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Plant Diversity and Associated Income in Agroforestry Systems of Ayodhya district, Uttar Pradesh

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Abstract: This study was undertaken in the Ayodhya district of Uttar Pradesh to understand the plant species diversity and the associated income in the agroforestry systems. Five agroforestry systems namely, agrisilviculture system, agrisilvihorticulture system, agrihorticulture system, silvipastoral system and aquasilviculture system were found in this district which altogether recorded 95 plant species belonging to 76 genera which consisted of 12 tree species and 83 herb species. The maximum plant species was in agrisilviculture system with 74 plant species (7 tree species and 67 herb species) belonging to 60 genera, followed by agrisilvihorticultural system, aquasilviculture, silvipastoral and agrihorticulture. The Sorenson's Similarity index revealed that all the agroforestry systems had a very low degree of similarity between the vegetation species. The highest average productivity and average income of *Oryza sativa* (Paddy) was in agrisilviculture (40.53 q/ha and Rs. 1,01,325/-) and of *Triticum aestivam* (wheat) was in agrihorticulture and agrisilviculture (27.53 q/ha and Rs. 55,060/-). The agroforestry systems of this region have conserved a high amount of plant diversity. Shifting towards a more diverse plant species cultivation by including indigenous tree species will be recommended for future conservation practices.

Keywords: Agrisilviculture, Agrihorticulture, Aquasilviculture, Agrisilvipastoral, Plant species richness, Silvipastoral

Agroforestry is a land use system which integrates trees on farms to produce diverse products sustainably. It is a complex association of multi-functional and uneven-aged trees and crops (Sanchez, 1995). They are sustainable alternatives to monocultural agriculture system (Tscharntke et al 2010). Agroforestry is a traditional practice in India as in many parts of the world. In Uttar Pradesh, agroforestry systems such as silvipastoral systems, agrisilvicultural systems and agrihorticulture are more commonly practiced. Recently, there has been drastic loss in biodiversity. Plant diversity is reducing at a great speed due to human pressures and climatic factors. As agroforestry is an integrated land use system it can boost plant diversity and reduce habitat loss and fragmentation. The mixing of woody species with agricultural crops increases niche diversification and certain combinations complement each other. Plant diversity forms the basis for productivity and sustainability in any system. There are many reports which suggest that the agroforestry systems conserve biodiversity. Not only that, people depend on agroforestry systems for subsistence, income and other economic gains. The agroforestry systems also provide supplementary income from the tree crops. But in many systems the economic productivity has not been assessed. Therefore, there is a need to quantify the productivity and economic benefits of such agroforestry systems.

Mendez et al (2001) identified 324 plant species in ten different micro-zones of homegarden in Nicaragua. Zimik et al (2012) carried out a comparative study of homegardens of Assam and Arunachal Pradesh in terms of species diversity and plant utilization pattern. They found that species richness per homegarden varied greatly. A total of 268 species were identified in the studied homegardens with highest percentage of species in herb stratum (37%). Yashmita-Ulman et al (2021) reported a total of 516 plant species. Their study reported that homegardens had the highest species richness, followed by agrisilvicultural systems and the least was in tea gardens. Kaushik and Kumar (2003) worked out the economics of the Khejri (*Prosopis cineraria*)-based agroforestry system and found that higher returns were obtained when any of the fodder crops in sequence was grown in association with Khejri than in monocropping. Maximum net returns and benefit–cost ratio was obtained when pearl millet in kharif followed todia (*Brassica tournefortii*) in rabi under khejri trees. Grain crops, both in kharif and rabi also earned more profit when grown with khejri than alone in arid Haryana, India. Bijalwan et al (2009) reported the annual productivity of all tree species was 3775 kg $ha^{-1}yr^{-1}$ in northern aspect (site-N) and 3101 kg. $ha^{-1} yr^{-1}$ in southern aspect (site-S) of Garhwal Himalaya. Among the tree species *Grewia optiva* had the maximum productivity in both site-N and site-S, followed by *Melia azedarach, Quercus leucotrichophora* and *Celtis australis*. The average biological productivity of agricultural crops in northern aspect was 16% higher as compare to southern aspect under traditional agrisilviculture system. The northern aspect in traditional agrisilviculture system (Crop+tree) had a highest overall productivity i.e., 24% compared to the southern aspect (21%).

Most of the studies on agroforestry systems in Uttar Pradesh have been on carbon storage potential and crop productivity. But there are no studies in agroforestry systems on plant diversity, composition and associated income. So, this study attempts to assess and compare floristic compositions, structure and associated income in agroforestry systems in Ayodhya district, Uttar Pradesh.

MATERIAL AND METHODS

Study site: This study was conducted in the Ayodhya district of Uttar Pradesh which lies between 26.7730 ^{0}N and 82.1458 ^{0}E. This district is situated 93 m above MSL (Mean Sea Level). The climate of the district is tropical monsoon. The average temperature varies from 32 ^{0}C in summers to 16 ^{0}C in winters and the average annual rainfall is 1067 mm. The study area includes reserve forests, remnant vegetation patches, rivers, temple ponds, wetlands, gardens, agroforestry systems, paddy fields and human habitations.

A preliminary survey was conducted for two years (2021 to 2023) to identify the plant species diversity and associated income from these agroforestry systems practiced in Ayodhya district of U.P. which contain 11 blocks. From each block, 10 villages were selected and from each village 10 households were selected (Table 1). In totality, 1100 households in 11 blocks were surveyed in which 77 households were categorized into five agroforestry systems namely agrisilviculture system, agrisilvihorticulture system, agrihorticulture system, silvipastoral system, aquasilviculture system, agroforestry systems. In addition to the agroforestry systems, these villages also have other land use systems such as wetlands, grasslands, wastelands, orchards, forest patches, riverine systems etc. interspersing across the district.

Plant species diversity: To conduct plant species inventory, 10 x 10 m quadrants were used for trees, 5 x 5 m for shrubs and 1 x 1 m for herbs. Trees (>15 cm girth at breast height of 1.37 m,>3 m height), shrubs (<15 cm girth at breast height of 1.37 m, <3 m height), saplings (5-10 cm collar diameter at base, <1 m height) and seedlings (<5 cm collar diameter at base, <20 cm height) were considered for sampling (Khumbongmayum et al 2006). The herbaceous succulents, seedlings and climbers were considered as herbs. Girth at breast height (1.3 m aboveground) was measured with the help of a measuring tape. Height of the individual tree was measured using the range finder.

The following community parameters were calculated using the below given formulae:

i. Importance Value Index (IVI) for trees = Relative Frequency + Relative Density + Relative Dominance
ii. Importance Value Index (IVI) for shrubs and herbs = Relative Frequency + Relative Density
iii. Shannon Weiner index (Shannon and Weiner 1963)

$$H' = \sum_{i=1}^{s} \mathrm{p_i} \ln \mathrm{p_i}$$

where, p_i is often the proportion of individuals belonging to the 'i'th species in the dataset and 's' is the species richness. The values usually lies between 1 and 4 where 1 shows less diversity and 4 shows high diversity.

iv. Simpson's index (Simpson 1949)

This was calculated according to Simpson (1949) to measure the concentration of dominance (CD) of plant species.

$$\mathrm{CD} = \sum_{i=1} (\mathrm{pi})^2$$

where pi is the proportion of the IVI of the 'i'th species and IVI of all the species (ni/N). The values of Simpson's index is limited to 1 where 1 shows dominance by a single species.

v. Pielou's evenness index (Pielou 1966) = H'/$\log_{10}$N(S)

where H' is the Shanon Weiner Index of diversity and S is the total number of species.

vi. Sorenson's similarity coefficient (Sorenson 1948)

$$\text{Sorenson similarity coefficient} = \frac{2\mathrm{C}}{\mathrm{A+B}}$$

where C is the number of species common to both sites, A is the total number of species in site A, and B is the total number of species in site B. Sorenson's coefficient gives a value between 0 and 1, the closer the value is to 1, the more the communities have in common.

Production and income: Production of trees was calculated using values based on the region, questionnaire survey and local knowledge. The income generated by the economic plants each plant category was calculated using the product prices derived from local market surveys.

