference from	Residential Status	WPA (1972)
Order: Bucerotiformes; Family: Upupidae				
Eurassian Hoopoe* *Upupa epops*	A,B,C	3,4,5,13,14,16,19,23	S	
Order: Coraciiformes;				
Indian Roller *Coracias benghalensis*	C	13	R	
European Roller *Coracias garrulus*	A	6	S	IV
Order: Bucerotiformes; Family: Alcedinidae				
Common Kingfisher *Alcedo atthis*	A,B,C	3,12,23	R	IV
Order: Piciformes; Family: Picidae				
Himalayan Woodpecker *Dendrocopos himalayensis*	A,B	23	R	IV
Eurasian Wryneck *Jynx torquilla*	C	5,13,19	Br	IV
Scaly-bellied Woodpecker*Picus squamatus*	A,B,C	12	R	IV
Order: Passeriformes; Family: Vangidae				
Common Woodshrike *Tephrodornis pondicerianus*	A,B,C	12	R	
Family: Campephagidae				
Long-tailed Minivet * *Pericrocotus ethologus*	A,B	23	Br	IV
Scarlet Minivet* *Pericrocotus flammeus*	A		R	IV
Family: Laniidae				
Great Grey Shrike *Lanius excubitor*	B	23	V	
Isabelline Shrike *Lanius isabellinus*	C	19	W	
Long-tailed Shrike * *Lanius schach*	A,B,C	3,5,7,8,13,14,19,23	W	
Grey-backed Shrike *Lanius tephronotus*	A,B,C	11,12,13,14,19,23	Br	
Family: Dicruridae				
Ashy Drongo * *Dicrurus leucophaeus*	C	13,19,23	Br	IV
Black Drongo *Dicrurus macrocercus*	B,C	19,23	Isolated records	IV
Family: Oriolidae				
Indian Golden Oriole * *Oriolus kundoo*	A,B,C	19,23	S	IV
Family: Bombycillidae				
Eurasian Golden Oriole *Oriolus oriolus*	A,C	5,13,14,23	V	IV
Bohemian Waxwing *Bombycilla garrulus*	A	21	V	
Family: Rhipiduridae				
White-throated Fantail *Rhipidura albicollis*	A,C	7,12	R	
Family: Monarchidae				
Indian paradise flycatcher *Terpsiphone paradisi*	A,C	3,13	R	IV
Family: Corvidae				
Northern Raven * *Corvus corax*	A,B,C	1,4,7,12,13,14,16,19,23	R	
Large-billed Crow * *Corvus macrorhynchos*	A,B,C	7,13,14,16,19,23	R	
House Crow *Corvus splendens*	A,B,C	13,19,23	R	IV
Grey Treepie *Dendrocitta formosae*	C	14	R	IV
Spotted Nutcracker * *Nucifraga caryocatactes*	A,B,C	12	R	
Large- Spotted Nutcracker * *Nucifraga multipunctata*	A,B		R	
Alpine Cough* *Pyrrhocorax graculus*	A,B,C	1,7,11,12,13,14,16,17,19,23	R	

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Table 2. Checklist (observed during the survey and previous records) and direct sighting from Lahaul and Spiti district of Himachal Pradesh

Species name	Location in 2019	Reference from	Residential Status	WPA (1972)
Red-billed Chough * *Pyrrhocorax pyrrhocorax*	A,B,C	1,7,11,12,13,14,16,19,23	R	
Family: Paridae				
Fire-capped Tit *Cephalopyrus flammiceps*	A,C	5,6,19,23	Br	IV
Cinereous Tit *Parus cinereus*	A,B,C	23		IV
Great Tit * *Parus major*	A,B,C	3,12,14,23	R	IV
Green-backed Tit *Parus monticolus*	A,B	23	R	IV
Coal Tit * *Periparus ater*	A,B,C	23	R	IV
Rufous naped Tit * *Periparus rufonuchalis*	A,B,C	3,7,14,23	R	IV
Family: Aegithalidae				
White-browed Tit-Warbler *Leptopoecile sophiae*	C	19,23	R	
Family: Cettidae				
Aberrant Bush Warbler *Horornis flavolivaceus*	A,B	23	R	
Brown-flanked Bush-Warbler * *Horornis fortipes*	C	13,14	R	
Family: Phylloscopidae				
Tickell's Leaf Warbler * *Phylloscopus affinis*	A,B,C	3,7,12,19,23	Br	
Lemon-rumped warbler * *Phylloscopus chloronotus*	A,B,C	23	Br	
Common Chiffchaff * *Phylloscopus collybita*	A,C	3,5,6,7,8,13	W	
Sulphur-bellied Warbler * *Phylloscopus griseolus*	A,C	1,3,7,12,13,14,19,23	Br	
Hume's Leaf Warbler *Phylloscopus humei*	C	19	Br	
Yellow browed Warbler *Phylloscopus inornatus*	A	6	W	
Large-billed Leaf Warbler *Phylloscopus magnirostris*	A	3,7	Br	
Green Warbler *Phylloscopus nitidus*	C	5	PM	
Western Crowned Warbler *Phylloscopus occipitalis*	A,B	3,23	Br	
Blyth's Leaf Warbler * *Phylloscopus reguloides*	A		Br	
Mountain Chiffchaff *Phylloscopus sindianus*	A,C	19,23	Br	
Greenish Warbler *Phylloscopus trochiloides*	A,B,C	19,23	Br	
Family: Hirundinidae				
Red-rumped Swallow *Cecropis daurica*	C	14	R	
Asian House-Martin * *Delichon dasypus*	C	13,14,19,23	R	
Common House-Martin *Delichon urbicum*	A,C	1,3,19,23	S	
Barn Swallow * *Hirundo rustica*	B,C	13,19,23	Br	
Eurasian Crag-Martin * *Ptyonoprogne rupestris*	A,B,C	1,3,13,14,19,23	R	
Grey-throated Martin *Riparia chinensis*	C	14	R	
Family: Alaudidae				
Eurasian Skylark *Alauda arvensis*	A,C	3,5,13,19	W	IV
Oriental Skylark * *Alauda gulgula*	A.C	7,13,14	R/W	IV
Lesser Short-toed Lark *Alaudala rufescens*	C	13		IV
Hume's Short-toed Lark *Calandrella acutirostris*	A,B.C	1,3,5,12,19,23	S	IV
Greater Short-toed Lark *Calandrella brachydactyla*	C	5,6,19	W	IV
Horned Lark * *Eremophila alpestris*	A,B,C	1,3,5,7,10,12,13,14,16,17,19,23	R	IV
Family: Pycnonotidae				
Black Bulbul *Hypsipetes leucocephalus*	A,B	23	R	IV
Himalayan Bulbul *Pycnonotus leucogenys*	B	23	R	IV

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Table 2. Checklist (observed during the survey and previous records) and direct sighting from Lahaul and Spiti district of Himachal Pradesh

Species name	Location in 2019	Reference from	Residential Status	WPA (1972)
Family: Acrocephalidae				
Blyth's Reed Warbler *Acrocephalus dumetorum*	C	19	W/PM	
Booted Warbler *Iduna caligata*	C	19	W	
Family: Sylviidae				
Yellow-eyed Babbler *Chrysomma sinense*	A,C	12	RM	IV
Common Whitethroat *Sylvia communis*	C	19	PM	
Lesser Whitethroat *Sylvia curruca*	A,B.C	12,19,23	Br	
Family: Leiotrichidae				
Bar-throated Minla *Chrysominla strigula*	A,B,C	23	R	
Streaked Laughingthrush * *Trochalopteron lineatum*	A,B,C	3,7,12,23	R	
Variegated Laughingthrush * *Trochalopteron variegatum*	A,B,C	3,7,12,23	R	
Family: Zosteropidae				
Whiskered Yuhin *Yuhina flavicollis*	B	23	R	IV
Oriental White-eye *Zosterops palpebrosus*	A,B,C	19,23	R	IV
Family: Regulidae				
Goldcrest * *Regulus regulus*	A,B,C	6,19,23	R	
Family: Troglodytidae				
Eurasian Wren *Troglodytes troglodytes*	A,B	3,23	R	IV
Family: Cinclidae				
White-throated Dipper * *Cinclus cinclus*	A,B,C	1,3,4,5,7,12,13,19,23	R	
Brown Dipper * *Cinclus pallasii*	A,B,C	3,4,7,12,19,23	R	
Family: Sittidae				
White-cheeked Nuthatch * *Sitta leucopsis*	A,B	19	R	
Wallcreeper * *Tichodroma muraria*	A,B,C	3,4,7,19,23	R	
Family: Certhiidae				
Eurasian Treecreeper *Certhia familiaris*	B	23	R	
Bar-tailed Treecreeper * *Certhia himalayana*	A,B	6,7,23	R	
Hodgson's Treecreeper * *Certhia hodgsoni*	B	23	R	
Family: Sturnidae				
Rosy Starling *Pastor roseus*	A,C	3,5,6,19	W	
Brahminy starling *Sturnia pagodarum*	A	3	MR	IV
Common Starling *Sturnus vulgaris*	C	19,23	W	
Family: Turdidae				
Grandala *Grandala coelicolor*	C	19,23	R	
Black-throated Thrush * *Turdus atrogularis*	A,B,C	6,19,23	W	IV
Tibetan Blackbird *Turdus maximus*	A,C	6,19	R	
Red-throated Thrush *Turdus ruficollis*	A,C	5,12	W	IV
Tickell's Thrush *Turdus unicolor*	A,C	23	S	IV
Mistle Thrush **Turdus viscivorus*	A,B,C	3,12,23	R	IV
Family: Muscicapidae				
Siberian Rubythroat *Calliope calliope*	C	19	W	
Himalayan Rubythroat *Calliope pectoralis*	A,B,C	3,12,14,19,23	Br	

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Table 2. Checklist (observed during the survey and previous records) and direct sighting from Lahaul and Spiti district of Himachal Pradesh

Species name	Location in 2019	Reference from	Residential Status	WPA (1972)
Bluethroat *Cyanecula svecica*	C	14,19,23	S	
Little Forktail *Enicurus scouleri*	A,B	23	R	
Verditer Flycatcher *Eumyias thalassinus*	A,B,C	23	S	IV
Rusty-tailed Flycatcher *Ficedula ruficauda*	A,C	3,12	S	IV
Ultramarine Flycatcher * *Ficedula superciliaris*	A,B	23	S	IV
Slaty-blue Flycatcher * *Ficedula tricolor*	C	23	S	IV
Blue Rock-Thrush * *Monticola solitarius*	A,B,C	1,3,7,13,19,23	R/W	IV
Dark-sided Flycatcher * *Muscicapa sibirica*	A,B,C	12	Br	IV
Blue Whistling-Thrush * *Myophonus caeruleus*	A,B,C	19,23	R	IV
Red-tailed Wheatear * *Oenanthe chrysopygia*	A		W	
Desert Wheatear *Oenanthe deserti*	A,B,C	3,5,12,13,14,19,23	Br	
Isabelline Wheatear *Oenanthe isabellina*	C	19	W	
Pied Wheatear *Oenanthe pleschanka*	A,B,C	3,5,7,12,18,19,23	Br	
Blue-capped Redstart * *Phoenicurus coeruleocephala*	A,B,C	1,3,7,19,23	R	
Guldenstadt's Redstart * *Phoenicurus erythrogastrus*	A,B,C	3,11,13,16,19,22,23	R/W	
Blue-fronted Redstart *Phoenicurus frontalis*	A,B,C	3,7,12,14,23	Br/R	
Plumbeous Water Redstart *Phoenicurus fuliginosus*	A,B,C	1,6,14,19,23	Br/R	
White-capped redstart* *Phoenicurus leucocephalus*	A,B,C	3,4,7,12,13,14,19,23	Br/R	
Black Redstart * *Phoenicurus ochruros*	A,B,C	1,3,7,8,12,13,14,16,17,19,23	Br/R	
Pied bush chat *Saxicola caprata*	C	3	R	
Grey Bushchat *Saxicola ferreus*	A,C	14,23	Br/R	
Common Stonechat *Saxicola torquatus*	C	5	Br	
Orange-flanked Bush-robin *Tarsiger cyanurus*	C	14	R	
Himalayan Bush-robin * *Tarsiger rufilatus*	A,B,C	23	Br/R	
Family: Passeridae				
Black-winged Snowfinch * *Montifringilla adamsi*	A,B,C	1,3,11,12,13,14,16,19,23	R	IV
White-winged snowfinch *Montifringilla nivalis*	A,C	3,5		
White-rumped snowfinch *Onychostruthus taczanowskii*	C	13	R?/W	
Russet Sparrow *Passer cinnamomeus*	A,C	14,23	R	
House Sparrow * *Passer domesticus*	A,B,C	1,3,5,7,12,13,14,16,19,23	R	
Blanford's Snowfinch *Pyrgilauda blanfordi*	C	19	R?W	
Family: Estrildidae				
Scaly-breasted Munia *Lonchura punctulata*	C	1,23	R	IV
Family: Prunellidae				
Black-throated Accentor *Prunella atrogularis*	A,B,C	23	W	
Alpine Accentor * *Prunella collaris*	A,B,C	3,16,18,23	R	
Brown Accentor *Prunella fulvescens*	C	13,19,23	R	
Altai Accentor *Prunella himalayana*	C	19	W	
Robin Accentor * *Prunella rubeculoides*	C	1,3,13,19,23	R	
Rufous-breasted Accentor * *Prunella strophiata*	A,B,C	3,7,11,12,16,19,23	R	
Family: Motacillidae				
Tawny Pipit *Anthus campestris*	C	19	W	IV

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Table 2. Checklist (observed during the survey and previous records) and direct sighting from Lahaul and Spiti district of Himachal Pradesh

Species name	Location in 2019	Reference from	Residential Status	WPA (1972)
Olive-backed Pipit *Anthus hodgsoni*	A	6	Br	IV
Richard's Pipit *Anthus richardi*	A	3	W	IV
Rosy Pipit * *Anthus roseatus*	A,C	3,5,7,13,19,23	Br	IV
Buff-bellied Pipit * *Anthus rubescens*	C	19	W	IV
Water Pipit *Anthus spinoletta*	C	19	W	IV
Upland Pipit *Anthus sylvanus*	C	13	R	IV
Tree Pipit * *Anthus trivialis*	A,B,C	5,12,14,19,23	R/W	IV
White Wagtail * *Motacilla alba*	A,B,C	1,3,5,7,12,13,14,16,19,23	Br	
Grey Wagtail * *Motacilla cinerea*	A,B,C	3,5,7,8,13,14,19,23	Br	
Citrine Wagtail * *Motacilla citreola*	A,B,C	1,3,4,5,7,13,14,16,17,19,23	Br	
Western Yellow Wagtail * *Motacilla flava*	C	5,6,16,19	W	
White-browed Wagtail *Motacilla maderaspatensis*	C	13	R	
Family: Fringillidae				
Mongolian Finch *Bucanetes mongolicus*	C	19	R	
Spectacled Finch **Callacanthis burtoni*	A,C	12,19,23	R	
Eastern Goldfinch *Carduelis caniceps*	A,C	3,5,7		
Common Rosefinch * *Carpodacus erythrinus*	A,B,C	1,3,5,7,8,13,14,19,23	Br	IV
Blyth's Rosefinch *Carpodacus rhodochlamys*	A,C	3,5,7,12,14,19,23	PrM / R	
Red-fronted Rosefinch *Carpodacus puniceus*	A,B,C	1,3,4,5,12,13,19,23	R	IV
Pink-browed Rosefinch *Carpodacus rodochroa*	A,B,C	1,3,12,23	R	IV
Great Rosefinch * *Carpodacus rubicilla*	A,B,C	1,3,5,11,12,13,14,19,23	R	IV
Streaked Rosefinch *Carpodacus rubicilloides*	C	5,19	R	IV
Himalayan White-browed Rosefinch *Carpodacus thura*	A,B,C	12	R	IV
Yellow-breasted Greenfinch * *Chloris spinoides*	A,B,C	3,14,19,23	R	IV
Brambling *Fringilla montifringilla*	B	23	W	IV
Brandt's mountain finch * *Leucosticte brandti*	A,B,C	1,3,12,14,16,18,23	R	IV
Plain Mountain-Finch * *Leucosticte nemoricola*	A,B,C	1,3,7,11,13,14,16,19,23	R	IV
Twite * *Linaria flavirostris*	C	5,12,14,16,19,23	R	
Red Crossbill *Loxia curvirostra*	A,B,C	3,12	R	
White-winged Grosbeak *Mycerobas carnipes*	A,B,C	3,7,12,23	R	IV
Spotted-winged Grosbeak *Mycerobas melanozanthos*	A,C	12	R	IV
Dark-breasted Rosefinch *Procarduelis nipalensis*	A,B,C	12,23	R	IV
Orange Bullfinch *Pyrrhula aurantiaca*	A,B,C	23	R	IV
Red-headed Bullfinch *Pyrrhula erythrocephala*	A,B	3,23	R	IV
Red-fronted Serin * *Serinus pusillus*	A,B,C	1,3,5,7,12,13,14,16,19,23	R	IV
Family: Emberizidae				
Red-headed Bunting *Emberiza bruniceps*	A,C	5,12,19	W	IV
Rock Bunting * *Emberiza cia*	A,B,C	1,3,5,7,8,12,13,14,16,19,23	R	IV
Ortolan Bunting *Emberiza hortulana*	C	19	V	
Pine Bunting *Emberiza leucocephalos*	B	23	W	IV
Black-headed Bunting *Emberiza melanocephala*	A,B,C	12	W	IV
Little Bunting *Emberiza pusilla*	C	19	W	IV
Chestnut Bunting *Emberiza rutila*	C	19	W	IV
Chestnut breasted Bunting *Emberiza stewarti*	A,B,C	23	Br	IV

Note: Location of the recorded birds given as A, B, C, details of the locations given in Table 2. Reference from the literature survey as numerical digits viz. 1,2…23. Residential status abbreviation: R- Residential; W- Winter visitor; PM- Passage migrant; V- Vagrant; R/W- Resident or Winter Visitor; WR- Widspread resident; Br-Breeding; RR- Rare resident; PrM- Partially migrating; ?- Uncertain records. *Denotes species observed by our team members; # Denotes NT-Near Threatened, ## denotes VU-Vulnerable and ### denotes EN-Endangered and "no #" denotes LC-Least Concerned.

Reference from. 1. Whistler (1923) 2. Baker (1923) 3. Whistler (1925) 4. Lowndes (1929) 5. Koelz (1937) 6. Koelz (1939) 7. Alexander (1951) 8. Mahajan (1974) 9. Manjrekar and Mehta (1999) 10. Mishra (2000) 11. Singh (2001) 12. Mahabal A. (2005) 13. Tak PC and Paliwal R.(2008) 14. Thakur and Mattu (2011) 15. Pande et. al (2013) 16. Rana et. al. (2014) 17. Singh (2014) 18. Jha (2014) 19. Himachal State Govt. (2016) 20. Rana (1995) 21. Rawal et. al. (2017) 22. Sangha et. al. (2017) 23. eBird.org

***Lesser Cuckoo* (*Cuculus poliocephalus* Latham, 1790):** A single juvenile individual was observed in Udaipur (32.74061˚ N, 76.63191˚ E; 2688 m a.s.l.) repeatedly feeding on caterpillars in an agricultural field at Madgra Village in Udaipur from 26 August 2018 to 30 September 2018. Identified bird to species from its comparatively small size grey head and body, yellow colour on the mandibles and feet, white barred lines in under parts. This species is known to breed in the Himalayas but has not been reported before in this region.

***Scarlet Minivet* (*Pericrocotus flammeus speciosu* Latham, 1790):** This species was observed often in pairs in Udaipur (32.75835˚ N, 76.44506˚ E; 2768 m a.s.l.) perched on the *Cedrus deodara* in the month of August 2018. It is a resident bird of the Himalayas but being reported for the first time. It was identified from the scarlet colour of the male with isolated red markings on its secondaries and yellow colour of the female.

***Blyth's Leaf-warbler* (*Phylloscopus reguloides* Blyth, 1842):** Individuals of the species were observed often (September, 2018) in the infringement areas of Udaipur (32.68565˚ N, 76.67893˚ E; 3105 m a.s.l.). Individuals were sighted while hopping and perching on the *Pinus wallichiana* and *Cedrus deodara* trees. It was identified from its long pale yellow supercilium, dark olive lores and eye-stripe throat and whitish underparts, tinged yellow, becoming pale yellow on undertail-coverts.

Red-tailed Wheatear (*Oenanthe chrysopygia* De Filippi, 1863): Six individuals of the species in pea agricultural fields in Spiti (32.47119˚ N, 77.71643˚ E; 4172 m a.s.l.) between the months of August-September, 2018 were observed. The species is known as winter visitor to North-West India but un-reported from the Lahaul and Spiti landscape. We identified the species from its brown upper body, uniform pale brown underparts rufous-orange rump (visible in flight) and rufous sides to tail.

Although there were numerous inventories were documented since 1920's by Baker et al (1923) and Whistler (1923) up to 2017 by Rawal et al. form different localities of the landscape but none of the studies provided the complete observed avifauna of the landscape. The compiled checklist of 278 species indicates maximum species from Spiti even after being barren and rugged terrains, but that could be due to clear long-distance visibility. Only single study (Editor-Director 2005) that unveils the distribution of avifaunal assemblage in the whole landscape but reported only 74 species. We recorded 102 species (12 orders, 36 families and 71 genera) between July & September during our survey and enlisted 236, 178 and 133 species from Spiti followed by Lahaul and Udaipur respectively. In the presented checklist highest number of species belongs to order Passeriformes represented by 34 families and 174 species, Charadriiformes by 6 families and 24 species, Accipitriformes by Accipitridae and 17 species and Anseriformes by Anatidae and 13 species, Galliformes by Phasianidae and 07 species were among the most abundant in the region.

Out of three localities (*i.e.*, Lahaul, Udaipur and Spiti), Spiti was the most studied locality with 13 published literatures among which seven publication highlighted checklist of species (Whistler 1923, Koelz 1937, Singh et al 2003, Tak and Paliwal 2008, Thakur et al 2011, Rana et al 2014) and six articles are on new sighting records (Rana

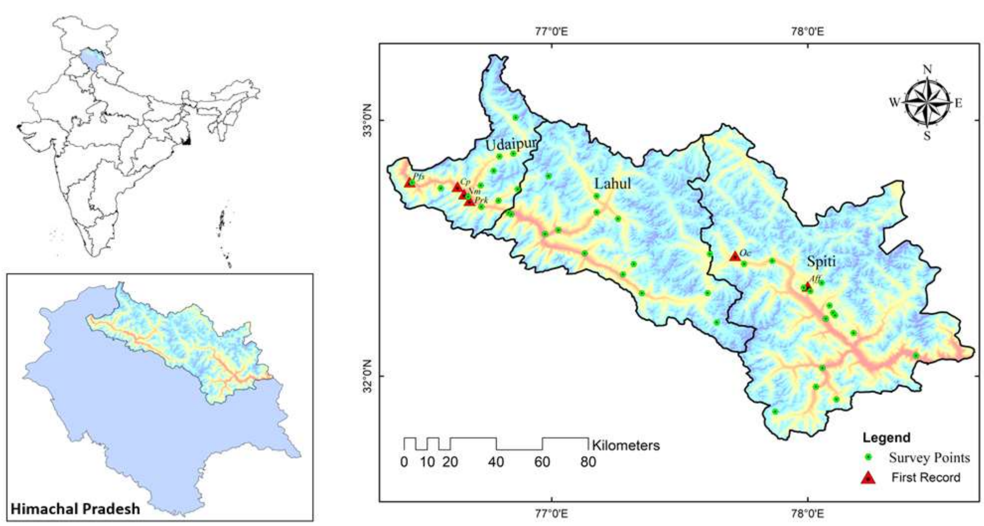

Fig. 1. Map showing the study sites (Lahaul, Udaipur and Spiti) and locations of new records of birds (*Aff-Asio flammeus, Nm-Nucifraga multipunctate, Cp-Cuculus poliocephalus, Pfs-Pericrocotus flammeus, Pr-Phylloscopus reguloides, Oc-Oenanthe chrysopygia are marked with triangles*)

1995, Manjrekar and Mehta 1999, Mishra 2000, Jha 2014, Singh et al 2014, Sangha et al 2017). In Lahaul, six observational records were available among which four records (Whistler 1925, Lowndes 1929, Koelz 1939, Alexander 1951) were on enlisting species and two articles (Baker 1923, Rawal et al 2017) on new records from the area. The eBird observations (accessed on 30 November 2018) by several observers from different localities also contributed significantly to the existing avifaunal assemblage to all the three localities and also incorporated in the presented checklist.

Spiti Valley in the Indian trans-Himalayan region is known to harbor a fragile ecosystem (both flora and fauna) (Rodgers and Panwar 1988), known for its scanty vegetation. The vegetation of this area has been broadly classified as dry alpine steppe (Champion and Seth 1968) where the average altitude ranges from 3900 m – 4300 m. Very little is known about the trans-Himalayan steppe grasslands which supports a fair number of wildlife including avifauna (Rawat 1998), and this seems to be grievously true till date. These grasslands are home to some of the globally threatened birds' species*viz*., White-headed Duck (*Oxyura leucocephala*) (EN), Steppe Eagle (*Aquila nipalensis*) (EN), Pallas's Fish Eagle (*Haliaeetus leucoryphus*) (EN), Ferruginous Duck (*Aythya nyroca*) (NT), Cinereous Vulture (*Aegypius monachus*) (NT), Himalayan Griffon (*Gyps himalayensis*)(NT), Northern Lapwing (*Vanellus vanellus*) (NT), Lammergeier (*Gypaetus barbatus*) (NT) and Curlew Sandpiper (*Calidris ferruginea*) (NT) *etc*. But the population status and understanding of their ecological needs are not only lacking but also never been a subject of study. The most legit and possible reason for this being the terrain of the landscape itself which is inaccessible due to its ruggedness. Vast stretches of wilderness with no human habitation makes it impossible to carry out prolonged studies. However, some accessible and resource rich areas like the Kibber Wildlife Sanctuary provides habitat to most of the above-mentioned raptors and vultures. On the other hand, water bodies and streams of Pin Valley National Park and Chandertal Wildlife Sanctuary provide foraging and resting ground to water birds distributed in the area. Common Teal (*Anas crecca*), Mallard (*Anas platyrhynchos*), Bar-headed Goose (*Anser indicus*), Tufted Duck (*Aythya fuligula*), Gadwall (*Mareca Strepera*) were few among the migratory birds reported from the landscape.

Lahaul Valley on the other hand is bestowed with dense forest patches of Himalayan birch (bhojpatra) and Himalayan cedar (Deodar/ Devdar). Summers lasting from May to

Fig. 2. Image showing the topography of Lahaul (1a and 1b), Udaipur (2a and 2b) and Spiti (3a and 3b)

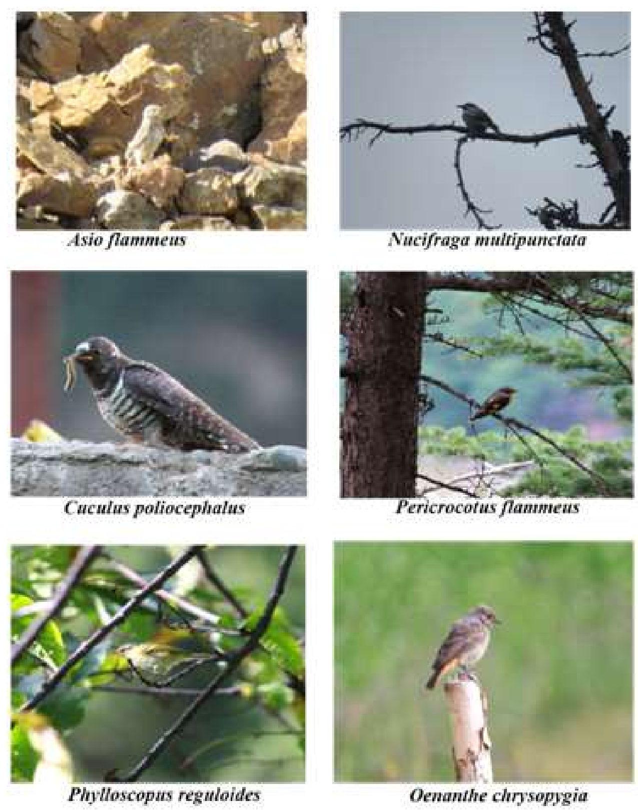

Fig. 3. Showing the images of six new records of species from the Lahaul, Udaipur and Spiti administrative blocks

October provides lush green agricultural fields and apple orchards, it is drained by the rivers Chandra and Bhaga. There are considerable agglomerations of settlements in the valleys. However, not many research articles are published from this valley which abides by the fact that no protected areas have been established so far in Lahaul Valley. Bohemian Waxwing (*Bombycilla garrulus*) was the last addition to the checklist of the Lahaul and Spiti by Rawal et al (2017). The species known resident from Kazakhstan, Mongolia, Japan, Russia and United States whereas the species is supposed to be winter visitor to the landscape (Grimmett et al 2015). Considering the dearth of information on the avifauna of this opulent landscape this document proves to be of great importance in compiling the baseline data.

In the face of modern-day pressures on already feeble wildlife, feral dogs in Spiti Valley are emerging as a threat (Kumar and Paliwal 2015). They are known to hunt on the livestocks of people living there and are seen chasing foxes, hunting on blue sheep, hare and fawns of Ibex (Kumar and Paliwal 2015). We also observed a pack of feral dogs chased a pair of Rudy shelduck (*Tadorna ferruginea*). Out of our six new records of bird from the landscape, Lesser Cuckoo (*Cuculus poliocephalus*) and Blyth's leaf Warbler (*Phylloscopus reguloides*) are known to breed in the Lahaul valley whereas Red-tailed Wheatear (*Oenanthe chrysopygia*), observed in the Spiti Valley is a winter visitor (Rasmussen and Anderton 2012,Grimmett et al 2015). Remaining three species *viz., Nucifraga multipunctata*, *Pericrocotus flammeus* and *Asio flammeus* are known to be residents in an area (Grimmett et al 2015).

Besides this, few bird species *i.e.*, Chukar Partridge (*Alectoris chukar*), Snow Pigeon (*Columba leuconota*), Common Hoopoe (*Upupa epops*), Yellow-billed Chough (*Pyrrhocorax graculus*), Red-billed Chough (*Pyrrhocorax pyrrhocorax*), Black Redstart (*Phoenicurus ochruros*), Common Rosefinch (*Carpodacus erythrinus*), were among the commonest species which were sighted throughout the study period in different time scale and locality.

It is well known fact that the exact and detailed data on species occurrences are fundamental requirement for the evaluation of conservation and emphasizing on management considerations(Saini et al 2017). This article provides a complete checklist of birds and information on their distribution, current conservation status, migratory/residential information and population trend. We hope our study will help researchers in the future and conservation managers to conserve birds in this region. We recommend further update by conducting systematic and detailed surveys studies in the region as this information are crucial for preparing conservation and management action plans.

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Assessment of Faunal Diversity and the Influencing Factor for Bird Diversity in Panchase Protected Forest of Kaski District, Nepal

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Abstract: There exists limited information on biodiversity including faunal diversity and habitat conditions in protected forests (PF) of Nepal; thus, we aimed to assess the faunal diversity and the associated habitat characteristics of birds in the Bharang area of Panchase protected forest. The point count method was used for assessing bird diversity. Mammals were searched in existing trials through direct field observations and indirect signs like scat, urine, scratch, bites pugmarks. Shannon-Wieners index, Margalef's Richness Index, and Pielou's evenness were used to determine the respective effect of various biophysical factors associated with the richness of bird species. In total, 121 (summer 50, winter 71) bird species were recorded in 50 sample plots and 6 mammals were identified using transect survey. The Shannon-Wiener diversity index was calculated as 3.14 and 4.04, Margalef's richness index as 9.44 and 14.53, and Pielou's evenness index as 0.75 and 0.80 for summer and winter, respectively. These indices proved the study area to be rich in species distribution. In addition, aspect and season are found to be the main influencing factor for bird diversity. Species richness is found to be higher in the case of birds than mammals. Similar research on an elaborate scale is highly recommended all over the Panchase area such that the local level to policy level conservation implications is to be addressed.

Keywords: Biodiversity, Habitat, Influencing factors, Margalef's richness index, Shanon-weiner index

Globally around 1.75 million species have been discovered and formally named to date (Larsen 2017). Most animals species including 8 million are estimated to exist, almost 10000 bird species and 4640 mammals are recognized (Octenjak et al 2020). Nepal is exemplified as a biodiversity-rich country that represents a significant share of global biodiversity (Garima and Singh 2019). Nepal occupies about 0.1 percent of the global land area but harbors 3.2 percent and 1.1 percent of the world's known flora and fauna, respectively (Poudel et al 2021). This includes 5.2, 9.5, 5.1 and 8.2 percent of the world's known mammals, birds, gymnosperms, and bryophytes respectively (Aryal et al 2019). Panchase is an important mid-mountain ecological zone of Nepal (Baral et al 2017) and is considered as a biological corridor between Chitwan National Park and Annapurna Conservation area. A total of 613 species belonging to 393 genera and 111 families have been documented from Panchase Protected Forest (Bhandari et al 2018). Bird species diversity and richness are associated with the distribution and presence of field margins, forest edges, habitat fragmentation, habitat quality, landscape changes, landscape structure, farming systems, type of vegetation, and climate (Basnet et al 2016). A recent study indicated that temperature, precipitation, habitat resources, and the level of disturbances influenced bird species' diversity and richness in the mid-hills (Pandey et al 2020). A total of 915 bird species has been recorded in Nepal (Baral and Inskipp 2020). The reason for richness in avian diversity is due to amazingly diverse climatic and topographical variations within the country that have provided a variety of forest and ecosystem types (Poudel et al 2021). Out of the 915 species recorded in Nepal, one is endemic, one has been introduced by human and 42 species are globally threatened. Apart from being the bird only found in Nepal, Spiny Babbler is also the smallest bird that is found in Nepal (Bastola 2021). The major habitat of birds includes forests, wetlands, and grassland (Browder et al 2021). Similarly, out of 212 mammals species, 49 species (23%) are listed as nationally threatened of which 9 (18%) are critically endangered species, 26 (53%) endangered species, and 14 (29%) vulnerable species (Amin et al 2018). Over sixty percent of Nepal's ungulates are threatened and almost half of Nepal's carnivores face extinction (45% threatened) (Amin et al 2018).

Panchase has the greatest diversity of ecosystems and species in Nepal (Baral et al 2017). It is home to the number of endangered wildlife species including Himalayan black bear (*Ursus thibetanus*), the common leopard (*Panthera* *pardus*), and eight species of bats (Bhandari et al 2018). To understand the function of ecosystems, the composition of birds and mammals needs to be assessed as each flora and fauna has a specific and important role in maintaining ecosystems (Miloslavich et al 2018). When forest habitats are protected, faunal tourism can be promoted that can contribute to the rural economy of poor people (Girma et al 2017, Gupta et al 2019). Pandey et al (2016) have conducted on policy and socio-economic aspects of protected areas in Nepal; however, the role of protected forests in conservation of faunal diversity is often neglected. It is essential to think not only about the utilization of forest resources but also about maintaining biological diversity and conserving the threatened faunal species in the protected forest. Thus, long-term monitoring of biodiversity should be performed in the forest ecosystem of Nepal that will help to develop strategies and management plans for conserving the biodiversity. In this study, we explored the contribution of protected forest to the faunal composition and species richness, followed by determining the associated habitat characteristics for bird species. To the best of our knowledge, such information is lacking in Nepal, therefore we believe that this study helps to fill such gaps, which can ultimately give an important contribution to a better understanding of birds and mammals of the Panchase Protected Forest, and also provide basic information necessary for the conservation of avifauna of this area.

MATERIAL AND METHODS

Study area: The study was carried out in the Panchase Protected Forest which is situated at the junction of three districts namely Kaski, Parbat, and Syangja. It is situated in the mid-hills of Nepal, west of Pokhara, at longitudes between 83^{0} 45' and 83^{0} 57' E and at latitudes between 28^{0} 12' and 28^{0} 18' N. Altitude ranges from 815 m at Harpan River to 2517 m at the peak of Panchase hill whereas the forest ranges from 1450 m to the peak and receives more than 5000 mm precipitation per year (Chikanbanjar 2015). Varieties of terrain types and climatic variations ranging from upper tropical to moist temperate have allowed Panchase to give rise to a great diversity of ecosystems and species. Hill Sal forest, pine forest, Schima-Castanopsis forest, and Oak forest are the major types of forests in the Panchase area.

Data Collection

Preliminary field visit: During this visit, consultations and discussions were made with the committee members of Panchase protected forest and some users of the Bharang area of Panchase protected forest regarding the objective of this study and planning for major field observations and measurements. The general information about the biophysical characteristics and socio-economic characteristics of users was obtained during this visit.

Bird survey using point count method: Bird species in the study area was surveyed using the point count method (Ralph et al 1995). For the point count method, points were laid at a distance of 200 m along the existing trails within the forest area. The distance between two consecutive stations was maintained at 200 m to avoid double counting. The bird species seen and heard within 20 m of radius were counted for a period of 10 minutes (Ralph et al 1995, Hostetler and Main 2001). To minimize disturbance during the count, a waiting period of 3 to 5 min before counting was applied. The data collection was carried out for 5 hours a day from 6:30 to 10 am in the morning and from 4:30 to 6 pm in the afternoon as during the time interval; the activities of the birds are prominent and avoided performing point counts in days with rain and stronger wind. Photographs and calls were used to identify the conspicuous birds whereas others have been identified with the aid of binoculars and spotting scope. The birds have been identified at the species level with the help of experienced bird watchers and experts as well as from guide of Helm Field Guide "Birds of Nepal" (Nepali version), and details like the number of individuals of particular bird species recorded on the special datasheet.

Mammals survey using transect survey: The transect survey method was used to monitor and collect the fecal samples of mammals in the study area. This survey is a widely used method to monitor and collect fecal samples of bigger-sized mammals (Pokharel et al 2015, Kunwar et al 2016). The walking trails and existing paths that course through the forest and cover all the habitats were chosen as transect lines. The animals sighted within 50 m on each side

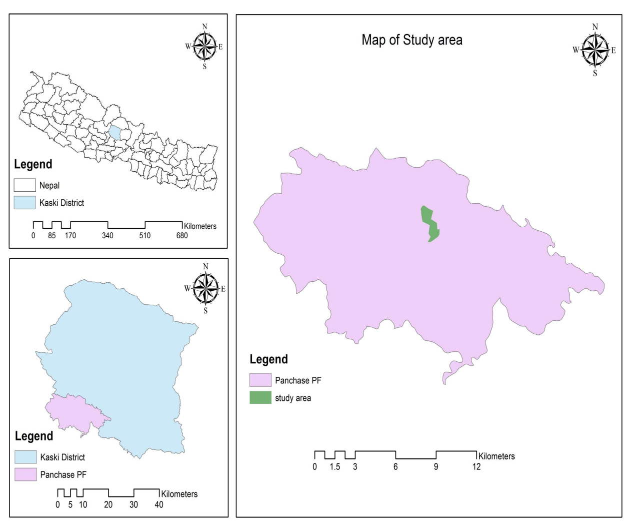

Fig. 1. Location map of study area

of transect and evidence of its presence such as pellets, hairs, footprints observed within 5 m in each side of the transect were recorded. Two people, mostly local people acquainted with the forests were involved in searching for signs of target species. GPS coordinates were recorded wherever we observed the direct and indirect signs of the species to prepare a distribution map using Arc GIS. For habitat assessment, habitat use plots (U) were laid out in areas that contain signs (fecal pellets, hair, footprints, resting sites, etc.) of mammal species. Furthermore, habitat parameters such as slope, altitude, crown cover, ground cover, and land features were recorded from the same plots. The quadrate size was selected as suggested by Schemnitz (1980) for vegetation analysis: 10×10 m^2 for the tree layer (plants above 3 m height and 5 cm DBH), 4×4 m^2 for the shrub layer (woody plants below 3 m height), and 1×1m^2 plots for herbs (plants up to 1 m height). In each plot, the different parameter of the trees was recorded (e.g., DBH, height, crown cover, ground cover, number of trees, frequency of shrubs and herbs, signs of other animals, and other anthropogenic pressures (Joshi et al 2019).