RESULTS AND DISCUSSION

Plant diversity of agroforestry systems: Altogether the five different agroforestry systems recorded 95 plant species (76 genera, 29 families) which consisted of 12 tree species (12 genera, 6 families) and 83 herb species (66 genera, 24 families) (Fig. 1 and Table 2 and 3). The maximum plant species was in agrisilviculture system, followed by agrisilvihorticultural system, aquasilviculture, silvipastoral

Table 1. GPS location of the villages surveyed in Ayodhya District

Block name	Village name	No. of households	Latitude	Longitude
Milkipur	Baripara	10	26.564202°	81.871700°
Milkipur	Ranapur	10	26.583643°	81.959009°
Milkipur	Bansapur	10	26.562927°	81.900045°
Milkipur	Bhitaura	10	26.742714°	81.017765°
Milkipur	Banwa	10	26.595456°	81.329646°
Milkipur	Sidhauna	10	26.550323°	81.883049°
Milkipur	Chirauli	10	26.632867°	81.900787°
Milkipur	Sari	10	26.596110°	81.879465°
Milkipur	Tikra	10	26.621463°	81.914771°
Milkipur	Bawan	10	26.569380°	81.857996°
Sohawal	Mirpur Kanta	10	26.716515°	81.982337°
Sohawal	Sadhu Ka Purwa	10	26.712986°	81.960270°
Sohawal	Khirauni	10	26.739146°	81.989076°
Sohawal	Sodhiyawan	10	26.692996°	82.010478°
Sohawal	Bishunpur Sara	10	26.716076°	82.021122°
Sohawal	Gopinathpur	10	26.748260°	81.988163°
Sohawal	Pilkhanwa	10	26.758623°	81.954976°
Sohawal	Tandwa	10	26.680346°	81.808784°
Sohawal	Gaurakurmiyan	10	26.739081°	81.972609°
Sohawal	Rampur Grant	10	26.725898°	81.921803°
Harigatonganj	Semra	10	26.559876°	82.011077°
Harigatonganj	Sidhaura	10	26.524173°	81.980669°
Harigatonganj	Nimdi	10	26.566036°	81.948340°
Harigatonganj	Devgiri	10	26.530400°	81.961133°
Harigatonganj	Chikhri	10	26.546718°	82.028039°
Harigatonganj	Harigatonganj	10	26.557190°	82.009841°
Harigatonganj	Bhitari	10	26.556070°	81.977499°
Harigatonganj	Jamua	10	26.636083°	82.017150°
Harigatonganj	Lakshmanpur grant	10	26.514220°	82.077785°
Harigatonganj	Paruwa	10	26.636667°	82.090833°
Mawai	Jamoli	10	26.7555291°	81.541438°
Mawai	Rewna	10	26.527393°	81.911661°
Mawai	Hariharpur	10	26.277565°	81.808056°
Mawai	Rampur Godra	10	26.303056°	81.914444°
Mawai	Ganeshpur	10	26.6420777°	81.675186°
Mawai	Badlapur	10	26.112778°	81.865647°
Mawai	Sheodhara	10	26.7614589°	81.579757°
Mawai	Saidpur	10	26.621944°	81.740278°
Mawai	Para garib shah	10	26.556596°	82.239422°
Mawai	Padera	10	26.520978°	82.270551°
Rudhuali	Sandwa	10	26.721729°	81.654335°
Rudhuali	Rampur janak	10	26.6686009°	81.6137517°
Ruduali	Sunwa	10	26.608056°	81.691389°
Rudhuali	Sahapur	10	26.7564232°	81.754368°
Rudhuali	Kurhasadat	10	26.789458°	81.7623754°
Rudhuali	Haleemnagar	10	26.807222°	81.807778
Rudhuali	Kura sadat	10	26.784722°	81.732545°
Rudhuali	Seewan	10	26.825124°	81.709167°
Rudhuali	Khandpipra	10	26.839444°	81.768056°
Rudhuali	Manapur	10	26.724675°	81.770885°
MayaBazar	Mirzapur	10	26.727778°	81.783332°
MayaBazar	Rasoolpurkhurd	10	26.682778°	81.834722°
MayaBazar	Ichauliya	10	26.810556°	81.733333°
MayaBazar	Amauni	10	26.595833°	82.305556°
MayaBazar	Belwari khan	10	26.588889°	82.386667°
MayaBazar	Gauhaniya	10	26.566944°	82.535556°
MayaBazar	Maya Bhikhi	10	26.648889°	82.343889°

Cont...

Table 1. GPS location of the villages surveyed in Ayodhya District

Block name	Village name	No. of households	Latitude	Longitude
MayaBazar	Raja pur	10	26.606389°	82.368611°
MayaBazar	Ratanpur	10	26.631462°	82.332316°
MayaBazar	Uniyar	10	26.640833°	82.438611°
Bikapur	Arwat	10	26.617222°	82.301944°
Bikapur	Kanakpur	10	26.641389°	82.341667°
Bikapur	Toniya	10	26.726111°	82.147222°
Bikapur	Toro mafi	10	26.588333°	82.166667°
Bikapur	Umarnipipri	10	26.574722°	82.168611°
Bikapur	Newnapurab	10	26.493333°	82.180278°
Bikapur	Askaranpur	10	26.561111°	82.103611°
Bikapur	Bhawapur	10	26.648056°	82.171389°
Bikapur	Kalyanpur	10	26.613056°	82.313611°
Bikapur	Jalal pur	10	26.763889°	81.799722°
Masodha	Chandpur	10	26.612778°	82.140278°
Masodha	Baintikala	10	26.481389°	82.163333°
Masodha	Dewapur	10	26.546389°	82.129722°
Masodha	Pora	10	26.686667°	82.096389°
Masodha	Ragghupur	10	26.799722°	81.882222°
Masodha	Mohiuddinpur	10	26.705278°	82.013889°
Masodha	Sariyawa	10	26.722222°	82.077778°
Masodha	Sakhupara	10	26.710833°	82.095556°
Masodha	Amauna	10	26.698333°	82.078611°
Masodha	Bhaipur	10	26.704722°	82.021667°
Purabazar	Ganja	10	26.735556°	82.181667°
Purabazar	Madna Uparhar	10	26.699994°	82.297585°
Purabazar	Fatehpurmumtjabad	10	26.6255612°	82.282222°
Purabazar	Kutubpur	10	26.687875°	82.268578°
Purabazar	Rajepur Uparhar	10	26.7073655°	82.271828°
Purabazar	Takpura	10	26.766111°	82.202778°
Purabazar	Takpura	10	26.766111°	82.202778°
Purabazar	Hainsa	10	26.564167°	82.494722°
Purabazar	Ashifbagh	10	26.759444°	82.238889°
Purabazar	Kachhauli	10	26.659444°	82.2025124°
Purabazar	Shahjahanpur	10	26.766111°	82.196111°
Amaniganj	Hainsa	10	26.564167°	82.494722°
Amaniganj	Ranopali	10	26.758889°	82.203611°
Amaniganj	Jagdishpur	10	26.744167°	82.123889°
Amaniganj	Padkiya	10	26.651389°	81.843889°
Amaniganj	Raipatti	10	26.662778°	81.846667°
Amaniganj	Ranikpur	10	26.554444°	81.718889°
Amaniganj	Baghaura	10	26.863056°	81.387222°
Amaniganj	Gahnag	10	26.648611°	81.845556°
Amaniganj	Ram purgauhaniya	10	26.78475°	82.170109°
Amaniganj	Pal pur	10	26.827222°	81.690833°
Tarun	Jaisinghmau	10	26.566899°	82.257970°
Tarun	Baherpur	10	26.584455°	82.258555°
Tarun	Bhaisuli	10	26.540563°	82.305661°
Tarun	Tarun	10	26.547222°	82.244722°
Tarun	Para ram	10	26.476944°	82.222314°
Tarun	Karnaipur	10	26.532255°	82.270889°
Tarun	Charawan	10	26.547222°	82.213889°
Tarun	pichhaura	10	26.556111°	82.297778°
Tarun	Paliachalpur	10	26.551117°	82.307840°
Tarun	Saraimanodhar	10	26.458611°	82.2551452°

Table 2. Vegetation species richness of selected agroforestry systems in Ayodhya District