Data Analysis

Bird abundance and diversity analysis: Nomenclature and classification of birds were performed by using Birdlife International (2017). Their global status and population trend followed IUCN Red List Series (2017) whereas national status and migratory status followed the National Red List Series of Nepal's Birds (Inskipp et al 2016). The relative abundance of each observed bird species observed was determined by using the equation:

Relative abundance (%) = n/N ×100

Where, n= numbers of individuals of particular recorded species, N= total number of individuals of recorded species

Besides, the abundance status was assessed as per the criteria of Khan and Ali (2014):

Very common if seen on 75-100% of visits. Common if seen on 50-74% of visits, uncommon if seen on 25-49% of visits. Rare if seen on <25% of visits. Complete checklists of bird species were compiled

Species diversity: Species diversity of bird was determined using Shannon-Wiener's index (Odum 1971) (H´), Margalef's Richness Index (Margalef 1958) and Pielou's Evenness Index (Pielou 1996).

Shannon-Wieners Index

$$H' = -\sum \frac{ni}{N} \ln \frac{ni}{n}$$

Where, ni= number of individuals of ith species, N= total number of all individuals. ln= natural logarithm. The value of the index ranges from 1.5 (low species richness and evenness) to 5.0 (high species evenness and richness).

$$H = \frac{S - 1}{\ln N}$$

Where, S= total number of species, N = total number of individuals encountered ln= natural logarithm. Higher the value of 'R', higher will be the species richness.

Pielou's evenness index

e= H'/ ln S

Where, S= total number of species, H= Shannon-Weaver diversity index

The value of 'e' ranges from 0 to 1 with 1 being complete evenness i.e., species are equally distributed throughout the habitat.

Margalef's richness index

$$H = \frac{S - 1}{\ln N}$$

Where, S= total number of species, N = total number of individuals encountered ln= natural logarithm. Higher the value of 'R", higher will be the species richness.

Factors influencing bird diversity: The presence and absence of direct and indirect sightings of birds was taken as a dependent variable while vegetation type, floral composition, elevation, season, anthropogenic disturbances, distance from water sources, and distance from settlements and aspect was taken as independent or predict variables. Besides, with the help of all recorded GPS coordinates, the habitat distribution map of major faunal species was prepared using Arc GIS 10.3. All the required statistical tests were performed by using "R x 64 3.0.1. Though we recorded different habitat parameters at the locations where we recorded different signs of mammal species, but we did not analyze them due to less number of mammal species and sample sizes for this study.

RESULTS AND DISCUSSION

A total of 121 bird species was recorded in 50 sample plots. Similarly, only 6 mammals was recorded using transect survey. Among the recorded bird species, 50 species of birds were recorded in summer while 71 species of birds were recorded in winter. A total of 35 bird species was recorded only in winter and 14 bird species were recorded only in summer, whereas 36 bird species were recorded in both summer and winter (Table 1). The highest number of birds was found in the order Passeriformes in both the summer and winter seasons (Table 2).

Species Richness of Bird

Species richness in the summer season : A total of 50 species of bird belonging to 7 orders and 21 families were recorded during the summer season only (Table 3). The highest numbers of species (32) was represented in the order Passeriformes along with 13 families (Fig. 2). Other major orders in terms of species richness were Piciformes (2 families and 6 species), Columbiformes (1 family and 5 species), Cuculiformes (1 family and 2 species), Strigiformes (2 family and 2 species), Galliformes (2 species and 2 families) and all these species were in a mixed forest of Panchase.

Species richness during the winter season : A total of 71 species of bird belonging to 7 orders and 25 families were recorded during the winter season only (Table 4). The highest number of species (52) was represented in the order Passeriformes along with 17 families (Fig. 3). Other major orders in terms of species richness were Piciformes (2 families and 7 species), Strigiformes (2 family and 5 species), Columbiformes (1 family and 4 species, Galliformes (1 species and 1 family), Psittaciformes (1 species with 1

Table 1. Birds observed in both summer and winter season

Common name	Scientific name	Family
Black Bulbul	*Hypsipetes leucocephalus*	Pycnonotidae
Black lored tit	*Machlolophus xanthogenys*	Stenostridae
Blue Whistling Thrush	*Myophonus caeruleus*	Muscicapidae
Blue-throated Barbet	*Megalaima asiatica*	Megalaimidae
Bronzed Drongo	*Dicrurus aeneus*	Dicruridae
Common Green Magpie	*Cissa chinensis*	Corvidae
Common Tailorbird	*Orthotomus sutorius*	Sylviidae
Crested Serpent Eagle	*Spilornis cheela*	Accipitridae
Fulvous-breasted Woodpecker	*Dendrocopos macei*	Picidae
Great Barbet	*Megalaima virens*	Megalaimidae
Greater Yellownape	*Picus flavinucha*	Picidae
Grey Treepie	*Dendrocitta formosae*	Corvidae
Grey-headed Woodpecker	*Picus canus*	Picidae
Grey-headed Canary Flycatcher	*Culicicapa ceylonensis*	Muscicapidae
Grey-hooded Warbler	*Phylloscopus xanthoschistos*	Sylviidae
Hill Partridge	*Arborophila torqueola*	Phasianidae
Himalayan Bulbul	*Pycnonotus leucogenys*	Pycnonotidae
Large Cuckooshrike	*Coracina macei*	Campephagidae
Lesser Racquet-tailed Drongo	*Dicrurus remifer*	Dicruridae
Lesser Yellownape	*Picus flavinucha*	Picidae
Long-tailed Minivet	*Pericrocotus ethologus*	Campephagidae
Maroon Oriole	*Oriolus traillii*	Oriolidae
Mountain Bulbul	*Ixos mcclellandi*	Pycnonotidae
Orange-bellied Leafbird	*Chloropsis hardwickii*	Chloropseidae
Oriental Turtle Dove	*Streptopelia orientalis*	Columbidae
Plumbeous Water Redstart	*Rhyacornis fuliginosa*	Muscicapidae
Spangled Drongo	*Dicrurus hottentottus*	Dicruridae
Speckeled Wood Pigeon	*Columba hodgsonii*	Columbidae
Spotted Owlet	*Athene brama*	Strigidae
Streak-breasted Scimitar Babbler	*Pomatorhinus ruficollis*	Timaliidae
Velvet-fronted Nuthatch	*Sitta frontalis*	Sittidae
Wedge-tailed Green Pigeon	*Treron sphenurus*	Columbidae
Whiskered Yuhina	*Yuhina flavicollis*	Timaliidae
White-capped Water Redstart	*Chaimarrornis leucocephalus*	Muscicapidae
White-crested Laughingthrush	*Garrulax leucolophus*	Timaliidae
White-throated Laughingthrush	*Garrulax albogularis*	Timaliidae

Table 2. Most abundant bird species with frequency of occurrence

Common name	Scientific name	Frequency	Status
Ashy Bulbul	*Hemixos flavala*	12	Least Concern
Ashy drongo	*Dicrurus leucophaeus*	4	Least Concern
Barred cuckoo Dove	*Macropygia unchall*	2	Least Concern
Black Bulbul	*Hypsipetes leucocephalus*	11	Least Concern
Black chinned Babbler	*Stachyridopsis pyrrhops*	2	Least Concern
Black Drongo	*Dicrurus macrocercus*	52	Least Concern
Black -lored tit	*Machlolophus xanthogenys*	4	Least Concern
Black throated tit	*Aegithalous concinnus*	2	Least Concern
Blue throated barbet	*Megalaima asiatica*	80	Least Concern
Blue whistling thrush	*Myophonus caeruleus*	36	Threatened
Bronzed drongo	*Dicrurus aeneus*	17	Least Concern
Common green magpie	*Cissa chinensis*	18	Least Concern
Common hawk -cuckoo	*Hierococcyx varius*	87	Least Concern
Common Tailorbird	*Orthotomus sutorius*	3	Least Concern
Creasted kingfisher	*Orthotomus sutorius*	2	Least Concern
Crested serpent eagle	*Spilornis cheela*	11	Least Concern
Crimson sunbird	*Aethopyga siparaja*	8	Least Concern
Emerald dove	*Chalcophaps indica*	10	Least Concern
Fulvous breasted woodpecker	*Dendrocopos macei*	10	Least Concern
Great Barbet	*Megalaima virens*	81	Least Concern
Greater Yellownape	*Chrysophlegma flavinucha*	10	Threatened
grey-headed canary-flycatcher	*Culicicapa ceylonensis*	5	Least Concern
Grey headed woodpecker	*Picus canus*	13	Least Concern
Grey Treepie	*Dendrocitta formosae*	102	Least Concern
Grey-hooded Warbler	*Phylloscopus xanthoschistos*	3	Least Concern
Hill partridge	*Arborophila torqueola*	3	Threatened
Himalayan bulbul	*Pycnonotus leucogenys*	12	Least Concern
Indian cuckoo	*Cuculus micropterus*	9	Least Concern
Kalij Pheasant	*Lophura leucomelanos*	4	Least Concern
Large cuckoo shrike	*Coracina macei*	5	Least Concern
Lesser racket Tail	*Dicrurus remifer*	21	Least Concern
Lesser Yellownape	*Picus chlorolophus*	1	Least Concern
Long tailed board bill	*Psarisomus dalhousiae*	3	Least Concern
Long tailed minivet	*Pericrocotus ethologus*	5	Least Concern
Maroon oriole	*Oriolus traillii*	8	Least Concern
Mountain bulbul	*Ixos mcclellandii*	11	Least Concern
Oriental turtle dove	*Streptopelia orientalis*	3	Least Concern
Plumbeous water red stout	*Rhyacornis fuliginosa*	2	Least Concern
Spangled drongo	*Dicrurus bracteatus*	27	Least Concern
Spotted Dove	*Spilopelia chinensis*	3	Least Concern
Short billed minivet	*Pericrocotus brevirostris*	2	Least Concern
Velvet fronted mithaten	*Sitta frontalis*	4	Least Concern
Verditer flycatcher	*Eumyias thalassinus*	8	Least Concern
Wedge tailed great pigeon	*Treron sphenurus*	4	Least Concern
Whiskerd Yunina	*Yuhina flavicollis*	7	Least Concern
White Throated laughing thrust	*Garrulax albogularis*	9	Least Concern
White Creasted laughing thrush	*Garrulax leucolophus*	50	Least Concern

Table 3. Bird species encountered in summer only

Common name	Scientific name	Family
Crested Serpent Eagle	*Spilornis cheela*	Accipitridae
Black throated tit	*Aegithalos concinnus*	Aegithalidae
Crested kingfisher	*Megaceryle lugubris*	Alcedinidae
Large Cuckooshrike	*Coracina macei*	Campephagidae
Long-tailed Minivet	*Pericrocotus ethologus*	Campephagidae
Crimson sunbird	*Aethopyga siparaja*	Chloropseidae
Orange-bellied Leafbird	*Chloropsis hardwickii*	Chloropseidae
Barred cuckoo dove	*Macropygia unchall*	Columbidae
Emerald dove	*Chalcophaps indica*	Columbidae
Oriental Turtle Dove	*Streptopelia orientalis*	Columbidae
Speckeled Wood Pigeon	*Columba hodgsonii*	Columbidae
Wedge-tailed Green Pigeon	*Treron sphenurus*	Columbidae
Common Green Magpie	*Cissa chinensis*	Corvidae
Grey Treepie	*Dendrocitta formosae*	Corvidae
Common hawk cuckoo	*Hierococcyx varius*	Cuculidae
Indian cuckoo	*Cuculus micropterus*	Cuculidae
Ashy drongo	*Dicrurus leucophaeus*	Dicruridae
Black drongo	*Dicrurus macrocercus*	Dicruridae
Bronzed Drongo	*Dicrurus aeneus*	Dicruridae
Lesser Racquet-tailed Drongo	*Dicrurus remifer*	Dicruridae
Spangled Drongo	*Dicrurus hottentottus*	Dicruridae
Long tailed broadbill	*Psarisomus dalhousiae*	Eurylaimidae
Blue-throated Barbet	*Megalaima asiatica*	Megalaimidae
Great Barbet	*Megalaima virens*	Megalaimidae
Blue Whistling Thrush	*Myophonus caeruleus*	Muscicapidae
Grey-headed Canary Flycatcher	*Culicicapa ceylonensis*	Muscicapidae
Plumbeous Water Redstart	*Rhyacornis fuliginosa*	Muscicapidae
Verditer flycatcher	*Eumyias thalassinus*	Muscicapidae
White-capped Water Redstart	*Chaimarrornis leucocephalus*	Muscicapidae
Maroon Oriole	*Oriolus traillii*	Oriolidae
Hill Partridge	*Arborophila torqueola*	Phasianidae
Kalij pheasent	*Lophura leucomelanos*	Phasianidae
Fulvous-breasted Woodpecker	*Dendrocopos macei*	Picidae
Greater Yellownape	*Picus flavinucha*	Picidae
Grey-headed Woodpecker	*Picus canus*	Picidae
Lesser Yellownape	*Picus flavinucha*	Picidae
Ashy bulbul	*Hemixos flavala*	Pycnonotidae
Black Bulbul	*Hypsipetes leucocephalus*	Pycnonotidae
Himalayan Bulbul	*Pycnonotus leucogenys*	Pycnonotidae
Mountain Bulbul	*Ixos mcclellandi*	Pycnonotidae
Velvet-fronted Nuthatch	*Sitta frontalis*	Sittidae
Black lored tit	*Machlolophus xanthogenys*	Stenostridae
Spotted Owlet	*Athene brama*	Strigidae
Common Tailorbird	*Orthotomus sutorius*	Sylviidae
Grey-hooded Warbler	*Phylloscopus xanthoschistos*	Sylviidae
Black chinned babbler	*Cyanoderma pyrrhops*	Timaliidae
Streak-breasted Scimitar Babbler	*Pomatorhinus ruficollis*	Timaliidae
Whiskered Yuhina	*Yuhina flavicollis*	Timaliidae
White-crested Laughingthrush	*Garrulax leucolophus*	Timaliidae
White-throated Laughingthrush	*Garrulax albogularis*	Timaliidae

Table 4. Birds encountered in winter only

Common name	Scientific name	Family
Crested Serpent Eagle	*Spilornis cheela*	Accipitridae
Mountain Hawk Eagle	*Nisaetus nipalensis*	Accipitridae
Red-headed Vulture	*Sacrogyps calvus*	Accipitridae
Large Cuckooshrike	*Coracina macei*	Campephagidae
Long-tailed Minivet	*Pericrocotus ethologus*	Campephagidae
Scarlet Minivet	*Pericrocotus flammeus*	Campephagidae
Orange-bellied Leafbird	*Chloropsis hardwickii*	Chloropseidae
Rufous-bellid Nitava	*Niltava sundara*	Cinclidae
Ashy Wood Pigeon	*Columba pulchricollis*	Columbidae
Oriental Turtle Dove	*Streptopelia orientalis*	Columbidae
Speckeled Wood Pigeon	*Columba hodgsonii*	Columbidae
Wedge-tailed Green Pigeon	*Treron sphenurus*	Columbidae
Common Green Magpie	*Cissa chinensis*	Corvidae
Grey Treepie	*Dendrocitta formosae*	Corvidae
Large-billed Crow	*Corvus microrhynchos*	Corvidae
Red-billed Blue Magpie	*Erocissa erythrorhyncha*	Corvidae
Bronzed Drongo	*Dicrurus aeneus*	Dicruridae
Crow-billed Drongo	*Dicrurus annectans*	Dicruridae
Lesser Racquet-tailed Drongo	*Dicrurus remifer*	Dicruridae
Spangled Drongo	*Dicrurus hottentottus*	Dicruridae
Black-headed Munia	*Lonchura malacca*	Estrildidae
White-rumped Munia	*Lonchura striata*	Estrildidae
Blue-throated Barbet	*Megalaima asiatica*	Megalaimidae
Great Barbet	*Megalaima virens*	Megalaimidae
Grey Bushchat	*Saxicola caprata*	Muscicapidae
Grey-headed Canary Flycatcher	*Culicicapa ceylonensis*	Muscicapidae
Little Forktail	*Enicurus scouleri*	Muscicapidae
Plumbeous Water Redstart	*Rhyacornis fuliginosa*	Muscicapidae
Rufous-gorgeted Flycatcher	*Ficedula strophiata*	Muscicapidae
Small Niltava	*Niltava macgrigoriae*	Muscicapidae
White-capped Water Redstart	*Chaimarrornis leucocephalus*	Muscicapidae
Black-throated Sunbird	*Aethopyga saturata*	Nectarinidae
Fire-tailed Sunbird	*Aethopyga ignicauda*	Nectarinidae
Green-tailed Sunbird	*Aethopyga nepalensis*	Nectarinidae
Mrs Gould's Sunbird	*Aethopyga gouldiae*	Nectarinidae
Maroon Oriole	*Oriolus traillii*	Oriolidae
Black-lored Tit	*Parus xanthogenys*	Paridae
Great Tit	*Parus major*	Paridae
Green-backed Tit	*Parus monticulus*	Paridae
Hill Partridge	*Arborophila torqueola*	Phasianidae
Crimson-breated Woodpecker	*Dendrocopos cathpharius*	Picidae
Fulvous-breasted Woodpecker	*Dendrocopos macei*	Picidae
Greater Yellownape	*Picus flavinucha*	Picidae

Cont...

Table 4. Birds encountered in winter only

Common name	Scientific name	Family
Grey-headed Woodpecker	*Picus canus*	Picidae
Lesser Yellownape	*Picus flavinucha*	Picidae
Slaty-headed Parakeet	*Psittacula himalayana*	Psittacidae
Black Bulbul	*Hypsipetes leucocephalus*	Pycnonotidae
Grey-bellied Tesia	*Tesia cyaniventer*	Pycnonotidae
Himalayan Bulbul	*Pycnonotus leucogenys*	Pycnonotidae
Mountain Bulbul	*Ixos mcclellandi*	Pycnonotidae
Yellow-bellied Fantail	*Chelidorhynx hypoxantha*	Stenostridae
Chestnut-billed Nuthatch	*Sitta cinnamoventris*	Sittidae
Velvet-fronted Nuthatch	*Sitta frontalis*	Sittidae
Asian Barred Owlet	*Glaucidium cuculoides*	Strigidae
Spotted Owlet	*Athene brama*	Strigidae
Buff-barred Warbler	*Phylloscopus pulcher*	Sylviidae
Chestnut-crowned Wabler	*Seicercus poligenys*	Sylviidae
Common Tailorbird	*Orthotomus sutorius*	Sylviidae
Greenish Warbler	*Phylloscopus trochiloides*	Sylviidae
Grey-cheeked Warbler	*Seicercus poliogens*	Sylviidae
Grey-hooded Warbler	*Phylloscopus xanthoschistos*	Sylviidae
Grey-sided Bush Warbler	*Cettia brunnifrons*	Sylviidae
Black-chinned Yuhnia	*Yuhina nigrimenta*	Timaliidae
Streak-breasted Scimitar Babbler	*Pomatorhinus ruficollis*	Timaliidae
Whiskered Yuhina	*Yuhina flavicollis*	Timaliidae
White-browed Scimitar Babbler	*Pomatorhinus schisticeps*	Timaliidae
White-crested Laughingthrush	*Garrulax leucolophus*	Timaliidae
White-throated Laughingthrush	*Garrulax albogularis*	Timaliidae
Red-headed Trogon	*Harpactes erythrocephalus*	Trogonidae
Blue Whistling Thrush	*Myophonus caeruleus*	Muscicapidae
Orientle White-eye	*Zosterops palpebrosus*	Zosteropidae

family), Trogoniformes (1 species and 1 family) and all these species were in mixed forest of Panchase.

Species diversity: The Shannon-wieners index ranged from 1.5 to 5. The index was 3.14 in summer whereas 4.04 in the winter season which means the species richness and evenness of birds are high in both the season in the study area (Table 5). The higher the value of Margalef's Richness Index, the higher will be the species richness. The index was 9.44 in summer and 14.53 in the winter season which means the species richness is comparatively high in the winter season than in summer. The Pielou's evenness index ranged from 0 to 1 in which 1 means complete evenness that displays species are equally distributed throughout the habitat. The index was 0.75 in summer and 0.80 in the winter season which means the species are more even and distributed equally in the study area.

Habitat factors influencing the bird diversity: The histogram with a prominent mound in the center and similar tapering to the left and right giving a bell-shaped means the data is normally distributed (Fig. 4).

The bird diversity was found to be good in case of normality check. Thus, the assumption is fulfilled (Fig. 5). The data was*significant at p<0.01 and ** at p<0.001 at 5% level of significance which shows that aspect and season are the

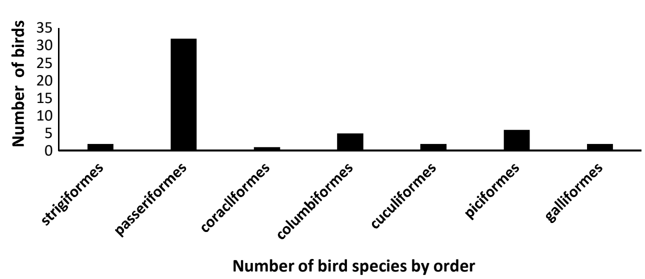

Fig. 2. Numbers of bird species with orders in summer season

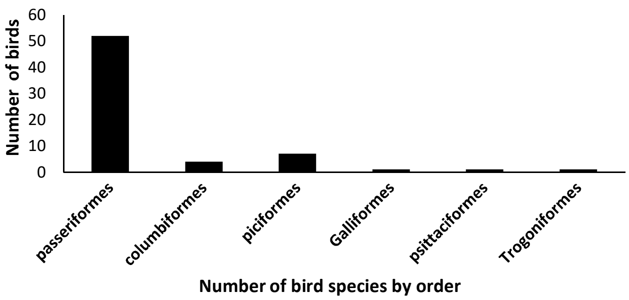

Fig. 3. Number of birds with their orders in winter season

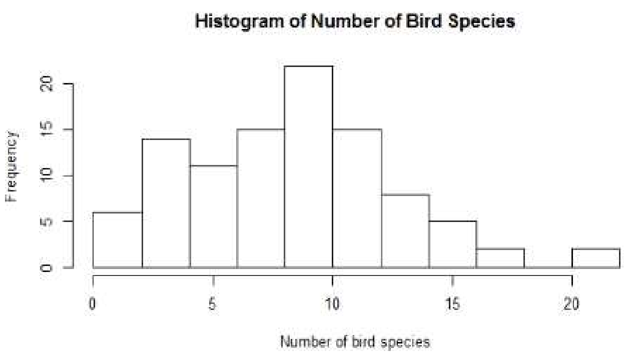

Fig. 4. Histogram showing number of bird species

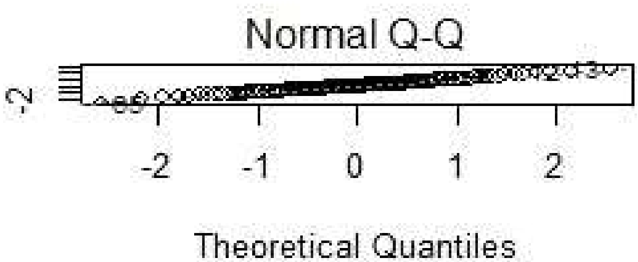

Fig. 5. Normality curve

Table 5. Species diversity in two different seasons

Season	Shannon-wieners index	Margalef's richness index	Pielou's evenness index
Summer	3.14	9.44	0.75
Winter	4.04	14.53	0.80

Table 6. F-test for habitat characteristics

Variable	df	F-value	P-value
Aspect	6	2.25	0.44*
Season	1	8.79	0.003**

main influencing factor of bird diversity (Table 6).

Habitat Distribution Map of Major Bird and Mammal Species

Habitat distribution map of mammals: Figure 6 shows the habitat distribution map of mammals species found in the study area. Only six species of mammals were found namely common leopard *(Panthera pardus*), barking deer (*Muntiacus vaginalis*)*,* Hodgson's flying Squirrel (*Petaurista magnificus*), Porcupine (*Hystrix indica*), Rhesus monkey (*Macaca mulatta*), and Jungle Cat (*Felis chaus*) (Table 7). As barking deer was in most of the area but the number is less in between the map is due to less presence of deer in landslide areas as well as most sloppy land.

Habitat distribution map of bird : The numbers of birds are

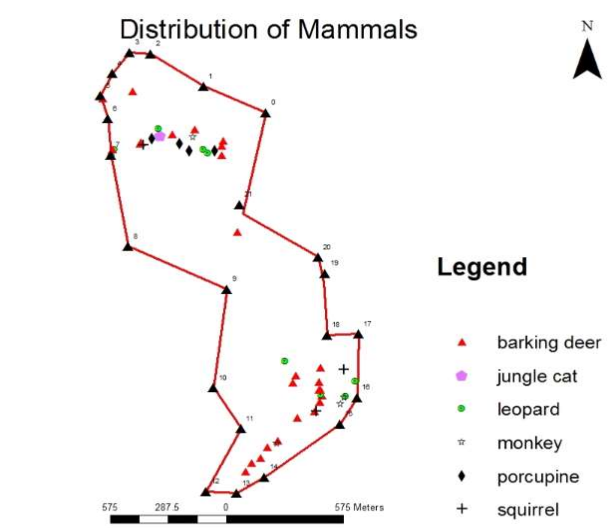

Fig. 6. Distribution map of recorded mammal's species

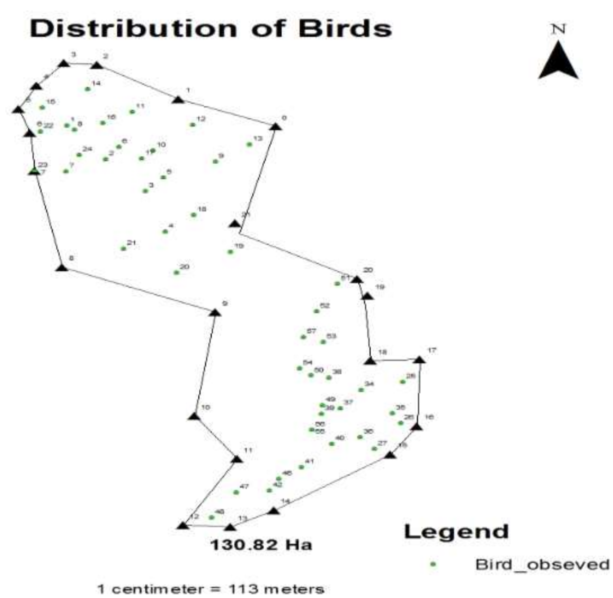

Fig. 7. Distribution map of bird's species in study area

Table 7. List of mammal species

Common name	Sscientific name	Frequency of occurrence	Signs type	Status
Common leopard	*Panthera pardus*	13	Scat, urine scent, scratch	Vulnerable
Barking deer	*Muntiacus vaginalis*	34	Pellet, hoofmarks, biting mark, call, direct sighting	Least concern
Hodgsons flying squirrel	*Petaurista magnificus*	3	Direct sighting	Least concern
Porcupine	*Hystrix Indica*	4	Direct sighting	Least concern
Rhesus monkey	*Macaca mulatta*	4	Direct sighting	Least concern
Jungle cat	*Felis chaus*	1	Direct sighting	Least concern

found in lesser number in summer season than in winter season (Fig. 7).

Panchase is a rich area in biodiversity, possessing a total of 310 plants, out of which about 100 species are NTFPs and 113 orchids and also 152 species of birds belonging to 10 order and 26 families with a total number 2722 were recorded (Baral et al 2017). Baral et al (2017) observed that higher richness of birds in Panchase was in the natural forest area which is similar to this study. Although the study area was small and surveys were carried out within a short period, higher species richness was observed (H=3.19 and E= 0.75). It may be high due to more species with single individuals and 2 individuals in the study area. The high species richness might be attributed to diverse habitat conditions and seasonality in environmental factors like light, temperature, humidity, precipitation, food availability, vegetation, the flowering of plants, etc. (Djordjevic and Tsiftsis 2022). The bird community of any given habitat changes seasonally (Neate-Clegg et al 2020). Species richness was higher during the winter season than in summer. Habitat becomes dry and unproductive in other places during the winter season while the riparian habitat is pristine and productive throughout the year which has contributed to have greater diversity during winter. Thus, seasonal variation highly affects the species richness and distribution of birds (Elsen et al 2021). Rieger et al (2022) reported flowering in the early winter assure food availability could be contributed to high species richness for birds. An assemblage of many migratory birds in the winter season due to favorable ecological and climatic conditions has also contributed to high species richness. However, Neupane et al (2020) found fewer species during the winter season in Ghunsa valley of Kanchenjunga Conservation Area because landscapes above 3,000 m were covered by snow in the winter season. Birds were highly territorial for breeding activity in the summer season which had reduced its detection (Hager and Craig 2014). Thus, fewer species might have observed this season as compared to the winter season which is similar to present results. The next reason is that during the summer season there occurs a heavy rainfall that destroys the bird's breeding and feeding ground as mentioned by Desgranges et al (2006). Bird abundance and characteristics differ with garden ecoregion; urban cover also influenced bird species composition while bird trait distribution is influenced by the urban cover, ecoregion, and grass cover (Mayorga et al 2020). The landscape matrix also acts as a source of habitat for a large set of species differently related to human transformed environments (e.g., synanthropic and generalist species; Grass et al 2021). Moreover, the habitat edges developing in the interface between matrix and wetland are landscape structures that influence bird diversity and composition depending on season and habitat characteristics (Klingbeil and Wilig 2016) which is similar to the result of this study as well. In human-transformed and fragmented landscapes, wetlands are usually small, isolated, and surrounded by a human-altered matrix (urbanized or cultivated) that may act as a source of disturbance increasing the edge effect on these remnant fragments (Van Schalkwyk 2015). The high species richness of birds in the study area represents diversity in the habitat which is due to great variations in altitude, slope, and aspect. There are twenty-four species of mammals and one hundred fifty-two species of birds species recorded in the entire region of Panchase (Baral et al 2017). The high habitat diversity of birds could be another reason for harboring many threatened bird species (Sreekar et al 2021). Bird species diversity in different habitat types in and around North Nandi Forest, Kenya, reported a significant difference in bird abundance across habitats (Bett et al 2016).

CONCLUSION

Out of 121 bird species, 36 species were recorded in both seasons within the study area. A total of 50 species of birds were recorded in summer belonging to 7 orders and 13 families, and 71 species of birds are recorded in the winter season belong to 7 orders and 17 families. Order Passeriformes was found as the dominant order in both the season which comprises 32 species in the summer season and 52 species in the winter season. Only 6 species of mammal were identified in the forest through direct sighting, pugmark, biting of grass and footmark. Number of species of mammals is very less as compared to birds. Though the richness of bird species was found higher in winter season than in the summer season, the distribution of species was also found to be normal. Aspect and season are the major factors that influence bird diversity because breeding birds prefer lower forests with cooler temperatures at mid-day (presumably to avoid summer overheating stress), less unpredictable weather, and where trees develop leaves earlier (suggesting that birds, particularly those that prey on folivorous insects, would be able to breed early in the season). Such vital information about the faunal species and the associated habitat factors in the protected forest will help to develop strategies and plans to protect the faunal species and their habitats, which has also potential to initiate faunal tourism in Nepal for the benefit of local communities.

Conflicts of Interest: Authors have no competing financial, professional, or personal interests from other parties or in publishing this manuscript.

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First Photographic Record of Himalayan Brown bear from Kanawar Wildlife Sanctuary, Himachal Pradesh, India

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Abstract: The Himalayan brown bear (HBB) population is restricted to a few geographical areas of India, Pakistan, and Nepal. In India, the HBB was distributed in the fragmented pockets of Jammu-Kashmir, and Ladakh UT, followed by Himachal Pradesh and Uttarakhand. HBB is a high conservation priority species in India and is listed as Schedule I of the Indian Wildlife Protection Act (1972). However, in the recent past, the extant population of HBB has been under tremendous threats such as accelerated habitat fragmentation, human encroachment, and climate change. The present study was conducted in the Kanawar wildlife sanctuary (KWS), situated in the Kullu district of Himachal Pradesh, using camera trapping and trail sampling. The study reports the first photographic evidence of HBB from the Kanawar wildlife sanctuary (KWS). Further, we recommend a long-term assessment and population estimation of HBB in and around KWS to conserve the species.

Keywords: Camera trap, Conservation, Opportunistic records, Schedule I, Brown bear

The brown bear (*Ursus arctos*) is polytypic species with a wide range of distribution in most of Europe, North America to northern and Central Asia (McLellan et al 2017). The Himalayan brown bear subspecies of brown bear occupies a high elevation zone of the Himalayan region (Aryal et al 2012). The species distribution is confined to Northern areas in Pakistan and the north and North Western Himalayan regions of India (Sathyakumar 2006, Abbas et al 2015). In Indian Himalayan Region (IHR), the species is distributed in high altitude scrub, sub-alpine, and alpine meadows and are in very low densities in the elevation range of 2500m to 5000m (Su et al 2018, Sharief et al 2020). Brown bear is the least concerned, according to IUCN. However, the population of Himalayan brown bear (HBB) (*Ursus arctos isabellinus*) is considered endangered on the IUCN Red List under criteria D (McLellan et al 2017). It is also listed as Appendix I of CITES (GOI, 1992) and on Schedule I of the Indian Wildlife Protection Act (1972) as amended in 2003 (Mohanta et al 2014). The population of HBB is vulnerable due to three major prevailing threats in its entire distribution range, *viz* climate change, habitat encroachment/ degradation, and conflict with humans (Su et al 2018, Sharief et al 2020, Mukherjee et al 2021). Even though the species population is declining, HBB is the least studied carnivore species in IHR (Rathore 2008, Sharief et al 2020). Several methods exist, such as sign survey (direct and indirect sign), camera trapping, and non-invasive genetic method to study carnivore species in the IHR (Joshi et al 2020). The species demands urgent attention and conservation for its long-term survivability. The present study was conducted to assess the biodiversity of the sanctuary.

MATERIAL AND METHODS

Study area: Kanawar Wildlife sanctuary is situated in Kullu district (Himachal Pradesh) is extended from North latitude N 32˚ 00' 21" to longitude E 77˚ 18' 11", East latitude N 31˚ 54' 40" and longitude E 77˚ 27' 03", South latitude N 31˚ 53' 25" and longitude E 77˚ 24' 41", West latitude N 31˚ 57' 25" and longitude E 77˚ 15' 04" elevation ranging between 1800m to 4817m with a total area of 107.29 sq. km (Fig. 1). Kanawar wildlife sanctuary has diverse habitats and species. The area's climate is typically temperate, sub-alpine, and alpine types. The primary habitat is temperate and sub-alpine forests dominated by broad-leaved deciduous, western mixed conifers forest, and alpine meadows mainly dominated by alpine shrubs and herbaceous species (Devi K et al 2019). The rainfall varies from 733 to 1733 mm with an average rainfall of 1090 mm, and the temperature of the district ranges from as low as -2˚C to -5˚C in January to 25˚C to 37˚C in June (Suman Jangra and Mohan Singh 2011, Thakur et al 2019).

Camera trapping and sign survey: As a part of Project "Pilot project on Biodiversity corridor" in selected protected areas of Himachal Pradesh, deployed seven camera traps (Spypoint-11Force-D) in Kanawar wildlife sanctuary (Fig. 2), and traversed eight trails of 1-2 km in length in between April to July 2021. Camera traps are placed 15–30 cm above the ground and 3–5 m away from the trail or expected areas of animal movement for 15 days (Joshi et al 2020). Necessary information such as type of forest, disturbance, distance from water and human settlement, geo-coordinates, and elevation was recorded for every camera trap location and digging signs.

RESULTS AND DISCUSSION

The total sampling effort of 105 camera trap nights (7 camera traps) and eight trails of 19 km walked in Kanawar wildlife sanctuary. Unfortunately, HBB was captured only in one camera trap location (N 31.949425, E 77.355522) (Fig. 1). Also, the digging signs were recorded during the trail walk, apart from the camera trap. HBB capture was in the sub-alpine forest with dominated trees *Picea smithiana* and *Quercus semecarpifolia;* the other tree species in the area are *Aesculus indica, Prunus sp., Rhododendron campanulatum*.

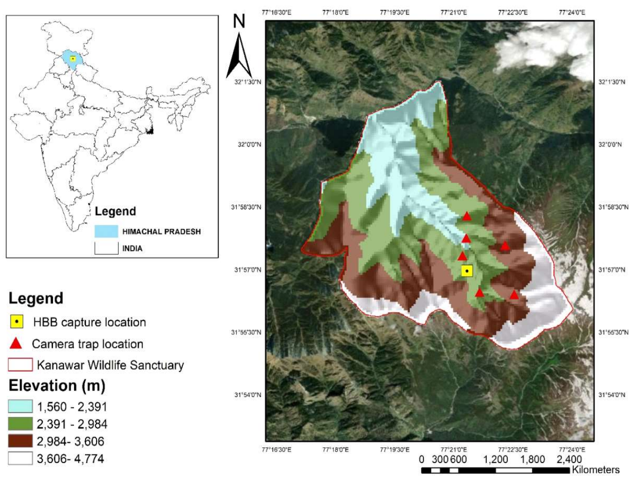

Fig. 1. Map of study area showing capture location of Himalayan brown bear and camera traps sites

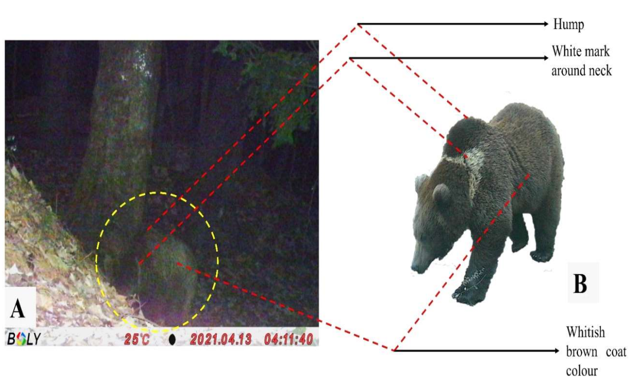

Fig. 2. Camera trap record of HBB from Kanawar Wildlife sanctuary (A) Yellow circle shows the original image (B) Image shows the prominent identification character of HBB

The present study reports the first confirmed presence record of HBB from the Kanawar wildlife sanctuary. The literature revealed that HBB is reported in 10 protected areas of Himachal Pradesh (Sathyakumar 2001, Rathore 2008). The distribution of HBB occurs in a narrow elevation range between 2500-5000 m. Due to its prevailing threats, the population of the species is declining, which demands urgent conservation. The study suggests that suitable habitats of HBB will decrease by more than 70% from its entire distribution range by 2050 (Mukherjee et al 2021). The study has also highlighted that most of the suitable habitats within the Kanawar Wildlife sanctuary may get lost due to climate change impact (Mukherjee et al 2021). Furthermore, the retaliatory killing of HBB by humans due to bear-human conflict is another threat to the declining population of HBB. Studies revealed that species are sensitive to anthropogenic disturbances (Sharief et al 2020). The present study confirms the presence of HBB from Kanawar Wildlife Sanctuary, which is a new record from that area. Knowing the increasing threats of HBB in IHR, strongly suggest monitoring of HBB population in Kanawar Wildlife Sanctuary for conservation and management implications.

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Taxonomic and Trophic Characterization of Benthic Macroinvertebrate Fauna in Headwaters of Eastern Himalaya

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***Abstract*:** A taxonomic and functional feeding group study of benthic macroinvertebrate fauna was conducted in the headwater streams/Kholas of eastern Himalaya *viz.* Rimbi Khola and its tributaries (Nambu Khola, Limbuni Khola and Lingsur Khola). Samples were collected twice in a year during January and February 2017. Standard methods were adopted for the samples collection and identification of benthic macroinvertebrate fauna. The density ranged from 264 to 792 indiv.m^{-2} among Rimbi Khola and tributaries. Seventeen taxa were recorded with highest taxonomic richness in Rimbi Khola compare to the tributaries. Leptoceridae was most abundant taxa in all streams/Kholas followed by Hydropsychidae, Leptophlebidae and Baetidae. CCA identified discharge, current velocity and substratum as most important factors in streams/Kholas, while different characteristics taxa were determined by PCA. These variations in environmental variables and characteristics taxa were attributed to proximate factors in each river. However, trophic characterization indicated that all the streams/Kholas were dominated by shredders. Collectors were second most abundant trophic group in the Rimbi, Limbuni and Lingsur Khola, while scraper in Nambu Khola. Nambu Khola was different from rest of the streams due to variation in dominant trophic groups, because of open stream system and high periphytonic growth.