Order	Family name	Common name	Scientific name	Habit	System				
					Agrisilviculture	Agrisilvihorticulture	Agrihorticulture	Silvipastoral	Aquaculture
Sapindales	Meliaceae	Bakain	*Melia azedaraah*	Tree	*				
Myrtales	Myrtaceae	Guava	*Psidium guajava*	Tree		*			
Sapindales	Meliaceae	Mahogany	*Swietenia macrophylla*	Tree	*				
Sapindales	Anacardiaceae	Mango	*Magnifera indica*	Tree	*	*	*		
Myrtales	Myrtaceae	Safeda	*Eucalyptus hybrid*	Tree	*	*		*	
Fabales	Fabaceae	Shisham	*Dalbergia sissoo*	Tree	*				
Myrtales	Myrtaceae	Jamun	*Syzygium cumini*	Tree			*		
Lamiales	Lamiaceae	Teak	*Tectona grandis*	Tree	*	*			
Sapindales	Simaroubaceae	Tree of heaven	*Alianthus excelsa*	Tree	*				
Fabales	Fabaceae	Kanjii	*Pongamia pinnata*	Tree					*
Fabales	Fabaceae	Kassod	*Cassia siamea*	Tree					*
Sapindales	Meliaceae	Neem	*Azadirachta indica*	Tree					*
Fabales	Fabaceae	Alyce clover	*Alysicarpus monilifer*	Herb	*	*		*	
Poales	Poaceae	Annual rabbits foot grass	*Polypogonmon speliensis*	Herb				*	
Alismatales	Araceae	Arbi	*Colocasia escluenta*	Herb	*				
Malpighiales	Euphorbiaceae	Asthma herb	*Euphorbia hirta*	Herb	*	*		*	
Asterales	Asteraceae	Asthmaweed	*Conyza bonariensis*	Herb	*				
Poales	Poaceae	Barnyard grass	*Echinochloa crus galli*	Herb	*	*			*
Poales	Poaceae	Bermuda grass/Doob grass	*Cynodon dactylon*	Herb	*	*	*	*	*
Poales	Poaceae	Brachiaria ramosa	*Brachiaria ramosa*	Herb	*				
Solanales	Solanaceae	Brinjal	*Solanum melongena*	Herb	*		*		
Poales	Poaceae	Browntop millet	*Brachiaria ramosa*	Herb	*	*	*		*
Malvales	Malvaceae	Burbush	*Triumfetta rhomboidea*	Herb	*				*
Solanales	Convolvulaceae	Bush morning glory	*Ipomoea carnea*	Herb					*
Brassicales	Brassicaceae	Cardamine hirsuta	*Cardamine hirsuta*	Herb	*				
Brassicales	Cleomaceae	Celandine spider flower	*Cleome chelidonii*	Herb	*				

Cont...

Table 2. Vegetation species richness of selected agroforestry systems in Ayodhya District

Order	Family name	Common name	Scientific name	Habit	System				
					Agrisilviculture	Agrisilvihorticulture	Agrihorticulture	Silvipastoral	Aquaculture
Plantae	Poaceae	Chari	*Sorghum bicolar*	Herb				*	
Asterales	Asteraceae	Coatbuttons	*Tridax procumbens*	Herb	*			*	
Caryophyllales	Amaranthaceae	Common lambsquarter	*Chenopodium album*	Herb	*	*	*		
Caryophyllales	Portulacaceae	Common purslane	*Portulaca oleracea*	Herb	*	*			
Malvales	Malvaceae	Common wireweed	*Sida acuta*	Herb	*	*		*	
Asterales	Asteraceae	Congress grass	*Parthenium hysterophorus*	Herb	*				
Oxalidales	Oxalidaceae	Creeping wood sorrel	*Oxalis corniculata*	Herb			*		
Poales	Poaceae	Crowfoot grass	*Dactyloctenium aegyptium*	Herb	*	*	*	*	
Caryophyllales	Amaranthaceae	Devil's horsewhip	*Achyranthes aspera*	Herb	*	*			*
Poales	Poaceae	False amaranth	*Digitaria arvensis*	Herb	*		*		
Asterales	Asteraceae	False daisy	*Elipta alba*	Herb	*				
Solanales	Convolvulaceae	Field bindweed	*Convolvulus arvensis*	Herb	*	*		*	
Asterales	Asteraceae	Flossflower	*Ageratum houstonianum*	Herb	*				
Rosales	Cannabaceae	Hemp	*Cannabis sativa*	Herb	*	*			
Poales	Poaceae	Giant reed	*Arundo donax*	Herb	*			*	
Asterales	Asteraceae	Goat weed	*Ageratum conyzoides*	Herb	*				*
Poales	Poaceae	Goosegrass	*Eleusine indica*	Herb	*				*
Poales	Cyperaceae	Grass-like fimbry	*Fimbristylis miliacea*	Herb					*
Brassicales	Brassicaceae	Hairy bittercress	*Cardamine hirsuta*	Herb	*				
Poales	Cyperaceae	Haspan flats edge	*Cyprus haspan*	Herb	*	*			
Caryophyllales	Aizoaceae	Horse pursalane	*Trianthema portuacastrum*	Herb		*	*	*	
Poales	Poaceae	Indian muraina grass	*Ischaemum indicum*	Herb					*
Poales	Poaceae	Johnson grass	*Sorghum halapense*	Herb	*	*			
Poales	Poaceae	Jungle Rice	*Echinochloa colona*	Herb	*	*			
Caryophyllales	Amaranthaceae	Khaki weed	*Alternanthera pungens*	Herb		*			*
Poales	Poaceae	Kleberg's bluestem	*Dichanthium annulatum*	Herb	*	*			
Poales	Poaceae	Knot grass	*Paspalum distichum*	Herb					*
Poales	Poaceae	Large crabgrass	*Digitarias anguinalis*	Herb	*	*			

Cont...

Table 2. Vegetation species richness of selected agroforestry systems in Ayodhya District

Order	Family name	Common name	Scientific name	Habit	System				
					Agrisilviculture	Agrisilvihorticulture	Agrihorticulture	Silvipastoral	Aquaculture
Poales	Poaceae	Little seed canary grass	*Phalaris minor*	Herb	*				
Poales	Poaceae	Maize	*Zea mays*	Herb	*	*	*		
Poales	Cyperaceae	Motha/Purple nutsedge	*Cyperus rotundus*	Herb	*	*	*	*	
Poales	Cyperaceae	Mullimbimby couch	*Cyperus brevifolius*	Herb					*
Solanales	Convolvulaceae	Mustard	*Brassica spp.*	Herb	*				
Solanales	Convolvulaceae	Obscure morning glory	*Ipomoea obscura*	Herb	*				
Asterales	Asteraceae	Oligochaeta	*Volutarella divaricata*	Herb	*				
Asparagales	Amaryllidaceae	Onion	*Allium cepa*	Herb	*				
Poales	Cyperaceae	Pale galingale	*Cyperus eragrostis*	Herb	*				
Lamiales	Acanthaceae	Panicled peristrophe	*Peristrophe paniculata*	Herb		*			
Asterales	Asteraceae	Para cress flower	*Blainvillea acmella*	Herb		*			
Solanales	Solanaceae	Patato	*Solanum tuberosum*	Herb	*				
Brassicales	Brassicaceae	Pepper grass	*Lepidium sativam*	Herb	*				
Gentianales	Apocynaceae	Pergularia	*Pergularia daemia*	Herb	*				
Fabales	Fabaceae	Pig's senna	*Cassia absus*	Herb	*	*			
Poales	Cyperaceae	Poorland flat sedge	*Cyperus compressus*	Herb	*	*			
Caryophyllales	Amaranthaceae	Prostrate globe-amaranth	*Gomphrena decumbens*	Herb		*			
Zygophyllales	Zygophyllaceae	Puncture vine	*Tribulus terrestris*	Herb	*		*		
Poales	Poaceae	Purple chloris	*Chloris barbata*	Herb	*				
Asterales	Asteraceae	Red tassel flower	*Emilia sonchifolia*	Herb	*				
Poales	Poaceae	Rice	*Oryza sativa*	Herb	*	*			
Poales	Cyperaceae	Ricefield flat sedge	*Cyperus iria*	Herb	*	*		*	
Solanales	Convolvulaceae	Roundleaf bindweed	*Evolvulus nummularius*	Herb	*				
Poales	Poaceae	Running grass	*Brachiaria reptans*	Herb	*	*		*	*
Ericales	Primulaceae	Scarlet pimpernel	*Anagallis arvensis*	Herb	*				
Fabales	Fabaceae	Showy pigeonpea	*Atylosia scarabaeoides*	Herb	*				
Caryophyllales	Amaranthaceae	Slender amaranth	*Amaranthus viridis*	Herb	*			*	
Solanales	Convolvulaceae	Slender dwarf morning glory	*Evolvulus alsinoides*	Herb		*			

Cont...

Table 2. Vegetation species richness of selected agroforestry systems in Ayodhya District

Order	Family name	Common name	Scientific name	Habit	System				
					Agrisilviculture	Agrisilvihorticulture	Agrihorticulture	Silvipastoral	Aquaculture
Caryophyllales	Amaranthaceae	Spiny pigweed	*Amaranthus spinosus*	Herb	*	*			
Asterales	Asteraceae	Spiny sowthistle	*Sonchus asper*	Herb		*	*	*	
Poales	Poaceae	Sugercane	*Saccharum officilarum*	Herb	*	*			
Malpighiales	Euphorbiaceae	Three-leaved caper	*Croton bonplandianum*	Herb				*	
Caryophyllales	Polygonaceae	Toothed dock	*Rumex dentatus*	Herb	*				
Commelinales	Commelinaceae	Tropical spiderwort	*Commelia benghalensis*	Herb	*		*		
Fabales	Fabaceae	Urad	*Vigna munga*	Herb	*				
Poales	Cyperaceae	Variable flats edge	*Cyperus difformis*	Herb	*				
Poales	Poaceae	Wheat	*Triticum aestivum*	Herb	*	*			
Poales	Cyperaceae	White head spike sedge	*Cyperus kyllingia*	Herb	*	*			
Poales	Poaceae	Wild oat	*Avena ludoviciana*	Herb	*				
Lamiales	Orobanchaceae	Witch weed	*Striga asiatica*	Herb	*	*			
Poales	Poaceae	Yellow foxtail	*Setaria glauca*	Herb	*				

Cont...

and agrihorticulture (Fig. 1 and Table 2 and 3). The maximum number of families with genera and maximum number of species with families was recorded in agrisilviculture system and the minimum was recorded in agrihorticulture system (Table 3). The maximum number of tree species was recorded in agrisilvicultural system and the minimum was recorded in silvipastoral system (Table 4). The maximum number of herb species was recorded in agrisilviculture and the minimum in agrihorticulture (Table 4).

The highest vegetation species richness was in the agrisilviculture, followed by agrisilviculture, silvipastoral, aquasilviculture and the least in agrihorticulture (Table 5). This plant species in agrisilviculture system in the current study is lower than the plant diversity (101 plant species) and vegetation species richness (69.33) found in the same system in Assam (Yashmita-Ulman et al 2021) and higher than that of swidden agroforestry system of Peru (Wezel and Ohl 2005). These differences in species richness might be due to the people's preferences for tree species, topographic and climatic factors of the study sites. The highest tree species richness was recorded in agrisilviculture system, followed by agrisilvihorticulture, aquaculture and agrihorticulture and silvipastoral system (Table 5). The maximum tree density was in agrisilviculture, agrisilvihorticulture, silvipastoral, agrihorticulture and aquaculture (Table 5). The maximum tree basal area was recorded in silvipastoral system, followed by agrisilviculture, agrisilvihorticulture, agrihorticulture, aquasilviculture (Table 5). The maximum tree Shannon Weiner Index was in agrisilvihorticulture, followed by agrisilviculture, agrihorticulture, aquaculture (Table 5). The maximum tree Simpson's Dominance index was in silvipastoral, agrihorticulture, aquaculture, agrisilvihorticulture, agrisilviculture (Table 5). The tree evenness index was the highest for the agrisilvihorticulture, followed by agrisilviculture, and aquasilviculture (Table 5).

The highest tree stand density was recorded in silvipastoral system and lowest was recorded in both agrihorticulture and aquasilviculture system (Fig. 2). The highest basal area was recorded in silvipastoral system and the lowest was recorded in both agrihorticulture and aquaculture system (Fig. 2). *Eucalyptus* spp. in silvipastoral system recorded the highest tree density and the lowest was recorded by *Dalbergia sissoo* in agrisilviculture system (Table 6). The highest tree basal area was recorded in *Eucalyptus* spp. in silvipastoral system and the lowest was recorded in *Magnifera indica* in agrisilviculture system (Table 6). *Eucalyptus* spp., *Tectona grandis, Swietenia macrophylla, Melia azedaraah,* and *Ailanthus excelsa* were the top five ranked woody species with the highest IVI values.

Table 3. Overall families with genera and species richness of selected agroforestry systems in Ayodhya District

Family	Overall		Agrisilvicultural		Agrihorticulture		Agrisilvihorticulture		Silvipastoral		Aquasilviculture	
	No. of genera	No. of species	No. of genera	No. of species	No. of genera	No. of species	No. of genera	No. of species	No. of genera	No. of species	No. of genera	No. of species
Acanthaceae	1	1					1	1				
Aizoaceae	1	1			1	1	1	1				
Amaranthaceae	5	6	3	4	1	1	5	5			2	2
Amaryllidaceae	1	1	1	1								
Anacardiaceae	1	1	1	1	1	1	1	1	1	1		
Apocynaceae	1	1	1	1								
Araceae	1	1	1	1					1	1		
Asteraceae	9	10	7	8	1	1	2	2	1	1	1	1
Brassicaceae	2	3	2	3								
Cannabaceae	1	1	1	1			1	1				
Cleomaceae	1	1	1	1								
Commelinaceae	1	1	1	1	1	1						
Convolvulaceae	4	6	4	4			2	2			1	1
Cyperaceae	2	9	1	7	1	1	5	5			2	2
Euphorbiaceae	2	2	1	1			1	1	1	1		
Fabaceae	6	7	5	5			2	2	4	4	2	2
Lamiaceae	1	1	1	1			1	1	1	1		

Table 4. Tree and herb wise families with genera and species richness of selected agroforestry systems in Ayodhya district

Family	Overall		Agrisilvicultural		Agrihorticulture		Agrisilvihorticulture		Silvipastoral		Aquasilviculture	
	No. of genera	No. of species	No. of genera	No. of species	No. of genera	No. of species	No. of genera	No. of species	No. of genera	No. of species	No. of genera	No. of species
Tree												
Anacardiaceae	1	1	1	1	1	1	1	1				
Fabaceae	3	3	1	1							2	2
Lamiaceae	1	1	1	1			1	1				
Meliaceae	3	3	2	2							1	1
Myrtaceae	3	3	1	1	1	1	1	2	1	1		
Simaroubaceae	1	1	1	1								
Total	12	12	7	7	2	2	3	4	1	1	3	3
Herb												
Acanthaceae	1	1					1	1				
Aizoaceae	1	1			1	1	1	1	1	1		
Amaranthaceae	5	6	3	4	1	1	5	5	1	1	2	2
Amaryllidaceae	1	1	1	1								
Apocynaceae	1	1	1	1								
Araceae	1	1	1	1								
Asteraceae	9	10	7	8	1	1	2	2	2	2	1	1
Brassicaceae	2	3	2	3								
Cannabaceae	1	1	1	1			1	1				
Cleomaceae	1	1	1	1								
Commelinaceae	1	1	1	1	1	1						
Convolvulaceae	4	6	4	4			2	2	1	1	1	1
Cyperaceae	2	9	1	7	1	1	1	5	1	2	2	2
Euphorbiaceae	2	2	1	1			1	1	2	2		
Fabaceae	4	4	4	4			2	2	1	1		
Malvaceae	2	2	2	2			1	1	1	1	1	1
Orobanchaceae	1	1	1	1							1	1
Oxalidaceae	1	1			1	1						
Poaceae	20	25	17	21	5	5	11	13	6	6	6	7
Polygonaceae	1	1	1	1								
Portulacaceae	1	1	1	1			1	1				
Primulaceae	1	1	1	1								
Solanaceae	1	2	1	2	1	1						
Zygophyllaceae	1	1	1	1	1	1						
Total	65	83	53	67	13	13	29	35	16	17	14	15

The IVI, tree density, tree basal area, Shannon Weiner index, Simpson's Dominance index, Pielou's Evenness index suggest that these systems are dominated by a few tree species such as *Eucalyptus* spp*., Tectona grandis, and Mangifera indica* (Table 5 and 6). The farmers prefer these trees as the timber of these trees fetch high market price.

The maximum herb species richness was in agrisilviculture, followed by agrisilvihorticulture, silvipastoral, agrihorticulture, aquaculture (Table 5). The maximum herb density was in agrisilvihorticulture, followed by silvipastoral system, agrisilviculture, agrihorticulture and aquaculture (Table 5). The highest herb density was recorded by *Triticum aestivum* and the lowest was recorded in *Amaranthus viridis* in agrisilviculture system (Table 6). The maximum herb Shannon Weiner Index was recorded in agrisilviculture system, followed by agrisilvihorticulture, aquaculture, agrihorticulture and silvipastoral (Table 5). The maximum herb Simpson's dominance index was in silvipastoral system, followed by agrisilvihorticulture, agrihorticulture (Table 5). The highest herb evenness index was in aquasilviculture, agrihorticulture, agrisilvihorticulture (Table 5). The Sorenson's Similarity index revealed that all the agroforestry systems had a very low degree of similarity between the vegetation species (Table 7). Shastri *et al.* (2002) also found very low similarity between different agroforestry systems in Karnataka.