Keywords: Eastern Himalaya, Discharge, Leptoceridae, Shredders, CCA

The aquatic invertebrates have the ability to clean rivers as they utilize the organic and detritus matter. The benthic invertebrates can be used as a barometer of overall biodiversity in aquatic ecosystems (Chatzinikolaou et al 2006). They are an important and integral part of any aquatic ecosystem as they form the basis of the trophic level. The negative effects of pollution in the community structure can in turn affect trophic relationships. The study of structural composition of benthic macroinvertebrate fauna is governed with various physico-chemical factors such as water quality, sediments quality and biological factors i.e., competition and predation. In view of above global studies, various Indian studies have been conducted in the rivers and streams of different geographical regions like Himalaya (Nautiyal et al 2015, Semwal and Mishra 2019, Mishra and Prasad 2020), the central Highlands (Mishra and Nautiyal 2013, Mishra and Nautiyal 2016, Nautiyal et al. 2017, Mishra and Nautiyal 2017) and in the Western Ghat (Dinakaran and Anbalagan 2010, Santosh et al 2014). Though many studies have been performed in the lesser Himalayan region, but very sparse study is available in the rivers of north east Himalaya and especially in the rivers proposed for hydroelectric project. Therefore, a study was conducted on the Rimbi Khola (a tributary Rangit River basin) at proposed dam site (Rangit-II Hydroelectric project) to determine the structural and functional composition of the benthic invertebrate fauna and this will be a reference study after commencement of hydroelectric project.

MATERIAL AND METHODS

Study area: The Rimbi Khola originates from Lachhmi Pokhari and Lam Pokhari lakes as Chhinjyum Khola and drains the forested areas through Pale Khola on its left bank and Longman Khola on its right bank (Fig. 1). The Rimbi Khola receives water from a number of streams like Nambu Khola, Limbuni Khola and Lingsur Khola before drain in to Rathong Chhu (tributary of the Rangit river Basin).The total catchment area of Rimbi Khola above the proposed dam site is 120 sq km. The catchment is of small size and fan shaped. The Rimbi Khola catchment has a good forest cover and about 8094.95 hectare of the catchment area is covered with forest to the proposed dam site. However, the major part (49.78%) of the total forest land is covered with open forest. The dense forest covers only 17.56% of the total forest around the catchment area to the dam site. The alpine scrub covers 9.37%, while 5.97% is under scrub of the total catchment. A large part (11.17%) of the catchment is under barren/ rocky land and snow/ glaciers covers. There are few lakes viz. Lachhami Pokhari, Lam Pokhari, Sukia Pokhari and Ghuniah Pokhari are famous, covers only 0.16 hectare of the catchment (Anonyms 2009). A rapid survey of the Rimbi Khola and its tributaries was made up and downstream of the proposed dam site. The sampling was performed at 4 sites (RK1 to RK4) in the Rimbi (Fig. 1). The macroinvertebrate community (with proven indicator value) was sampled for determining density, taxonomic composition, abundances and trophic characterization by using standard methods.

The extensive samples were collected during the lean season twice in a year (January and February 2017) which is most productive period because of low depth and high penetration of the sunlight, which cause high density of the producers and ultimately the consumers. Twenty quadrates were collected from each sampling locations. The physico-chemical parameters like water temperature (Mextech, multi meter), pH (Hanna portable digital meters) and conductivity (LABTRONICS model – LT23) were recorded at each station. The dissolved oxygen (DO), alkalinity, hardness, nitrate and phosphate were measured by titrimetric Winkler's method (APHA 1992).The sampling rationale, process and analysis were performed by using standard protocol (Mishra and Nautiyal 2011, Nautiyal et al 2017). The standard methods were used to determine trophic category (Ramusino et al 1995, Cummins et al 2005).

RESULTS AND DISCUSSION

The physical and chemical characteristics varied among the station in the Rimbi Khola and its tributaries. The water temperature slightly decreased from RK1 (13.5°C) to RK4 (13°C). However, pH and conductivity increased from 6.9 to 7.3 and 50 to 60 (µC), respectively from RK1 to RK4.The hardness decreased from RK1 (60.15 mg/l) to RK4 (30.08 mg/l). The alkalinity decreased from RK1 (55.04 mg/l) to RK3 (47.87 mg/l) and increased at RK4 (55.04 mg/l). The value of dissolved oxygen was almost same among the stations (Table 1). However, in tributaries the pH, conductivity and hardness were observed to be high in Limbuni Khola

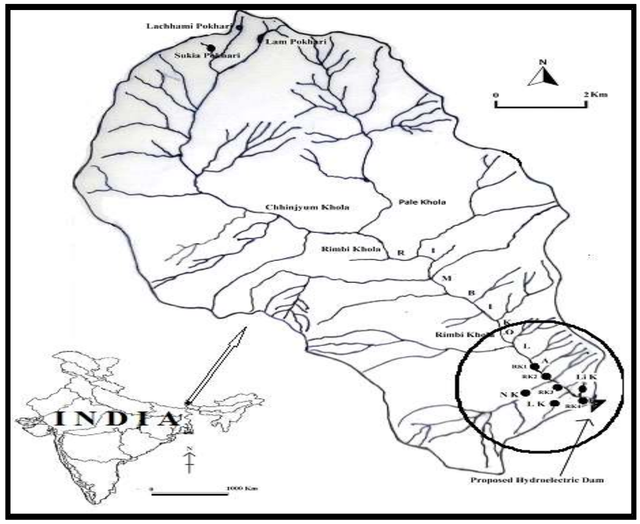

Fig. 1. Location of Rimbi Khola drainage system in India. The encircle portion indicate the sampling point at the Rimbi Khola and tributaries. Acronyms: RK1, RK2, RK3, RK4 –Rimbi Khola stations, NK-Nambu Khola, LK-Limbuni Khola, LiK-Lingsur Khola

Table 1. Geographical locations and physico-chemical characteristic at various stations

Parameters	Rimbi Khola				Tributaries		
	RK1	RK2	RK3	RK4	NK	LK	Lik
Latitude (°N)	27°19'9.9"	27°21'33.9"	27°18'45"	27°19'58.5"	27°19'10.2"	27°18'41.7"	27°20'5"
Longitude (E)	88°09'37"	88°11'55.4"	88°10'59"	88°13'43"	88°09'38"	88°10'47"	88°13'5.5"
Altitude (m asl)	1432	1405	1275	1040	1454	1345	1180
River depth range (m)	0.35-1.5	0.45-.95	0.20-0.50	0.30-0.70	0.25-0.45	0.15-.45	0.13-0.40
Sampling Depth (m)	0.35	0.45	0.45	0.40	0.25	0.30	0.28
River width (m)	30	30	50	34	20	11.5	18
Width water channel (m)	19	9	20	20	1-8	5	6.3
Transparency (%)	100	100	100	100	100	100	100
Current velocity (ms^{-1})	0.819	0.46	0.936	0.63	0.232	0.621	0.94
Discharge (m^3s^{-1})	2.722	4.055	4.055	5.895	0.408	0.264	0.694
Substratum	R	RBC	RBC	RB	R	RBC	R
WT °C	13.5	12	12	13	12	14.5	15
pH	6.9	6.6	6.9	7.3	6.8	7.2	6.6
Conductivity µC	50	50	65	60	60	80	50
Alkalinity (ppm)	55.038	55.04	45.87	55.04	64.21	55.04	64.21
Hardness (ppm)	60.150	60.15	37.59	30.08	45.11	67.67	37.59

Rimbi Khola (RK 1-RK4), Nimbu Khola (NK), LK (Limbuni Khola), LiK (Lingsure Khola). Acronyms: R- Rock, B- Boulder, C-Cobble compared to Nambu Khola and Lingsur Khola.

Benthic macroinvertebrate fauna: The benthic macroinvertebrate density increased from RK1 (264 indiv.m^{-2}) to RK2 (506 indiv.m^{-2}) to RK3 (792 indiv.m^{-2}) but suddenly decreased at RK4 (440 indiv.m^{-2}) in the Rimbi Khola. However, among the tributaries, the highest density was observed in Ligsure Khola (737 indiv.m^{-2}) followed by Nambu Khola (682 indiv.m^{-2}) and Limbuni Khola (385 indiv.m^{-2}). Though all these streams belong to 1st order in headwater zone but their total density varied. The variation in the total density among the headwater streams was also evident in USA (Viosca 2007). Total 17 taxa (richness) were recorded, 15 from Rimbi Khola and 12 taxa from tributaries. Out of these, 9 taxa were common between Rimbi Khola and tributaries. The richness was highest in the Rimbi Khola (15) compared Limbuni Khola (8), Lingsur khola (6) and Nambu Khola (Table 2). The highest richness and density in the Rimbi Khola as compared to the tributaries, attributed to increase in the in the stream order, discharge and substrate heterogeneity. The reduction of substrate particle size provides variety of substrate for various taxa (Molokwu et al 2014, Mishra and Nautiyal 2016).

The Leptoceridae was most abundant taxa among all the water bodies. However, in Rimbi Khola, Chironomidae (25%) was second most abundant taxa followed by Baetidae Heptageniidae, Leptophlebiidae while Hydropsychiidae (29%) was second most abundant taxa followed by Heptageniidae (19%) in the tributaries (Fig. 2). The faunal assemblage patterns varied in Rimbi Khola as Leptoceridae-Chironomidae-Leptophlebiidae (RK1), Leptoceridae - Heptageniidae (RK2), Leptoceridae (RK3)and Leptoceridae-Baetidae-Heptageniidae-Chironomidae (RK4), but almost same in the tributaries i.e. in Nimbu Khola (LPR-HP-HY), Limbuni Khola (LPR-HY-HP) and Lingsur Khola (LPR-HY). The abundance of Leptoceridae at all the stations of Rimbi Khola and also in tributaries was attributed to the identical temperature profile as the ambient temperatures are moderated by subtropical humid climate. The fluctuations are also minimized in the snow fed streams as they receive constantly cold waters. Similarly, the taxa Heptageniidae was persist in most of the assemblages among the stations of Rimbi Khola and tributaries, was attributed to decline of water depth, favour more growth of bottom flora. Trichoptera were abundant in the streams of Mandakini basin in west Himalaya (Nautiyal et al 2015).

Table 2. Taxonomic richness and distribution of benthic macroinvertebrate fauna in the Rimbi Khola and its tributaries

Taxa	Rimbi Khola	Tributaries		
		Nambu Khola	Limbuni Khola	Ligsur Khola
Taxonomic richness	15	5	8	6
Brachycentridae			+	
Hydropsychiidae	+	+	+	+
Hydroptilidae	+			
Leptoceridae	+	+	+	+
Limnephilidae	+			
Psychomyiidae	+		+	
Baetidae	+	+		+
Ephemerellidae	+	+		
Heptageniidae	+	+	+	+
Leptophlebiidae	+		+	+
Chironomidae	+			
Simulidae	+			+
Tabanidae	+			
Perlidae	+		+	
Psephenidae	+			
Hemiptera	+			
Lepidoptera			+	

Trophic characterization: The trophic categories of benthic macroinvertebrate fauna among the headwater streams indicated that shredders were most dominant category in all the streams; Rimbi Khola (58.5%), Nambu Khola (70%), Limbuni Khola (Fig. 3). However, the second dominant category was gathering collectors in the Rimbi Khola (27%) followed by Lingsur Khola (11%), Scrapers (18%) in Nambu Khola and filtering collectors (35%) in Limbuni Khola. The dominance of shredders among the streams was attributed to availability of food as coarse particulate organic matter

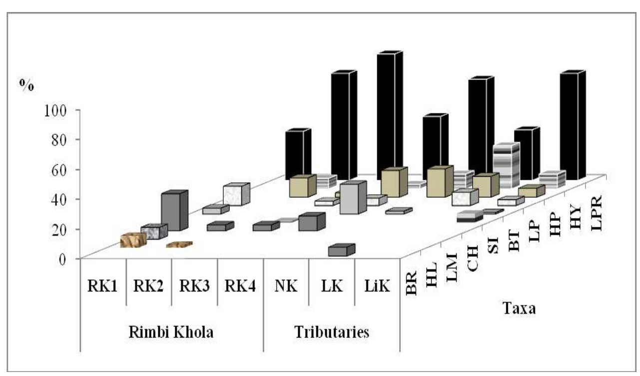

Fig. 2. Taxonomic compositions of abundant benthic macroinvertebrate taxa (5%) at different stations in Rimbi Khola and tributaries. Acronyms: BR-Brachycentridae, HL-Helidae, LM-Limnephildae, CH-Chironomidae, SI-Simulidae, BT-Baetidae, LP-Leptophlebiidae, HP-Heptagenidae, HY-Hydropsychidae, LPR-Leptoceridae. The rest are similar as Figure 1.

(CPOM). The detritus feeders (caddis fly) were abundant in the streamlets discharging into the Rimbi as they flow through forested area. Functionally, the abundance of gathering collectors is known to utilize fine particulate organic matter (FPOM) which is available after the degradation of CPOM. The collectors (gathering + filtering) were observed to be most abundant trophic group in the Rimbi and its two tributaries (Limbuni and Lingsur), while in tributary of Rimbi Khola (Nambu khola), scraper was second most dominant group. The variation in second dominant trophic group of Nambu Khola from rest of the streams was due to presence of open stream system, which allows the penetration of sun rays for periphytonic growth.

The grazing and detritus food chains exist in the headwaters of the Bhagirathi river (Nautiyal 2010). The abundance of shredders was also evident in the headwaters

Table 3. Canonical correspondence analysis along with taxon –environmental correlation

Axes	1	2	3	4	Total inertia
Eigenvalues	0.346	0.254	0.186	0.101	0.999
Taxon-environment correlations	1.000	1.000	1.000	1.000	
Cumulative percentage variance of taxon data	34.6	60.0	78.6	88.8	
Sum of all eigenvalues					0.999
Sum of all canonical eigenvalues					0.999
Conditional Effects					
Variables	λ-Value	P-value	F-Value	% Variation	
Discharge	0.21	0.268	1.33	24	
Current velocity	0.24	0.204	1.77	21	
Substrate	0.18	0.266	1.48	18	
Conductivity	0.14	0.378	1.19	14	
pH	0.14	0.324	1.62	14	
Sampling depth	0.09	.000	0.00	9	

Variable indicates percentage of variation cause in the distribution of benthic macroinvertebrate fauna

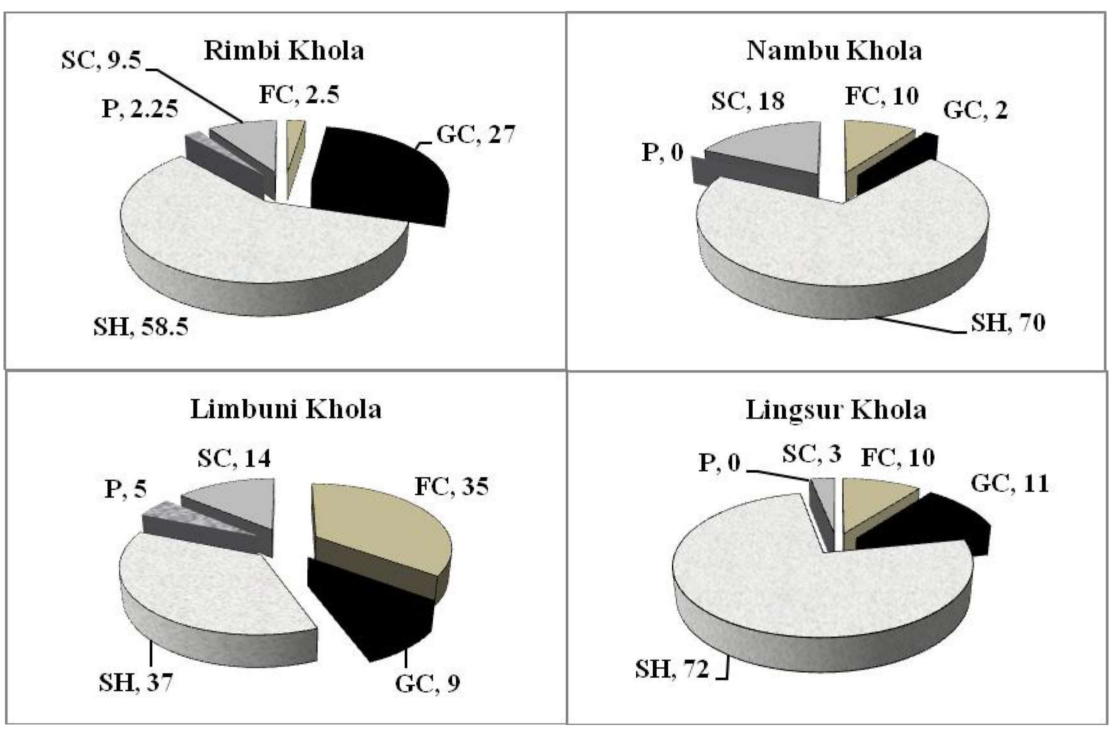

Fig. 3. Percentage of functional feeding groups (FFG) in the river Rimbi Khola and its tributaries. Acronyms: SH-Shredder, SC-Scraper, GC-Gathering Collector, FC-Filtering Collector, P-Predator

of Vindhyan river, the Paisuni (Mishra and Nautiyal 2011). According to the River Continuum Concept (RCC, Vannote et al 1980), the streams/rivers is categorized into headwaters (orders 1-3), medium-sized streams (4-6), and large rivers (>6). The Rimbi Khola and its tributaries were shredder dominated belong to 1-2 order and represent the headwater category. The predominance /dominance of shredders support to RCC model.

Role of environmental variables and characteristic taxa: Factors governing distribution of invertebrate fauna in Rimbi Khola and its tributaries were explored through CCA. The cumulative percentage variance of taxon - environmental variation for CCA axes 1 and 2 was 44.4 and 21.6 %, respectively. Among the array of variables, discharge, current velocity, substrate (and conductivity were most important variables responsible for invertebrate distribution (Table 3). The taxa associated with discharge were Chironomidae, Baetidae, Leptophlebidae, while Heptageniidae - Leptoceridae were associated with current velocity and Hydropsychidae with substrate (Fig. 4). In the lesser Himalayan river, forest type, land use and substratum were most important factors for invertebrate distribution (Nautiyal et al. 2015). Current velocity was most important variables in the rivers of Vindhyan mountain region (Mishra and Nautiyal 2011). In PCA, the eigen values for axis 1 (λ_1=0.513) and 2 (λ_2=0.164) explained cumulative variance in taxonomic composition and taxon-environmental

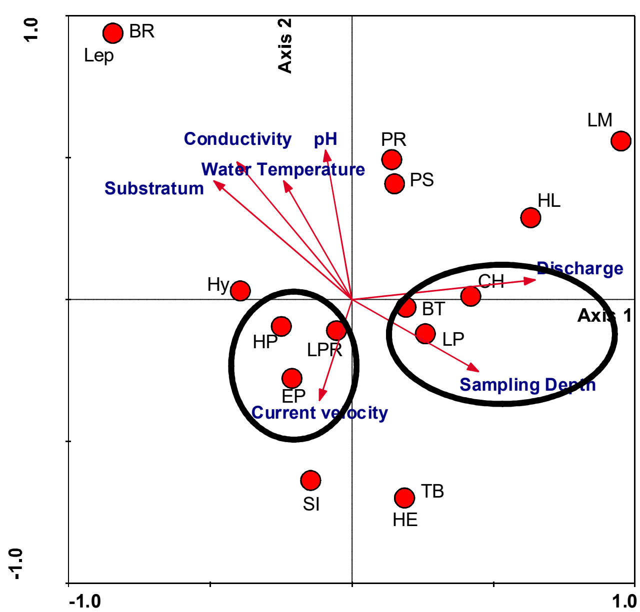

Fig. 4. Relationship between benthic macroinvertebrate fauna and environmental variables. Acronyms: PS-Psephenidae, EP-Ephemerellidae, TB-Tabanidae, PR-Perlidae. The rest are similar as Figure 2.

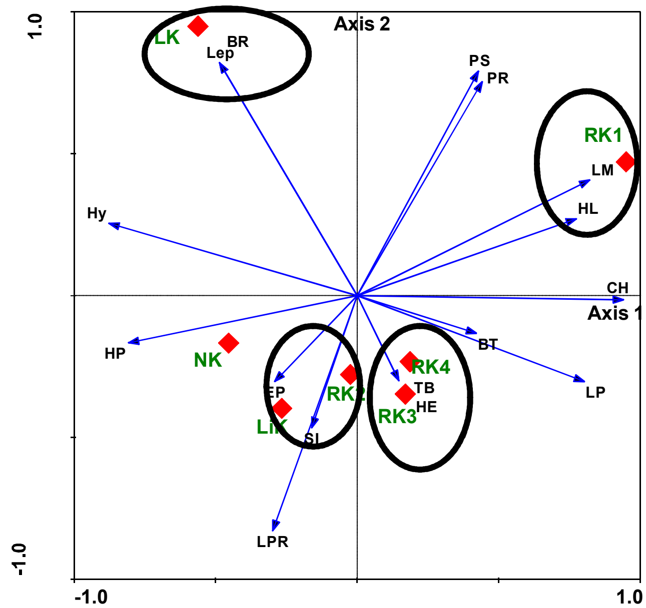

Fig. 5. Characteristics benthic macroinvertebrate taxon at each station of Rimbi Khola and tributaries. The rest are similar as Figure 2. .

relationships in the stream and caused 51.3 and 16.4% variation in the taxon-site relationship, respectively. The characteristic taxa varied at different stations like Limnephilidae - Hydroptilidae at RK1, Tabanidae - Helidae at RK3 and RK4, Brachycentridae – Lepidoptera at LK and Ephemerilidae at LiK (Fig. 5).

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Adsorption Iron Ions from Aqueous Solution onto Duck Feathers Modification using $Na_2S_2O_5$

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***Abstract*:** Adsorption Iron Ions from Aqueous Solution onto Duck Feathers Modification using $Na_2S_2O_5$ has been done. The objectives were to study modification reactions, to determine adsorption capacity. Modification duck feather adsorbents done by 2 g of duck powder was dissolved in 25 mL $Na_2S_2O_5$ with a concentration of 0.025, 0.100, 0.175, 0.250, 0.325, 0.400 and 0.475M, then placed on hotplate stirrer at 50° C and stirred for 24 hours. The reaction mechanism was studied through functional group analysis using Fourier Transform Infrared (FTIR) spectroscopy**.** The results showed that adsorption capacity of Fe (III) by adsorbent of duck feather optimum with 3.25M and adsorption capacity before and after modification were 83.333 mg/g and 129.870 mg/g.

Keywords: Adsorption capacity, Iron, Duck feather, $Na_2S_2O_5$

The increase of duck breeding efforts can lead to increase produce duck feather waste. Based on data from the Livestock Service Office of South Kalimantan Province in 2019, the number of ducks is 4,786,370 population, are generated can be estimated that a total of 239 tons of duck feather waste. These duck feather to be used as adsorbents for absorbing metals in industrial wastewater. Related studiess on chicken feather modified with 6% CH_3OH and 2% HCl, adsorption capacity As(III) of 90,6 mg/g (Khosa, Wu and Ullah, 2013) and duck feathers on Fe(III) modified with 25% CH_3OH and 4% HCl adsorption capacity of 125 mg/g (Utami, Cahyono and Susanto, 2020). Duck feathers modified with NaOH have a relatively high concentration of Cu^{2+} and Cr^{6+} adsorption capacity (Xiangyu Jin, Lu Lu, Haibo Wu, Qinfei Ke, 2013). The adsorption capacity of Pb by chicken feathers is 1.9 g/L and by duck feathers 2.3 g/L (Kumari and U. Kiran Babu 2011). Copper adsorption with Dromaius novaehollandiae feathers and chitosan composite maximum adsorption was found to be 93.91% (Kumari and Sobha 2015). Sodium sulfide is used as a less expensive chemical substitute for keratin extraction. Research on modification of keratin with Na_2S and immersion in isopropyl alcohol or weak acid (13 to 50% increase) or by crosslinking of formaldehyde or glutaraldehyde (24 to 40% increase) (Poole and Church 2015). Pb^{2+} ion adsorption using $Na_2S_2O_5$ modified chicken feathers resulted in an adsorption capacity of 11.16 mg/g (Kong et al 2014). Cu adsorption on electroplating waste using modified $NaOH/Na_2SO_3$ chicken feathers resulted in an adsorption capacity of 38.43 mg/g (Sunarto 2014). Modification of keratin with sodium meta-bisulfite showed that the optimum level was 0.2 M with a yield of 87.6% (Ji et al 2014).

Previous research on the reaction mechanism of keratin using sodium sulfide includes the extraction of keratin from wool through sulfitolysis, with the formation of cysteine (R-S-H) (Aluigi et al 2014). The results of studies of keratin from rabbit hair showing typical absorption at 3420, 1650, 1543, 1240, and 685 cm^{-1}, showing NH(II), amide III (CN) and amide IV, respectively (Wang et al 2018). The results of another study showed the presence of disulfide bonds in keratin and formed SH (Zhang et al 2013) and disulfide bonds (S-S) from being completely broken down in hydrolysis (Hamouche et al 2018). For this reason, it is necessary to conduct research on the modification of keratin from duck feathers using $Na_2S_2O_5$ and study the reaction mechanism and study its adsorption.

MATRIAL AND METHODS

Preparation duck feather powder: One kg of duck feather is washed with detergent and water, then dried in the sun and the smell is gone, then heated with oven for 24 hours at 50°C to remove the remaining water content. The duck feather is milled using grinder and sieved using a 40 mesh sieve. 20 g of duck powder was soaked with 300 ml of 0.1 M HCl and 300 mL petroleum ether for 24 h, then washed with distilled water, then filtered using a Buchner filter. The obtained residue is dried with oven at 60°C. The results obtained were analyzed by FTIR.

Modification of duck feather adsorbents: 2 g of duck powder was dissolved in 25 mL $Na_2S_2O_5$ with a concentration of 0.025; 0.100; 0.175; 0.250; 0.325; 0.4000; and 0.475M, then placed on hotplate stirrer at 50°C and stirred for 24 hours. The mixture is filtered using a Buchner filter and washed with distilled water to neutral. A total of 0.25 g of duck powder was done by adsorption test on Fe 100 ppm solution. The mixture is homogeneous using a magnetic stirrer for 100 minutes, and then filtered using a Buchner filter. The results obtained were analyzed by Atomic Absorption Spectrophotometer (AAS-GBC Avanta Ω) in Industry Research and Standardization Center Banjarbaru, South Kalimantan and analyzed by Fourir Fourier Transform Infrared (FTIR 8201PC Shimadzu, Japan) spectra of samples were recorded in a wide range of wave number from 400 to 4000 cm^{-1} in Laboratory of Organic Chemistry and Biochemistry Department of Chemistry, Gadjah Mada University.

Determination of adsorption capacity of Fe by adsorbent duck feather: Fe solution of concentration 100, 150, 200, 250, and 300 ppm with pH 6. 50 mL Fe solution was pipetted into 5 provided erlenmeyer. A total of 0.25 g duck adsorbent is inserted into each erlenmeyer. The mixture was stirred using a magnetic stirrer for 100 minutes of contact time filtered using a Buchner filter. The results obtained were analyzed by Atomic Absorption Spectrophotometer (AAS-GBC Avanta Ω) in Industry Research and Standardization Center Banjarbaru, South Kalimantan the same procedure is performed on the adsorbent of duck that has not been activated. Furthermore, the adsorption capacity was calculated using the Langmuir and Freundlich Isotherm.

Data analysis: The data obtained based on the parameters studied and made the graph to know the amount of adsorbed metal (M), obtained from the difference of concentration M before and after adsorption by duck modified adsorbent from the measurement of Atomic Absorption Spectrophotometer. The data is then incorporated into the equation:

Ishoterm Langmuir

$$\frac{C_e}{q_e} = \frac{1}{b.K_L} + \frac{C_e}{b} \quad (1)$$

where, qe = the amount adsorbed per unit weight of adsorbent ($mg.g^{-1}$) Ce = the equilibrium concentration ($mg.L^{-1}$) K_L = the Langmuir adsorption constant, indicating the binding energy between adsorbent and adsorbate ($L.mg^{-1}$)

b = Maximum adsorption capacity ($mg.g^{-1}$)

Ishoterm Freundlich

Log qe = log KF + 1/n log Ce (2)

where, qe = Number of adsorbat adsorbed ($mg.g^{-1}$) Ce = Concentration of solution at equilibrium ($mg.L^{-1}$) KF and 1 / n = Freundlich constants of the adsorbent adsorption capacity and intensity.

RESULTS AND DISCUSSION

Adsorption of metal ions by fibrous materials such as keratin can be increased by treating it with a certain chemical, such as by adding $Na_2S_2O_5$. Activation is carried out using a 0.025; 0.100; 0.175; 0.250; 0.325; 0.400; and 0.475M. The activation of duck feather adsorbents aims to increase the number of ligands and form complexes with ferrous metal ions. The relationship between $Na_2S_2O_5$ and adsorption of duck feather adsorbent is shown in Figure 1.

From the picture, it can be seen that at the $Na_2S_2O_5$ concentration after 0.175M the condition of the adsorption capacity is stable until the concentration is 0.325M. At a concentration of .400, this begins to decrease, indicating that the $Na_2S_2O_5$ concentration is excessive, so it is no longer able to bind to keratin. The reaction that occurs between keratin and $Na_2S_2O_5$ is estimated to be seen in Figure 2, where cysteine thiol and S-sulfonation residues will occur. Sodium sulfite (SO_3-2), bisulfite (HSO_3-), and sulfite ($S_2O_5$2-) are the main sulfite compounds present in aqueous solutions and can be used for sulfitolysis. First, hydrosulfite and hydroxyl ions are formed from the reaction of sodium sulfite with water in Figure 4. In the next step, hydroxyl ions break disulfite bonds and form dehydroalanin and perthiocysteine as a propos (Poole and Church 2015).

The dehydroalanin formed from this reaction is very reactive and forms lanthionin and lysinoalanine through a cross linking with cysteine and lysine (Fig. 6). This cross-linking reaction can improve the mechanical properties of the final regeneration product, however, sodium sulfite treatment can damage the backbone of proteins, and therefore, optimizing the extraction conditions is an important step to maintain the structure of keratin (Shavandi *et al.*, 2008). Thiol

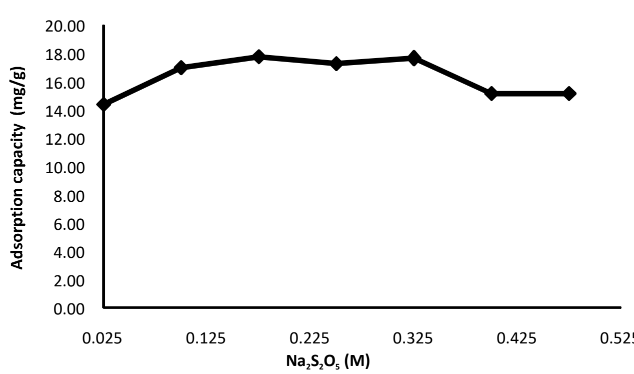

Fig. 1. Relationship between $Na_2S_2O_5$ concentration and Fe adsorption capacity

R-S-S-R + $Na^{2+}S_2O_5^{2-}$ ↔ R – S^- + R – S – $S_2O_5^-$

Keratin Sodium disulfite Cystein thiol Cystein S –Sulphonate

Fig. 2. Sulfitolysis cystin reaction by sulfite

groups (-SH) play a definite role during the grafting of the Monomer methyl Acrylate (MMA) onto the wool by providing sites for grafting. Thiol groups present on the cysteine aminoacids have been reported as the preferred sites for grafting monomers by some researchers. (Shavandi and Ali 2019). Fourier Transform Infrared Spectrophotometer is used to identify functional groups keratin from duck feather before and after modification with $Na_2S_2O_5$, and duck feather adsorbents after contact with Fe^{3+}. FTIR spectra results can be seen in Figure 7. Identify functional groups in FTIR spectra of adsorbent duck feathers before modification, after modification with $Na_2S_2O_5$, and after the adsorption process with Fe, showed in Table 1**.**

Based on Table 1, the infrared spectrum of the duck feather before modification shows an absorption of 3266 cm^{-1} indicating a symmetrical OH stretching vibration range of NH, strengthened by the presence of buckling N_2H which absorbs near 1531 cm^{-1}, SH stretching vibrations appear at 2276 cm^{-1}, 1629 cm^{-1} which marks the absorption of C=O carboxylic acid, Absorption 1235 cm^{-1} marks the stretching vibration of CO carboxylic acid and 1072 which marks CN, Based on the results of FTIR spectra it can be concluded that in duck feathers there are keratin groups, similar to research on chicken feathers and rabbit fur (Sa'adah N, R. Hastuti 2013), (Wang et al 2018).

Infrared spectrum of duck feather after modification shows 1618.80 cm^{-1} absorption which indicates the presence of C=O carboxylic acid absorption. This is reinforced by the O-H vibration in the wave number 3266.17 cm^{-1}. The FTIR spectra of the $N_{a2}S_2O_5$ modified duck feathers are in agreement with the results of keratin studies from rabbit hair

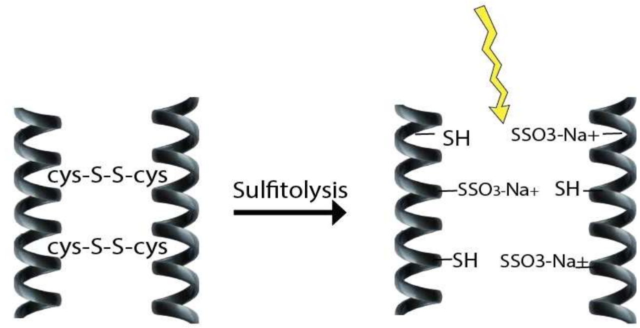

Fig. 3. Schematic diagram of the sulfitolysis reaction which breaks the strong disulfide bonds of keratin fibers

$$NaS_2O_5 + H_2O \longrightarrow 2Na^+ + HS_2O_5^- + OH^-$$

Fig. 4. Reaction of the formation of hydroxyl and hydroxyl ions from the reaction of sodium disulfite with water

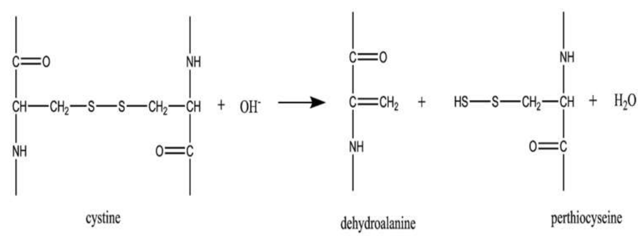

Fig. 5. Hydroxyl ion reaction breaks dehydroalanine sulfite bonds

Table 1. Analysis of functional groups in FTIR spectra of adsorbent duck feathers before modification, after modification with $Na_2S_2O_5$, and after the adsorption process with Fe

Adsorbent before modification (cm^{-1})	Adsorbent after modification (cm^{-1})	Adsorbent after the adsorption process with Fe (cm^{-1})	Reference wave numbers (cm^{-1})	Cluster function prediction
3266.42	3265.17	3273.98	3000 - 3700	O-H
2276.00	2276.00	2337.72	2300-2700	S-H
		2108.64		
1629.27	1618.80	1630.65	1500 - 1900	C=O
1531.56	1536.03	1522.60	1500 - 1650	N-H
1450.01	1448.21	1448.03	1340-1470	C-H
1396.09				
1341.74	1229.26			
1235.79	1201.46	1229.81	1000 - 1300	C-O
	1159.60			
	1077.52			
1072.32	1023.44	1023.32	900 - 1300	C-N

showing typical absorption at 3420, 1650, 1543, 1240, and 685 cm^{-1}, showing NH(II), amide III (CN) and amide IV (Wang et al 2018). The results of keratin studies from rabbit hair showing characteristic absorption at 3420, 1650, 1543, 1240, and 685 cm^{-1}, respectively showing NH(II), amide III (C-N) and amide IV. Keratin modified with a deep eutectic solvent (DES) mixture of choline chloride and oxalic acid shows the absorption of amide I, which may be associated with lower amounts of α-helical crystallites. In addition, new weak absorption bonds appear in 1317, 1170, and 1124 cm^{-1} absorption of hydrogen-bound NH groups (amide A, stretch NH), amide I (C = O stretch), amide II (flexible NH), amide III (CN stretching), and IV amide. Absorption bands appear at 1317, 1170, and 1124 cm^{-1}, which are associated with disulfide bonds in keratin and form SH (Zhang et al 2013) Disulfide bonds (S-S) from are broken down thoroughly in the hydrolysis. Keratin modified by Na_2S, the FTIR results showed that the carboxylic acid group in the sample was at wave numbers 1261 and 1262 cm^{-1}. Amides at 3369 and 3376 cm^{-1}, and at wave numbers 2361 cm-1 indicate the presence of amines (Yilmaz et al 2019). Keratin extraction with NaOH shows that the main structures of amide I, amide II and amide III are maintained, meaning that the peptide bond (−CONH) is not greatly affected in the process of base hydrolysis (Hamouche et al 2018). At wave numbers 3265 cm^{-1}, there are OH and NH (amide A) stretches, and at 2916 cm^{-1} is associated with symmetrical stretch CH_3 vibrations, while amide I is connected mainly to C=O stretch vibrations and occurs in the range (1700-1600 cm^{-1}). Modification of keratin from chicken feathers using methanol in an atmosphere acid showed that there was a change in peak sharpness at 1653 cm-1 and a significant change at 1738 cm^{-1} where esterification occurred in O of the carbonyl group, in the range of characteristic absorption (1750-1717 cm^{-1}) (Khosa, Wu and Ullah, 2013).

In duck feather adsorbents that have been contacted with Fe solution, the O-H vibration occurs at a wave number of 3425.58 cm^{-1}. The S-H stretching vibration appeared at 2276.00 cm^{-1}. The bending N-H velocity of NH_2 appeared at 1531.56 cm^{-1}. In the duck feather adsorbent that has been contacted with Fe solution, the bending N-H vibration appears at the wave number 1522.60 cm^{-1}. The C-O stretching of the ester appeared at 1159.60cm^{-1} and shifted to 1235.79 cm^{-1}. The C-N vibration gave the absorption at wave number 1023.32 cm^{-1} and shifted to 1072.32 cm^{-1}. Alteration of the functional summits of the functional groups is suspected to have proved the interaction at the time of the amodification process and when contacted with Fe^{3+}. This ligand will donate the free electron pairs and occupy the empty orbitals in the sub duster of the ferrous metal (central metal ion). Donation of ligand pairs of electrons to iron metal ions results in covalent coordination bonding. The possible scheme of Fe metal bond with keratin is shown in Figure 5. Like structure (an intrachain complex in wool keratin) could be formed if two carboxyl groups of two neighboring protein chains matched. Taking into account the expected value of such fragments frequency (50 per g of wool), Cu(II) uptake associated with the carboxylic residues can reach 150–300 μmoles/g of wool (Nikiforova, Kozlov and Islyaikin 2019).

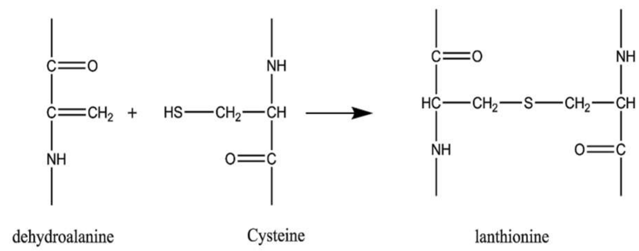

Fig. 6. Arrangement of lanthioalanin by the addition of cysteine to dehydroalanin

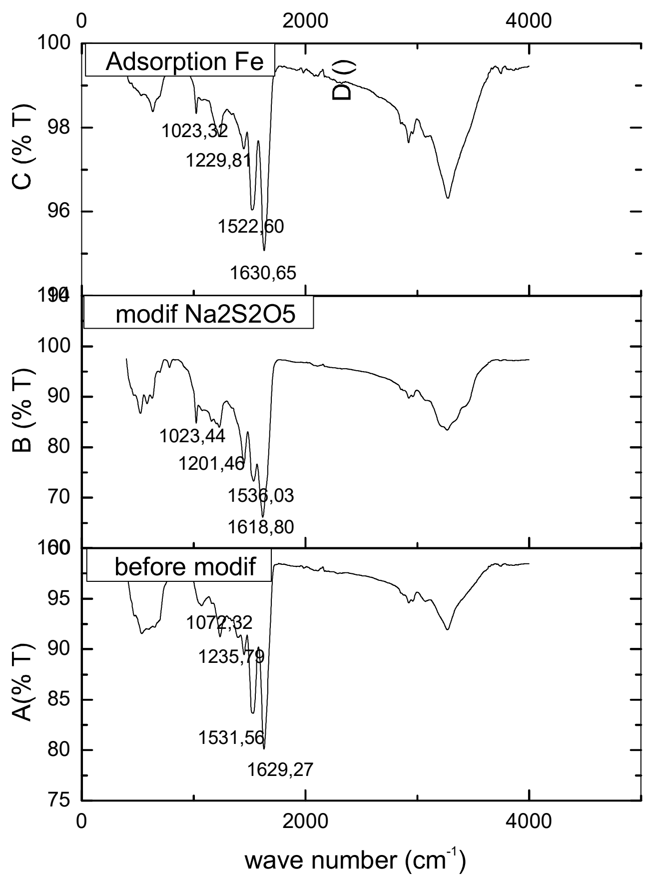

Fig. 7. FTIR spectra of duck finger adsorbent (A) before modification, (B) after modification with $Na_2S_2O_5$ and (C) after Fe^{3+} adsorption

Determination of adsorption isotherm model is done to know the process of adsorption of the iron metal ion by duck adsorbent modification with $N_{a2}S_2O_5$. The adsorption isotherm model used to describe the adsorption process on the solid surface, Langmuir and Freundlich isotherms. The adsorption capacity of Fe metal ions by duck adsorbent before and after modification is shown in Figure 9 and 10.

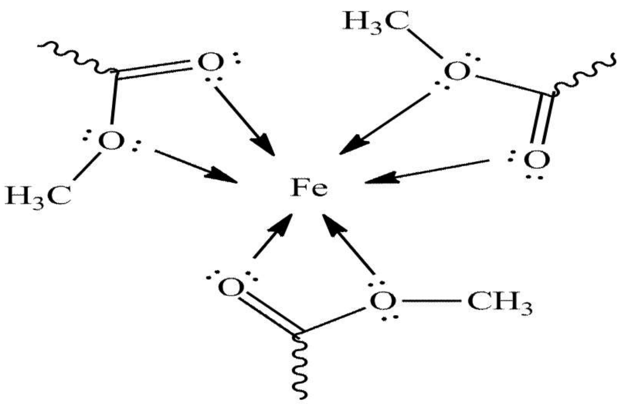

Fig. 8. Schematic possibilities of Fe metal bond with keratin

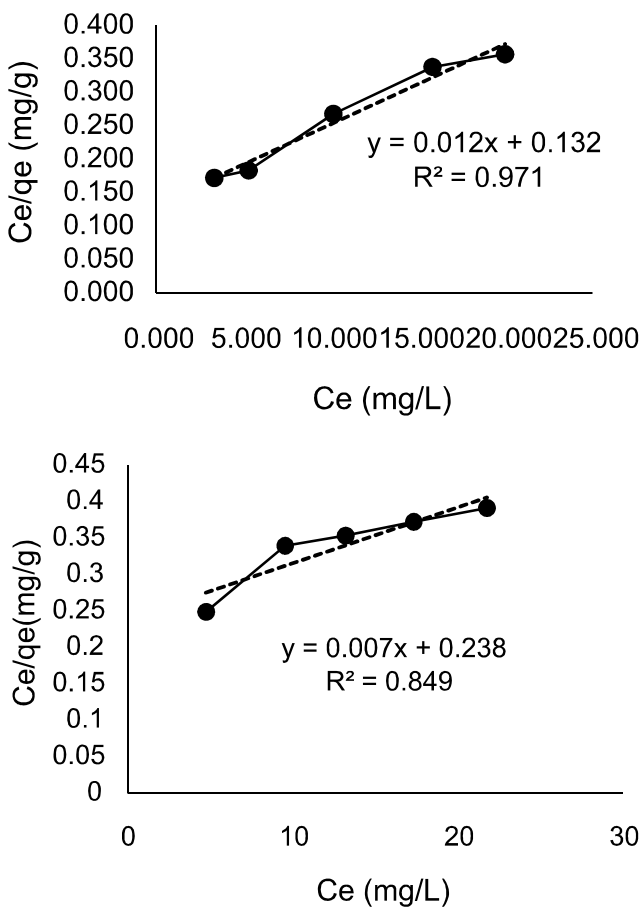

Fig. 9. Langmuir isotherm adsorption of Fe duck feather adsorbent before and after modification $Na_2S_2O_5$

Figures 9 and 10 shows that the adsorption of Fe on duck feather follows Langmuir and Freundlich isotherms. The adsorption ion of iron metal by adsorbent of duck feather follows the equation having the value of R^2 close to number 1. The result of the comparison of R^2 value indicates that the Langmuir and Freundlich isotherms equation have R^2 value close to 1. This suggests that the duck's adsorbent surface is homogeneous and adsorb only one adsorbate molecule for each of its adsorbent molecules, as well as the Langmuir isotherm, in general, would be preferable to apply to chemical adsorption. The adsorption of Fe by chitosan (Radnia, Ghoreyshi and Younesi, 2011), Rice Hush ash (RHA) (Zhang et al 2014) iron by fly ash from coal (Irawan and Rumhayati 2014), and kaolin based nanocomposite (Shaban, Hassouna and Nasief, 2017) also follows Langmuir and Freundlich isotherms. Isotherm Langmuir > Temkin >Freundlich biosorption iron using oil palm biomasses (Khosravihaftkhany and Morad 2013). The research of adsorption dye textile by father according isotherm Langmuir and Freundlich are tantrasine and malachite green, azo dye amido black 10B, and azo dye brilliant yellow and textile dyes by hen feathers performed by (Mittal, Thakur and Gajbe 2012) (Mittal, Thakur and Gajbe 2013). Methylene

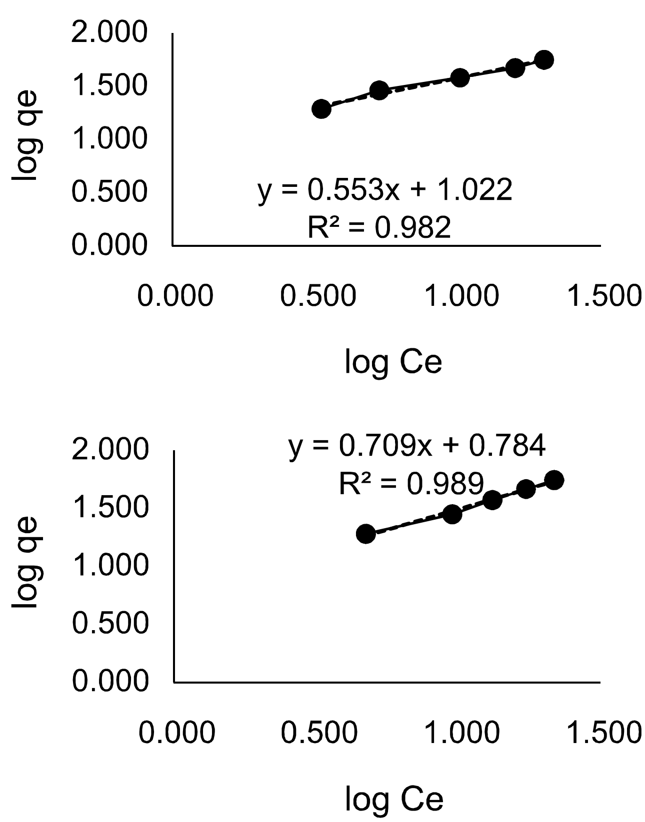

Fig. 10. Freundlich isotherm adsorption of Fe duck feather adsorbent before and after modification $Na_2S_2O_5$

Table 2. Data of equations and ditermination coefficients (R^2) for adsorption of Langmuir isotherms and Freundlich isotherms

Adsorbent	Isotherm Langmuir Equation	q_{max} (mg/ g)	R^2
Before modification	y = 0.012x + 0.1323	83.333	0.972
Modification $Na_2S_2O_5$	y = 0.0077x + 0.2383	129.870	0.849

blue by feather keratin (Chowdhury and Saha 2012a), amoxicillin by chicken feather carbon (Li et al 2017). To calculate the adsorbent adsorption capacity of modified $Na_2S_2O_5$ duck further obtained from Langmuir and Freundlich isotherm equation obtained from the relationship between Ce log and log qe. The results of the equation and the ditermination coefficient (R^2) and the adsorption capacity (qmax) for the adsorption can be seen in Table 2.

Table 2 shows the graph of the relationship between Ce/me to the concentration of the iron solution. so that the value of adsorption capacity for Fe^{3+} ions by $Na_2S_2O_5$ modified duck further that 129.870 mg/g. Compared with previous studies conducted. Where the adapted capacity of the chicken feather is 6% CH_3OH and 2% HCl in As (III) only 96.00 mg/g (Khosa, Wu and Ullah, 2013). The other research results adsorption of copper with Dromaius novaehollandiae feathers and chitosan composite. was adsorption capacity of 18.78 mg/l (Kumari and Sobha, 2015). The adsorption of lead using biopolymer feather chicken on lead (Pb) of adsorption capacity was 1.9 g/l. and lead (Pb) adsorption by duck feather adsorption capacity was 2.3 g/l (Kumari and Babu 2011). Adsorption of Methylene Blue by chicken feather with the adsorption capacity of 134.76 mg /g (Chowdhury and Saha, 2012b). Maximum sorption capacity (q_{max}) by chicken feather and magnetized activated carbon were 76.3, 56.5, 113.3, 32.6, and 45.5 respectively for Cd^{2+}, Cu^{2+}, Pb^{2+}, Ni^{2+} and Zn^{2+}, showing the suitability of the new sorbent for water and waste-water treatment usages (Rahmani-sani et al 2020). The maximum adsorption capacities were ACF powder-hydrolyzed feathers (PHF) bio-modifier and *Trapa natans husks* (TH) are 50.2 mg/g for Cd(II) and 46.7 mg/g for Ni(II)) were higher than those of AC (33.8 mg/g for Cd(II) and 31.15 mg/g for Ni (II) (Yin et al 2019).

CONCLUSIONS

Analysis of keratin from duck feathers using $Na_2S_2O_5$ showed that keratin had formed which was indicated by a shift in wavenumber in the FTIR spectrum. Adsorption capacity of Fe(III) by adsorbent of duck feather modified with $Na_2S_2O_5$ optimum with 3.25M and adsorption capacity before & after modification were 83.333 mg/g and 129.870 mg/g. Adsorption of Fe on duck feather modified with $Na_2S_2O_5$ follows Langmuir and Freundlich isotherms. Duck feathers that have been modified with $Na_2S_2O_5$ can be used as adsorbents in wastewater containing heavy metals

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Biochemical Composition of Few Commercially Important Food Fishes of River Sutlej in Punjab

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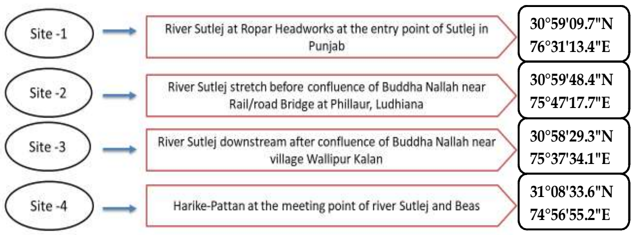
***Abstract*:** River Sutlej is under pronounced pressure of anthropogenic activities and this study was devised to check whether the prevailing environmental conditions of the Sutlej have affected the biochemical composition of the few commercially important food fish species. The study was conducted from February 2018 to June 2019 to evaluate the biochemical composition of fish flesh (Protein, lipids, carbohydrates, ash, and moisture) of commercially important fish species *Labeo rohita, Cyprinus carpio, Sperata seenghala,* and *Wallago attu* owing to their high consumer preference. Fish samples were collected from four different designated sites i.e., Ropar Headworks, River Sutlej before confluence of Buddha Nallah at Phillaur, River Sutlej after the confluence of Buddha Nallah at Wallipur Kalan and Harike-Pattan where river Sutlej meets with Beas in plastic zipper bags (in triplicate) at two-monthly intervals . The percent moisture, lipid, protein, ash and carbohydrates ranged from 75.25-81.45, 1.13-2.50, 13.59-19.89, 1.20-1.59 and 1.12-1.15 at Ropar Headworks, River Sutlej before confluence of Buddha Nallah at Phillaur, River Sutlej after the confluence of Buddha Nallah at Wallipur Kalan and Harike-Pattan, respectively. However, protein, lipid, and carbohydrates were lower at River Sutlej after the confluence of Buddha Nallah at Wallipur Kalan as compared to other sites irrespective of fish species and seasons which might be due to the direct discharge of polluted water interfering with normal physiology of fish affecting the biochemical profile.

***Keywords*:** Biochemical composition, Fishes, Punjab, Sutlej, Buddha Nallah

Fish is considered an important source of high-quality balanced and easily digestible protein profile, besides being a rich source of polyunsaturated fatty acids (Shamsan and Ansari 2010). The changes in proximate composition of fishes are often related to their habitat and thus affecting their nutritive value (Devi and Vijayaragahwan 2001). Protein and lipids are traditionally used as an indicator of the nutritional value as well as the physiological condition of the fish (Moghaddam et al 2007, Aberoumad and Pourshafi 2010). Proximate composition of fishes ranges between 65-90% water, 10-22% protein, 1-20% lipid and 0.5-05% minerals (Nair and Mathew 2000). It depends mostly on the season, size, age, sex, reproductive cycle, and breeding season (Singh et al 2016, Muchtadi et al 2016) as well as environmental factors such as temperature, habitat, availability, and source of food (Herawati et al 2018). The river Sutlej being a major source of capture fisheries in Northern India, possess a great diversity of fish species. But in recent times, due to natural and anthropogenic sources, it is under pronounced pressure of pollution which has deteriorated its water quality (Pandiarajan et al 2019) to the extent that it is unfit for any use. The deteriorating water quality of river Sutlej is a matter of concern (Kaur and Singh 2017) since it might be affecting the nutritional profile of fish species caught for human consumption by degrading their habitat as well as food sources. Habitat degradation due to pollution (Hussain et al 2018) as well as alterations in the ecology of a water body has a profound influence on changes in the proximate composition of fish muscles (Padmawati and Kumari 2006). The nutrient profile of fish flesh plays an important role in indicating their nutritional status as well as physiological health and is significantly affected by their food & feeding habits, habitat conditions and prevailing environmental factors. Since anthropogenic interferences are largely affecting the habitats of aquatic fauna, thus it becomes imperative to examine the biochemical composition of fishes collected from an ecologically sensitive aquatic environment to have a fair knowledge of any alteration in its biochemical profile. Therefore, keeping this in view, the present study was designed to evaluate the biochemical composition of few commercially imported fish species collected from river Sutlej which is a major freshwater source of capture fisheries in Punjab.

MATERIAL AND METHODS

Study area: The River Sutlej is one of the major sources of capture fisheries in Northern India. It originates southwest of the Tibetan lakes of Rakasthal and Mansarover, enters the plains of Punjab at Ropar, flowing via the industrial city of Ludhiana, and finally meets with river Beas at Harike-Pattan. For the present study, the whole stretch of river was divided into four sites as given below:



Sample collection: Samples were collected from the above-designated sites in triplicate at two-monthly intervals from February 2018 to June 2019. *Labeo rohita, Cyprinus carpio, Sperata seenghala* and *Wallago attu* were selected for the study, being commercially important, preference by consumers, and their greater contribution towards the faunal diversity of river Sutlej. Fish samples were collected in plastic zippers and brought to the laboratory in insulated boxes under iced condition sand were immediately analyzed at the College of Fisheries, Guru Angad Dev Veterinary & Animal Sciences University (GADVASU), Ludhiana for biochemical composition as per standard protocols.

Biochemical composition analysis: The biochemical composition of the species under study was analyzed for moisture, ash, total protein, lipid, carbohydrate following standard methodology (AOAC 2000). The protocol being followed is tabulated as below:

Parameter	Protocol
Moisture	Moisture was estimated by heating samples in an oven at 100 ± 5°C to a constant weight and loss of weight considered as moisture.
Ash	Ash was determined by the ignition of a known weight of sample at about 550 °C in a muffle furnace till all carbon has been removed.
Total protein	Sample digestion was done with protein digestion system- KEL PLUS (model no-KES 12L), distillation with KEL PLUS-Classic DX Model (Pelican, make) and titrated with standard acid (0.1 N sulphuric acid). The amount of N obtained was multiplied by a factor of 6.25 to calculate the Total Protein content of sample
Lipid	The ether extract was estimated by Soxhlet extraction apparatus SOCS PLUS, (Model-SCS 6)
Carbohydrate	Carbohydrates (%) = 100 – (% Lipid + % TP + % Ash + % Moisture)

Table 1. Biochemical composition (%) of flesh of *L. rohita* during the study period

Month	Site	Moisture	Lipid	Protein	Ash	Carbohydrate
February 2018	S-1	77.85^{a}	2.40^{a}	17.15^{a}	1.48^{a}	1.12^{a}
	S-2	77.58^{a}	2.23^{b}	17.55^{a}	1.46^{a}	1.18^{a}
	S-4	78.08^{a}	2.52^{a}	16.72^{b}	1.56^{a}	1.12^{a}
April 2018	S-1	78.29^{a}	2.24^{b}	16.98^{a}	1.38^{b}	1.11^{a}
	S-2	78.43^{a}	2.16^{b}	16.83^{a}	1.40^{b}	1.18^{a}
	S-4	78.88^{a}	2.56^{a}	15.83^{b}	1.58^{a}	1.15^{a}
June 2018	S-1	76.82^{a}	2.92^{a}	17.83^{a}	1.25^{b}	1.18^{a}
	S-2	77.63^{a}	2.62^{b}	17.25^{b}	1.38^{b}	1.13^{a}
	S-4	77.93^{a}	2.48^{c}	16.98^{c}	1.49^{a}	1.12^{a}
December 2018	S-1	76.89^{a}	2.87^{a}	17.80^{a}	1.28^{b}	1.16^{a}
	S-2	77.07^{a}	2.58^{b}	17.85^{a}	1.32^{b}	1.18^{a}
	S-4	77.32^{a}	2.38^{c}	17.70^{a}	1.45^{a}	1.15^{a}
February 2019	S-1	76.43^{a}	2.99^{a}	18.25^{a}	1.21^{c}	1.12^{a}
	S-2	76.70^{a}	2.82^{b}	17.98^{b}	1.32^{b}	1.18^{a}
	S-4	76.30^{a}	2.48^{c}	17.85^{b}	1.41^{a}	1.16^{a}
April 2019	S-1	77.97^{a}	2.42^{b}	17.12^{a}	1.37^{a}	1.12^{a}
	S-2	78.05^{a}	2.31^{c}	16.98^{b}	1.48^{a}	1.18^{a}
	S-4	78.09^{a}	2.60^{a}	16.72^{b}	1.40^{a}	1.19^{a}
June 2019	S-1	77.20^{a}	2.78^{a}	17.58^{a}	1.32^{b}	1.12^{a}
	S-2	77.72^{a}	2.61^{b}	17.12^{a}	1.37^{b}	1.18^{a}
	S-4	78.12^{a}	2.48^{c}	16.82^{b}	1.43^{a}	1.15^{a}

*Values with different alphabetical superscripts (a, b, c..) differ significantly between the sites within a month (in a column) (P< 0.05)
Fish samples were not available at Site-3

Statistical analysis: Duncan Multiple Range Test (DMRT) was applied to find out the significant differences in biochemical composition parameters with SPSS-16 software package.

RESULTS AND DISCUSSION

The *L. rohita* percent moisture, lipid, protein, ash and carbohydrate varied from 76.30-78.88, from 2.16-2.99, 15.83-18.25, 1.21-1.58 and 1.11-1.19, respectively (Table 1) whereas in *C. carpio*, it ranged from 76.92-82.67, 1.20-2.82, 12.20-18.23, 1.22-1.98 and 1.12-3.15, respectively (Table 2). Similarly, for *S. seenghala*, the percent moisture, lipid, protein, ash and carbohydrate varied from 74.63-81.40, 1.37-2.78, 13.02-20.44, 1.15-1.89 and 1.01-3.08, respectively (Table 3) while as for *W. attu*, varied from 74.59-81.69, 1.32-2.81, 13.10-20.82, 1.11-1.35 and 1.02-2.18, respectively (Table 4). Comparatively, the highest moisture (80.87 %) was recorded in *W. attu* at S-3, lipid (2.72 %) in *S. seenghala* at S-3, protein (19.89 %) in *W. attu* at S-1, ash (1.59 %) in *C. carpio* at S-4 and carbohydrates (1.18 %) in *C. carpio* at S-1 (Table 5). The variation in the moisture may depend upon the size and maturity stage of the fish while as higher protein content is mainly found during the pre-spawning periods since more protein assimilation is required for the process of vitellogenesis and to cope up the energy requirement of fast body metabolic rate. (Bakhtiyar and Langer 2018).

Bakhtiyar and Langer (2018) observed that in *L. rohita* protein content may vary with age but doesn't differ significantly during the different month and lipids also varied with the maturity stage of fish while as moisture content may decrease or increase as per the size of the fish rather than

Table 2. Biochemical composition (%) of flesh of *C. carpio* during the study period

Month	Site	Moisture	Lipid	Protein	Ash	Carbohydrate
February 2018	S-1	77.26^b	2.12^a	17.90^a	1.59^b	1.13^b
	S-2	77.56^b	1.80^b	17.85^a	1.64^a	1.15^b
	S-3	82.30^a	1.66^c	12.23^c	1.43^b	2.18^a
	S-4	78.2^b	1.98^a	16.98^b	1.72^a	1.12^b
April 2018	S-1	77.09^b	2.17^a	17.89^a	1.60^b	1.15^b
	S-2	78.28^b	1.89^b	16.88^c	1.78^a	1.17^b
	S-3	82.01^a	1.29^c	13.20^d	1.35^c	2.12^a
	S-4	78.03^b	2.02^a	17.25^b	1.42^c	1.18^b
June 2018	S-1	77.46^b	2.13^a	17.68^a	1.58	1.15^b
	S-2	77.69^b	1.78^c	18.10^a	1.26^b	1.17^b
	S-3	81.70^a	1.20^d	13.22^b	1.26^b	2.12^a
	S-4	77.36^c	1.95^b	17.92^a	1.52^a	1.15^b
December 2018	S-1	76.92^b	2.18^a	18.08^a	1.57^b	1.25^a
	S-2	77.08^b	2.13^a	17.91^a	1.68^a	1.20^a
	S-3	80.13^a	1.90^b	12.20^b	1.49^c	1.18^a
	S-4	77.48^b	1.87^b	17.98^a	1.52^b	1.15^a
February 2019	S-1	77.25^b	2.24^a	18.10^a	1.22^b	1.19^b
	S-2	77.31^b	2.08^b	18.23^a	1.24^b	1.14^b
	S-3	82.67^a	1.46^c	12.31^c	1.28^b	2.18^a
	S-4	77.35^b	2.12^b	17.82^b	1.59^a	1.12^b
April 2019	S-1	76.97^b	2.39^a	17.96^a	1.48^a	1.20^b
	S-2	77.76^b	2.25^b	17.28^a	1.52^a	1.19^b
	S-3	81.09^a	1.42^c	13.77^b	1.37^b	3.15^a
	S-4	77.93^b	2.18^b	17.23^a	1.48^a	1.18^b
June 2019	S-1	78.09^b	2.02^b	16.83^a	1.87^b	1.19^b
	S-2	78.64^b	1.95^b	16.25^a	1.98^a	1.18^b
	S-3	81.29^a	2.82^a	13.85^b	1.62^c	2.12^a
	S-4	78.95^b	1.88^c	16.12^a	1.89^b	1.16^b

*Values with different alphabetical superscripts (a, b, c..) differ significantly between the sites within a month (in a column) (P< 0.05) any seasonal impact. Protein is one of the major constituents of fish which can vary depending on the time of year, environmental condition, stage of maturity of the gonads, state of nutrition, and age. The highest values of protein in February and June may be ascribed to the more accumulation of the protein for facilitating enhanced metabolic rate and process of vitellogenesis besides the abundance of natural food, desirable temperature, and other suitable environmental parameters during these months. The decline of muscle protein during the spawning and post-spawning phase may be attributed to its channelization towards ovaries to meet the energy requirement of fish for the process of vitellogenesis in conformity with the findings of Dabhade et al (2009). Relatively higher values of protein were recorded in *S. seenghala* and *W. attu* as compared to *L. rohita* and *C. carpio.* Relatively lower values of protein and lipid along with slightly higher values of moisture were observed at S-3 during the present study reflecting an inverse relationship between them. Ozyurt and Polat (2006) and Memon et al (2010) also reported similar results in the Indus river fishes and sea bass, respectively. The lipid content was <5 (%) which indicated that fishes were lean which is in corroboration with findings of Bennion and Scheule (2000). Total lipid composition in fish varies more than any other nutrient component (Thakur et al 2003) which was observed in present study also. Lower values of carbohydrates were recorded at S-3 as compared to other sites under study which may be attributed to chemical stress owing to higher pollution load which might have led to the depletion of stored carbohydrates. Similar findings have

Table 3. Biochemical composition (%) of flesh of *S. seenghala* during the study period

Month	Site	Moisture	Lipid	Protein	Ash	Carbohydrate
February 2018	S-1	75.28^{b}	2.34^{a}	20.03^{a}	1.20^{b}	1.15^{b}
	S-2	76.27^{b}	2.22^{a}	18.98^{a}	1.34^{a}	1.19^{b}
	S-3	81.40^{a}	1.37^{c}	13.22^{c}	1.34^{a}	2.10^{a}
	S-4	77.31^{b}	1.98^{b}	18.25^{b}	1.38^{a}	1.08^{c}
April 2018	S-1	75.66^{b}	2.56^{b}	19.36^{a}	1.24^{b}	1.18^{b}
	S-2	75.95^{b}	2.42^{c}	19.23^{a}	1.22^{b}	1.18^{b}
	S-3	80.87^{a}	2.78^{a}	13.02^{c}	1.18^{b}	2.10^{a}
	S-4	76.65^{b}	2.12^{d}	18.72^{b}	1.40^{a}	1.11^{b}
June 2018	S-1	74.63^{b}	2.62^{a}	20.44^{a}	1.15^{c}	1.16^{b}
	S-2	75.45^{b}	2.43^{b}	19.66^{b}	1.32^{b}	1.14^{b}
	S-3	79.96^{a}	2.50^{b}	13.87^{c}	1.59^{a}	2.08^{a}
	S-4	75.47^{b}	2.24^{c}	19.98^{b}	1.29^{b}	1.08^{c}
December 2018	S-1	74.88^{c}	2.68^{a}	20.24^{a}	1.19^{b}	1.01^{c}
	S-2	75.11^{b}	2.61^{a}	19.84^{a}	1.29^{a}	1.15^{b}
	S-3	80.24^{a}	2.72^{a}	13.96^{b}	1.20^{b}	3.08^{a}
	S-4	76.24^{b}	2.21^{b}	19.12^{a}	1.25^{a}	1.18^{b}
February 2019	S-1	75.86^{b}	2.51^{a}	19.23^{a}	1.38^{b}	1.02^{c}
	S-2	75.16^{b}	2.38^{b}	19.99^{a}	1.29^{c}	1.18^{b}
	S-3	79.94^{a}	2.11^{c}	13.95^{b}	1.89^{a}	2.11^{a}
	S-4	76.31^{b}	2.18^{c}	19.11^{a}	1.28^{c}	1.12^{b}
April 2019	S-1	75.05^{b}	2.46^{a}	20.12^{a}	1.18^{c}	1.19^{b}
	S-2	75.27^{b}	2.28^{b}	19.96^{b}	1.31^{a}	1.18^{b}
	S-3	80.94^{a}	1.58^{c}	13.92^{c}	1.25^{b}	2.31^{a}
	S-4	76.06^{b}	2.12^{b}	19.38^{b}	1.28^{b}	1.16^{b}
June 2019	S-1	75.43^{b}	2.23^{a}	19.88^{a}	1.27^{c}	1.19^{b}
	S-2	76.39^{b}	2.12^{a}	18.92^{b}	1.41^{b}	1.16^{b}
	S-3	80.95^{a}	1.38^{b}	13.94^{c}	1.61^{a}	2.12^{a}
	S-4	77.16^{b}	2.02^{a}	18.29^{a}	1.39^{b}	1.14^{b}

*Values with different alphabetical superscripts (a, b, c..) differ significantly between the sites within a month (in a column) ($P < 0.05$)

Table 4. Biochemical composition (%) of flesh of *W. attu* during the study period

Month	Site	Moisture	Lipid	Protein	Ash	Carbohydrate
February 2018	S-1	76.18^{b}	2.28^{a}	19.26^{a}	1.20^{a}	1.08^{c}
	S-2	76.66^{b}	2.14^{a}	18.95^{a}	1.12^{a}	1.03^{c}
	S-3	81.59^{a}	1.33^{b}	13.50^{b}	1.23^{a}	2.01^{a}
	S-4	77.32^{b}	2.23^{a}	18.15^{a}	1.18^{a}	1.12^{b}
April 2018	S-1	75.28^{b}	2.21^{a}	20.21^{a}	1.12^{b}	1.18^{b}
	S-2	75.40^{b}	2.19^{a}	19.98^{a}	1.28^{a}	1.15^{b}
	S-3	81.69^{a}	1.32^{b}	13.55^{c}	1.18^{b}	2.16^{a}
	S-4	76.98^{b}	2.10^{a}	18.51^{b}	1.23^{a}	1.18^{b}
June 2018	S-1	75.91^{b}	2.40^{a}	19.33^{b}	1.21^{a}	1.15^{b}
	S-2	75.17^{b}	2.25^{a}	20.29^{a}	1.15^{b}	1.14^{b}
	S-3	81.56^{a}	1.58^{c}	13.50^{d}	1.18^{b}	2.18^{a}
	S-4	77.12^{b}	2.02^{b}	18.55^{c}	1.16^{b}	1.15^{b}
December 2018	S-1	75.81^{b}	2.38^{a}	19.34^{a}	1.26^{b}	1.21^{a}
	S-2	76.16^{b}	2.12^{a}	19.20^{a}	1.32^{a}	1.20^{a}
	S-3	81.51^{a}	2.18^{a}	13.10^{b}	1.32^{a}	1.11^{b}
	S-4	76.24^{b}	2.25^{a}	18.98^{a}	1.35^{a}	1.18^{b}
February 2019	S-1	74.62^{b}	2.68^{a}	20.33^{a}	1.18^{b}	1.19^{b}
	S-2	75.22^{b}	2.38^{b}	20.23^{a}	1.15^{b}	1.02^{c}
	S-3	80.13^{a}	2.21^{b}	13.72^{c}	1.11^{b}	2.15^{a}
	S-4	75.05^{b}	2.56^{a}	19.91^{b}	1.30^{a}	1.18^{b}
April 2019	S-1	74.77^{b}	2.81^{a}	19.98^{a}	1.32^{a}	1.12^{b}
	S-2	75.06^{b}	2.58^{b}	20.06^{a}	1.19^{b}	1.11^{b}
	S-3	80.82^{a}	2.02^{d}	13.81^{c}	1.19^{b}	2.16^{a}
	S-4	76.58^{b}	2.30^{c}	18.61^{b}	1.33^{a}	1.18^{b}
June 2019	S-1	74.59^{c}	2.25^{a}	20.82^{a}	1.15^{c}	1.19^{b}
	S-2	75.50^{c}	2.18^{a}	19.92^{a}	1.22^{b}	1.18^{b}
	S-3	80.49^{a}	2.12^{a}	13.66^{c}	1.25^{b}	2.08^{a}
	S-4	77.17^{b}	2.13^{a}	18.26^{b}	1.32^{a}	1.12^{b}

*Values with different alphabetical superscripts (a, b, c..) differ significantly between the sites within a month (in a column) ($P< 0.05$)

been reported by Vijayavel and Balasubramanian (2006). Higher pollution load at S-3 could also be a reason for the lower nutritional profile of fish flesh since stressful conditions might have interfered with the normal physiology of fish thereby inhibiting the conversion of food material into energy. Kaur (2016) also reports similar trend. Variations reported in biochemical composition could also be attributed to the amount of food intake, season, size, and habitat of fish species (Deka et al 2012, Begum et al 2012). Exposure to environmental stressors may have an adverse effect on physiological health and nutritional value of fish (Kafilat et al 2013). The biochemical composition of fishes collected from different designated sites does not differ significantly except at S-3 where relatively lower values were recorded. This might be attributed to environmental stressors, altered physicochemical parameters of water, lesser availability of food, and interfered fish physiology due to higher pollutant load which eventually diminished the nutritional value of fish flesh that could have a pronounced effect on the human health benefits of fish flesh.

CONCLUSION

The biochemical composition of all the fishes under study was within the range as reported by several researchers however, at Site-3 where Buddha Nallah meets river Sutlej and carries along it the industrial effluents, the bio-chemical profile of fish species was altered as evident from the lower values of protein, lipid, and carbohydrates. This might be due to severity of pollution affecting the normal fish physiology and hampering the efficient conversion of food into body

Table 5. Comparative biochemical composition (%) of flesh of selected fish species during the study period

Site	Fish	Moisture	Lipid	Protein	Ash	Carbohydrate
S -1	*L. rohita*	$77.95^{a,2}$	$2.06^{c,5}$	$17.53^{b,3}$	$1.33^{b,3}$	$1.13^{a,1}$
	C. carpio	$77.29^{a,2}$	$2.17^{b,4}$	$17.77^{b,3}$	$1.55^{a,1}$	$1.18^{a,1}$
	S. seenghala	$75.25^{b,2}$	$2.48^{a,2}$	$19.68^{a,1}$	$1.23^{c,4}$	$1.12^{a,1}$
	W. attu	$75.30^{b,2}$	$2.43^{a,2}$	$19.89^{a,1}$	$1.20^{c,4}$	$1.16^{a,1}$
S -2	*L. rohita*	$77.59^{a,2}$	$2.47^{a,2}$	$17.36^{b,3}$	$1.39^{b,3}$	$1.17^{a,1}$
	C. carpio	$77.76^{a,2}$	$1.98^{c,5}$	$17.85^{b,3}$	$1.58^{a,1}$	$1.17^{a,1}$
	S. seenghala	$75.65^{b,2}$	$2.35^{a,3}$	$19.51^{a,1}$	$1.31^{b,3}$	$1.16^{a,1}$
	W. attu	$75.59^{b,2}$	$2.26^{b,3}$	$19.80^{a,1}$	$1.20^{c,4}$	$1.11^{a,1}$
S -3	*C. carpio*	$81.45^{a,1}$	$1.63^{c,6}$	$13.79^{a,5}$	$1.45^{a,2}$	$1.05^{a,2}$
	S. seenghala	$80.10^{a,1}$	$2.72^{a,1}$	$13.59^{b,5}$	$1.48^{a,2}$	$1.02^{a,2}$
	W. attu	$80.87^{a,1}$	$1.73^{b,5}$	$13.74^{a,5}$	$1.21^{b,4}$	$1.02^{a,2}$
S -4	*L. rohita*	$77.81^{a,2}$	$2.50^{a,2}$	$16.94^{c,4}$	$1.47^{b,2}$	$1.14^{a,1}$
	C. carpio	$77.90^{a,2}$	$2.10^{c,4}$	$17.32^{b,3}$	$1.59^{a,1}$	$1.15^{a,1}$
	S. seenghala	$76.45^{b,2}$	$2.12^{c,4}$	$18.97^{a,2}$	$1.32^{c,3}$	$1.12^{a,1}$
	W. attu	$76.63^{b,2}$	$2.22^{b,3}$	$18.71^{a,2}$	$1.26^{c,4}$	$1.15^{a,1}$

*Values with different alphabetical superscripts (a, b, c) differ significantly between the fish species within the site and with different numerical superscripts (1, 2, 3) differ significantly between the sites ($P < 0.05$)

nutrients besides non-availability of natural food a key precursor for accumulation of body nutrients. This waterbody being a major source of capture fisheries in Punjab, further studies are warranted to find the impact of pollution on the biochemical profile of fish species.

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Effect of Protective Polymers and Storage Temperatures on Shelf Life of Cyanobacterial Liquid Formulation

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Abstract: The aim of the study was to determine the influence of protective polymers and storage temperatures to prolong the shelf life of cyanobacterial liquid formulation of efficient bio-fertilizer strains of cyanobacteria using different protective polymers and storage conditions. In this experiment, the protective polymers used in formulations were dimethyl sulfoxide (DMSO), glycerol and polyvinyl pyrrolidone (PVP) along with gum Arabic, CMC and Tween-20. The formulation without the addition of protective polymers and no pH was maintained as control. Storage temperature of formulated liquid biofertilizers of cyanobacteria were 4^0C, 28^0C and 40^0C for a period of 18 months (540 days). Most of the formulations showed cell count $2.3x10^4$to $3.2\ x10^4$cells mL^{-1}, cell dry weight 1.98 to 2.51 mg mL^{-1}, total chlorophyll 3.50 and 6.05 µg mL^{-1} and nitrogenase activity 2.07 to 3.52 µmol C_2H_4 mg^{-1} chl h^{-1} at all the three stored temperatures after 18 months of incubation. The performance of developed liquid formulations was good with protective polymeric additives than without them. The liquid biofertilizer formulation using combination of protective polymers showed maximum cell count and other metabolic activities as compared to alone. At 4^0C, liquid inoculant formulated with DMSO and glycerol provided the greatest protection to cells of cyanobacteria and surviving and other growth-promoting ability whereas at 28^0C and 40^0C, PVP in addition to glycerol retained maximum surviving and other metabolic activities. The combinations of protective polymers *viz*. DMSO (3%) and glycerol (2%) at 4^0C and PVP (2%) and glycerol at 28^0C and 40^0C showed the best conditions to improve shelf life of cyanobacterial liquid biofertilizer formulations. It can be recommended to maintain the shelf life of liquid formulations for storage period of 18 months.

Keywords: Protective polymers, Formulation, DMSO, PVP, Glycerol

The success of any biofertilizer in the field depends on the quality of bioformulations. Microbial based nutrient inputs have emerged as the potential alternative for the productivity, reliability and sustainability of the global food chain. Although, carrier-based biofertilizers are in vogue since long and their use has proved beneficial both for crop yields as well as soil health, but, these are still not popular with the farming community because of varying reasons and mixed results and the main concern has been the viability of the microorganisms. The biofertilizer inoculants should survive up to field application and the shelf-life of inoculants is very important. To overcome such problems with carrier-based biofertilizers; liquid biofertilizers have been developed which seem to be the only alternative for cost-effective sustainable agriculture. Different approaches have been available for the development of liquid formulations of microorganisms, but the basic aim is to have storage stability, easy delivery, increased persistence and protection from harmful and damaging environmental factors. The success of a biofertilizer is dependent upon the survival of the microbial strain in the soil and it is a big challenge (Xavier et al 2004). The selection of ideal polymer is based upon several properties like complex chemical nature, solubility in water and nontoxicity which prevents microorganisms in the soil from rapid degradation. Liquid inoculant formulations may use various broth cultures amended with agents that promote cell survival in the package and after application to soil. It contains not only the desired microorganisms and their nutrients but also special cell protectants or additives that promote for longer shelf life and tolerance to adverse conditions (Hegde et al 2008). Many kinds of polymers such as sodium alginate, methyl cellulose, trehalose, arabinose, gum arabic, starch, glycerol, polyethylene glycol (PEG), polyvinyl pyrrolidone and DMSO (Criste et al 2014) have been used for inoculant production because of their ability to limit heat transfer, their good rheological properties and high water activities (Pindi and Satyanarayna 2012, Daniel et al 2013). These polymers, are normally used as additives or cell protectants. Hence, we evaluated the optimum concentration of polymers, surfactants and adjuvants for liquid formulation that could sustain the bacterial shelf life. So, a breakthrough is necessary for the current inoculant technology to reinforce the shelf life and field efficacy of biofertilizers in India to make them commercially viable and acceptable to the farmers. Therefore, the present study was conducted to increase the survival of the liquid formulations of cyanobacterial inoculants by the addition of different polymers like gum arabic, CMC, glycerol, polyvinyl pyrrolidone and DMSO.