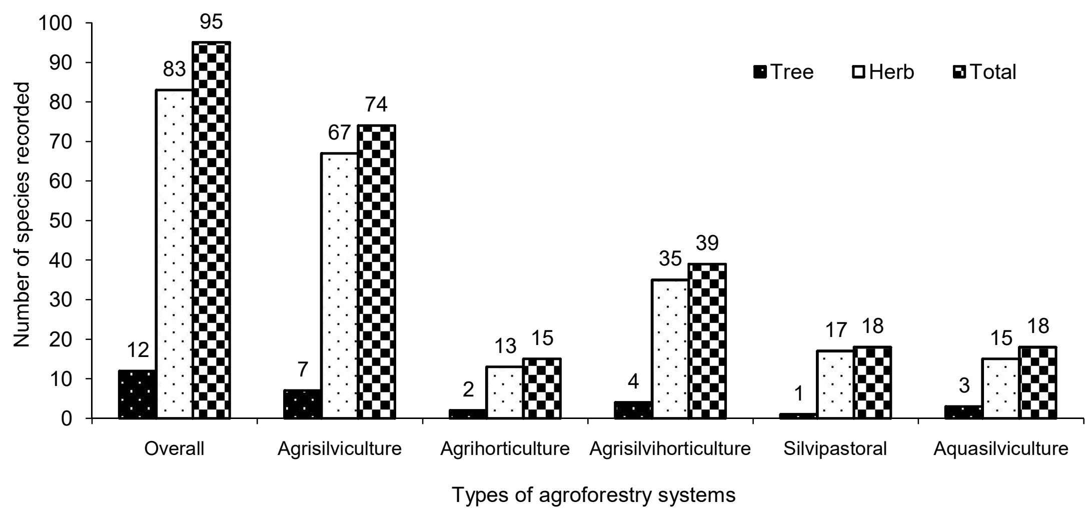

Fig. 1. Vegetation species richness of selected agroforestry systems in Ayodhya district

Table 5. Community characteristics of selected agroforestry systems in Ayodhya district

Parameters	Types of agroforestry systems studied				
	Agrisilviculture	Agrisilvihorticulture	Agrihorticulture	Silvipastoral	Aquasilviculture
	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Vegetation species richness	42.72±3.92	19±4.50	9.5±0.5	12	10±2
Tree species richness	2.72±0.30	2.66±0.66	1	1	2±1
Tree density (individuals ha^{-1})	35210.81±34812.22	427.77±14.69	250±50	375±75	250±50
Tree Basal area (m^2 ha^{-1})	1.79±0.06	1.68±0.25	0.92±0.03	2.11±0.65	0.81±0.09
Tree Shannon Weiner index	0.78±0.12	0.85±0.17	0.33±0.33	0	0.34±0.34
Tree Simpson's Dominance index	0.46±0.07	0.46±0.05	0.76±0.24	1	0.75±0.25
Tree Evenness index	0.67±0.08	0.94±0.03	0	0	0.31±0.31
Herb Species richness	40±3.751	16.33±3.84	8.5±0.5	11	8±1
Herb density (individuals ha^{-1})	382820.72±18068.32	1702638.89±1275557	152500±32500	453125±60625	152500±5000
Herb Shannon Weiner index	1.25±0.04	0.97±0.13	0.84±0.005	0.72±0.05	0.86±0.08
Herb Simpson's Dominance index	0.10±0.01	0.19±0.06	0.17±0.005	0.34±0.03	0.15±0.04
Herb Evenness index	0.34±0.005	0.35±0.02	0.39±0.009	0.29±0.02	0.41±0.01

Table 6. Stand density, basal area and IVI of selected agroforestry systems in Ayodhya district

Scientific name	Agroforestry systems														
	Agrisilviculture			Agrisilvihorticulture			Agrihorticulture			Silvipastoral			Aquasilviculture		
	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI
Tree															
Alianthus excelsa	17.59	0.06	13.34												
Azadirachta indica													50	0.05	60
Cassia siamea													50	0.2	80
Dalbergia sissoo	15.74	0.05	11.76												
Eucalyptus hybrid	266	1.13	198.04	250	0.98	169.84				400	2.33	300			
Magnifera indica	0.92	0.01	1.22	83.33	0.35	66.95	200	0.75	230						
Melia azedaraah	22.22	0.12	18.98												
Pongamia pinnata													200	0.55	220
Psidium guajava				66.66	0.15	41.53									
Swietenia macrophylla	26.85	0.09	19.58												
Syzygium cumini							50	0.15	70						
Tectona grandis	48.14	0.19	37.11	33.33	0.1	22.34									
Herb															
Achyranthes aspera	1417		1.67	2500		1.91									
Ageratum conyzoides	500		0.39												
Ageratum houstonianum	1000		1.04												
Allium cepa	1917		1.54												
Alternanthera pungens				1667		1.70									
Alysicarpus monilifer	583		0.67	833		1.50				4166		3.73			
Amaranthus spinosus	333		0.34	1250		1.60									
Amaranthus viridis	83		0.28							7500		7.29			
Anagallis arvensis	2417		1.93												
Arundo donax	250		0.32							5000		3.91			
Atylosia scarabaeoides	583		0.90												

Cont...

Table 6. Stand density, basal area and IVI of selected agroforestry systems in Ayodhya district

Scientific name	Agroforestry systems														
	Agrisilviculture			Agrisilvihorticulture			Agrihorticulture			Silvipastoral			Aquasilviculture		
	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI
Avena ludoviciana	333		0.61												
Blainvillea acmella				2500		1.91									
Brachiaria ramosa	833		1.00	1250		1.60	2500		6.18						
Brachiaria reptans	1750		1.49	3750		3.51				5000		6.77			
Brassica nigra	2750		2.01												
Cammelina benghalensis	917		0.49												
Cannabis sativa	1667		2.00	2500		1.91									
Cardamine hirsuta	1917		1.54												
Cassia absus	500		0.39	3333		2.11									
Chenopodium album	2167		2.12	3750		3.51	3750		7.00						
Chloris barbata	3333		2.69												
Cleome chelidonii	417		0.63												
Colocasia escluenta	1917		1.27												
Commelina benghalensis	5417		3.74				10000		11.10						
Convolvulus arvensis	4500		4.30	6667		6.82				1666		6.06			
Conyza bonariensis	583		0.93												
cpyprus rotundus	16000		11.70												
Croton bonplandianum										4166		3.73			
Cynodon dactylon	20083		14.85	21250		12.99	41250		36.14	18333		18.15			
Cyperus compressus	1667		1.47	2500		3.20									
Cyperus difformis	417		0.36												
Cyperus eragrostis	500		0.39												
Cyperus haspan	1667		1.73	1667		1.70									
Cyperus iria	2917		2.58	10417		5.14				1666		3.20			
Cyperus kyllingia	417		0.36	2083		1.80									
Cyperus rotundus				8750		7.33	6250		8.64	8333		7.47			

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Table 6. Stand density, basal area and IVI of selected agroforestry systems in Ayodhya district

Scientific name	Agroforestry systems														
	Agrisilviculture			Agrisilvihorticulture			Agrihorticulture			Silvipastoral			Aquasilviculture		
	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI
Dactyloctenium aegyptium	5750		4.09	4167		3.61	8750		10.28	4166		3.73			
Dalbergia sissoo	85		0.28												
Dichanthium annulatum	500		0.39	3333		2.11									
Digera arvensis	3500		2.47				1250		5.36						
Digitaria sanguinalis	1833		1.51	1667		1.70									
Echinochloa colona	11917		7.77	5833		5.32									
Echinochloa crusgalli	1000		1.04	10833		5.25									
Eclipta alba	500		0.65												
Eleusine indica	1750		1.49												
Emilia sonchifolia	667		0.43												
Euphorbia hirta	1167		0.82	1667		1.70				2500		3.38			
Evolvulus alsinoides				417		1.40									
Evolvulus nummularius	500		0.65												
Gomphrena decumbens				1250		1.60									
Ipomoea obscura	417		0.36												
Lepidium sativum	167		0.30												
Magnifera indica				833		1.50	2500		6.18						
Oryza sativa	57000		20.62	38750		13.38									
Oxalis corniculata							3750		7.01						
Parthenium hysterophorus	750		0.45												
Pergularia daemia	417		0.36												
Peristrophe paniculata				2083		3.10									
Phalaris minor	1000		1.30												
Polypogon monspeliensis										2500		3.38			

Cont...