MATERIAL AND METHODS

Cyanobacterial cultures used in the study: Four cyanobacterial cultures *viz*., *Anabaena variabilis* (CCC441), *Nostoc muscorum* (*CCC442*), *Tolypothrix tenuis* (CCC443) and *Aulosira fertilissima* (CCC444) and one P-solubilizing cyanobacterial culture *i.e., Westiellopsis prolifica* (CCC474) maintained in Culture Collection of Cyanobacteria (CCC) at CCUBGA, Indian Agricultural Research Institute, New Delhi were taken for the study.

Preparation of formulations: Different liquid formulations were prepared using combination of carriers (vegetable oil) at 1%, emulsifiers (Tween-20) at 2% and thickening agents (Gum arabic and CMC) at 5 % with different concentrations (1.5 - 5 %) of protective agents (DMSO, PVP and Glycerol). The experiments were carried out in 100 ml plastic bottle containing amended liquid formulation. A total 10 formulations with triplicates were prepared (Table 1). Initially, pH of all the formulations except F10 were adjusted to 7 by adding drop by drop 1 N NaOH or 1 N HCl. Late log phase (14 days old) cultures of the Cyanobacterial mixture (2.5% v/v) were inoculated into each formulation and stored at three different temperatures 40C, 28^{0}C and 40^{0}C in refrigerator, room condition and incubator respectively. These formulations were characterized for shelf life and metabolic activities up to 18 months at different time intervals *viz*. 30, 60, 90, 150, 180, 270, 360, 450 and 540 days.

Table 1. Different combinations of protective agents used in formulations

Formulations	Protective agents	Concentration (%)
F1	DMSO	3
F2	DMSO	5
F3	Glycerol	3
F4	Glycerol	5
F5	PVP	1.5
F6	PVP	3
F7	Mixture (DMSO+ Glycerol)	3+2
F8	Mixture (PVP+ Glycerol)	2+2
F9	No Protective agents	-
F10	No protective agents, No pH maintained	-

Revival of culture from formulation: Ten ml formulation containing culture was taken in a pre-sterilized centrifuge tube and centrifuged at 5000 X g for 10 minutes. The cell pellet was washed with sterilized distilled water, centrifuged and finally re-suspended in 1ml sterilized BG-11 medium. This re-suspended cell pellet was used as inoculum to inoculate 100 ml nitrogen-free BG-11 medium taken in 250 ml conical Erlenmeyer flasks. The flasks were incubated in a culture room at 28 ± 2^{o}C under a light intensity of 52-55 μmol photon m^{-2} s^{-1} and L: D cycles of 16:8 hours. Representative samples of cyanobacterial cultures were taken after 21days of incubation for analysis of cell population, total chlorophyll, dry weight and nitrogen fixation by ARA.

Cell population: Cell population counting was done using the Neubauer chamber of the Haemocytometer having depth of 0.100 mm and 1 mm^2 area. 10 ml culture was taken in a test tube and homogenized for 60 seconds. 1ml of this was taken through a micropipette (1000μl) and added in 9 ml sterilized BG-11 (N^- medium) and homogenised for 1 minute. This homogenized and diluted culture was taken through micropipette and onto the Neubauer chamber and covered with a coverslip. Cells were observed under compound microscope OLYMPUS-CX40 and counting was performed. Total chlorophyll was estimated by methanol extraction method (McKinney 1941) while Nitrogenase activity was determined by ARA (Acetylene Reduction Assay) given by Hardy et al 1973.

Statistical analysis: Completely randomized design (CRD) was used for experimental data analysis and critical difference (CD) was calculated at 5% level of significance with the help of SPSS-16.0 statistical package.

RESULTS AND DISCUSSION

Shelf life of liquid formulations: Formulations containing protective polymers showed higher viability and metabolic activities of cyanobacteria than formulations without protective polymers. Similarly, Girisha et al (2006) also reported that liquid formulation of cowpea rhizobia prepared with PVP as an osmoprotectant also had higher shelf life than those without PVP amendment. All the formulations amended with additives stored at refrigerator showed higher viable count and other metabolic activities of cyanobacteria as compared to formulations without additive. As compared to initial cell population of 3.5 X 10^4 cells mL^{-1} that was added to each formulation, all formulations maintained a cell population of 2.3x10^4 to 3.2 x10^4 cells mL^{-1} after 18 months of incubation across all the stored temperature. The combination of protectants gave better result than when they were used alone across the stored temperature. Formulations which contained DMSO and combination of DMSO and glycerol as protective polymers showed higher shelf life and other metabolic activities at 4^0C whereas at 28^0C and 40^0C, formulations contained PVP and combination of PVP and glycerol showed higher shelf life and other metabolic activities. At 4^0C, maximum cell population of 3.2 x10^4 cells mL^{-1} was observed in formulation F7 containing mixture of protective polymers *i.e.*, DMSO and glycerol whereas at 28^0C and 40^0C, formulation F8 containing mixture of PVP and glycerol maintained maximum cell population of 3.0x10^4 and 2.9x10^4 cells mL^{-1} respectively. However, a cell population of 2.9x10^4 and 2.8x10^4 cells mL^{-1} was maintained in formulations F1 and F2 containing protective polymers DMSO only at concentration of 3 and 5% respectively and 2.7x10^4 and 2.8 x10^4 cells mL^{-1} in formulation F3 and F4 containing protective polymers glycerol only at concentration of 3 of 5% respectively after 18 months of incubation at 4^0C (Table 2). Similar results were observed at 28^0C and 40^0C in those formulations containing PVP and glycerol alone. A cell population of 2.8x10^4 and 2.9 x10^4 cells mL^{-1} and 2.7x10^4 and 2.9 x10^4 cells mL^{-1} was observed in formulations F5 and F6 containing PVP at concentration of 1.5% of 3% respectively at 28^0C (Table 3) and formulations F5 and F6 at 40^0C after 18 months of incubation (Table 4). In case of cell dry weight, maximum cell dry weight was observed in culture revived from formulation F7 (2.51 mg mL^{-1}) followed by formulation F2 kept at 4^0C after 18 months of incubation (Table 5). On the other hand, at 28^0C culture from formulation F8 recorded maximum dry weight of 2.20 mg mL^{-1} followed closely by that of formulation F5 (Table 6) whereas at 40^0C, maximum cell

Table 2. Cell count (x 10^4) of BGA culture mixture as revived from selected formulations kept at 4^0C

Treatment	Cell count (x 10^4) at different months of storage									
	1	2	3	4	5	6	9	12	15	18
F1	3.2	3.2	3.2	3.1	3.1	3.1	3.0	2.9	2.9	2.9
F2	3.3	3.2	3.2	3.2	3.2	3.2	3.2	3.1	3.1	2.8
F3	3.2	3.1	3.1	3.1	3.0	3.1	3.0	2.8	2.8	2.7
F4	3.3	3.2	3.2	3.1	3.1	3.1	3.1	3.0	2.8	2.8
F5	3.3	3.3	3.2	3.2	3.1	3.2	3.1	3.0	3.0	2.7
F6	3.4	3.4	3.3	3.3	3.1	3.1	3.0	3.0	3.0	2.7
F7	3.4	3.4	3.3	3.3	3.3	3.3	3.3	3.2	3.2	3.2
F8	3.2	3.1	3.1	3.1	3.0	3.1	3.0	3.0	3.0	2.9
F9	3.3	3.2	3.1	3.1	3.1	3.1	3.0	2.7	2.6	2.6
F10	3.2	3.2	3.1	3.0	3.0	2.8	2.8	2.7	2.6	2.4
CD (P = 0.05)	NS	0.005	0.009	0.127	0.008	0.112	0.107	0.124	0.105	0.12

Cell count of culture revived on 0 day of storage: 3.4 X 10^4

Table 3. Cell count (x 10^4) of BGA mixture as revived from selected formulations kept at 28^0C

Treatment	Cell count (x 10^4) at different months of storage									
	1	2	3	4	5	6	9	12	15	18
F1	3.1	3.1	3.0	3.1	3.1	3.1	3.1	2.9	2.8	2.6
F2	3.1	3.0	3.0	3.0	2.9	2.8	2.8	2.7	2.7	2.5
F3	3.3	3.3	3.2	3.2	3.1	3.1	3.0	2.8	2.8	2.8
F4	3.4	3.3	3.2	3.2	3.1	3.1	3.0	3.0	2.9	2.9
F5	3.4	3.3	3.2	3.2	3.1	3.2	3.1	3.0	2.9	2.8
F6	3.4	3.3	3.3	3.2	3.2	3.1	3.0	3.0	2.9	2.9
F7	3.2	3.1	3.1	3.0	3.0	2.9	2.9	2.8	2.7	2.7
F8	3.4	3.3	3.3	3.3	3.2	3.2	3.1	3.1	3.0	3.0
F9	3.3	3.2	3.1	3.1	3.0	2.9	2.9	2.7	2.7	2.7
F10	3.2	3.2	3.1	3.1	3.0	2.9	2.8	2.7	2.7	2.5
CD (p=0.05)	0.004	0.018	0.102	0.121	0.087	0.112	0.094	0.102	0.023	0.131

Cell count of culture revived on 0 day of storage: 3.4 X 10^4

Table 4. Cell count (x 10^4) of BGA culture mixture as revived from selected formulations kept at 40^{0}C

Treatment	Cell count at different months of storage									
	1	2	3	4	5	6	9	12	15	18
F1	3.2	3.2	3.0	3.0	2.8	2.7	2.6	2.6	2.6	2.4
F2	3.3	3.2	3.2	3.2	3.2	2.7	2.5	2.5	2.4	2.3
F3	3.2	3.1	3.1	3.1	3.0	3.1	3.0	3.0	2.7	2.7
F4	3.3	3.2	3.2	3.1	3.1	3.1	3.0	2.9	2.9	2.8
F5	3.3	3.3	3.2	3.2	3.1	3.2	3.0	2.8	2.8	2.7
F6	3.4	3.4	3.3	3.3	3.2	3.2	3.0	3.0	2.9	2.9
F7	3.4	3.2	3.1	3.1	3.0	3.0	2.8	2.8	2.7	2.5
F8	3.2	3.1	3.1	3.1	3.0	3.1	3.0	3.0	3.0	2.9
F9	3.3	3.2	3.0	3.0	3.0	2.7	2.7	2.5	2.5	2.5
F10	3.2	3.0	3.0	2.9	2.9	2.7	2.5	2.5	2.5	2.4
CD (p=0.05)	0.009	0.138	0.01	0.127	0.114	0.134	0.107	0.124	0.135	0.128

Cell count of culture revived on 0 day of storage: 3.4 X 10^4

Table 5. Cell dry weight (mg mL^{-1}) of BGA mixture as revived from selected formulations kept at 4^{0}C

Treatment	Cell dry weight (mg ml^{-1}) at different months of storage									
	1	2	3	4	5	6	9	12	15	18
F1	2.01	1.92	1.89	1.81	1.89	1.97	2.04	2.26	2.31	2.42
F2	2.12	1.97	1.92	1.86	1.98	2.07	2.19	2.38	2.42	2.51
F3	1.94	1.91	1.82	1.94	2.03	2.12	2.2	2.35	2.3	2.24
F4	1.98	1.87	1.84	1.89	1.97	2.1	2.28	2.41	2.36	2.28
F5	1.92	1.89	1.86	1.91	1.97	2.08	2.18	2.31	2.4	2.37
F6	1.97	1.95	1.9	2.01	2.14	2.22	2.32	2.4	2.51	2.46
F7	2.14	2.03	1.97	2.01	2.13	2.2	2.27	2.32	2.39	2.47
F8	2.08	1.98	1.91	1.96	2.01	2.1	2.16	2.2	2.28	2.34
F9	1.91	1.87	1.81	1.88	1.95	2.01	2.12	2.03	1.98	1.93
F10	1.89	1.8	1.76	1.83	1.87	1.92	1.98	2.14	1.97	1.86
CD (p=0.05)	0.101	0.123	0.084	0.072	0.107	0.157	0.132	0.054	0.124	0.093

Cell dry weight on 0 day of storage: 2.18 mg ml^{-1}

Table 6. Cell dry weight (mg mL^{-1}) of BGA mixture as revived from selected formulations kept at 28^{0}C

Treatment	Cell dry weight (mg ml^{-1}) at different months of storage									
	1	2	3	4	5	6	9	12	15	18
F1	1.98	1.94	1.90	1.85	1.89	1.93	2.09	2.12	2.02	1.97
F2	1.92	1.89	1.84	1.80	1.83	1.97	2.13	2.19	2.22	2.01
F3	2.06	1.94	1.98	2.02	2.05	2.11	2.18	2.22	2.13	2.04
F4	2.11	1.99	2.08	2.13	2.11	2.17	2.26	2.31	2.28	2.2
F5	2.04	1.96	1.99	2.05	1.98	1.92	1.88	1.93	1.99	2.16
F6	2.10	2.02	2.09	2.15	2.12	2.17	2.23	2.3	2.22	2.20
F7	2.09	1.97	1.93	1.96	2.00	2.12	2.19	2.12	2.03	1.97
F8	2.13	2.01	2.11	2.17	2.14	2.11	2.19	2.24	2.28	2.20
F9	1.94	1.89	1.83	1.79	1.75	1.81	1.89	1.92	1.87	1.82
F10	1.91	1.85	1.80	1.76	1.82	1.87	1.95	1.90	1.87	1.82
CD (p=0.05)	0.084	0.141	0.067	0.092	0.160	0.109	0.148	0.083	0.116	0.197

Cell dry weight on 0 day of storage: 2.18 mg ml^{-1}

dry weight was observed in formulation F8 *i.e.* 2.47 mg mL^{-1} followed by formulation F6) after 18 months of incubation (Table 7). Similar results were observed in case of total chlorophyll content. The total chlorophyll varied between 3.50 and 6.05 µg mL^{-1} amongst different formulations and across different temperatures. Maximum total chlorophyll content (5.21 µg mL^{-1}) was observed in culture revived from formulation F7 containing mixture of protective polymers *i.e.,* DMSO and Glycerol kept at 4^0C (Table 8) whereas at 28^0C (Table 9) and 40^0C (Table 10) formulation F8 showed maximum total chlorophyll content 5.06 µg mL^{-1} and formulation 4.84 µg mL^{-1} respectively after 18 months of incubation. Most of the formulations showed nitrogenase activity in terms of ARA in the range of 2.07 to 3.52 µmol C_2H_4 mg^{-1} chl h^{-1} at all the three stored temperatures after 18 months of incubation. At 4^0C, culture revived from formulation F7 showed maximum nitrogenase activity *i.e.* 3.52 µmol C_2H_4 mg^{-1} chl h^{-1} (Fig. 1) whereas that of formulation F6 and F8 at 28^0C and formulation F8 at 40^0C showed maximum nitrogenase activity of 2.81 and 2.62 µmol C_2H_4 mg^{-1} chl h^{-1} respectively after 18 months of incubation (Fig. 2 and Fig. 3). The results are similar to Velineni and Brahmaprakash (2011) and Sahai and Chandra (2009) who observed higher shelf life of PSB (*Pseudomonas* sp.) in liquid inoculants stored under refrigerated conditions as compared to room conditions. A similar finding was reported by Zdenek Hubalek (2003) who observed that DMSO is the most successful cryoprotective agent than glycerol and PVP at frozen storage of microorganisms. Adriana Criste et al. (2014) also studied the efficiency of cryoprotectants *viz*. DMSO (5% and 10%),

Table 7. Cell dry weight (mg mL^{-1}) of BGA mixture as revived from selected formulations kept at 40^0C

Treatment	Cell dry weight (mg ml^{-1}) at different months of storage									
	1	2	3	4	5	6	9	12	15	18
F1	1.91	1.89	1.82	1.84	1.88	1.96	2.07	2.21	2.28	2.05
F2	1.86	1.82	1.84	1.88	1.92	2.01	2.18	2.25	2.31	2.26
F3	1.92	1.87	1.79	1.86	1.92	2.01	2.19	2.37	2.48	2.54
F4	1.88	1.85	1.76	1.84	1.92	2.14	2.23	2.29	2.38	2.49
F5	1.92	1.84	1.88	1.90	1.94	2.02	2.21	2.27	2.41	2.37
F6	1.90	1.86	1.90	1.95	1.99	2.14	2.19	2.34	2.48	2.41
F7	1.98	1.92	1.91	1.96	1.99	2.1	2.19	2.2	2.28	2.34
F8	1.90	1.93	1.97	2.02	2.07	2.20	2.29	2.32	2.39	2.47
F9	1.89	1.85	1.76	1.82	1.91	2.09	2.17	2.23	2.07	1.89
F10	1.90	1.83	1.78	1.74	1.79	1.87	1.91	1.89	1.85	1.80
CD (p=0.05)	0.072	0.094	0.126	0.083	0.009	0.091	0.102	0.114	0.083	0.148

Cell dry weight on 0 day of storage: 2.18 mg ml^{-1}

Table 8. Total chlorophyll (µg ml^{-1}) in BGA mixture as revived from selected formulations kept at 4^0C

Treatment	Total chlorophyll (µg ml^{-1}) at different months of storage									
	1	2	3	4	5	6	9	12	15	18
F1	5.74	5.46	5.55	5.63	5.75	5.86	5.92	5.96	5.44	5.10
F2	5.66	5.60	5.83	5.88	5.91	5.99	5.89	5.71	5.26	4.48
F3	6.05	5.59	5.50	5.54	5.69	5.75	5.20	5.31	5.20	4.65
F4	5.80	5.44	5.47	5.50	5.64	5.68	5.71	5.82	5.37	5.08
F5	5.64	5.52	5.63	5.73	5.99	5.91	5.69	5.43	4.47	4.16
F6	5.55	5.43	5.45	5.66	5.75	5.69	5.43	5.34	4.38	4.05
F7	6.01	5.92	5.98	6.05	6.19	6.23	6.27	6.31	5.49	5.21
F8	5.99	5.78	5.90	5.98	6.09	6.15	5.98	5.44	4.57	4.19
F9	5.70	5.41	5.80	5.93	5.76	5.27	4.46	4.22	4.13	4.01
F10	5.50	5.27	5.43	5.46	5.24	4.63	4.39	4.19	4.00	3.87
CD (p=0.05)	0.10	0.08	0.08	0.05	0.08	0.08	0.11	0.16	0.19	0.12

Total chlorophyll on 0 day of storage: 6.97 µg mL^{-1}

ethylene glycol (5 and 10%) and glycerol (5 and 10%) on long term microorganisms conservation and they found that among the four cryoprotectants, the best results were given by DMSO or combination of DMSO and glycerol utilization. Santhosh (2015) reported that liquid biofertilizers formulated with PVP in addition to glycerol at the rate of 0.5% retained maximum number of colonies *of Rhizobium, Azotobacter, Azospirillum* and PSB at 28±2 °C for a period of 180 days. Daniel et al (2013) and Gopal and Baby (2016) have reported the liquid inoculants formulations using 2% PVP and 0.1% CMC promoted long term survival of *B. megaterium, Azospirillum* and *Azotobacter* after 480 days at 30^{0}C. Our results are also similar to Mugilan et al (2011) reported better survival of PSB (*Pseudomonas striata*) in liquid formulation amended with PVP and glycerol stored at room temperature. Aarti Yadav et al (2017) observed higher shelf life of PSB in inoculants amended with PVP (2%, 1%) followed by glycerol (2% and 1%) during the storage at room temperature. Kumaresan and Reetha (2011) reported that liquid *Azospirillum* bioinoculant formulated with glycerol (10 mM) followed by gum arabica (0.3%) and PVP (2%) promoted long term survival of *Azospirillum* and supported 10^{8} cells/mL up to 11 months of storage under ambient temperature (28°C to 32°C). Babu et al (2002) also found higher population of *Azospirillum* due to the addition of PVP at both 1 and 2% levels. Similarly, Sherawat et al. (2015) reported that liquid inoculants prepared in amended additives such as glycerol, PVP, GA media showed higher viable count in comparison to inoculants prepared in YEMB (control) at 180 days of storage. Sridhar et al (2004) developed liquid inoculant of

Table 9. Total chlorophyll (µg mL^{-1}) in BGA mixture as revived from selected formulations kept at 28^{0}C

Treatment	Total chlorophyll (µg ml^{-1}) at different months of storage									
	1	2	3	4	5	6	9	12	15	18
F1	5.31	5.19	5.04	4.9	4.84	4.87	4.76	4.6	4.12	4.09
F2	5.15	4.99	4.93	4.72	4.65	4.56	4.44	4.3	3.97	3.92
F3	6.01	5.74	5.76	5.8	5.81	5.86	5.99	5.94	5.66	5.01
F4	5.83	5.63	5.5	5.56	5.6	5.71	5.81	5.94	5.26	4.5
F5	5.77	5.72	5.75	5.79	5.8	5.82	5.85	5.87	5.17	4.73
F6	5.62	5.57	5.63	5.65	5.69	5.61	5.34	5.43	4.89	4.25
F7	5.42	5.31	5.39	5.41	5.21	5.06	4.96	4.89	4.78	4.37
F8	5.84	5.75	5.79	5.82	5.87	5.98	6.01	5.8	5.33	5.06
F9	5.98	5.48	5.44	5.5	5.58	5.5	5.29	4.98	4.46	4.18
F10	5.53	5.33	5.29	5.44	5.02	4.98	4.52	4.15	3.92	3.5
CD (p=0.05)	0.17	0.15	0.16	0.18	0.20	0.15	0.18	0.15	0.16	0.12

Total chlorophyll on 0 day of storage: 6.97 µg mL^{-1}

Table 10. Total chlorophyll (µg ml^{-1}) in BGA mixture as revived from selected formulations kept at 40^{0}C

Treatment	Total chlorophyll (µg ml^{-1}) at different months of storage									
	1	2	3	4	5	6	9	12	15	18
F1	5.54	5.46	5.31	5.1	4.85	4.53	4.44	4.12	3.9	3.83
F2	5.51	5.44	5.17	4.99	4.44	4.23	4.14	3.98	3.52	3.46
F3	5.7	5.67	5.6	5.39	5.5	5.58	5.39	5.09	4.43	4.27
F4	5.66	5.52	5.43	5.28	5.3	5.33	5.15	4.94	4.32	4.24
F5	5.74	5.68	5.63	5.4	5.41	5.47	5.22	5.04	4.45	4.33
F6	5.76	5.71	5.66	5.48	5.59	5.6	5.45	5.13	4.82	4.7
F7	5.57	5.54	5.41	5.14	4.91	4.85	4.69	4.48	3.96	3.92
F8	6.01	5.86	5.8	5.69	5.75	5.81	5.75	5.32	5.00	4.84
F9	5.62	5.54	5.44	5.25	5.04	4.91	4.59	4.3	3.92	3.88
F10	5.51	5.44	5.23	5.07	4.57	4.44	4.22	4.11	3.8	3.5
CD (p=0.05)	0.12	0.11	0.17	0.13	0.18	0.12	0.09	0.06	0.08	0.05

Total chlorophyll on 0 day of storage: 6.97 µg mL^{-1}

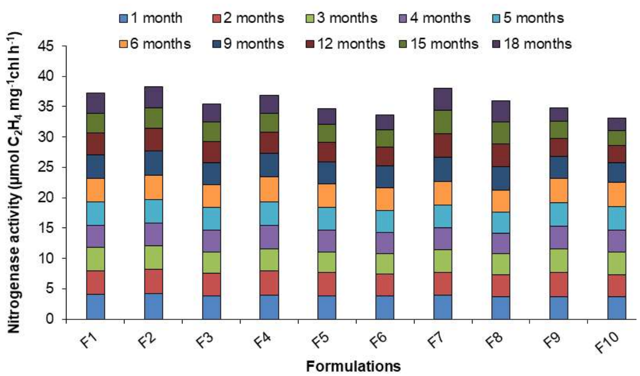

Fig. 1. Nitrogenase activity in terms of ARA (µmol C_2H_4 mg^{-1} chl h^{-1}) in BGA culture mixture as revived from selected formulations kept at 4^0C

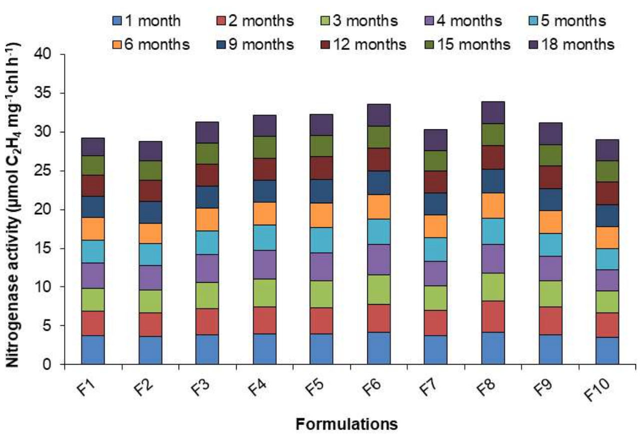

Fig. 2. Nitrogenase activity in terms of ARA (µmol C_2H_4 mg^{-1} chl h^{-1}) in BGA culture mixture as revived from selected formulations kept at 28^0C

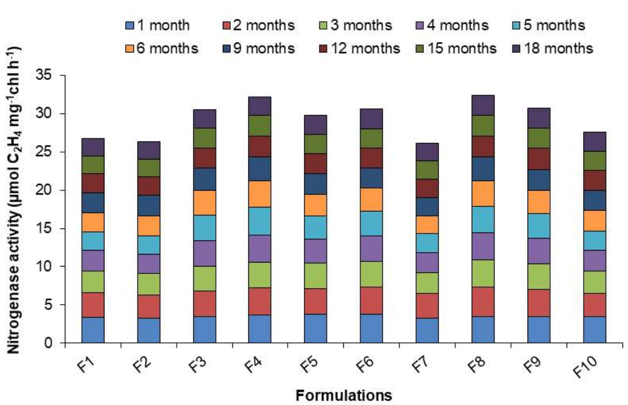

Fig. 3. Nitrogenase activity in terms of ARA (µmol C_2H_4 mg^{-1} chl h^{-1}) in BGA culture mixture as revived from selected formulations kept at 40^0C

Bacillus megaterium using osmoprotectants PVP, glycerol and glucose and found supported higher viable population up to 6 months storage period than formulations where they used alone. *Mahdi* et al (2010) also reported that the shelf-life of liquid formulations could be as high as two years.

Temperature is important for the shelf life of microbial products and can affect their normal activity. Cultures used in these formulations normally grow at 28^0C±2°C but since the temperature in many parts of our country goes around 10^0C in winter and above 40°C in summer so it was necessary to test the formulations at these lower and higher temperature. Liquid biofertilizer preparations contain special cell protectants or additives which promote longer shelf life and tolerance to adverse conditions (Hegde 2008). A number of types of polymers has been used for inoculant preparations because of their ability to limit heat transfer, good rheological properties and high water activities. Polymers like sodium alginate, gum arabic, dimethyl sulfoxide (DMSO), polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA) and polyethylene glycol (PEG) have normally been used as thickener/adhesive or protectants (Leo Daniel et al*.,* 2013). Higher survival under refrigerated conditions may be due to the fact that low temperature in refrigerator allows no or little growth with less utilization of nutrients during storage making them available to the organism in optimum concentration for longer period. Low temperature in refrigerator also protects the cell death in inoculums. Glycerol and DMSO decrease the freezing-point of water and biological fluids by colligative action (up to 46^0C and 73^0C respectively). Therefore lessen the concentration of salts dissolved in solutions, in turn inhibiting osmotic shock and bind intracellular water colligatively which prevents excessive dehydration, reduces salt toxicity and prevents the formation of large ice crystals within the cell (Mugilan et al 2011, Singleton et al 2002) Glycerol has high water binding capacity and may protect cells from the effect of desiccation by reducing the rate of drying (Lorda and Balatti 1996). In contrast, during storage of inoculants at room conditions where temperature may go beyond 30^0C, growth of organism is allowed creating depletion of nutrients and accumulation of toxic metabolites (Tittabutr et al 2007). In the present study, formulations were also kept at higher temperature to study the shelf life and other metabolic activities of inoculated culture. Hoe and Rahim (2010) reported that liquid formulations can tolerate the temperature as high as 55°C. Similar reports were observed by many researchers (Panlada et al 2007, Vendan and Thangaraju 2006). In the present study, at higher storage temperature PVP and combination of PVP and glycerol polymeric additives maintain higher shelf life and other metabolic activities. At higher temperature, PVP act as stabilizing polymers in reducing the extent of protein precipitation or coagulation of cells (Deaker et al 2004, Gaind et al 1990). PVP help in maintaining the higher viable count as bacteria do not use these polymers as an energy source, these polymers have other properties supporting the growth and survival of cells. PVP is believed to detoxify the fermentation medium by complexing with the phenolic-type, shelf-limiting toxins in the medium. (Temprano et al 2002, Errington et al 2002) PVP also has colloidal stabilization and adhesive properties with high water holding capacity that appears to slow down the drying rate of media, thus maintaining the moisture level in the media and maintain water around the cells for their metabolism (Singleton et al 2002, Deaker et al 2004, Tittabutr et al 2007). This polymer is absorbed in a thin molecular layer on the surface of the individual colloidal particles resulting in a stabilized suspension that prevents coalescence of cells, which might block the O_2 and nutrient diffusion from media to cells. PVP has also ability to limit heat transfer. These might be the mechanisms that improve the survival of cyanobacterial cells in formulations stored at higher temperature.

CONCLUSIONS

Present study revealed that the performance of developed liquid formulations was good with protective polymeric additives than without them. The liquid biofertilizer formulation using combination of protective polymers showed maximum cell count and other metabolic activities as compared to alone. At 4^0C, liquid inoculant formulated with DMSO and glycerol provided the greatest protection to cells of cyanobacteria and surviving and other growth promoting ability whereas at 28^0C and 40^0C, PVP in addition to glycerol retained maximum surviving and other metabolic activities. Hence, the combinations of protective polymers viz. DMSO (3%) and glycerol (2%) at 4^0C and PVP (2%) and glycerol at 28^0C and 40^0C showed best conditions to improve shelf life of cyanobacterial liquid biofertilizer formulations. It can be recommended to maintain the shelf life of liquid formulations for storage period of 18 months. Further, in-depth analysis of the formulations is needed to understand the role of protectants for maintenance under various storage conditions and needs to be standardised.

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Sedimentological Model for Underground Tigris Terraces Channel in Sallahuddin Oil Refinery, Beji, Iraq

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Abstract: The study area is located structurally southwest Makhulplunge. Quaternary deposits overlying unconformably Fat'ha and Injanah Formations. The Quaternary deposits beneath Sallahuddin oil refinery display topsoil and the third stage of Tigris river terraces. The latter is represented by braided to meandering river channels composed of; conglomerates, sandstones, and siltyclaystones. The conglomerate reflects channel deposits and longitudinal bars, whereas the sandstones and siltyclaystones represent the channel levee with occasional crevasses play. From the available data of 81 boreholes and vertical pits and trenches, a depositional model was built for the main and subsidiary channels, the water flows during flood times only due to its relatively higher altitude compared with the main channel. During groundwater levels rise due to pipe leakage of industrial acid water and the infiltration of the surface runoff water, the foundations of the civil and industrial buildings of oil refinery could be affected. In conclusion, this impact will change the lithology by the dissolution of the cementing materials of the conglomerates and hence, the geotechnical characteristics of the conglomerate and other lithology will decline to result in building foundation disturbances.

Keywords: Sedimentological model, Underground channel, Tigris terraces, Sallahuddin oil refinery, Beji

The results of the study of sub-surface fluvial sequences and the relationship of the facies with each other in terms of overlap and extension, by comparing them with classic facies are used to give a concept about the nature of the sedimentary environment (Colombera and Mountney 2019). The study of fluvial sediments is complicated by lateral, vertical, upstream and downstream accumulations of river sediments (Zang et al 2020). Distinguishing and describing sub-surface fluvial sequences based on reginal correlations and mapping, that help identify the characteristics of the faciess and their relationships with each other and reflect the sedimentary environment (Rizqi and Purnomo 2021). The Sallahuddin Oil Refinery is located north of Beji city and southwest Fat'ha area in nearly flat terrain areas (Fig. 1). On the behalf of the project "Geophysical investigation of cavities and leakage areas in Sallahuddin Oil Refinery(SOR), Beji" the idea of accomplishing the sedimentologic model of the underlying rockshasarisen. The seismic survey conducted by revealed the presence of an ancient Tigris river meander channel at the internal fence of the SOR. The channel is directed due west and bends to the south (Al-Saigh 2007). Most of the civil and industrial buildings were established above this channel. Eighty-one boreholes were conducted in a network covered SOR. Their logs reflect a stage of Tigris river terraces beneath the refinery and surrounding areas to the south in an attempt to speculate the movement and distribution of the groundwater. The elevation differences approach about six meters within the study area. The valleys were directed due southwest and deviated to the south to connect with the present Tigris river channel.

Structurally, the area is located in the northeastern edge of the Samarraa syncline and southwest Makhul anticline according to the geologic and tectonic map of Iraq (Fig. 1). The river terraces have overlain Fat'ha and Injanah Formations in an unconformable way. These terraces were party covered by soil. NEDECO Company conducted a study on Al-Tharthar valley in 1959 for oil exploration including the study area (NEDECO 1959). They performed a structural map for the area restricted by Al-Qayyarah in the north, Baghdad in the south, Hemrin, and Makhul Mountains in the east, and Tharthar basin in the west, their study revealed; Fat'ha Formation foundin the core of both Hemrin and Makhul anticlines and their extensions, in addition to the Al-Jazera east Al-Tharthar basin. On the contrary, the outcrops of the Injana Formation restricted to the limbs of Makhul and Hemrinanticlines and in the uppermost part of the Samarraa syncline (Jassim and Buday 2006). The Injana Formation extends to the south runs parallel to the eastern bank of Al-Tharthar lake.

The most prominent geomorphological features in the area are Makhul and Hemrin mountains. The V-shaped valley is a characteristic feature of the limestone layers forming flat iron topography. The valley's gradients decrease towards the west - southwest and become low to the very low gradient at (SOR) near Samaraa syncline axis (Fig. 2). The valley system in the area attains NE-SW direction west of the area confined between the Makhul anticline and the axis of the Samaraa syncline. The valley patterns are sub-dendritic to sub parallel. A geomorphologic work conducted assured that the present study area is related to the Tigris river terraces that were deposited at the Pleistocene.

During the study of the Fat'ha area, in the Tigris river left four stages of terraces, the oldest stage is the highest one and the youngest is the lowest one (the present river stage). The terraces formed mostly of conglomerate forming elongated lenses (tens to hundred meters in length) as outcrops. The covered 3rd terrace stage beneath the refinery was almost found as groundwater channel paths. The terraces are composed mostly of conglomerate, sandstone, siltyclaystone, and claystone. The oldest two terraces stages (1st and 2nd) are characterized by the presence of longitudinal and transverse bars which indicate braided river channel type. The (3rd and 4th) stages represent the meandering channel according to (AL-Jubouri et al 2001) as the case of the present rivers stage. The thicknesses of these stages range between 3 meters for the first stage near Al-Fat'ha to more than 10 meters for the 3rd stage (studied stage beneath SOR).

MATERIAL AND METHODS

Eighty-one boreholes were dug in a network fashion covered (SOR) and surrounding areas. The borehole network constitutes nine lines directed in NE – SW direction, and nine boreholes on every line. The distance between lines is about 200 m and the distance between boreholes on the same line is about 500 m (Fig. 3). The boreholes range in depth (6-12 m). The boreholes lithology is described in detail. Correlation of boreholes on every line performed forming groups. These groups correlated with others to estimate the real geological and depositional model beneath the studied refinery. Besides, many vertical pits have about 3 m depths found within the area were also described.

RESULTS AND DISCUSSION

The study of every single borehole and their stratigraphic correlation revealed that the questioned refinery (SOR) is located on the quaternary deposits. The soil thickness ranges between (1->5m.), constitutes of top transported soil few centimeters of fine to a very fine grain size of loss sands, and the flood plain deposits. It displays flood plains of the third stage of the Tigris river terrace. This stage furnished the ground of the foundation of the SOR. The flood plain deposits after severe weathering exposed changes gradually to residual soil. This type of soil is characterized by brown to yellowish-brown color, friable nature, and secondary gypsum coexisted with the soil together. The gypsum behaves as cementing material for the soil and dissolves easily with water. Variegated plant roots and rootlets co-existed on the upper part of the soil horizon. The recent soil of the present stage of the Tigris River has about 13m. as maximum thickness, covers whole the flood plains deposits. It is composed of silty claystone and fine sandstone beds. This bed intercalates lenses of conglomerate not exceed 1m thick, pinch out laterally, appear in the vertical pits. Often, these conglomerate lenses have flattened lower surfaces. The prevailing colors for these deposits are brown and yellowish-brown, they are medium tough except for some fine sandstones lenses. Their bedding surface is unclear in most of the vertical pits.

The conglomerate represents the channel lag deposits of the 3rdstagechannels of the Tigris river terraces, where the coarse pebbles are laid down at the bottom of the channel followed by medium-size pebbles and then fine pebbles at the top forming fining upward size gradations. It intercalates lenses of sandstones and silty claystone 0.5-1m.in length and a few centimeters to 0.5 m in thicknesses. The dominant cementing materials constitute secondary gypsum, which grows around the pebbles as a satin spar. In the whole borehole sections, there were very thin lenses of secondary gypsum a few millimeters thick (Figs. 4-13). The 3rd Tigris river terrace stage can be divided into three lithofacies according to lithologic variations; the conglomerate, the sandstone and the silty claystone.

Conglomerate lithofacies: The correlation diagrams (Figs. 4-13) showed that the maximum thickness of the studied boreholes reaches about 12m, some boreholes do not penetrate the conglomerate layer completely, though the thickness could increase to 15m. It can be compared by the same terrace stage (3rd) in Mosul, Hammam Al-Alil, and Sherqat area (AL-Jubouri et al 2001). The pebbles of these lithofacies range in diameter between a few millimeters to 15cm. It is composed mainly of metamorphic rocks like schist, gneiss, marble, and quartzite, and a subordinate amount of sedimentary rocks like limestone, sandstone, and chert with scarce pebbles of igneous origin. The pebbles are mostly rounded to sub-round. The gneiss and schist pebbles are almost discoid and flattened in shape, but the other components mostly have good sphericity and badly sorted. This lithofacies refer to the dominance of longitudinal gravel bars, where the area between the bars is filled with finer-grained sizes, deposited in cases of low discharges (Saikia 2017). The parallel gravel bars and their bifurcating channel,

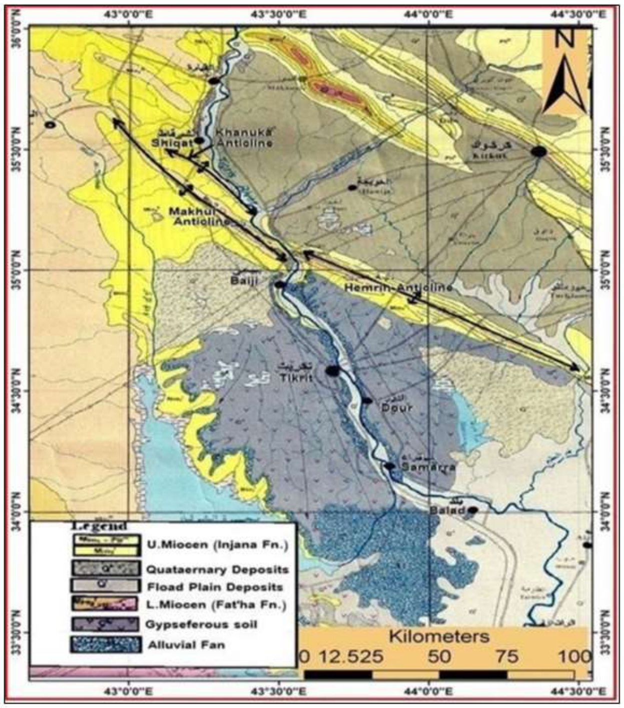

Fig. 1. Geological and tectonic map of the studied area. (General Company for Geological Survey and Mining)

and the presence of sandstone beds on their edges can be explained as transversal bars migrate laterally toward the channel between bars (Sigdel and Sakai 2016). During a flooding storm, the channel incised and then the river laid down its bedload in a relatively short period (Nehyba and Roetzel, 2015). The sediments were almost badly sorted. The depositional cycle appeared to be incomplete and not clear (Colombera and Mountney 2019). Often, the dominant matrix of these sediments are mixtures of clay, sand, and silt with different percentages, giving rise to weakening rock strength, washing clay easily (Rits et al 2016) and consequently producing groundwater passages along with the terraces. The conglomerate lithofacies can be divided into four lithosubfacies according to their matrix and cementing materials;

Clayey conglomerate lithosubfacies: The matrix and cementing materials are composed of clay with little calcium carbonate and secondary gypsum. Consequently, this lithosubfacies is mostly friable and loose which caused pebble collapse during drilling in some boreholes.

Silty conglomerate lithosubfacies: The matrix and cementing materials constitute calcium carbonate and low to

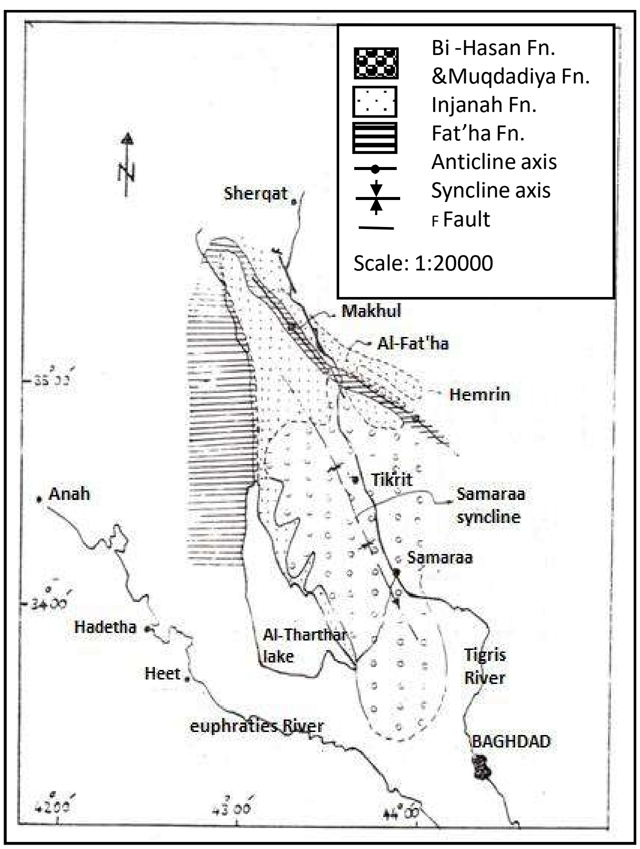

Fig. 2. Geomorphological map of the middle of Iraq (NEDECO Co., 1959)

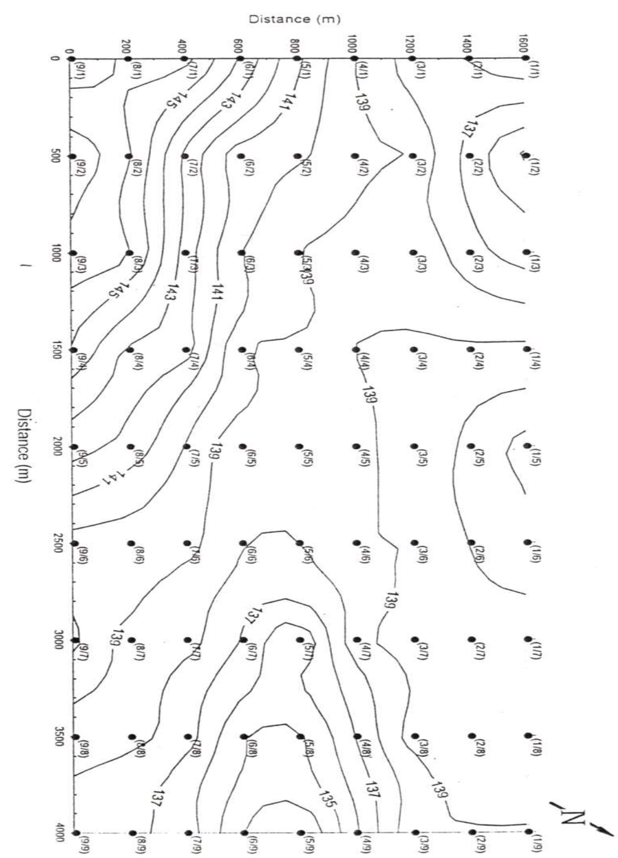

Fig. 3. The topographic map and Borehole locations (watertable elevations m.a.s.l.)

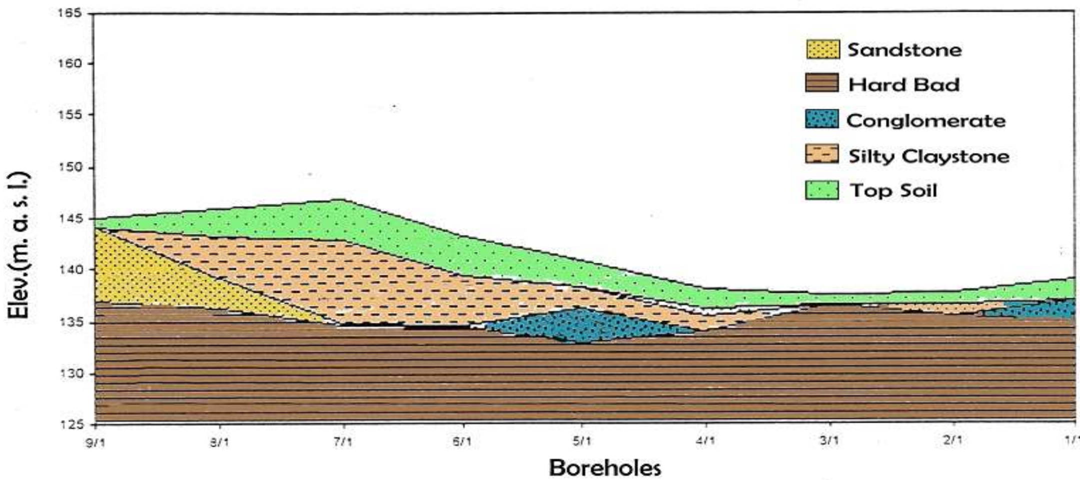

Fig. 4. The correlated geological section of wells (group 1) along the nine traverses.

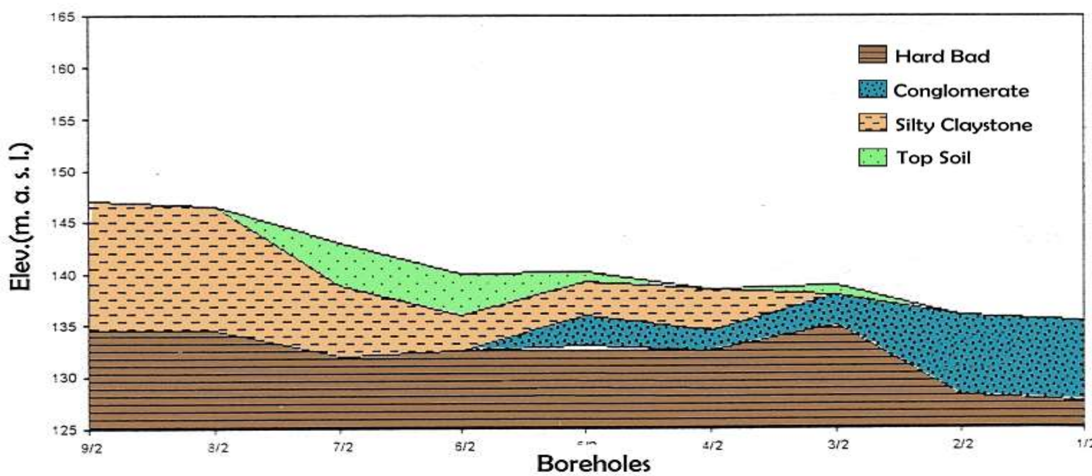

Fig. 5. The correlated geological section of wells (group 2) along the nine traverses.

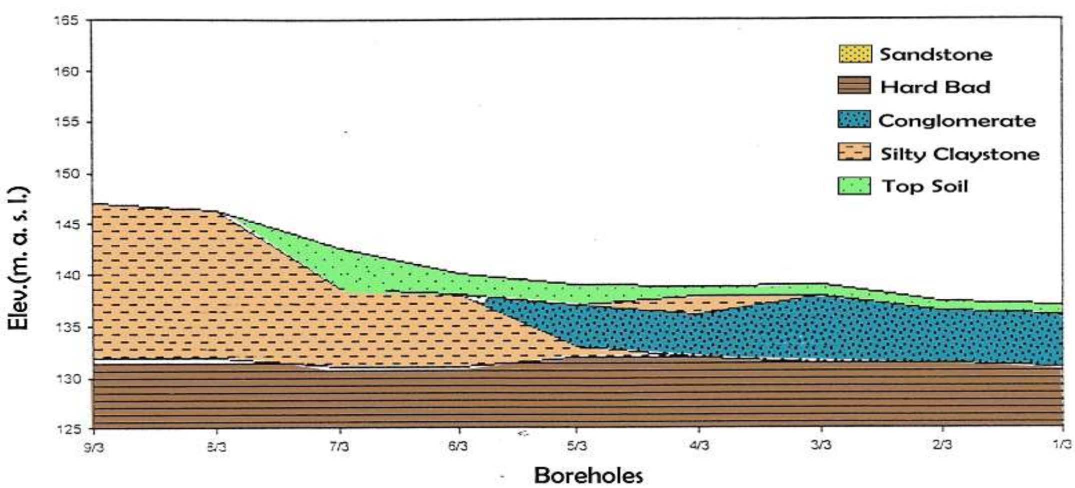

Fig. 6. The correlated geological section of wells (group 3) along the nine traverses.

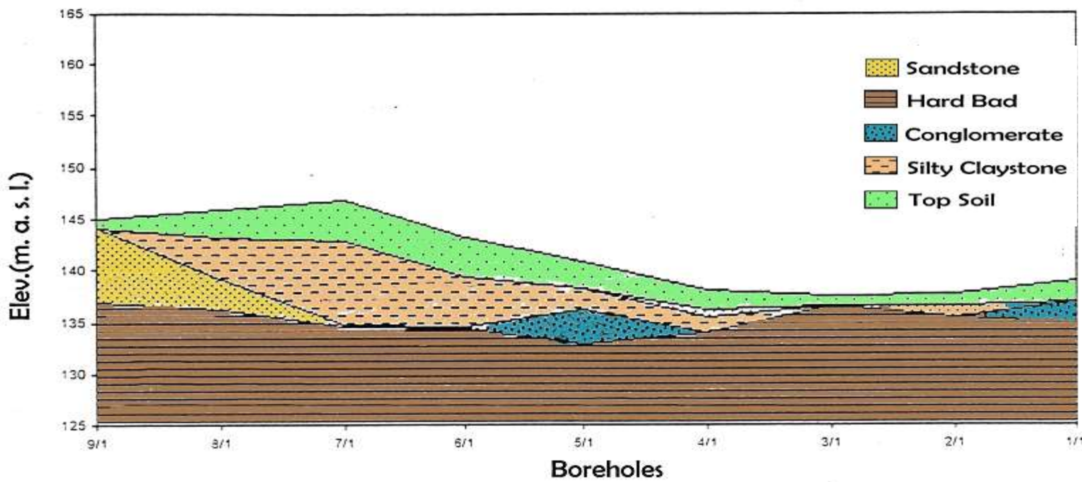

Fig. 7. Correlated geological section of wells along the nine traverses(group 4)

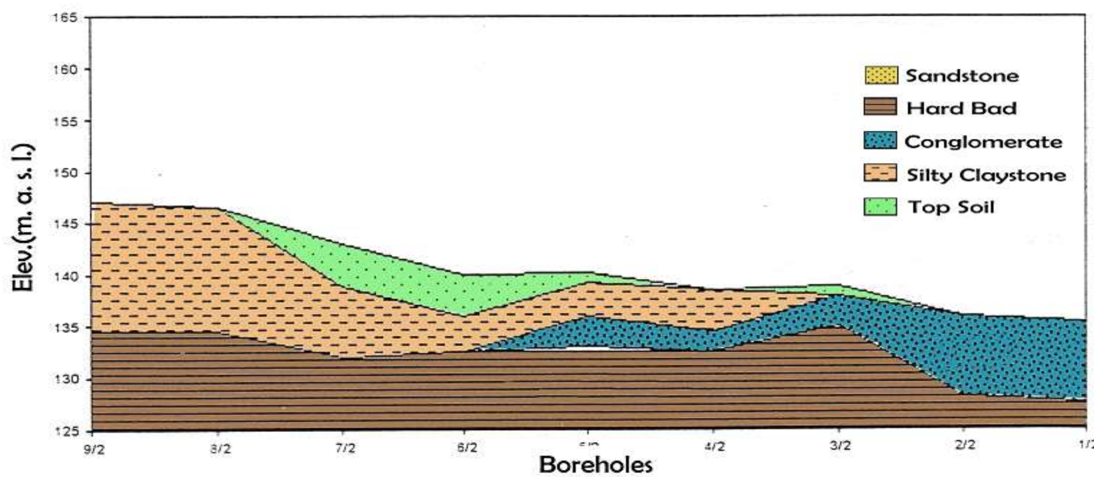

Fig. 8. Correlated geological section of wells along the nine traverses(group 5)

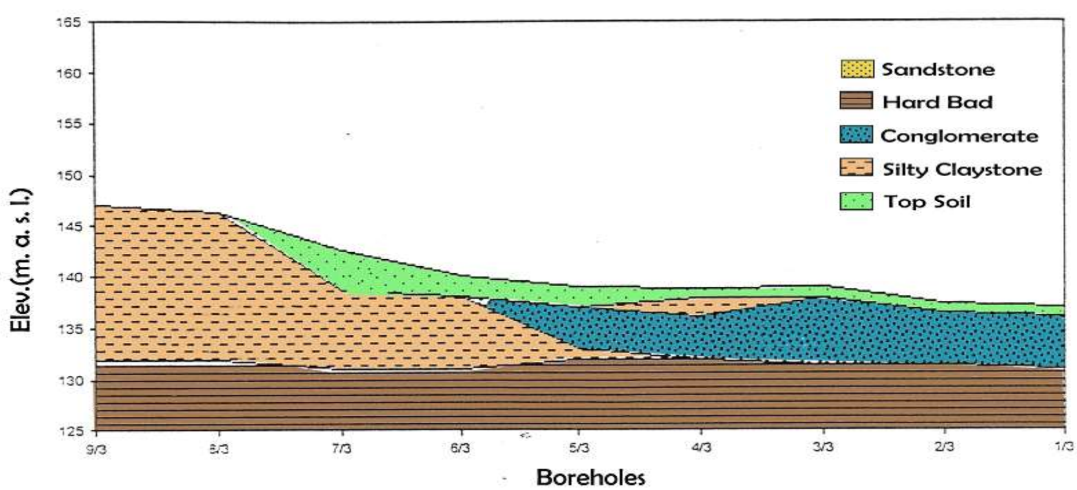

Fig. 9. Correlated geological section of wells (group 6) along the nine traverses.

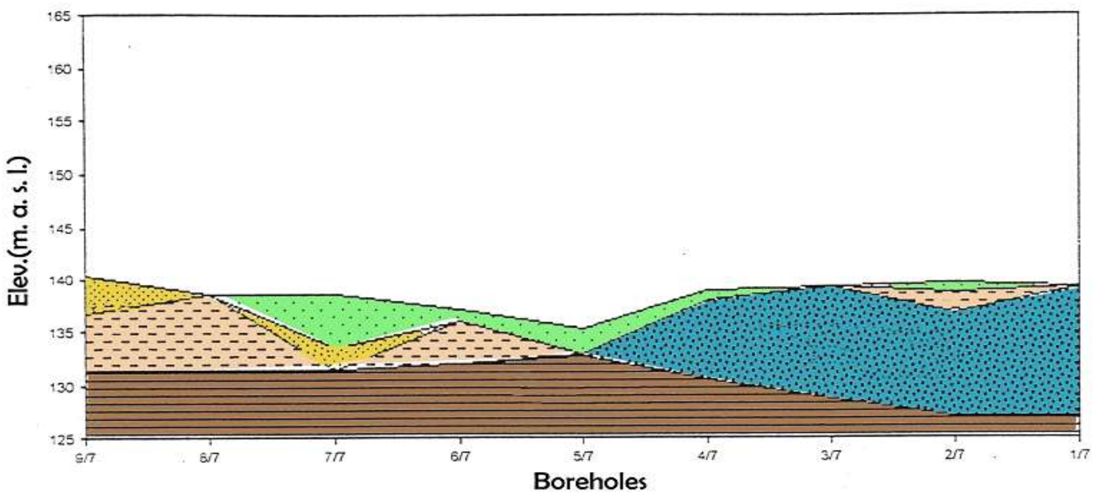

Fig. 10. Correlated geological section of wells) along the nine traverses(group 7)

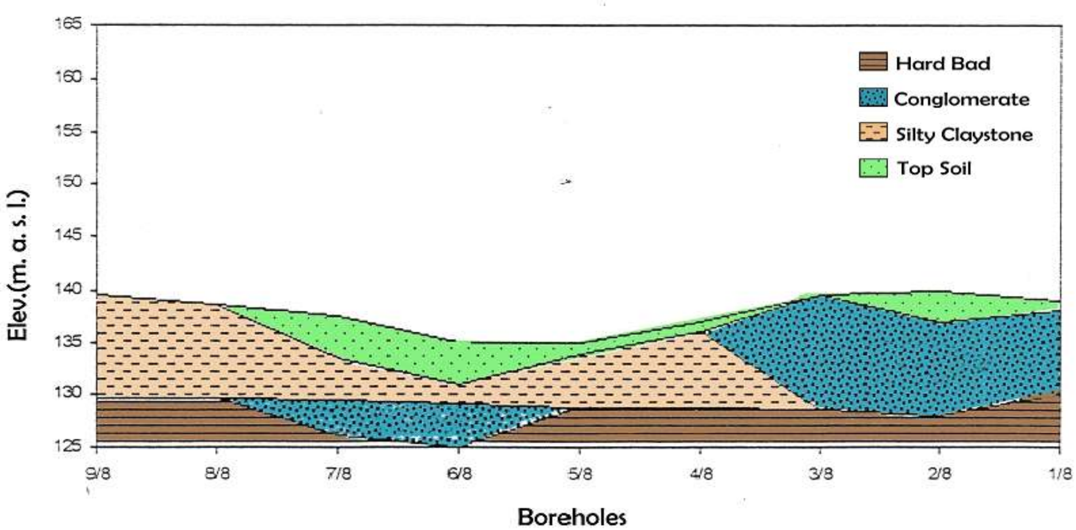

Fig. 11. The correlated geological section of wells along the nine traverses(group 8)

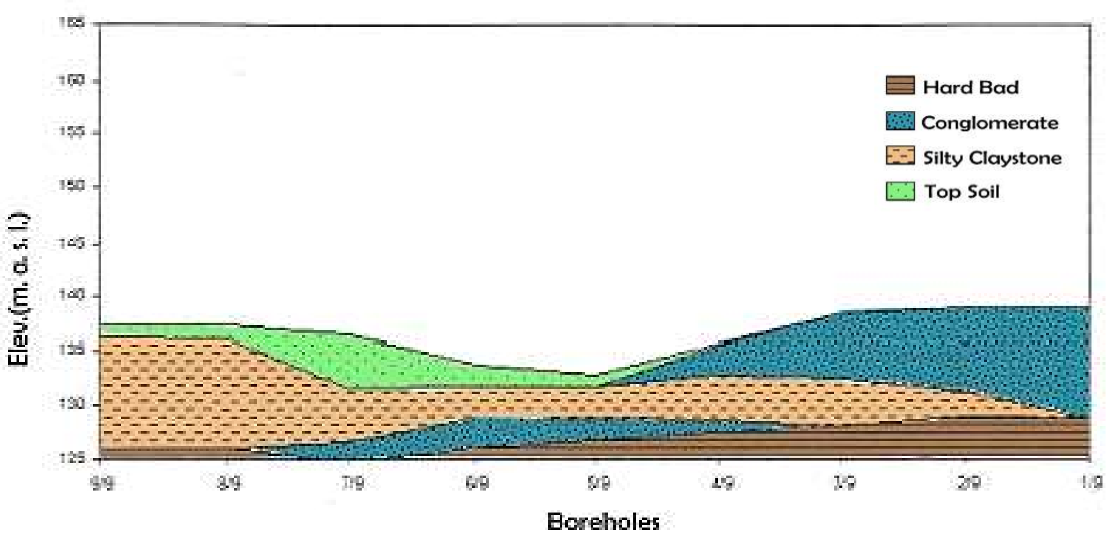

Fig. 12. Correlated geological section of wells along the nine traverses(group 9)

medium amount of secondary gypsum, therefore this lithosubfacies is mostly friable – medium tough.

Sandy conglomerate lithosubfacies: The matrix constitutes fine sand and silt, and again the cementing materials composed of calcium carbonate and a medium amount of secondary gypsum, produce medium tough – tough lithosubfacies.

Washed conglomerate lithosubfacies: It is rarely observed, represents the lower part of the channel, always washed and free of matrix. The main cementing materials are calcium carbonate with a subordinate amount of secondary gypsum.

The lateral extension of the conglomerate lithofacies exceeds 1200 m within (SOR). It changes laterally to clayey conglomerates, silty conglomerates sandy conglomerate, and even to washed conglomerates (Olusola and Samuel 2012). The change depends on the sediment load and the depositional scheme of the river (Sigdel andSakai 2016). The slopeis represented by the difference in elevations between the Fat'ha area and SOR.

***Sandstone lithofacies*:** This lithofacies persists subordinately in comparison with the silty clayey lithofacies (Fig. 6). Its thickness ranges between 2–7m. Laterally changed to silty claystonelithofacies and its extension does not exceed 400m. Secondary gypsum grows near the top of the soil profile.

Silty claystone lithofacies: The lithofacies found in all boreholes. The thickness reaches 12m (Figure 7). Lenses of sandstones do not exceed 0.5 m intercalate this lithofacies. The silty claystone facies changed laterally to bad-sorted sandstone lithofacies, the width of the lithofacies reached more than 1400m. Secondary gypsum found within the upper part of the facies probably due to capillary action, ruled by thearid to semi-arid climate dominated the study area.

Depositional model: Lithofacies map (Figure 14) and isopach map of conglomerate (Figure 15) show allthelithological components like conglomerate, sandstone, and silty claystone. The questioned third terraces stage beneath SORis composed of two main channels. The first one is dissected by sandstone, silty claystone levees. It speared as a narrow elongated levee directly proportional with the paleo-river channel width 212-764m. Levees formed mainly of silty claystone and even conglomerate facies separate the two channels, the first and second leaving some small crevasse splay connected the two main channels.

This condition appears to be low sinuosity with restricted flood plain displays by the silty claystone facies (Sigdel and Sakai 2016, Colombera and Mountney 2019). The maximum depth of the first channel No.1 is (4 m), whereas the second channel No.2 is wide and continuous, with width ranges (411-1235m), and the depth exceeds (14 m). The second channel is considered as the main permanent channel of the river during annual normal discharges. The paleo-flow direction from NE to SW (Figure 16).

Paleo - hydrogeology: Throughout the interpretation of Figures (14, 15, and 16), it seems that the estimated depositional model built (Fig. 16) for the studied area beneath SOR is composed of two channels. Channel 1 is the subsidiary channel, which displays dissected parts of conglomerate rocks; part (1a) includes two lines of boreholes (8 and 9) representing conglomerate rocks, connected with the main channel 2 by crevasse splay. A sandstone bar is separated between the two channels, and part (1b) is composed of sediments and clayey siltstone bar. Every one of these parts represents an underground aquifer for water derived from, precipitations and seeped industrial water, produced from leakage of industrial water pipes. These

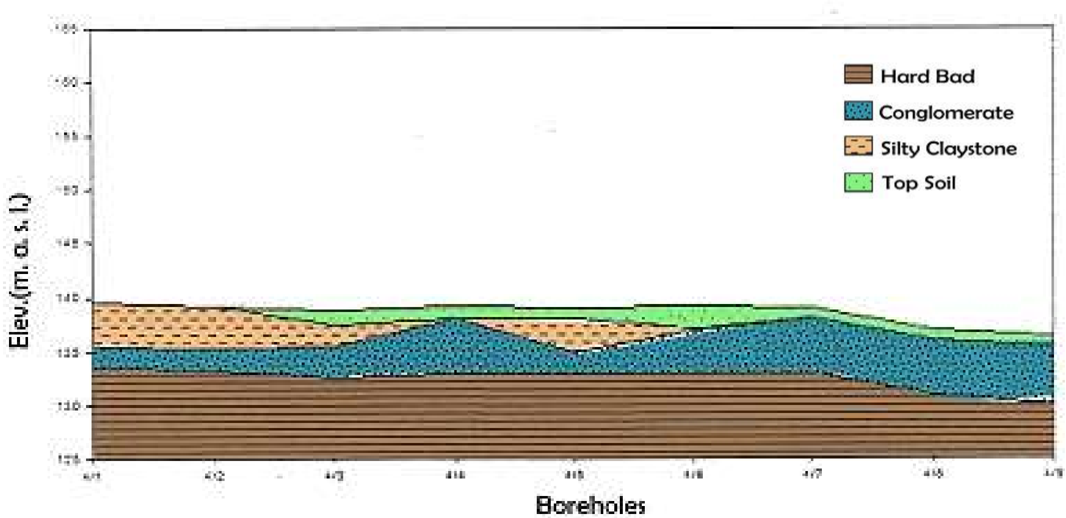

Fig. 13. Correlated geological section of wells (1 - 9) along the traverse No. 4.

aquifers are not connected with the main channel2 except with a few small connections, so it serves and attain water and no discharge occurs. In addition to, any water supply to this channel raise the groundwater table level GWTL for the storage water (Fig. 17) during the period (2000-2001), in this context, part (1a) of channel. 1 mark an increase in GWTL reach 40 cm. On the contrary, a decrease in GWTL took place between part (1b) and part (1c) reach to about (2-4 m), due to the direct connection of the two main channels by small crevasse splays at those parts.

The groundwater table levels increase in the channel (2) and decrease in the channel (1), (Fig. 18). However, the trials of lowering the GWTL fail unless pumping operation to the ground surface took place. In this sense, the discharge pumping operations could cause dangerous impacts on both civil and industrial buildings in the area. On condition that the

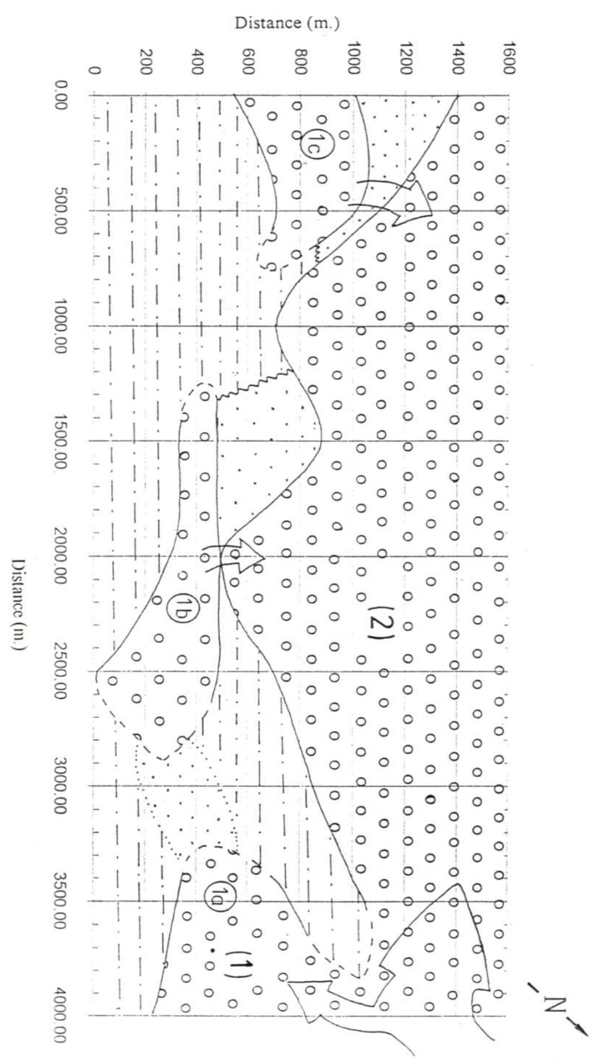

Fig. 14. The lithofacies map of the 3rd river terraces beneath the refinery area

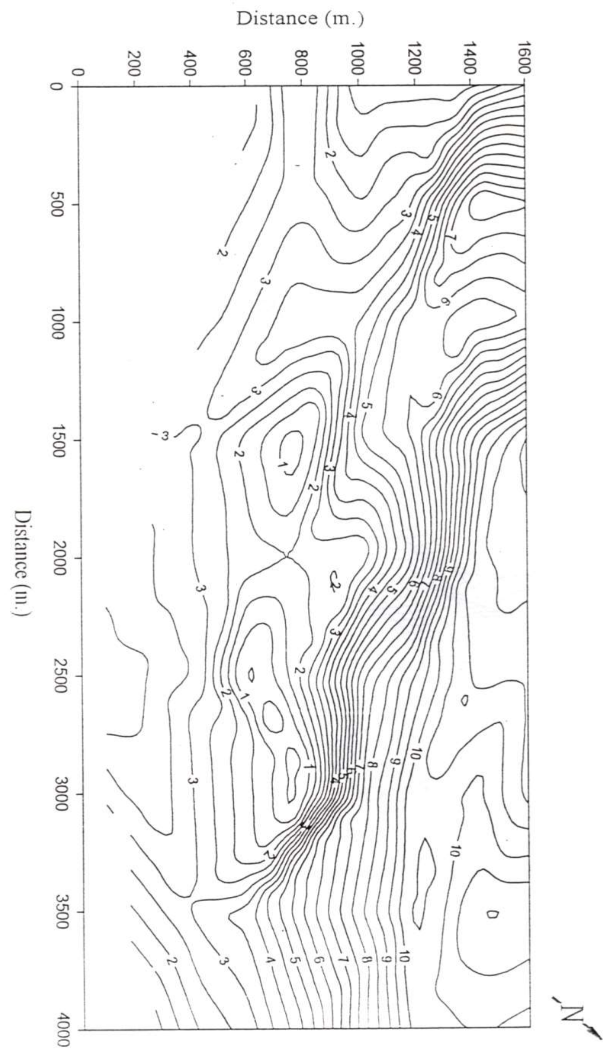

Fig. 15. The thickness isopach map of the conglomerate lithofacies

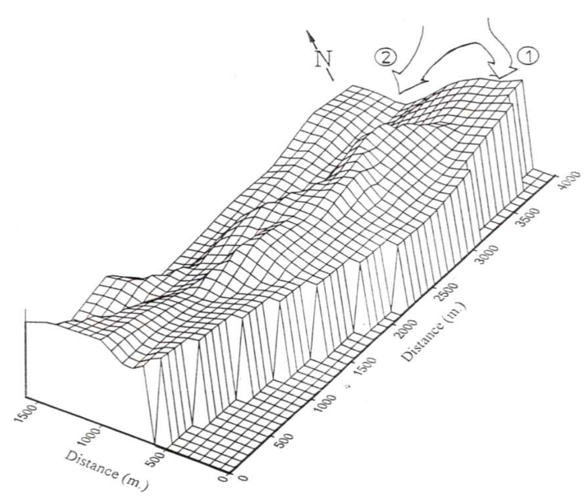

Fig. 16. Depositional model (3D) of the accent Tigris river channel beneath the refinery area

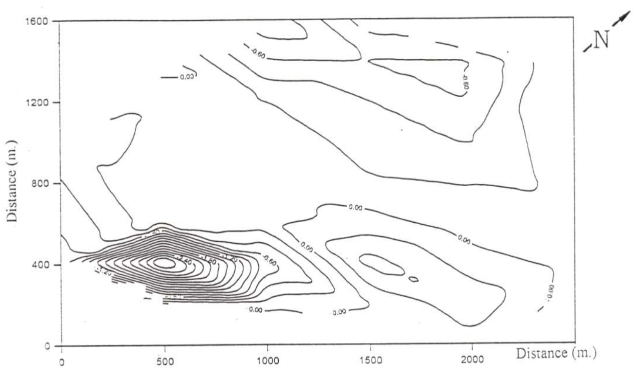

Fig. 17. Difference of water table elevations in the studied area (Al-Jabari 2001)

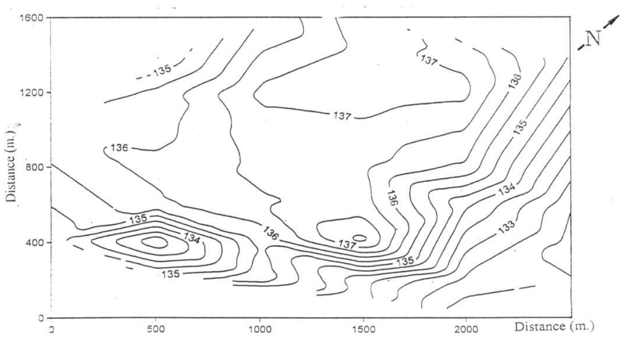

Fig. 18. Map of the water table elevations through the 1st six months of 2001 in the studied area (Al-Jabari 2001)

pumping must be slow and gradual for certain selected boreholes to minimize the impact on the foundation of the buildings. Another risk, the industrial seeped water stored in these dissected channels takes its time to dissolve all the carbonate cementing materials, which causes these rocks to be more friable and decrease the efficiency of the natural ground to bear the load of the buildings. The rise of GWTL in these secondary channels cannot be treated unless the shutdown of the seeped industrial water happens. Then the programming discharge must begin from these channels. After that, grouting of certain materials and for long time intervals must take place, to recover some of the lost cementing materials or at least to fill the ground mass among the gravels. Despite the continuous dissolution of the cementing and binding materials, there are natural discharges for the groundwater to the lower adjacent valleys, and the ground levels of their boreholes could decrease directly after the repairs of the broken and perforated industrial water pipes.

CONCLUSIONS

The Quaternary deposits represent braided and low sinuosity river channels with restricted flood plain deposits. It constituted alternations of conglomerate rocks, sandstone, and clayey siltstone. These deposits reflect the ancient paleo-channel of the third terrace stage of the Tigris River. The conglomerate reflects the main channels, sandstone, and silty claystone represent the deposits of the restricted flood plains. The estimated depositional model is mainly made up of conglomerate rocks, forming the main channel, continuously discharged groundwater, and secondary channel dissected by sand bars bearing groundwater only in flooding events. The presence of conglomerate beds beneath the refinery building has a significant impact on the building foundation of the refinery due to its industrial water contents. The long-term effect of the industrial acid water dissolved continuously the binding and cementing materials deteriorating the engineering properties and bearing capacity of the ground. There is need to conduct a study on certain materials as equivalent to the lost binding and cementing materials to afford the engineering and lithological support for the conglomerate rocks. Repair the industrial pipes during continuous monitoring of the GWTL in the boreholes. Conduct program for the pumping from selected boreholes, particularly in the channel (No. 1) part (1a) to the land surface, then transport the water by lined pipes to the valley area outside the refinery.

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Standardization of Jamun-Bael Blended Fruit Cheese and Physico-Chemical Analysis during Storage

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Abstract: Jamun and bael fruits are rich source of therapeutic and nutraceutical components but due to high perishability and seasonality of these fruits there is a need to develop a product which could be relished throughout the year and also possess high nutritional quality. The present investigation therefore, aimed at evaluating the physico-chemical quality of jamun-bael blended fruit cheese during ambient storage conditions. Jamun and bael pulp were blended in ratios of 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100 % for the development of fruit cheese. The storage studies of blended fruit cheese packed in butter paper followed by LDPE bags showed significant changes in physico-chemical properties during 90 days of storage. The L* and b* colour values of blended cheese decreased with the advancement of storage whereas a* value increased. Total soluble solids, reducing sugars and total sugars increased significantly in jamun-bael blended cheese. Titratable acidity (%) was found highest in 100 % jamun pulp. On the basis of overall acceptability, 40:60::jamun:bael was adjudged as superior among all the treatments.

Keywords: Fruit cheese, Jamun, Bael, LDPE, Nutritional quality

Fruit cheese is a chewable confectionary product which is relatively new to fruit processors. It is prepared by combining fruit pulp with sugar, pectin and acid, and cooked to achieve minimum total soluble solids of 68 %. Fruits like pineapple, papaya, banana, apple, pear and plum have been utilized for preparation of fruit cheese but yet there are many fruits like jamun and bael which are underutilized by the food processing industries (Sucheta et al 2017). Due to balanced sugar, acid and tannin content, jamun or black plum (*Syzygium cumini*) fruit has gained huge popularity among consumers. Jamun fruit have always been the choice of population suffering from diabetes as it is reported to have antidiabetic properties and is traditionally used to cure diabetes mellitus. The fruit has also gained attention due to some other medicinal properties including antibacterial, anti-inflammatory and preventing gastrointestinal problems and thus, has been entitled as herbal medicine by Ayurveda (Rahul et al 2019). The fruit has high antioxidant activity due to presence of anthocyanins (Bhatt et al 2020). It is also enriched with flavonoids, essential oils, phenolic compounds and other antioxidants (Patel and Rao 2014, Akhila and Hiremath 2018). Bael (*Aegle marmelos* Corr.) on the other hand, is a native fruit of India (Sarkar et al 2020) with different common names as Indian quince, golden apple, stone apple, bengal quince. It belongs to family *Rutaceae* and contains total soluble solids in range of 28-39 %, carbohydrates 19-21 %, sugar 11-17 %, fat 0.2 % and vitamin C as 7-21 mg/100g. The bael fruit protect against many chronic and severe diseases as the pulp contains many bioactive and functional compounds such as carotenoids, flavonoids, alkaloids, coumarins, terpenoid, phenolics and other antioxidants. Aroma of the bael fruit is really tempting and strong which is not destroyed even during processing. However, because of its hard outer coat (shell), mucilaginous texture and numerous seeds embedded in pulp, bael fruit is not much popular. Being excellent in flavour, palatability, nutritive and therapeutic values, the fruit shows potential for processing into functional food products (Ullikashi et al 2017). Therefore, the present investigation has been carried out to prepare jamun-bael blended cheese and its quality evaluation during ambient storage conditions.

MATERIAL AND METHODS

The present investigation was conducted at Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu during the year 2019-20. An attempt has been made in the present investigation to select an appropriate level of jamun and bael pulp for preparation of nutritious and high quality fruit cheese. The storage quality of the cheese was analysed with respect to the physico-chemical and sensory attributes.

The jamun pulp was extracted manually from the seeds and ground in a mixer to obtain fine pulp. The pulp so obtained was pasteurized at 80ºC for 1 minute and cooled.

Sodium benzoate @ 1 g/kg of the jamun pulp was added. Finally, jamun pulp was filled in sterile glass jars till further use. Ripened bael fruits with a uniform outer yellow coat were taken and washed thoroughly. The hard coat of bael fruit was half opened using hammer. The pulp was scooped out with seeds and fibers. The scooped pulp was homogenised (1:1 pulp and water) by blending it manually and passed through stainless steel sieve to remove seeds, and then heated to 80ºC for 1 minute with intermitted stirring followed by cooling and mixing of sodium benzoate @ 1 g/kg of pulp. The cooled pulp was filled in pre-sterilized glass bottles, corked and stored till further use.