Table 6. Stand density, basal area and IVI of selected agroforestry systems in Ayodhya district

Scientific name	Agroforestry systems														
	Agrisilviculture			Agrisilvihorticulture			Agrihorticulture			Silvipastoral			Aquasilviculture		
	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI	Density (ha^{-1})	Basal area (m^2 ha^{-1})	IVI
Portulaca oleracea	250		0.85	417		1.40									
Psidium guajava				833		1.50									
Rumex dentatus	917		1.28												
Saccharum officilarum	73000		27.07	75833		28.96									
Setaria glauca	750		0.71												
Sida acuta	750		0.45	2500		3.20				5000		3.91			
Solanum melongena	4000		3.12				18750		25.93						
Solanum tuberosum	4333		2.15												
Sonchus asper				833		1.50	8750		14.82	833		3.03			
Sorghum bicolar										398333		115			
Sorghum halapense	2250		1.88	5000		3.82									
Striga asiatica	1000		1.04												
Tectona grandis	90		0.28												
Trianthema portuacastrum	2167		2.39	1667		1.70	8750		14.82	2500		3.38			
Tribulus terrestris	250		0.32				5000		7.82						
Tridax procumbens	500		0.65							1666		3.20			
Triticum aestivum	105167		30.06	159167		44.17									
Triumfetta rhomboidea	1167		1.08												
Vigna munga	14833		6.94												
Volutarella divaricata	250		0.32												
Zea mays	8500		6.37	14583		12.66	31250		38.67						

Production and income from agroforestry systems: Out of the five agroforestry systems found in the study area, three systems namely agrisilviculture, agrihorticulture and agrisilvihorticulture are used for cultivating rice. The highest average productivity and average income of *Oryza sativa* was found in agrisilviculture, followed by agrisilvihorticulture and agrihorticulture system (Table 8). The highest average productivity and income of *Triticum aestivam* (wheat) was recorded in agrihorticulture and agrisilviculture, followed by agrisilvihorticulture (Table 8). *Triticum aestivam* is grown with *Eucalyptus* spp. and *Tectona grandis* in the study area. Kar et al (2022) has reported that in Madhya Pradesh, the productivity of *Triticum aestivam* grown with *Dalbergia sissoo* is 27.60 q ha^{-1}. The average productivity for *Brassica nigra* was highest in agrihorticulture system (Table 8). The average productivity and average income of *Eucalyptus* spp. was the highest in agrisilviculture system followed by agrisilvihorticulture system (Table 8). A similar study in Andhra Pradesh reported that the income generated by *Eucalyptus* spp. *after* four years in agrsilviculture system was Rs. 27, 440/-. Similarly, in agrisilviculture system, the average productivity and average income was the highest from *Tectona grandis*, followed by *Swietenia mahogany, Dalbergia sissoo* and *Ailanthus excelsa* (Table 8). In agrisilvihorticulture, the average productivity and average income was highest from *Emblica officinalis* followed by *Psidium guava* (Table 8).

Table 7. Sorenson's similarity index of selected agroforestry systems in Ayodhya district

Agroforestry systems	Agrisilviculture	Agrihorticulture	Agrisilvihorticulture	Silvipastoral	Aquasilviculture
Agrisilviculture	0.00				
Agrihorticulture	0.24	0.00			
Agrisilvihorticulture	0.54	0.33	0.00		
Silvipastoral	0.28	0.30	0.42	0.00	
Aquaculture	0.19	0.12	0.21	0.11	0.00

Table 8. Productivity and income from products sold from various agroforestry systems in Ayodhya district

Crop name	Agrisilviculture		Agrihorticulture		Agrisilvihorticulture	
Cereals	Productivity mean (quintal)	Income by sale of products (Rs.)	Productivity mean (quintal)	Income by sale of products (Rs.)	Productivity mean (quintal)	Income by sale of products (Rs.)
Oryza sativa	40.53	46875	23.75	18900	33	46200
Triticum aestivam	27.55	46862	30.75	39600	26.83	34800
Brassica nigra	4.16	13775	5.50	5700		
Cash crops						
Saccharum officinarum	176.50	42100				
Vegetables						
Solanum melongena	11.50	144				
Trees						
Eucalyptus globulus	9.02	20796.61			6.29	18560
Tectona grandis	1.56	74083.33				
Ailanthus excelsa	0.40	9533.33				
Swietenia mahogany	0.80	29142.85				
Dalbergia sissoo	0.53	20516.67				
Fruits (kg)						
Psidium guajava					108	1563
Emblica officinalis					330	3350
Magnifera indica			161.40	2869		

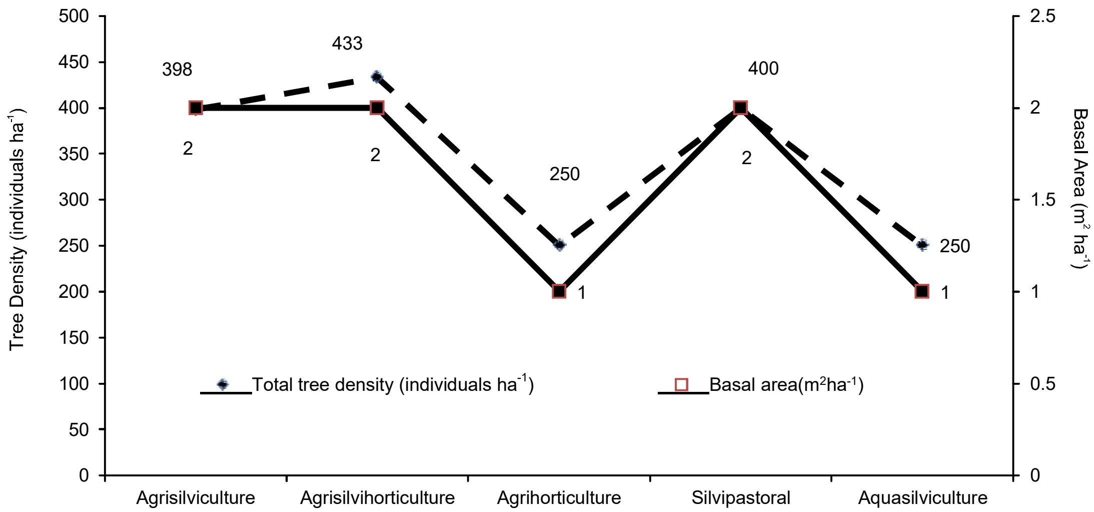

Fig. 2. Stand density (individuals ha^{-1}) and basal area (m^{2}ha^{-1}) of tree species in selected agroforestry systems in Ayodhya district

CONCLUSION

The five different agroforestry systems of this region have conserved a high amount of plant diversity. But still there is a scope for conserving more species as currently these systems seem to be more inclined towards having a few dominant species alone as they are preferred by the local farmers. These agroforestry systems are also associated with high income earning opportunities. Shifting towards a more diverse plant species cultivation by including indigenous tree species will be recommended for future conservation practices. Preference must be given to planting of multipurpose trees which provide multiple benefits including production, protection and income generation. Timely and scientific management practices are required for improvement of yield and system functioning.

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Assessing Diameter Growth in Conifers and Relation with Bioclimatic Variables under Temperate Conditions of Western Himalayas

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Abstract: The current study was taken to assess average annual growth rate put on different conifer species (*Abies pindrow, Cedrus deodara, Picea smithiana* and *Pinus wallichiana)* under varying climatic variables including temperature, precipitation and relative humidity over a period of 10 years using multiple regression approach. A general response of average increment per year under different diameter classes viz. 1 (10-30) cm, 2 (31-50) cm, 3 (51-70) cm and 4 (71-90) was also assessed using standard tree increment core method. Ninety-six core samples were extracted from the selected trees and analysed for tree ring widths according to standard dendrochronological procedures. *Cedrus deodara* showed highest average annual increment of 6.32mm, while as *Abies pindrow* showed the lowest annual increment with 4.31mm over the period. Average annual increment of diameter class 2 (31-50cm) was highest (5.53 mm) irrespective of species and sites while as the lowest was observed for class 3 (4.81 mm) and 4 (4.85 mm). Response of average annual increment to climatic variables was best explained for *Cedrus deodara* (R^2 = 0.62) while as diameter class 2 best explained the response to climatic variables with R^2 (0.92). The regression models developed for different species and diameter classes were validated through predicted models having close coherence with the observed values. The study addresses data gaps and offers potential to predict biomass carbon accumulation under the changing climatic conditions.

Keywords: Increment, Conifers, Bioclimatic variables, Wood core sample, Kashmir Himalayas

Tree growth also referred to as increment is one of the most important biophysical variables' that contributes to biomass accumulation (Vieira et al 2020). Tree growth prediction has become important in view of its direct relation to productivity and dynamics of forest stands (Condit et al 2006) responds to temperature changes across the latitudinal gradient in the same way it responds to altitudinal gradients in the mountainous areas (Lyu et al 2017). There is an inconsistency in tree growth responses to climate with varying geographic location, forest type, and tree species (Rahman et al 2018).

There is a considerable decrease in duration and rates of xylem cell production due to drought conditions resulting into declined wood production (Vieira et al 2020). Cambial activity is hugely influenced by the existing environmental and physiological conditions including phenological stage, soil water availability, precipitation, temperature levels and on the number of sunlight hours per day. These factors increase the rate of photosynthesis when present in optimum amount. Climatic conditions especially water availability triggers cambial activity that leads to increase in tree girth and other wood characteristics (Drew et al 2009, Sette Jr 2016). Diameter has been extensively used and monitored to study the increment of trees due to its more pronounced relation with the growth. Moreover, diameter can be measured easily with a higher accuracy compared to tree height (Ishihara et al 2016). Species based on existing environmental drivers, local adaptations and individual plasticity to climate respond to climate by adjusting the timing and extent of their phenology, growth and reproductive seasons (Diez et al 2012). Past growth dynamics at the tree treeline in response to changing climatic conditions and climatic variability are the ready references to know tree population dynamics (Jochner et al 2017).