The fruit cheese was prepared from jamun-bael blends as per the standard procedure given by Kumar (2017) with certain modifications using 1 kg blended pulp, sugar (750 g), butter (70 g), citric acid (3 g) and salt (3 g). These ingredients were kept constant in all the treatments with variation in only jamun and bael pulp ratios. 2% pectin was also added for proper setting of fruit cheese. The mixture of blended pulp, sugar, butter and citric acid was cooked with constant stirring to obtain desired consistency. Pectin dissolved in lukewarm water was added to the cooking mass. Finally, salt was mixed with the cooked mass, when it started leaving sides of the vessel. End point was judged by sheet test and total soluble solids (72ºBrix) were measured using hand refractometer. The product was finally spread on butter smeared trays (0.60 cm) and left at room temperature for setting and cooling. After setting of fruit cheese, suitable size pieces were cut out and finally wrapped and packaged in butter paper and LDPE bags (150 gauge), respectively. The prepared fruit cheese was stored under ambient (24.8±5.02º C and RH 62±5 %) conditions for a period of 90 days. The samples were analysed for various quality parameters at an interval of 30 days. All physical parameters were analysed by randomly selecting ten fruits from the bulk and the experiment was replicated thrice for recording the observations. After recording the initial weight of the fruits, the pulp and seeds were separated and weighed, to calculate pulp to seed ratio.Colour analysis of the prepared cheese was done by using Hunter Lab Colorimeter (Hunter Lab Colour Flex Reston VA, USA) after calibrating the instrument using method of Vargas et al (2009). Total soluble solids (TSS) were measured by using hand refractometer (Erma, Japan) and the results were expressed as Brix (ºB) according to standard procedure as given in (Ranganna 2014). Titratable acidity was determined by titration technique (AOAC 2012). The titrimetric method using 2,6-dichlorophenol indophenol dye was used to estimate ascorbic acid (AOAC 2012). Reducing and total sugars were determined as per the method mentioned by Ranganna (2014). The sensory evaluation of jamun-bael cheese was done by a panel of 10 semi-trained judges using the 9 point hedonic rating scale (scores assigned as 9-"like extremely" to 1- "dislike extremely") as described by Amerine et al (1965). The data obtained were analyzed statistically using factorial Completely Randomized Design (CRD) for interpretation of results through analysis of variance at critical difference of 5% (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

The weight of fresh whole jamun fruits, pulp and seed weight were 8.96 g/fruit, 656.50 g/kg fruit and 240.60 g/kg fruit, respectively (Table 1). The colour values of L*, a* and b* in fresh jamun were depicted as 12.11, 4.20 and -0.87, respectively which were in accordance with the findings of Ghosh et al (2017). The total soluble solids, titratable acidity, ascorbic acid, reducing sugars and total sugars in jamun was 14.89 ºBrix, 0.92 %, 19.29 mg/100 g, 7.08 % and 11.31 %, respectively which were in conformity to the findings earlier workers Ghosh et al (2017), Akhila and Hiremath (2018), Rahul et al (2019) and Dagadkhair et al (2017). The fruit weight, pulp weight and seed weight of bael was 620.86 g/fruit, 649.98 g/kg fruit and 20.67 g/kg fruit, respectively (Table 1). The colour values of L*, a* and b* in bael were be 55.63, 32.42 and 58.71, respectively which were in accordance with the findings of Sonawane et al (2020) and Singh et al (2014). The total soluble solids, titratable acidity, ascorbic acid, reducing sugars and total sugars were recorded as 36.78 ºBrix, 0.39 %, 11.72 mg/100 g, 8.29 % and 20.89 %, respectively in bael. The values were in line with Reena (2007), Nagar et al (2017) and Sharma et al (2019).

Table 1. Physico-chemical composition of fresh jamun and bael fruits

Physical parameter	Jamun	Bael
Fruit weight (g fruit^{-1})	8.96	620.86
Pulp weight (g kg^{-1} fruit)	656.50	649.98
Seed weight (g kg^{-1} fruit)	240.60	20.67
Pulp : Seed ratio	2.73	31.44
Hunter colour values		
L* (Lightness)	12.11	55.63
a* (Redness)	4.20	32.42
b* (Yellowness/blueness)	-0.87	58.71
Chemical parameter		
TSS (ºBrix)	14.89	36.78
Titratable acidity (%)	0.92	0.39
Reducing sugars (%)	7.08	8.29
Total sugars (%)	11.31	20.89

Colour is one of the significant factors which affect acceptability of food products by consumer. The colour value of jamun-bael blended fruit cheese was measured in terms of Hunter L* (lightness), a* (redness) and b* (yellowness/ blueness). In the Table 2, highest mean value of 31.23 (L*) and 18.01 (a*) was recorded in treatment T_6 (0:100::Jamun:Bael) and the lowest was recorded as 6.87 (L*) and 12.34 (a*) for treatment T_1 (100:0::Jamun: Bael). The mean value for b* was found to be highest (35.06) in treatment T_6 (0:100::Jamun:Bael) and lowest with -2.32 in treatment T_1 (100:0::Jamun:Bael) (Table 2). There was a significant increasing trend in L*, a* and b* values with the incorporation of bael. This might be due to increasing level of bael pulp which contained higher colour values. The treatment T_1 (100:0::Jamun:Bael) was found with negative b* value indicating absence of yellowness and high blueness due to the presence of 100 % jamun pulp as jamun is rich in anthocyanin pigment which cause colour modification of blended cheese. Garg et al (2019) blended jamun pulp with kiwifruit and apple pulp for preparation of nutritionally enriched blackberry jam also reported similar results. Kapoor (2015) reported similar trend due to supplementation of jamun powder in papaya bar. Storage studies of jamun-bael blended cheese revealed a significant decrease in mean L* and b* values from 21.07 to 16.50 and 17.44 to 15.76, respectively during 90 days of storage period. However, a* value increased from 14.16 to 16.56 during storage. The decrease might be due to increased rate of pigment degradation (anthocyanins) with storage and formation of brown pigment (melanoidins) by maillard reaction. Similar results were reported by Vukoja et al (2019) reported decrease in colour values of different cherry jams during storage. The highest mean TSS of 73.23 ºBrix was in T_6 (0:100::jamun:bael) and lowest as 72.75 ºBrix was recorded in T_1 (100:0::jamun:bael) (Table 3). There was significant increase in mean TSS values with storage from 72.00 to 73.96 ºBrix. The increase in TSS in all the treatments might be due to conversion of insoluble fraction into soluble fraction. The increase in total soluble solids might also be due to partial hydrolysis of polysaccharides into simple substances and due to loss of moisture as a result of evaporation from product or due to solidification of pulp constituents during storage (Sharma 2014). Kumar (2017) discussed similar increase in TSS with storage in aonla and papaya cheese and Elbelazi (2015) and Thakur et al (2017) found similar increasing trends in value added apple cheese and pomegranate appetizer, respectively.

The mean titratable acidity was highest in T_1 (100:0::Jamun:Bael) as 1.07 % and lowest in T_6 (0:100::Jamun: Bael) as 0.73 % (Table 3). The significant difference among treatments of jamun-bael cheese might be due to the amount of citric acid and pulp used in preparation of blended cheese. The mean titratable acidity decreased from 0.96 to 0.82 % during 90 days of storage which might be due to chemical interactions between organic constituents of the pulp induced by temperature and action of enzymes during storage. The decrease in acidity might also be contributed to the chemical degradation (Kumar et al 2017). Mewada et al (2013) also reported that with the advancement of storage period, acidity decreased in guava papaya mixed toffee. The maximum mean reducing and total

Table 2. Colour values of jamun-bael blended fruit cheese during storage

Treatment (Jamun:Bael)	L*					a*					b*				
	Storage period (Days)					Storage period (Days)					Storage period (Days)				
	0	30	60	90	Mean	0	30	60	90	Mean	0	30	60	90	Mean
T_1 (100 : 0)	8.29	7.73	6.37	5.11	6.87	11.07	11.69	12.45	14.16	12.34	-2.05	-2.27	-2.34	-2.61	-2.32
T_2 (80 : 20)	13.26	11.65	10.22	8.94	11.02	12.02	12.15	13.16	15.20	13.13	5.61	5.10	4.67	4.38	4.94
T_3 (60 : 40)	18.51	16.26	14.81	13.52	15.78	13.65	13.91	14.48	16.11	14.54	13.23	12.79	12.33	11.91	12.57
T_4 (40 : 60)	24.23	22.31	20.95	19.21	21.67	14.57	14.69	15.16	17.05	15.37	22.16	21.73	21.34	20.92	21.54
T_5 (20 : 80)	28.70	26.76	24.37	23.06	25.72	16.32	16.60	16.78	17.90	16.90	29.19	28.77	27.31	26.88	28.04
T_6 (0 : 100)	33.42	31.90	30.43	29.17	31.23	17.34	17.81	17.96	18.92	18.01	36.52	35.92	34.69	33.12	35.06
Mean	21.07	19.43	17.86	16.50		14.16	14.48	15.00	16.56		17.44	17.01	16.33	15.76	
Effects	CD ($p \leq 0.05$)					CD ($p \leq 0.05$)					CD ($p \leq 0.05$)				
Treatment (T)	0.03					0.004					0.05				
Storage (S)	0.03					0.003					0.04				
TxS	0.07					0.008					0.09				

Table 3. TSS (ºBrix) and titratable acidity (%) of jamun-bael blended fruit cheese

Treatment (Jamun:Bael)	TSS (ºBrix)					Titratable acidity (%)				
	Storage period (Days)					Storage period (Days)				
	0	30	60	90	Mean (Treatment)	0	30	60	90	Mean (Treatment)
T_1 (100 : 0)	72.00	72.31	73.12	73.58	72.75	1.15	1.08	1.04	1.01	1.07
T_2 (80 : 20)	72.00	72.45	73.28	73.74	72.87	1.07	1.02	0.99	0.94	1.01
T_3 (60 : 40)	72.00	72.56	73.41	73.87	72.96	0.98	0.93	0.87	0.83	0.90
T_4 (40 : 60)	72.00	72.64	73.52	74.03	73.05	0.91	0.88	0.83	0.75	0.84
T_5 (20 : 80)	72.00	72.70	73.69	74.21	73.15	0.85	0.81	0.76	0.72	0.79
T_6 (0 : 100)	72.00	72.77	73.80	74.35	73.23	0.80	0.76	0.71	0.65	0.73
Mean	72.00	72.57	73.47	73.96		0.96	0.91	0.87	0.82	
Effects	CD($p\leq0.05$)					CD($p\leq0.05$)				
Treatment (T)	0.04					0.03				
Storage (S)	0.03					0.02				
TxS	0.09					NS				

Table 4. Content of reducing sugars (%) and total sugars (%) of jamun-bael blended fruit cheese

Treatment (Jamun:Bael)	Reducing sugars (%)					Total sugars (%)				
	Storage period (Days)					Storage period (Days)				
	0	30	60	90	Mean (Treatment)	0	30	60	90	Mean (Treatment)
T_1 (100 : 0)	28.62	28.81	29.03	29.28	28.93	58.73	59.24	60.03	60.74	59.68
T_2 (80 : 20)	29.00	29.20	29.45	29.73	29.35	60.81	61.17	61.78	62.36	61.53
T_3 (60 : 40)	29.59	29.83	30.06	30.26	29.94	62.46	62.85	63.61	64.63	63.39
T_4 (40 : 60)	30.08	30.32	30.58	30.89	30.46	64.07	65.41	65.98	66.56	65.50
T_5 (20 : 80)	30.61	30.79	31.01	31.32	30.93	65.92	66.32	66.73	67.81	66.69
T_6 (0 : 100)	31.01	31.25	31.50	31.83	31.40	67.12	67.51	68.15	68.97	67.94
Mean	29.82	30.03	30.27	30.55		63.18	63.75	64.38	65.18	
Effects	CD($p\leq0.05$)					CD($p\leq0.05$)				
Treatment (T)	0.04					0.05				
Storage (S)	0.03					0.04				
TxS	0.07					0.09				

sugars of 31.40 and 67.94 %, respectively (Table 4) was recorded in treatment T_6(0:100::Jamun: Bael) and minimum of 28.93 and 59.68 %, respectively for T_1 (100:0::Jamun:Bael) which might be due to differences in composition of raw materials (Kumar 2017). The mean value of sugars for blended cheese increased during 90 days of storage period. The increase in sugars might be due to gradual loss of moisture, inversion of sucrose to glucose and fructose or due to breakdown of complex polysaccharides into simple sugars during storage. The results are in accordance with Sharma et al (2013) in apricot fruit bar and Vidhya and Narain (2011) in wood apple bar and jam.Maximum overall acceptability score of 8.05 was in T_4 (40:60::Jamun:Bael) followed by T_5 (20:80::Jamun:Bael) with score of 7.90 however, treatment T_1 (100:0:: Jamun:Bael) scored least with 7.21 followed by T_2 (80:20::Jamun:Bael) (Fig. 1). This might be due to higher sensory scores for T_4 (40:60:: Jamun: Bael) compared to other treatments. A general decrease in overall acceptability scores from 7.90 to 7.30 during 90 days of storage was observed however, the average score remained above acceptable limits (5.5) throughout the storage period. The decrease might be attributed to the change in objective characteristics like loss of colour pigments, breakdown of insoluble solids, change in sugar acid ratio and overall quality loss in product (Sood 2015). Rahul et al (2019) while carrying

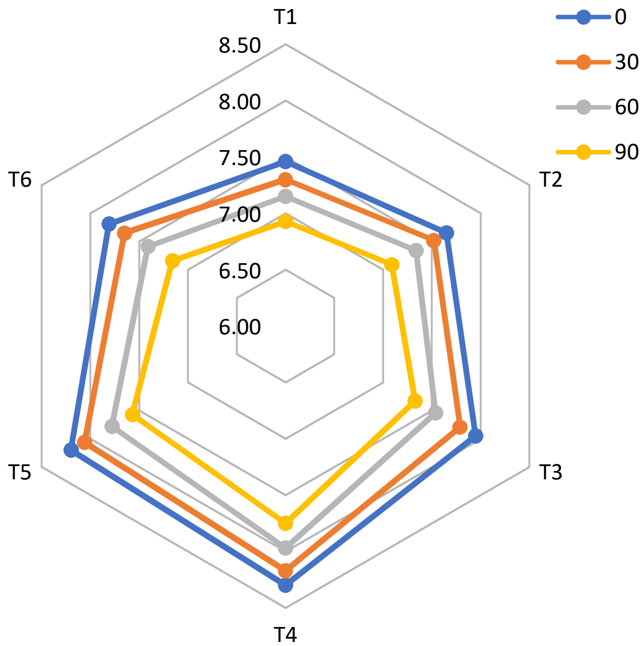

Fig. 1. Overall acceptability of Jamun-Bael blended fruit cheese

out storage studies on guava-jamun cheese reported a decrease in sensory score of different parameters, irrespective of treatments.

CONCLUSION

Jamun and bael fruits can be blended in appropriate ratio to develop fruit cheese with improved nutritional and sensory quality. Increase in bael percentage showed decreased titratable acidity whereas, increased reducing sugar (%) and total sugars % in fruit cheese. The stored cheese showed significant increase in total sugars, reducing sugars and non-enzymatic browning whereas decrease in carotenoids, total phenols and ascorbic acid. However, blended cheese prepared can be stored at ambient conditions for three months without much loss in nutritional and organoleptic quality. The findings of the present research could be useful for developing and improving the quality of functional products developed by fruit processing industries thus utilizing these underutilized fruit crops.

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Attitudinal Behaviour of Urban Habitations Towards Ecosystem Services in North Western Himalayas

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***Abstract*:** The importance of ecosystem services and its economics is well formed and emphasized by studies which can built correlation towards bridging the connection in terms of value behind and attitude towards the ecosystems surrounding urban areas. In the present study, assessed the attitudinal behaviour and perceptions of urban habitations towards ecosystem services through questionnaire surveys in Kangra district of Himachal Pradesh which is a hilly state of India situated in North western Himalayas. The sample areas were selected using survey conducted in Kangra district with a sample size of 180 respondents. Among the respondents, majority (71.11%) were in favour of non-payment for ecosystem services, whereas, only (28.89%) had shown their concern for payment. The 80.56% was aware about the importance of ecosystem services to environment. Among the six kinds of ecosystem services the highest was above Rs. 2000 per year for water conservation (46.15%) followed by Pollution decomposition (19.23%), soil retention (13.46%), Biodiversity conservation (11.53%), Carbon fixation (05.76%) and the lowest was Rs.400 per year per year for aesthetic existence (03.84%). The results illustrated the top limit of payments was Rs 2000 per year per capita, and people were willing to pay Rs. 426 per year per capita for water conservation, which was the highest among the six ecosystem services. Regarding the un-willingness the major was the low house hold income followed by that it was the duty of Government.

***Keywords*:** Ecosystem services, Water conservation, Urbanisation, Willingness to pay, Contigent valuation method

Ecosystem services (ES) are benefitted by human populations to a larger extent that comes from natural ecosystems (Chu et al 2020). Ecosystem services are a type of common pool resource which is a topic of consideration exceeding 20 years. There are broad range of quantifications methods for valuation of ecosystem services including biophysical, socio-cultural and economic valuations. Out of economic evaluation methods; social valuation can be an important tool in rendering user's willingness and demand for Ecosystem services (Liu 2020). More specifically, the contingent valuation method (CVM) has been used widely to capture socio-economic information that is relevant to ecosystem services. Contingent valuation methods are used when markets do not exist for environmental resources. The valuation is done here in hypothetical markets. This is computed by asking people's preference or willingness to pay for environmental benefit (WTP) or how much people are willing to accept as a compensation (WTA) for an environmental resource. CVM establishes how much people are willing to pay for maintaining services (West et al 2006).

Willingness to pay (WTP) is a survey-based method which is quite helpful in linking the value behind the ecosystem services (Bhandari et al 2016, van Riper et al 2017, Mayer and Woltering 2018) and can be used to know the attitude on resource use, ecological opinion of a place and the potential influence in socio-ecological system by the government (Ostrom 2009, Murtinho and Hayes 2017). WTP has three key elements which must be focused i.e., people, place and space (West et al 2006).

Payment for ES is a critical factor in knowing the management of common pool resources. (Fisher et al 2010). Contrary to recording the supply of ES, payment is embedded in demand of these ES and also includes people who have profited from the streaming of these services (Farley and Costanza 2010). A communal outcome on the payment of ES can augment the provisioning and regulating service on the supply sideways along with feedback return for operation on the demand side (Cook et al 2016, Plumb et al 2018).

The district Kangra is located on the southern slope of the Himalayas and it is the most populous district of Himachal Pradesh, which is a hilly state of India placed in North western Himalayas. Himalayas are large geographical fragile areas which are experiencing disruption in ecosystem services because of rapid and unplanned urbanization which is an inevitable worldwide trend. Himalayan states are responsible for bestowing crucial and gigantic ecosystem services to the country. Himalayas having special socio-ecological characteristics making the respective place to be an important place for scrutinizing the ecosystem values.

Considering the effects of fast urbanisation on ESs and their interactions could result in scientifically sound recommendations for urban planning that prioritize ES protection, long-term urban growth, and human well-being (Lyu et al 2018). The objective of the study was to analyse attitudes of urban habitatants towards ecosystem services and application of CVM for assessing their WTP in enhancing sustainable development along with various factors that drive their WTP.

MATERIAL AND METHODS

Study area: Kangra district is located between 31˚ 21′ to 32˚ 59′ North latitude and 75˚ 47′55″ to 77˚ 45′ East longitude. It has altitude that goes from 500 meters above mean sea level (amsl) to around 5000 meters above mean sea level (amsl). Mountain ranges, hills and valleys encompasses the district with snowfall reaching on Dhauladhar ranges (lesser Himalayan chain of mountains). It exhibits climate that vary from sub-tropical to sub-humid. December to February is followed by winters whereas summer prolongs from March to June while July to September are the rainy months in the district. The average maximum and minimum temperature ranges from 22.50 to 38.77^{0}C and 2.40 to 20.40^{0}C respectively. The district receives about 1751 mm average annual rainfall; majority of which appear during June to September.

Experimental details: The present study was conducted during the year 2019 and 2020 in the Department of Environmental Science, College of Forestry, YSP University of Horticulture and Forestry, Nauni-Solan, Himachal Pradesh, India (Fig. 1).

In order to achieve the objectives of the study survey were conducted in urban areas of Kangra district of Himachal Pradesh and assessment of the attitudinal behavior of urban habitations towards value of ecosystem services was done for enhancing environmental sustainability. Simple random sampling was worked to select the households. Face-to-face survey of households in the urban areas namely Municipal Corporation (Dharamshala), Municipal council (Palampur) and Nagar panchayat (Baijnath-Paprola) to determine their willingness was implemented. In total 180 household respondents were selected using a pre-structured and tested questionnaire. The questionnaire comprised three parts. Part 1 sought information on the respondent's socio-economic surroundings, comprising name, sex, age, education level, and occupation; household members and annual household income, among other things. Part-2 was designed to seek information on respondent's awareness and familiarity regarding willingness to pay. Part-3 was designed to evaluate the attitudes considering the significance of selected ecosystem services, payment for the services, associated satisfaction levels and various other factors which act as barriers or non- willingness in payment of ecosystem services.

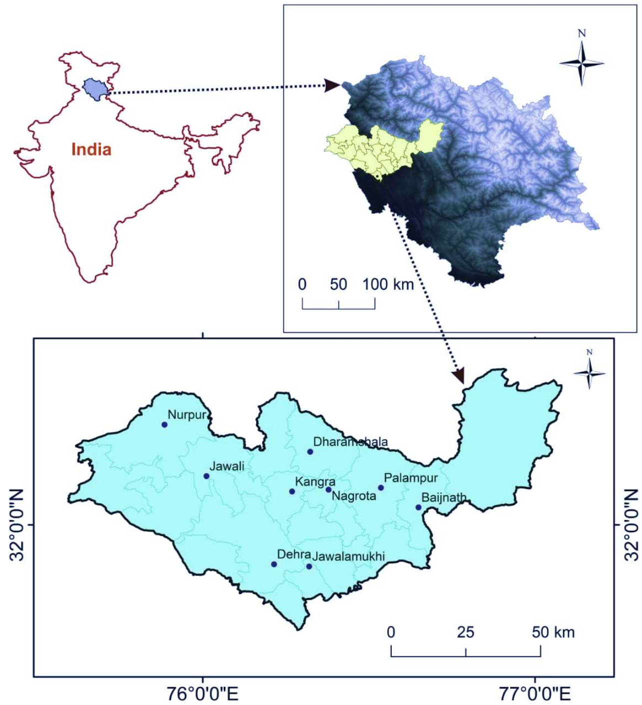

Fig. 1. Geo-referenced locations of the study area

Expected minimum willingness to pay (WTP): To calculate the WTP of urban habitations towards value of ecosystem services, ***traditional partition group processing method*** was applied using following formula (Wooldridge 2014, Feng et al 2018 and Chu et al 2020)

$$E(WTP) = \Sigma^{n}_{i=1} A_i \times P_i$$

Where,

E (WTP) = Expected minimum WTP of respondents

A_i = Degree of WTP (n options)

P_i = Probability of WTP

RESULTS AND DISCUSSIONS

Socio- demographic characteristics: The socio-demographic characteristics are given in Table 1. The majority of respondents (67.22 %) are aware off about ecosystem services. Half of the respondents understand WTP whereas, equally have not any familiarity about it. Substantial number of surveyed respondents (84.44%) believed that the environment has changed a lot with time for example droughts, rise in temperature, frequent rainfall and consequently the major global change. About 67.22% showed their willingness towards protecting and developing forests and urban green space around themselves. Almost

65% of respondents expressed their willingness to assist and participate in afforestation projects and allied activities. More than half of the respondents (65.56%) were aware about the land acquired by the government (forest department) for developing tree plantations.With respect to frequency of natural disasters, majority of the respondents (84.44%) believed that natural disasters have accelerated whereas, whereas, less than 20 % people have mixed thoughts about the occurrence of natural disasters either there is decrease (6.67%) or no change (8.89%). About 73.33% of respondents considered ecosystem services important for maintaining ecological environment. Overall, nearly half of the respondents (43.33%) has expressed their satisfaction whereas, above half of the respondents (52.78%) expressed their neutral attitude and mixed thoughts underlying to satisfaction towards ecosystem services. The majority of the respondents (71.11%) expressed their reluctancy whereas (28.89%) expressed their willingness to pay for ecosystem services (Table 3).

Table 1. Respondent's socio- demographic characteristics (N=180)

Variables	Description	Frequency	Proportion (%)
Gender	Male	119	66.11
	female	61	33.88
Age	16-25 years	28	15.56
	26-35 years	32	17.77
	36-45 years	54	30.00
	46-60 years	27	15.00
	60 and above	39	21.67
Educational qualification	Illiterate	02	01.12
	Up to Matric	35	19.44
	Plus two (+2)	24	13.33
	Graduate	74	41.11
	Post graduate and above	45	25.00
Occupation	Govt. employee	45	25.00
	Private sector employee	22	12.22
	Own Business	58	32.22
	Professional	29	16.12
	Any other	26	14.44
Annual household income	Below Rs. 100000	48	26.67
	Rs. 100000-300000	62	34.44
	Rs. 300000-500000	43	23.89
	Rs. 500000 and above	27	15.00
Family type	Joint	101	56.11
	Nuclear	79	43.89
No. of family members	1-2 members	12	06.66
	3-4 members	59	32.78
	5-6 members	73	40.56
	7 and above members	36	20.00

The willingness to pay for obtaining better ecosystem services in the study area is presented in the Table 4. The table above highlights the willingness to pay in the study area for better ecosystem services. The values in the questionnaire were based on intervals to describe the degree of willingness to pay. Using statistical conventions and the results of Feng et al. (2018) and He et al. (2018) by traditional partition group processing method, we calculated a respondent's WTP by taking the median value of each interval. Approximately 71.11 percent of the respondents demonstrated a preferential level of willingness to pay Rs. zero per year, with this group often exhibiting high levels of environmental awareness (Table 4). Over 90% of respondents picked above Rs.2000 per year, while Rs. 801-1200 per year and Rs. 1201-1600 per year placed second and third respectively. Only a minority of respondents selected Rs. 401-800 per year and Rs. 1601-2000 per year.

Following the distribution of the WTP obtained during the study, the following equation was used to calculate the average expected willingness to pay value:

$$E(WTP) = \sum_{i=1}^{6} A_i \times P_i$$

$$= 200 \times \frac{2}{180} + 600 \times \frac{6}{180} + 1000 \times \frac{10}{180} + 1400 \times \frac{8}{180} + 1800 \times \frac{2}{180} + 2000 \times \frac{24}{180}$$

$$= \text{Rs.}426.67/\text{year}$$

Where;

The WTP standard (six options) is denoted by Ai, and the probability of WTP is defined by Pi.

From the six kinds of ecosystem services measured, water conservation averages over Rs. 2000 per year (46.15%), followed by pollution decomposition, soil retention, biodiversity conservation, and carbon fixation and the lowest is Rs.400 per year for aesthetic existence (03.84%). Studying respondents' attitudes toward ecosystem services and the WTP to value and pay for improved ecosystems. The 71.11 percent of the respondents asked for free services and 28.89 percent expressed willingness to pay Rs. 426 per year for the improved ecosystem services. WTP values were found to be influenced by social, economic, and environmental factors.

With respect to the observation about willingness to pay because it provides tax incentives, it can be concluded that among the sample surveyed about more than fifty percent (53.85 %) were of no thought, whereas, nearly 10 per cent were strongly agreed about the fact (Table 5). A key indicator of environmental awareness is perception of ecosystem service importance, their satisfaction levels, and climate, disasters, and environmental changes.

Barriers/non-willingness in Payment of Ecosystem Services (ES)

Low income: The 78.13% of the respondents either agree or

Table 3. Willingness to pay for ecosystem services

Willingness to pay	No. of respondents	Percentage
Yes	52	28.89
No	128	71.11
Total	180	100.00

Table 2. Information pertaining to respondent's awareness regarding Willingness to pay (WTP)

Variables	Description	Frequency	Proportion (%)
Heard about ecosystem services	Yes	121	67.22
	No	59	32.78
Heard about WTP	Yes	90	50.00
	No	90	50.00
Importance of ecosystem services to environment	Importance	145	80.56
	Un-important	35	19.44
Change in today's environment	Change	152	84.44
	No change	28	15.56
Willingness to protect forests and urban green space	Yes	121	67.22
	No	59	32.78
Willing to assist in afforestation projects	Yes	117	65.00
	No	63	35.00
Land acquired by the government for ES	Yes	118	65.56
	No	62	34.44
Occurrence of natural disasters	Increase	152	84.44
	Decrease	12	06.67
	No change	16	08.89
Importance of ecosystem services to people	Very important	9	5.00
	Relatively important	123	68.33
	Un-important	0	0.00
	Neutral	48	26.67
Satisfaction with the ecosystem services	Very satisfied	4	2.22
	Relatively satisfied	74	41.11
	Unsatisfied	7	3.89
	Neutral	95	52.78

Table 4. Amount of payment for Willingness to Pay for ecosystem services in study area

Sr. No.	Payment card (Rs. /Year)	Ecosystem services	No. of respondents	Percentage
WTP = Yes				
1.	>2000	Water conservation	24	46.15
2.	1601-2000	Carbon fixation	02	03.84
3.	1201-1600	Soil retention	08	15.38
4.	801-1200	Pollution decomposition	10	19.23
5.	401-800	Biodiversity conservation	06	11.53
6.	1-400	Aesthetic existence	02	03.84
		Total	52	
WTP = No				
1.	0	Total	128	71.11
		Grand total	180	100.00

Following the distribution of the WTP obtained during the study, the following equation was used to calculate the average expected willingness to pay value :
E(WTP) = Rs.426.67/year

strongly agree that low income is a barrier in payment of ecosystem services followed by 10.15% were undecided on the statement. Further, the results of the goodness of fit test (X^2= 216.14, P< 0.05) indicate the test is highly significant.

Need: Majority of the respondents either agree or strongly agree 48.43%with the statement that they don't feel the need of payment for ecosystem services followed by 23.45% respondents undecided on the statement (Table 6). Finding of the analysis suggests that most of the respondents do not feel the need of payment. The result further indicates that the test of goodness of fit ($\chi^{\wedge 2}$ = 37.00, P< 0.05) was found to be significant.

Alternative investment preferences: The 67.96% of the respondents either agree or strongly agree with the statement that they prefer to invest in some other sources rather than paying for ecosystem services followed by 17.97% respondents who were undecided on the statement. Further, the results of the goodness of fit test ($\chi^{\wedge 2}$ = 151.61, P < 0.05) indicate the test is highly significant.

Unaware: The 54.68% neither agree nor disagree whereas 24.22% either agreed or strongly agreed with unaware variable of barrier in payment of ecosystem services. The result further indicates that the test of goodness of fit was found to be significant ($\chi^{\wedge 2}$= 96.92, P < 0.05).

No one suggested: The half of the respondents (52.34%) neither agree nor disagree with the statement that no one suggested about the ecosystem services followed by 33.59% of respondents who disagreed to it. The results further

Table 5. Information pertaining to agreement on payment of ecosystem services (ES)

Description	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree	Chi-square*
Willing to buy because it provides tax incentive to you	5 (9.62)	2 (3.84)	28 (53.85)	7 (13.46)	10 (19.23)	40.50
Environmental protection is such a big factor that compels to buy it	3 (5.76)	7 (13.47)	33 (63.47)	6 (11.53)	3 (5.77)	62.62
Willing to buy because it provides individual credit	4 (7.70)	3 (5.77)	29 (55.77)	8 (15.38)	8 (15.38)	43.58
Willing to buy because it provides honour recognition	7 (13.46)	8 (15.38)	25 (48.08)	7 (13.47)	5 (9.61)	26.08
Willing to buy because it is provided with the least cost	6 (11.54)	9 (17.31)	30 (57.69)	4 (7.69)	3 (5.77)	48.19
Willing to buy because it provides ecotourism offers	3 (5.77)	7 (13.46)	28 (53.85)	7 (13.46)	7 (13.46)	38.38

*P<0.05;Figures in the parentheses is in percentage

Table 6. Information pertaining to barriers/non-willingness in payment of ecosystem services (ES)

Barriers	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree	Chi-square*
Low Income	7 (5.46)	8 (6.26)	13 (10.15)	92 (71.87)	8 (6.26)	216.14
Don't feel the need.	22 (17.18)	14 (10.94)	30 (23.45)	50 (39.06)	12 (9.37)	37.00
Prefer to invest money in some other sources.	12 (9.38)	6 (4.69)	23 (17.97)	80 (62.50)	7 (5.46)	151.61
Unaware.	14 (10.94)	13 (10.16)	70 (54.68)	18 (14.06)	13 (10.16)	96.92
No one suggested.	18 (14.06)	25 (19.53)	67 (52.34)	10 (7.81)	8 (6.26)	90.83
Not taken by friends and relatives	6 (4.68)	10 (7.81)	12 (9.37)	23 (17.97)	77 (60.17)	135.20
Should be duty of government	8 (6.26)	7 (5.45)	8 (6.26)	15 (11.71)	90 (70.32)	204.11
Can be Entry fee of the scenic spots	13 (10.16)	11 (8.59)	86 (67.18)	15 (11.72)	03 (2.35)	181.38
Lack of reliability and flexibility	59 (46.09)	27 (21.10)	20 (15.63)	13 (10.15)	09 (7.03)	61.84
Not interested in ecological protection	43 (33.59)	50 (39.06)	12 (9.38)	13 (10.15)	10 (7.82)	58.02

*P<0.05;Figures in the parentheses is in percentage

Table 7. People's perception for the most appropriate option for form of payment for ecosystem services

Description	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree	Chi-square*
Taxation, environmental protection and natural resource protection	10 (7.81)	07 (5.47)	10 (7.81)	23 (17.97)	78 (60.94)	140.05
Public donations/property taxes in communities and utilities	09 (7.03)	12 (9.38)	23 (17.97)	31 (24.22)	53 (41.40)	48.72

*P<0.05;Figures in the parentheses is in percentage

indicate that the test of goodness of fit (χ^{2}= 90.83, P < 0.05) was found to be highly significant.

Not taken by friends and relatives: The 60.17 % respondents strongly agree with the statement that variable not taken by friends and relatives is a barrier in payment of ecosystem services followed by 9.37 % of respondents who were found to be undecided. Further, the test of goodness of fit (χ^{2}= 135.20, P < 0.05) was found to be significant.

Should be duty of government: The 82.03% of the respondents either agree or strongly agree with the statement that it should be the duty of government followed by 6.26% of respondents who had mixed thoughts for statement. The results further indicate that the test of goodness of fit (χ^{2}= 204.11, P < 0.05) was found to be highly significant.

Can be Entry fee of the scenic spots: The 67.18% respondents neither agree nor disagree with the statement whereas 14.07% either agree or strongly agree with the statement and believed that it can be entry fee of the scenic spots. The result further indicates that the test of goodness of fit (χ^{2}= 181.38, P < 0.05) was found to be significant.

Lack of reliability and flexibility: The 67.19 % of the respondents strongly disagree with the statement, followed by 17.18 % who either agree or strongly agree with it. The result further indicates that the test of goodness of fit (χ^{2}= 61.84, P < 0.05) was found to be significant.

Not interested in ecological protection: The 17.97 % of the respondents either agree or strongly agree with the statements whereas 39.06 % respondents were found strongly disagree with the concept of ecological protection and the test of goodness of fit (χ^{2}= 58.02, P < 0.05) was found to be significant. Based on the results of Chu et al (2020) and Liu (2020) who report ecosystem services are common pool resources that should be valued based on willingness to pay (WTP) and estimates of WTP for compensation in ecological protection strategies. As a result, top-limit payments were 1080.95 CNY/capita on average, and people wished to pay 172.40 CNY/capita for water conservation; the highest among ecosystem services. The difference in WTP values can be attributed to a range of economic, social, and environmental factors. Geographical diversity in the payment for ecosystem services exists across countries and regions.

The 60.94 % strongly agreed about the fact that payment for ecosystem services should be collected in the form of taxation, environmental protection and natural resource protection (Table 7). With respect to the perception of payment for ecosystem services in the form of Public donations/property taxes in communities and utilities, 41.40 per cent people strongly agreed.

CONCLUSION

Urban inhabitants of Kangra has mixed attitudinal behaviour which corresponds to less knowledge and awareness towards urban green space. Starting from the demand and participation for ecosystem services, this study not only identified the group of respondents willing to manage the common pool resources, but unveiled the driving forces of the WTP for ES. The importance of water conservation for all ecosystem services assessed during the study aligns with their preference for conserving ecosystem services. Urban dwellers have adhered to more of the self-centred approach rather than having a mutual concern for the environment. Consequently, it is recommended that a bottom-up adaptation of governance for managing common pool resources in urbanizing and developing regions alongwith there rises an urgent need to educate the urban dwellers regarding value of ecosystem and its services, so that policymakers and planners may undertake conservation programs to ensure judicious use of resources for sustainable urbanization in the region in order to maintain three pillars of sustainability i.e., social, economic and environmental.

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Comparison of ANN & RSM Approaches for Optimum Production of γ-Decalactone

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Abstract: γ-Decalactone is an important flavor compound widely used in food, diary and fragrances. Previously it was produced from fruits through chemical synthesis. Due to increase in demand for natural products by consumers, it has gained interest for its production through biotechnological way. The present study focusses on fermentation variables optimization for the production of γ-decalactone using *Sporidiobolus salmonicolor* via Response surface methodologies (RSM) and Artificial neural networks (ANN) using castor oil as substrate. The prediction abilities of RSM and ANN were compared based on error parameters namely Mean absolute error (MAE), Root mean square error (RMSE), chi-square (χ^2) and correlation coefficient (R^2) to suggest the best approach for modeling. The response variable (γ-decalactone production) was modelled and optimized as a function of four input variables (castor oil percentage, pH, incubation time and temperature). Training of ANN network was performed using a multilayer feed forward architecture with same experimental datasets used in RSM. Model predictions of both approaches were compared with the experimental values and reported that these are in good close agreement. The highest production of γ-decalactone 72.73 mg/l obtained at an optimum conditions of castor oil -29.68 %, pH -5.32, incubation time -99.89 h and temperature -23.22^{0}C. Hence, results were beneficial in using appropriately trained ANN over RSM for nonlinear fermentation systems.

Keywords: RSM, ANN, Fermentation, γ-decalactone and *Sporidiobolus salmonicolor*

γ-Decalactone is an important industrial flavour compound with a peachy aroma. It is approved as a food additive by FDA (Hamideh et al 2013). It has been widely used in dairy products, food, fragrances and beverages (Adelaide et al 2016, Jung-Ung et al 2013) and was produced from fruits through chemical synthesis, however, due to the increase in demand for natural flavors and has gained interest in producing through biotechnological processes using microorganisms (Dayana et al 2017). It is highly toxic for the microorganisms used for its production and accumulation in the medium depends on the difference between the formation and consumption rates by the yeast cells since lactones are toxic to the producing cells. In the last two decades, researchers have focused on the selection of suitable yeast strains, substrates, preliminary optimization of fermentation conditions, β-oxidation metabolic pathways, use of different mutant strains and mode of fermenter operation to enhance growth and production of γ-decalactone (Dayana et al 2017, Jolanta et al 2020, Eko et al 2020). However, there are few reports on the statistical optimization of fermentation conditions for γ-decalactone production. The aim of this work is to enhance γ-decalactone production and optimization of fermentation variables using statistical methods.

Castor oil is one of the main substrates used to produce γ-decalactone and it contains 86% of ricinoleic acid (Cao et al 2014, Dayana et al 2017). The ricinoleic acid transforms into γ-decalactone by yeast cells through the β-oxidation pathway (Adelaide et al 2016). The main limitation of industrial process development is lactone toxicity towards growing yeast cells. After a few hours of batch fermentation, yeast cells consume decalactone as a carbon source when a substrate is completely exhausted. Thus, results in a lower yield of γ-decalactone. One factor at a time (OFAT) method is laborious, time taken, unable to predict interaction effects and rarely guaranties the estimation of optimum conditions. The limitations of OFAT can overcome with the help of empirical methods, namely, RSM and ANN, in which levels of all desired factors can be varied simultaneously. RSM has been widely used in the medium optimization for fermentation systems (Suganthi et al 2015, Taswar et al 2017). RSM is a combination of several statistical techniques applied for model development, experimental design, determination of the influence of factors, and finding optimum values (Kalil et al 2000). The output responses in RSM are fitted according to second order polynomial equations. Thus, RSM can be considered to be the best choice for fermentation systems optimization. Recently, ANN has also been developed as an effective method for modeling of nonlinear systems due to its ability to learn from historical data and genetic structure (Kiran et al 2005). ANN does not need a predefined fitting function. It has the characteristics of universal approximation (Kiran et al 2008). Moreover, ANN provides better sensitivity analysis than RSM. The major objective of the present study was to maximize γ-decalactone production by *Sporidiobolus salmonicolor* using castor oil as substrate and to analyze modelling efficiencies of RSM and ANN for optimum production of γ-decalactone.

MATERIAL AND METHODS

Inoculum and culture conditions : *Sporidiobolus salmonicolor* (MTCC No. 485) was obtained from IMTECH, Chandigarh, India. It was rejuvenated on YM agar media at 30^0C for 2 days. A 3 ml of grown suspension was taken aseptically to a Erlenmeyer flask (250 ml) consists of 100 ml fermentation medium (castor oil-30 %, NH_4Cl-3 g/l, $CaCl_2.2H_2O$-1 g/l, KH_2PO_4-2 g/l, Tween 80-1 g/l, $FeSO_4.7H_2O$-0.5 g/l and $MgSO_4.7H_2O$-1 g/l) and incubated in rotating shaker at 180 rpm and 30^0C for 18–19 h until the cells enter the late exponential phase to obtain 10^6-10^7 cells/ml.

Biomass estimation: The biomass concentration was determined with the help of Neubauer's improved counting chamber method (Recombigen Laboratories, New Delhi) (Mather and Roberts 1998). The methylene blue method was used for estimating the viability of cells (Angelo and Donatella 1982).

Extraction and analysis of γ-decalactone: To estimate the lactones in the grown culture followed the method described by (Nelma et al 2011) after centrifugation, 2 mL of supernatant was taken and pH was varied to 2 with 1 N HCl. The lactone extraction was done with 2 ml of diethyl ether through sixty gentle shakings. After the partition of liquid phases, the diethyl ether phase was isolated and examined by gas chromatography (Analytical Technologies Limited, Baroda, India; model: GC2979 Plus) with a capillary column (300mmx280mmx270mm) using a helium carrier gas. The temperatures of the split injector and detector were set as 250^0C and 300^0C, respectively. The oven temperature varied from 60^0C to 145^0C at a rate of 5^0C/min and 145^0C to 180^0C at 2^0C/min.

RSM modeling: In previous studies, Plackett-Burman experimental design was performed to screen influential parameters on γ-decalactone production. Among eleven factors screened, five factors such as castor oil percentage, pH, incubation time, inoculum size and temperature had shown most influential effect on γ-decalactone production (Venkata Narayana et al 2019). However, inoculum size was eliminated due to a high p-value and less standard effect. Thus, the remaining four factors were considered for further optimization using RSM and ANN in this study. RSM based central composite design was adopted to determine the optimum values of screened parameters for γ-decalactone production. The parameters were designated as castor oil percentage (X_1), pH (X_2), incubation time (X_3) and temperature (X_4) (Table 1). The central values allocated for screened parameters based on preliminary experiments of OFAT were castor oil 30 %, pH 5, incubation time 100 h, and temperature 22.5^0C. In CCD, design of experiments was planned with four variables and each in five levels (-2, -1, 0, 1, 2) (Table 2). A total of thirty triplicate experimental runs were conducted in a randomized order, of which sixteen cube, eight axial and six center points as per CCD. The input variables were coded as per the equation (1) (Jamil et al 2018)

$$x_i = \frac{X_i - X_0}{\Delta X_i} \qquad (1)$$

where x_i denotes the coded form of input variable X_i and X_0 is the real value of input variable at the center and ΔX_i is the increment.

The process efficiency was estimated by analyzing the output response variable (Y) in relation to the input variables as represented by equation (2) (Jamil et al 2018)

$$Y = f(X_1, X_2, \ldots X_K) + e \qquad (2)$$

Where $X_1, X_2, \ldots X_K$ are input variables and e is the error.

Experimental data was fitted with second order polynomial equation and model terms were evaluated with Design Expert 8.0.7.1 software. Quadratic model equation is represented as per equation (3) (Jamil et al 2018)

$$Y = \beta_0 + \sum_{i=1}^{K}\beta_i X_i + \sum_{i=1}^{K}\beta_{ii} X_{ii}^2 + \sum_{i=1}^{K-1}\sum_{j=2}^{K}\beta_{ij} X_i X_j + e \quad (3)$$

Where Y is the response (γ-decalactone production), X_i

Table 1. Independent parameters (input variables) levels and ranges used in DOE

Independent parameters	Symbol	Levels and range				
		-2	-1	0	+1	+2
Castor oil (%)	X_1	10	20	30	40	50
pH	X_2	7	6	5	4	3
Incubation time (hr)	X_3	80	90	100	110	120
Temperature (^{0}C)	X_4	17.5	20	22.5	25	27.5

and X_j are coded input variables, β_0 is intercept & β_i, β_{ii}, β_{ij} are linear, square and interactive coefficients, respectively; k represents number of factors and *e* is the error, which is the difference of measured and observed values.

Quadratic model performance was examined with reference to R^2, predicted R^2 and adjusted R^2. The significance of model terms was confirmed with ANOVA. Factors interaction on the response was predicted from contour plots generated from regression models.

ANN modeling: The ANN feed forward architecture was employed to develop a nonlinear model between the output variable (γ-decalactone production) and four input variables (castor oil percentage, pH, incubation time and temperature) for the same data sets used in RSM. The input player consists of four neurons (input variables), while the output layer contains one neuron (response variable). The training of ANN network was performed with TRAIN tool using MATLAB 9.2 R2017a version. The process of data occurs in the forward direction from input to the output layers. The input data were scaled up by input neurons and then transferred to hidden layer through several weights. The weighted inputs are summed up via hidden layer neurons together with bias

Table 2. CCD DOE experimental runs along with experimental and predicted values of RSM and ANN for γ-decalactone production by S*poridiobolus salmonicolor*

Run No.	Castor oil % (X_1)	pH (X_2)	Incubation time (hr) (X_3)	Temperature (°C) (X_4)	Experimental γ-decalactone production (mg/L)	Predicted γ-decalactone production (mg/L)	
						RSM	ANN
1	30	5	120	22.5	47.38	46.60	46.98
2	20	6	90	20	34.29	34.96	34.32
3	40	6	110	25	44.67	45.15	44.81
4	30	5	100	27.5	50.36	50.28	50.42
5	20	6	90	25	43.58	43.16	43.52
6	30	3	100	22.5	38.95	39.61	38.73
7	40	6	110	20	28.94	29.03	28.99
8	20	6	110	25	47.29	47.49	47.01
9	30	5	100	22.5	73.23	71.74	72.67
10	40	4	90	25	35.68	34.59	36.70
11	30	5	100	22.5	69.83	71.74	71.27
12	30	5	100	22.5	71.68	71.74	71.48
13	40	6	90	25	50.27	51.17	50.27
14	50	5	100	22.5	8.25	8.28	8.26
15	30	5	80	22.5	46.74	47.10	46.92
16	20	4	110	25	38.35	38.77	38.44
17	20	4	110	20	42.75	42.27	44.96
18	20	6	110	20	37.74	38.83	37.92
19	30	5	100	22.5	70.23	71.74	71.23
20	30	5	100	22.5	72.64	71.74	73.79
21	40	4	90	20	30.85	31.08	30.92
22	30	5	100	22.5	72.83	71.74	71.92
23	10	5	100	22.5	16.73	16.28	17.86
24	40	6	90	20	35.93	35.51	35.72
25	30	7	100	22.5	53.83	52.75	53.52
26	30	5	100	17.5	38.46	38.11	38.36
27	20	4	90	20	37.23	36.75	37.12
28	40	4	110	25	30.47	30.22	30.10
29	20	4	90	25	32.46	32.79	32.62
30	40	4	110	20	25.83	26.25	25.97

as per equation (4) (Kiran et al 2008)

$$SUM = \sum X_i W_{ij} + \theta_j \qquad (4)$$

Where X_i is the input parameter, θ_j is bias and W_{ij} is connection weight.

The output-weighted sum is transferred to an activation function f (sum) as per equation (5) (Kiran et al 2008)

$$f(sum) = \frac{1}{1 + \exp(-sum)} \qquad (5)$$

In ANN training, predefined error in minimized by controlling the weights. The root-mean-squared error (RSME) is calculated as per the equation (6) (Kalil et al 2000)

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N} \sum_{n=1}^{M} (y_n^i - \widehat{y_n^i})^2}{MN}} \qquad (6)$$

Here 'i' is the pattern index, N is the no. of patterns, M is the output nodes number and y^i_n & y^i_n are the target and predicted responses of n^{th} node, respectively.

Comparison of ANN and RSM model abilities: The prediction capabilities and fitness of experimental data for both models were estimated by calculating RMSE (Kiran et al 2008), MAE (Youssefi et al 2009), χ^2 (Abuzer and Faruk, 2011) and R^2 (Rajendra et al 2009) as per the equations (6) – (9). In addition, the responses generated by ANN and RSM were represented in graphs against experiments as shown in Figure 2.

$$MAE = \frac{i}{n} \sum_{i=1}^{n} \left| Y_{i,e} - Y_{i,p} \right| \qquad (7)$$

$$\chi^2 = \sum_{i=1}^{n} \frac{(Y_{i,e} - Y_{i,p})^2}{Y_{i,p}} \qquad (8)$$

$$R^2 = \frac{\sum_{i=1}^{n} (Y_{i,p} - Y_{i,e})}{\sum_{i=1}^{n} (Y_{i,p} - Y_{i,e})^2} \qquad (9)$$

Where 'n' is no. of experiments, $Y_{i,e}$ is response for i^{th} experiment, $Y_{i,p}$ is predicted response for i^{th} experiment and Y_e is experimental average.

RESULTS AND DISCUSSION

RSM modelling: In this study, RSM modeling determines the influence of four input variables (castor oil %, pH, incubation time, and temperature) on γ-decalactone production. Thirty experimental runs were conducted using CCD, of which 16 cube points, 8 axial points and 6 central points. Model abilities were evaluated in terms of degrees of freedom, mean squares and sum of squares. RSM based CCD models were analyzed through the second-order polynomial regression equation and represented as follows

$Y = -1315.866 + 8.8486X_1 + 39.3135X_2 + 13.3129X_3 + 42.017X_4 + 0.1554X_1X_2 – 0.0258X_1X_3 + 0.0746X_1X_4 – 0.0413X_2X_3 + 1.2152X_2X_4 + 4.575E – 003X_3X_4 – 0.1486 X^2_1 + 6.3906X^2_2 – 0.0622X^2_3 – 1.101X^2_4$ (10)

Where Y is the response (γ-decalactone production), while X_1, X_2, X_3 and X_4 are castor oil %, pH, incubation time, and temperature, respectively. Equation (10) was represented in terms of coded factors and is used to compare relative influence of factors with coefficient of factors (Busra et al 2020). In this case, temperature (X_4) was the most effective factor and followed by pH (X_2), incubation time (X_3) and castor oil (X_1). Castor oil was the least influential factor.

The fitness of quadratic model was analyzed, adjusted R^2 and the coefficient of determination (R^2). The model is significant and ensures good goodness of fit. The R^2 value of 0.9978 indicates that the model explains more than 99% of data variability. The predicted R^2 of 0.9927 is in reasonably good agreement with adjusted R^2 of 0.9958 and shows the model's high significance. The significance of model terms was determined based on p-values. A Lower p-value (<0.05) refers to the greater significance of respective parameters (Busra et al 2020), X_1 (castor oil %), X_2 (pH), X_4 (temperature), X_1X_2 (interaction of castor oil % and pH), X_1X_4 (interaction of castor oil % and temperature) and X_2X_4 (interaction of pH and temperature) had shown significant effects on production of γ-decalactone. The interaction effects of input variables on the response were shown with contour plots. The oval shape represents the significant interaction between a pair of input variables. The γ-decalactone production rises as castor oil percentage varies from 20 % to 29.68%, beyond which the production decreased as an increase in the percentage of castor oil (Fig. 1) and it might be due to growth inhibition of yeast cells at high percentage of castor oil (Dayana et al 2017). As pH rises from 4.0 to 5.3, γ-decalactone production also increases up to optimum value beyond which production reduced (Dayana et al 2017). Other two variables also followed same pattern as incubation time reaches to 99.8 hr obtained highest production beyond which production decreased (Dayana et al 2017). Thus, the maximum γ-decalactone production predicted by the RSM model is 72.73 mg/L at optimum values (castor oil % -29.68, pH-5.32, incubation time-99.89 hr, and temperature-23.22°C).

ANN modeling: A multilayer perception with feed forward architecture ANN model was generated, which consists of four input nodes (castor oil %, pH, incubation time, and temperature) and one output node (γ-decalactone production). The RSM DOE data and the corresponding experimental response were used to train the ANN model network. Over parameterization is avoided by grading data into training, validation and testing. ANN model was optimized to minimize the error. Training of data was performed by varying neurons in the hidden layer for different

ANN parameters combination. The optimum number of hidden layers obtained was one. The transfer function generated was shown in equation (Dufosse et al 1999). The error was minimized based on trial-and-error method during the training of network. The ability of ANN model was proved by choosing parameter weights results in minimum value of RMSE. The correlation coefficient of 0.999 was obtained for γ-decalactone production.

Models validation: Model predictions of RSM and ANN were confirmed by conducting experiments in thrice at

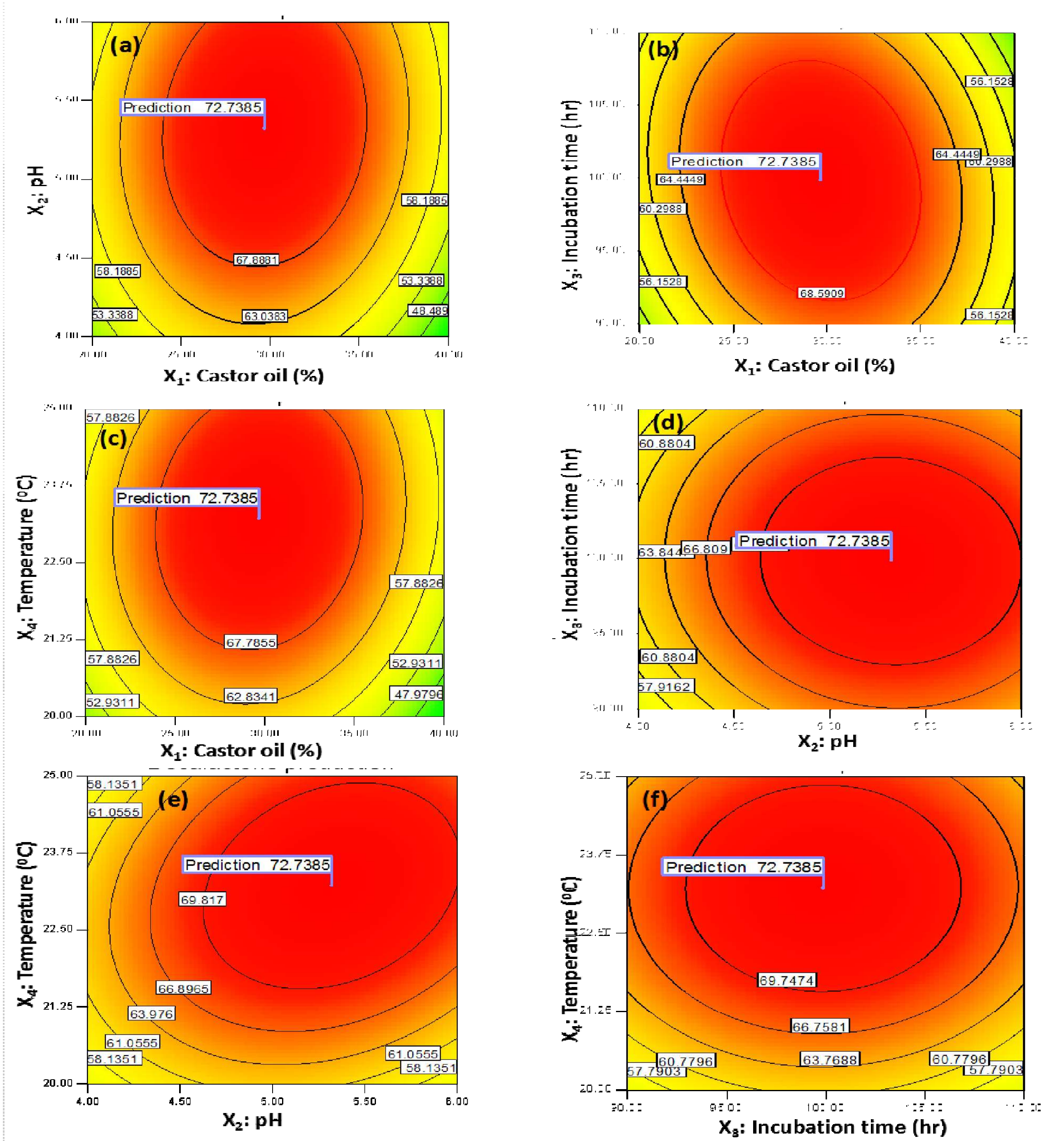

Fig. 1. Contour plots depicting interaction effect of input variables on γ-decalactone production by *Sporidiobolus salmonicolor*: Interaction effects of pH and castor oil (%) (a); incubation time and castor oil (%) (b); temperature and castor oil (%) (c); pH and incubation time (d); pH and temperature (e); incubation time and temperature (f)

optimum conditions. The predicted values of γ-decalactone production by RSM and ANN were 72.73 and 66.14 mg/L, respectively. The optimum input conditions predicted by RSM were castor oil-29.68 %, pH-5.32, incubation time-99.89 hr and temperature-23.22°C with the desirability of 0.992 for γ-decalactone production. The average of triplicate experiments for γ-decalactone production was 73.25 mg/L and it was reasonably close agreement with the response values predicted by models. The outstanding correlation coefficient of RSM and ANN confirms the validity of the models.

Optimum conditions and production of γ-decalactone: The input conditions for γ-decalactone production were optimized using RSM and ANN. The model predictions were validated with experiments. The final yield of γ-decalactone production was 72.73 mg/L at optimum conditions of castor oil-29.68%, pH-5.32, incubation time-99.89 hr and temperature-23.22°C. Earlier reports on the production of γ-decalactone revealed that a maximum production capacity of 131.8 mg/l with immobilized cells and 107.5 mg/l for free cells by *Sporidiobolus salmonicolor* CCRC 2195 after five days of cultivation and indicated that alginate immobilized cells are less susceptible to toxic effects than free cells (Shiow-Ling et al 1998). Gilles et al (1997) reported the production of γ-decalactone 1.8 ± 0.03 g/l with wild strain *Yarrowia lipolyitca* W29 and 5.5 g/l with mutant strain MTLY40-2p after 7 days of biotransformation. They revealed that mutant strain did not show any ability to degrade γ-decalactone.

Nama et al (2016) revealed that the γ-decalactone production of 62.2 mg/l with the same wild type strain used in this study and 81.9 mg/l with mutant strain UV3 after 96 h of fermentation and showed a 33% increase in production compared to wild type strain. Eko et al (2020) observed that γ-decalactone production of 282 mg/l through engineered oleaginous yeast *Yarrowia lipolyitca* from oleic acid in fed-batch fermenter. The yields of γ-decalactone reported above are slightly more compared to the current study. It is mainly due to the toxicity of lactone to producing cells and not genetically engineered strain. However, the fermentation conditions reported for γ-decalactone production in this study are in close agreement with (Dayana et al 2017) in which reported pH-5.0 and castor oil-30% using yeast *L. saturnus* CCMA 0243.

RSM and ANN models comparison: ANN and RSM models were evaluated for predictive abilities by taking the production of γ-decalactone as a case study. These models were compared based on error generated (RMSE, MAE, chi-square (χ^2) and R^2 from the predicted and experimental responses, as shown in Table 3. Thus, results revealed that ANN has good prediction ability compared to RSM for γ-decalactone production due to low error values of ANN and high correlation coefficient. Additionally, responses (γ-decalactone production) predicted by ANN in close agreement with experimental values than that of RSM (Table 2 & Fig. 2). The better prediction of ANN is mainly due to ANN can attribute to the universal approximation, while RSM is limited to a quadratic regression (Kiran et al 2008). ANN has better optimization and prediction abilities compared to RSM. ANN suitable for nonlinear systems for interactions higher than quadratic, whereas RSM is recommended for modeling new processes. Earlier studies reported a better prediction ability of ANN than RSM (Runni et al 2019, Hui-Chuan et al 2019). Thus, ANN proved to be a better prediction and optimization tool.

Table 3. ANN and RSM models comparison based on error parameters

Parameter	γ-decalactone production	
	RSM	ANN
Root mean squared error (RMSE)	0.777	0.617
Mean absolute error (MAE)	0.624	0.426
Chi-square (χ^2)	0.345	0.304
Coefficient of determination (R^2)	0.992	0.999

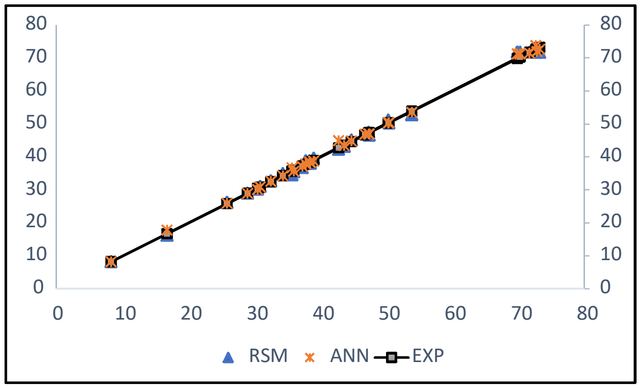

Fig. 2. Comparison of experimental values with the predicted data from ANN and RSM for γ-decalactone production

CONCLUSIONS

In this work modeling approaches of ANN and RSM were assessed for their predictive abilities by taking fermentative production of γ-decalactone as a case study. Optimized the input conditions for maximum γ-decalactone production by *Sporidiobolus salmonicolor* and validated model predictions against experiments. The maximum obtained production of γ-decalactone was 72.73 mg/L at optimum conditions of castor oil -29.68 %, pH -5.32, incubation time -99.89 h and temperature -23.22°C. ANN showed less error parameters and more predictive ability than RSM. Thus, it is showed that

ANN can be considered as alternative for RSM. And also, microbial strain S*poridiobolus salmonicolor* can be used as commercial strain for industrial production of γ-decalactone.

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Paddy Residue Management Alternatives in Punjab, India: An Economic Analysis

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Abstract: Farmer's choice of using crop residue management (CRM) method depends on different factors like the quantum of residue generated, accessibility and affordability of CRM solutions and other technical or marketing constraints. Considering the earnest need for well-managed CRM system with good potential for resource use efficiency, the present study was conducted in the Punjab state during the year 2020-21.Among the four prevalent strategies i.e. complete burning (CB), partial burning (PB), complete incorporation (CI) and complete removal (CR), the CI method proved to be the most cost-effective with highest returns (Rs.115274.2 per hectare) because of higher yield though CB was the most preferred by all the farm categories. CR raised the farmers' burden by Rs. 7200 per hectare in comparison to CB. Majority of the respondents faced technical problems related to lack of practical knowledge about operating machines, seed rate, moisture requirements and complicated methods followed by economical, managerial and marketing constraints. Steps like compensation to farmers by integrating the cost of paddy residue management in the MSP, assuring the availability of residue management machinery at reduced rates, improving custom hire services and extension services are needed to deal with the state's paddy straw management.

Keywords: Constraints, Economics, Input use, Management methods, Paddy residue

India is an agrarian economy. An average of 500 million tons of agricultural leftover is produced every year from various crop species, with rice accounting for the majority (34 Percent) of the residue (Bimbraw 2019). Globally, India ranks second in the production of rice, and stands first in the world in terms of paddy straw burning, accounting for around 27 per cent of the total rice residue burnt (Kumaret al 2019). In situ burning of agricultural residues produces not only greenhouse gases which contribute to global warming and particulate matter, but also plant nutrients like N, P and K which have negative effects on soil characteristics and cost money (Lohanet al 2018). Punjab produces approximately, 19 per cent wheat, 11 per cent of rice and 5 per cent of cotton of the country. Around 75 to 80 per cent of the area under paddy is machine-harvested, and approximately 95 per cent of paddy straw is burnt annually in the state (Singh et al 2018). In a study for NW India, the social cost of paddy straw burning was the highest for Punjab (Rs 1804 crores) (Kumar et al 2019). The annual monetary cost of crop residue burning is about Rs 800-2000 crore in terms of nutritional loss and Rs 500-1500 crore in the form of government subsidies on fertilizers to Punjab farmers (Alexakiet al 2019). Therefore, there is an earnest need for well-managed crop residue management (CRM) systems with good potential for resource use efficiency. Although, farmers are expected to use CRM machines for managing the stubble but still its burning has been a common way of managing crop residue even after the imposition of ban on stubble burning by the government. Therefore, it has become very important to find out the economically viable alternatives of paddy residue management. Keeping this in view, the present study was conducted to evaluate the production, management, present status and cost of paddy residue management alternatives along with the related problems faced by the farmers in the Punjab state.

MATERIAL AND METHODS

The present study was carried out in the South Western Punjab during the year 2020-21. Multi-stage random sampling technique was followed to draw a representative sample. At the first stage, two districts namely Sri Mukatsar Sahib and Firozpur were selected and at the second stage, two blocks from each selected district namely, Gidderbaha and Sri MukatsarSahib from district Sri Mukatsar Sahib and Ghalkhurd and Zira from Firozpur district were selected at random (Table 1, Fig. 1). At third stage, two villages from each selected block were chosen and hence total of eight villages were selected to carry out the study. A sample of five farmers from each category (i.e. small, medium and large farmers according to their operational holdings with upto 5acres, 5 to 15 acres and more than 15 acres, respectively) from each village was selected making a total sample 120 farmers.

Data were collected using pre-tested questionnaire regarding production and utilization of paddy residue, different residue management methods (RMMs) followed along with different constraints faced by farmers in the adopting the RMMs. Statistical techniques like percentage, average, etc. were worked out to analyses the data. Average mean score method was used to rank the problems faced by the respondents in paddy residue management. The different RMMs techniques used on the farms of selected farmers are as follows:

Complete Burning (CB): After harvesting of paddy, the loose straw and stubbles were cut using a straw cutter-cum spreader and the paddy straw was burnt completely. The field was then sown directly with zero till drill technique or prepared by ploughing for sowing wheat.

Partial Burning (PB): The loose straw produced with combine harvesting was burnt directly and wheat was sown in the remaining standing stubbles using tractor drawn zero till drill or after incorporation of standing stubbles by using various tractor drafted implements like disc harrow, cultivator, rotavator, etc. for the tillage.

Complete Incorporation (CI): After harvesting paddy, wheat was sown in the loose straw and standing stubbles using Super seeder, Happy seeder and Mulcher run by tractor. No separate preparatory tillage is required as the seed-bed preparation and sowing along with straw management is done in a single operation.

Complete Removal (CR): After paddy harvesting, straw was chopped with straw cutter cum spreader. Then the tractor run rotary rake is used to collect straw into windrows which is next turned into straw bales by tractor operated straw baler. The straw bales are then collected from the field manually and transported/stored and the field was prepared for sowing wheat.

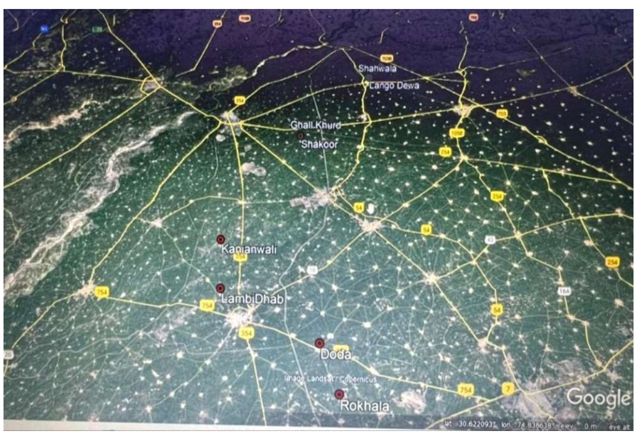

Fig. 1. Geographical distribution of the selected villages in the study

RESULTS AND DISCUSSION

Status of paddy residue management in Punjab: The complete burning (CB) emerged out to be the most preferred method by majority i.e. 68 farmers (48.95 % of total paddy area), followed by partial burning, complete removal and complete incorporation (Table 2). Farm category wise analysis indicated that among all the farm categories, CB was the most preferred method for paddy residue management. Large farmers opted for CR (21.91 %) while PB emerged to be the second most adopted RMM for small farmers (30.93 %) and medium category (17.78 %).

Input use under different residue management methods : The input use pattern for wheat following the paddy crop revealed that the seed rate was the highest (118.6 kg/Ha) on farms where wheat was sown after CI of paddy straw (Table 3), followed by PB,CB and CR. Respondents believed that paddy straw incorporation caused germination issues, necessitating the use of higher seed rates to compensate for the poor germination (Table 3). Plant protection costs were lower (Rs. 2525 and 2528) on farms following CB and CR technique respectively than on farms that used PB and CI (Rs. 2709.and Rs.2956 respectively). The fertiliser use ranged from 445 kg per hectare on farms using CI technique to 478 kg per hectare on fields following CB. Different studies indicate that unlike removal or burning of crop residue which put the adverse effect on soil climate and micro-organisms,

Table 1. Distribution of farmers selected for the study

District	Block	Village	Latitude	longitude
			N	E
Sri Mukatsar Sahib	Gidderbaha	Rokhala	30.29	74.64
		Doda	30.38	74.64
	Sri Mukatsar Sahib	LambiDhab	30.53	74.50
		Kanianwali	30.62	74.52
Firozpur	Ghal Khurd	Ghall Khurd	30.86	74.81
		Shakoor	30.83	74.76
	Zira	Lango Dewa	30.97	74.99
		Shahwala	31.00	75.04

incorporation of straw increases soil organic matter and N, P and K contents in soil (Adam 2013, Lohan et al 2018). According to PAU recommendations also, incorporation of paddy straw or its retention through Happy Seeder for more than three years helps in increasing the wheat productivity and improves soil health and from fourth year onwards, 20 kg urea can be saved per acre (PAU, 2021).

Comparison of time devoted to irrigation revealed that the irrigation hours were the highest for PB than other three methods of RRM. In terms of human labour involved, CR was most labour intensive (170 hours per hectare). In terms of machine labour though almost same time was involved in harvesting paddy with combine and making wheat straw (turi) yet the tractor use for CI method was the least. CR required higher machine use in order to collect straw and make bales for transportation (4 hours). The average yield was marginally high (52.7 auintals/Ha) for CI method in comparison to CB (51.5 quintals/Ha), PB (52.5 quintals/ha) and CR (52.2 quintals/ha). As a consequence, it can be concluded that straw management technique has no direct impact on wheat crop production, except in cases where wheat was seeded after paddy straw incorporation, which enrich soil by providing food to soil friendly organisms and aids in increasing soil fertility (Gill and Singh 2020) which benefit the farmers directly

Table 2. Paddy residue management methods followed by the farmers in Punjab

(Multiple response)

Residue management method/Farm category	Small		Medium		Large		Overall		
	No.	Area	No	Area	No.	Area	No.	Area	Residue
Complete burning (CB)	18	20.85 (54.65)	21	71.20 (49.44)	29	157.4 (48.07)	68	249.45 (48.95)	0.00
Partial burning (PB)	13	11.80 (30.93)	10	25.60 (17.78)	12	53.3 (16.28)	35	90.70 (17.80)	0.00
Complete incorporation (CI)	1	0.60 (1.57)	7	22.25 (15.45)	9	45.00 (13.74)	17	67.85 (13.31)	7011.25 (40.11)
Complete removal (CR)	4	4.90 (12.84)	10	24.95 (17.33)	17	71.75 (21.91)	31	101.60 (19.94)	10467.75 (59.89)
Total	36	38.15 (100.0)	48	144.0 (100.0)	67	327.45 (100.0)	151	509.60 (100.0)	17479.0 (100.0)

Note: I. Figures in parentheses are percentages from respective totals
ii. No crop residue from Paddy and Basmati was generated in case of CB and PB.
iii. Area in hectares and Crop residue is in Quintals;
Source: Field Survey

Table 3. Input use pattern under different paddy straw management techniques in Punjab

(Per hectare)

Particulars	Paddy straw management technologies							
	CB		PB		CI		CR	
	Q	V	Q	V	Q	V	Q	V
Seed (kg)	105.7	2848.0	111.6	3011.9	118.6	3218.2	106.0	2848.9
Fertiliser (kg)	478.2	5768.3	462.8	5571.9	445.3	5538.9	469.7	5571.7
Plant protection	0.0	2525.0	0.0	2709.6	0.0	2956.3	0.0	2528.8
Irrigation (hrs.)	50.7	708.9	76.7	1077.6	53.1	742.6	52.6	735.9
Human labor (hrs)	146.8	7683.9	153.1	8340.1	126.1	6648.7	170.0	12457.2
Combine harvesting (hrs)	1.5	3146.0	1.6	3096.6	1.7	3063.7	1.5	3128.1
Straw Reaper (hrs)	2.4	3978.8	2.6	4062.0	2.6	4090.2	2.5	4031.8
Tractor use (hrs)	14.1	5163.1	16.6	5915.7	7.8	5216.7	13.3	5151.0
Other machinery use*							4.0	3576.6
Yield (qtl)	51.5	101687.8	52.5	103638.1	52.7	104033.1	52.2	103021.0

Note: (i) CB, PB, CI, CR means complete burning, partial burning, complete incorporation and complete removal respectively , (ii) Q is the quantity and V is value in Rs., (iii) *other machinery use includes use of raker and baler.
Source: Field Survey

in terms of main product as well as the by-product of wheat crop through which farmer can get extra cash.

Cost-return structure of different residue management methods: The cost of machine use was the highest i.e.Rs 6355 per acre for CR and lowest i.e. Rs 4915 per acre for CB method (Table 4). Machine use was observed for tractors along with different implements for paddy straw management, sowing of the wheat crop, for transportation/marketing on farm and for manufacturing of wheat straw and combine harvester for harvesting of the wheat crop. For all of these farm activities, custom hiring rates and own machinery costs common in the research region were utilized to calculate the cost of machine usage.

The gross returns were the highest (Rs 115274.2 per Ha) on farms employing the CI and the lowest (Rs 112631.1 per Ha) on farms following CB. Furthermore, farmers that follow CR or PB obtained nearly identical gross returns with minor variations. Returns over variable cost (ROVC) were highest (Rs 82382.6 per Ha) on farms where wheat was sown following CI and lowest (Rs 72134.1 per ha) on farms that follows CR practices. CI technique, ROVC were roughly higher by about Rs 3000 per Ha than in conventional method of CB and this figure was only about Rs 140 per Ha for PB.In case of CR, the ROVC were about Rs 7200 per Ha less than for the CB method. In a similar study, the CI method using happy seeder proved to be the most cost-effective method of treating paddy straw before seeding wheat crops while other straw management techniques raised the burden on farmers from Rs 2000 to 5100 per hectare (Singh et al 2022).

In terms of share of different inputs used in wheat cultivation after adopting the RMMs, human labour (29.8%) and machine labour (38%) was the highest for CR method (Fig. 1); seed and plant protection chemicals use was maximum for CI (9.8%) while fertilizer use was maximum for CB (17.3%) and irrigation share (3.1%) was the highest for PB. Thus, the CI method not only reduces energy costs but also reduces adverse impact on the environment. In an earlier study for wheat sowing with happy seeder, reduced tillage operations as compared to the conventional method of sowing resulted in saving of time (95 minutes/ha) in comparison to conventional method of sowing (220 minutes/ha) along with saving of cost involved i.e. Diesel (Tiwari et al 2019).

Constraints in adoption of different residue management methods: Study of different problems faced by the respondents in following RMMs indicated that the technical problems related to lack of practical knowledge about operating machines, seed rate, moisture requirements and complicated methods occupied the first rank with an average mean score (AMS) of 158.80 followed by economic problems of high cost/hiring charges of implements and labour and price differences in subsidized and non-subsidized implements and management problems related to timely availability of required machinery/implements) respectively (Table 5). Marketing problems related to lack of nearby markets for selling the paddy residue, low demand for paddy residue had AMS 64.80 while the other problems such as lack of extension exposure, unwillingness and lack of interest scored the last rank with an AMS of 52.80. However,

Table 4. Cost-Return structure under different paddy straw management methods in Punjab

(Rs/Ha)

Particulars	Paddy straw management technologies			
	CB	PB	CI	CR
Human Labour	7683.9	8340.1	6648.7	12457.2
Machine labour	12287.9	13074.2	12370.6	15887.5
Seed	2848.0	3011.9	3218.2	2848.9
Fertilisers	5768.3	5571.9	5538.9	5571.7
Plant protection measures	2525.0	2709.6	2956.3	2528.8
Irrigation charges	708.9	1077.6	742.6	735.9
Interest on variable cost @ 9 percent for half the period of crop season	1432.0	1520.3	1416.4	1801.4
Total variable cost	33254.0	35305.5	32891.6	41831.3
Returns-main-product	101687.8	103638.1	104033.1	103021.0
Returns-by-product	10943.3	11185.4	11241.1	10944.4
Gross returns	112631.1	114823.6	115274.2	113965.4
Returns over variable cost (ROVC)	79377.1	79518.1	82382.6	72134.1
Difference of ROVC in comparison to complete burning		141.0	3005.6	-7243.0

Source: Field survey

Table 5. Ranking of the constraints faced by the farmers in management of crop residue in Punjab

Constraints	Total score	Average mean score	Rank
Technical	794	158.80	1
Economic	567	113.40	2
Management	487	97.40	3
Marketing	324	64.80	4
Others	264	52.80	5

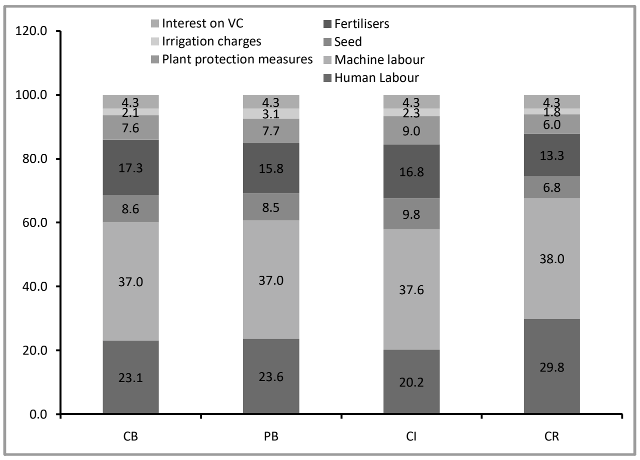

Fig. 1. Shares of inputs used in wheat cultivation after following different RRMs

in another study for Punjab the management related problems for paddy straw was ranked the highest followed by technical, financial and other issues (Roy et al 2018).

CONCLUSIONS

Among different paddy residue management practices followed by the farmers, complete burning has been the most common way of managing crop residue even after the imposition of ban on stubble burning by the Government while wheat sown after paddy residue incorporation method has proved to be time and cost saving without any compromise in terms of yield. Thus, there is a strong need to overcome the constraints in rapid adoption of different technologies for effective management of paddy residue to curb the practice of residue burning. Compensation for farmers by including the cost of paddy residue management in the minimum support price, ensuring the timely availability of residue management machines at subsidized rates, better custom hiring services and promoting the diversified uses of paddy straw in paper mills, energy generation plants, and other industries can prove to be better alternatives for addressing the state's paddy straw management problem

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