Besides the inherent factors of species that control growth rate, seasonality of cambial activity is influenced significantly by temperature (Drew et al 2009), photoperiod and precipitation (Marcati 2006, Drew et al 2009). Temperature and water availability are known to regulate growth and cambial activity (Drew et al. 2009). Thus tree ring analysis has been proven as a helpful technique to assess age and growth pattern of tree species over long time periods Rozendaal et al 2011).

Dendrochronology in the recent years has developed as a sophisticated science and its full potential is yet to be explored (Aryal et al 2018, Jawad and Ahmad 2021). Moreover, in context of REDD+, this quantification is prerequisite for assessment of forest biomass and carbon sequestration in turn. In addition, this relation helps to explore forest ecosystem capacity to adapt climate change (Wani et al 2019, Joshi et al 2022). Temperate Himalayan region is mainly composed of evergreen species including *Pinus wallichiana, Cedrus deodara, Abies pindrow and Picea smithiana*. They form the major strata that contribute to biomass carbon in the region and incremental data in these species is utterly lacking in the region (Wani et al 2019) and must be estimated using indirect methods in the absence of any reliable data. In this study, it was hypothesized that the increments put on by different conifers vary significantly in terms of diameter under a given set of climatic conditions.

MATERIAL AND METHODS

Study area: Special Forest Division, Tangmarg is spread across three districts of Jammu and Kashmir including Baramulla, Budgam and Srinagar (Fig. 1). The forest area primarily lies in district Baramulla between Longitude: 74˚16´E to 74˚46´E and Latitude: 33˚54´N to 34˚17´N as shown in Figure. 1 envisaging world famous skiing resort Gulmarg. It experiences pleasant weather in summer and severe cold in winter. Winter precipitation is mostly received in the form of snow by almost all parts of the district.

Sampling design and collection: Purposive random sampling technique was adopted within the study area. Core samples were collected from the dominantly identified species in the study area. Three sites viz. Site I or C1 (Baderkoot and Gogaldara), Site II or C2 (Kalantra and Baba Reshi) and Site III or C3 (Tangmarg and Gulmarg) were selected based on purposive random sampling from the study area. The sites varied from each other in terms of location an altitude with Site I (34° 02' 15.9" N; 74° 27' 26.7" E) at average altitude of 2206 m amls, Site II (34° 06' 02.4" N; 74° 23' 54.9" E) at an average altitude of 2011 m amsl and Site III (34° 02' 16.2" N; 74° 24' 28.5" E) at an average altitude of 2261 m amsl. From each site, core samples were taken from four conifers viz. Deodar (*Cedrus deodara)* (B1)*, Blue Pine (Pinus wallichiana)* (B2)*, Fir (Abies pindrow)* (B3) and *Spruce (Picea simithiana)* (B4).

With the help of increment borer, a small pencil sized piece of wood known as tree core, core sample or increment core was taken from the trunk of tree at breast height (1.37 metre above ground level). The trees were bored using 5 mm increment borer in accordance with (Jochner 2017). At least two cores were taken 90 degrees away at any point of measurement using increment borer. Core samples at each site were obtained in four diameter classes viz. 10-30, 31-50, 51-70 and (71-90) cm. The borer was inserted to a depth so that at least last 10 growth rings (i.e., from 2018 year of sampling to past 10 years of radial increments) were obtained on the radial core. Cores collected from 96 trees in the study area were immediately secured in core tubes with proper labelling and transferred to the laboratory for analysis. Annual radial increments from each core sample were recorded in synchronization with the respective tree DBH (diameter at breast height). Annual radial increments were doubled for calculation of annual growths or annual diameter increments or growth rate. Simple stereomicroscope was used for measurement of annual ring measurements using standard dendrochronological procedures (Fritts 1962). Repeated measurements were taken up to a resolution of 0.1 mm and then averaged for the final measurement.

Species, climate and growth: To identify the synergistic effect of the climatic variables on the increment, tried multiple regression analysis and generated models for prediction of diameter increment in different species. The response of increment was also assessed under different diameter classes for conifers in general. Prediction models were developed for different species and different diameter classes with the climatic variables as the dependent variables. Specifically, tree ring data and climate data was combined to figure out the following queries:

1. How the climatic variables of area determine the diameter increment of individuals in a species?
2. How different species respond to the same environmental factors prevailing in a locality?
3. How diameter increment varies in different diameter classes of the species?

Species wise diameter increments of trees from all the sites over a period of 10 years in corresponding diameter classes were tabulated and subjected to multiple regression analysis with average annual temperature (°C), mean annual precipitation (mm) and average annual relative humidity (%) as the independent variables using the climatic data of the region obtained from Indian Meteorological Department Srinagar. Coefficient of determination (R^2) was also calculated for each model. Graphically individual line fit and residual plots for different species and diameter classes were plotted to spot patterns and trends with individual climatic variables. These plots also represent the interaction of different multiple regression models and associated error with individual climatic variables.

RESULTS AND DISCUSSION

The diameter increments of *Cedrus deodara, Pinus wallichiana, Abies pindrow* and *Picea simithiana* over a period of 10 years reveals that the average diameter increment of *Cedrus deodara* ranges from 5.75 to 6.83 in 2014 and 2009 The average diameter increment of *Pinus wallichiana* ranges from 4.65 to 5.11 mm in 2009 and 2013

The average diameter increment of *Abies pindrow* ranges from 3.81mm (2018) to 4.74 mm (2014). The average diameter increment of *Picea smithiana* ranges from 4.07 mm (2017) to 4.93 mm (2013). Similarly among the diameter classes the increment under diameter ranged from 5.88mm (2013) under class 1 to 4.08mm (2018) under class 3. The diameter increment was found highest in *Cedrus deodara* (6.83 mm) for year 2014 while lowest was in *Abies pindrow* (3.83 mm) for 2018. Average annual temperature ranged from 7.71°C (2013) to 6.25°C (2009). Mean annual precipitation ranged from 4.86 cm (2015) to 1.05 cm (2010) while as average annual relative humidity ranged from 80.13 % (2013) to 64.78 % (2009).

In models, 'Y' denotes diameter increment (mm), 'β_0'denotes average annual temperature (°C), 'β_1' denotes mean annual precipitation (cm) and 'β_2' denotes average annual relative humidity (%) (Table 2). With these models, for any year annual diameter increment of any of these four species and in any diameter class in general can be calculated using the given set of climatic variables for the given region. Among the species, highest R^2 was for Deodar (0.62) followed by Fir (0.57) while as among different diameter classes the highest R^2 was estimated for D2 (0.92) followed by D3 (0.53) (Table 2). These higher values depict comparatively strong relations for the given set of variables. The models generated for each set of relations were also validated by generating predicted models for each species and all the diameter classes (Fig. 1 a-b). There was proximity between the predicted and observed values for each set of relation for species (Fig. 2a-o) with and diameter classes (Fig. 3a-l). Measures of central tendency for the diameter class 1-4 are shown in Figure 1 a-b.

The active growth period in Himalayan conifers ranges from March to October. Generally, the new shoots appear in March and early April. The xylem cells grow faster under optimum moisture conditions during the active growing season and hence are deemed important for annual growth in trees. Precipitation in the form of rain and snow is majorly received in winter months which makes the moisture available to plants in their early growing period. The conifer species indicate strong climatic signatures in ring-width measurement series, however, there is always a paucity of climatic data in mountainous region which makes it difficult to

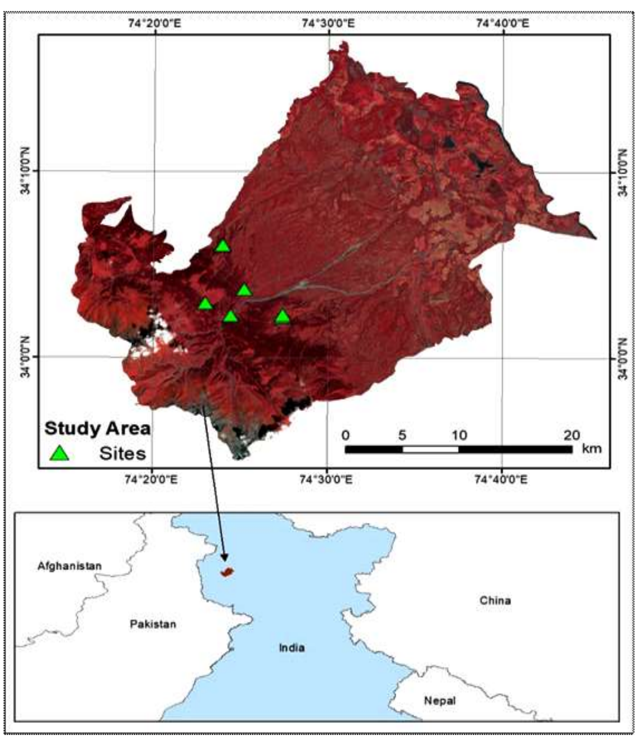
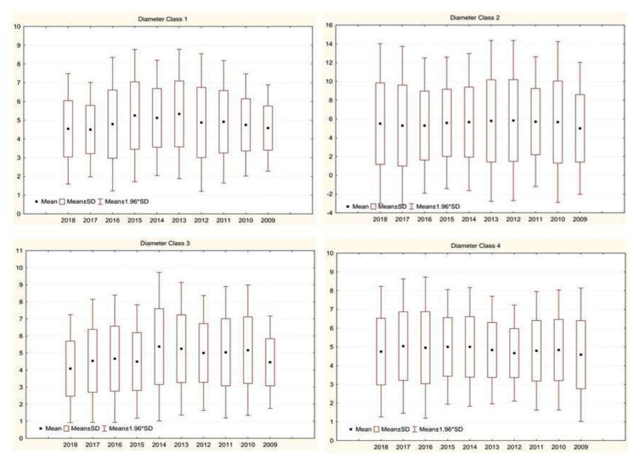
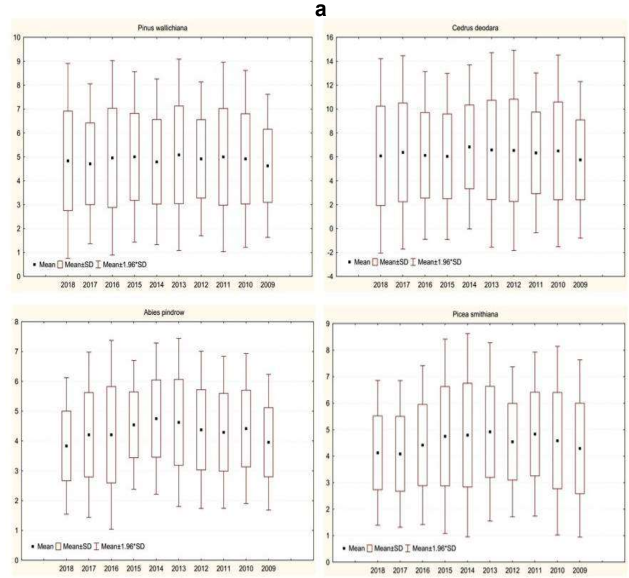


Fig. 1. Study area



a



b

Fig. 1. Statics for the diameter class 1-4

calibrate tree-ring data with climate (Dhyani et al. 2023). The diameter increment is generally considered as a growth indicator in trees. It depends on many factors which ranges from difference in genotypes among species to factors of locality affecting trees. Therefore, annual diameter increment can vary from species to species, among individuals in a species and annual year growths in an individual (Chen et al 2022).

The diameter class 1, 2 and 3 registered higher growth rates when the climatic variables were on higher side while only diameter class 4 showed lowest growth rate when the climatic variables were on lower side. There was strong correlation between diameter increment and climatic variables for diameter class 2 with R^2 of 0.92. The different diameter classes respond differently to climatic variables in terms of different growth responses. Merian and Lebourgeois (2011) also observed established a relation between tree diameter sizes and their climate growth responses. They further revealed that contrasting diameters of a species behave differently to climate-growth responses and large sized trees could be heavily influenced by climate change especially under xeric conditions. The reasons could be the variable resource availability per unit biomass across different sizes of the trees as the tree growth is liners to leaf biomass (Enquist et al 1999). However, this is true for symmetric competition and may not always be tree for asymmetric competition especially closed forests where smaller trees are consistently under shade of the dominant trees (Muller-Landau et al 2006). However, as explicit, the growth rates in the present study are averaged over a diameter class and doesn't reflect growth characteristics of individual trees subjected to crowdedness (tree density) (Fransson et al 2021) and level of disturbance (Muller-Landau et al 2006, Coomes et al 2011). Sette Jr (2016) reported that higher trunk growth rate was observed in larger trees than in other basal area classes in *Eucalyptus grandis,* while studying relationship between climatic growth rate, trunk growth rate and wood density. Gao et al (2020). In his study revealed that there was no significant difference in growth response to climate.

During 2013 to 2015, the climatic variables were on higher side and all the species registered highest diameter growth rate and from 2009 to 2010, the climatic variables were on lower side, only *Cedrus deodara* and *Pinus wallichiana* registered comparatively less diameter increment while as *Abies pindrow* and *Picea smithiana* continued to grow with average growth rate. Ram and Borgaonkar (2014) also observed climatic responses of tree rings of fir (*Abies pindrow*) in western Himalayas and concluded that relationship between diameter growth rate and climatic variables were significant positive r due to moisture availability through snow melt in the growing seasons when physiological processes are at its peak.

The current study indicates that different conifer species growing across the study area have different abilities to fix increments under a given set of climatic variables and varied biophysical parameters under different sites. Marquardt et al (2019) while studying climatic response of growth in Sky Island Ponderosa pines demonstrated that dendroclimatic response varies modestly between species and sites. Hughes et al (2019) also observed that diameter growth rate of pine was positively correlated with early summer total

Table 1. Species wise and diameter class wise diameter increment (mm) and climatic variables in a decade (2009-2018)

Year	Diameter increment (mm)					Diameter classes				Climatic variables		
	Dia Increment (mm) Deodar	Pine	Fir	Dia increment (mm) Spruce	Average annual diameter increment (mm)	Average annual diameter increment (mm) D1	Average annual diameter increment (mm) D2	Average annual diameter increment (mm) D3	Average annual diameter increment (mm) D4	Average annual temperature (°C)	Mean annual precipitati on (cm)	Average annual relative humidity (%)
2018	6.08	4.83	3.83	4.13	4.72	4.54	5.50	4.08	4.75	6.30	3.25	70.49
2017	6.38	4.71	4.21	4.08	4.84	4.50	5.38	4.54	5.04	6.87	3.79	74.73
2016	6.13	4.96	4.21	4.42	4.93	4.79	5.21	4.67	4.96	7.16	2.67	69.84
2015	6.04	5.00	4.54	4.75	5.08	5.25	5.67	4.50	5.00	6.30	4.86	76.78
2014	6.83	4.79	4.75	4.79	5.29	5.13	5.79	5.38	5.00	6.42	3.94	77.06
2013	6.58	5.08	4.63	4.92	5.30	5.33	5.88	5.25	4.83	7.71	2.77	80.13
2012	6.54	4.92	4.38	4.54	5.09	4.88	5.71	5.00	4.67	7.10	4.11	78.40
2011	6.33	5.00	4.29	4.83	5.11	4.92	5.75	5.04	4.79	7.40	2.67	79.66
2010	6.50	4.92	4.42	4.58	5.10	4.75	5.75	5.17	4.83	7.23	1.05	75.44
2009	5.75	4.63	3.96	4.29	4.66	4.58	5.04	4.46	4.58	6.25	4.89	64.78

precipitation in Spruce and Pine forests of northern European Russia. The current study results are in accordance with observations on species growth relations with temperature for *Pinus wallichiana* (Shah et al 2019), multi-species (Wettstein et al 2011) *P. abies* (Andreassen et al 2006), *Larix olgensis* (Shen et al 2016) and *Betula tortuosa* (Petrov et al 2019). Bolivian tropical lowland forests (Toledo et al 2011) *Larix decidua* (Carrer and Urbinati 2006). Ram and Borgaonkar (2014) reported that correlation coefficients between growth rate and temperatures in case of *Abies pindrow* were observed to be weaker but barely significant in fir (*Abies pindrow*) from Chandanwadi in Jammu and Kashmir, western Himalaya, India.

Rahman et al (2018) inferred stronger relations in the period 1986-2015 than during 1950-1985. Climate sensitivity changes were attributed to a warming trend in the recent decades. The increase in temperature at higher elevations increases growth, however climate change can possibly influence this forest growth dynamics positively or negatively (Jochner et al 2017). Climatic variables over a long period of time affect different biophysical factors and composition of the forests which have the potential to affect growth of the trees and the same has been established (Morin et al 2018). Using dendro-ecological approach, Latreille et al (2017) concluded that growth in Silver Fir is related to the current and previous year climatic variables and besides climatic variables other factors including competition, microsite conditions including soil moisture, slope steepness, solar radiation are related to species increment.

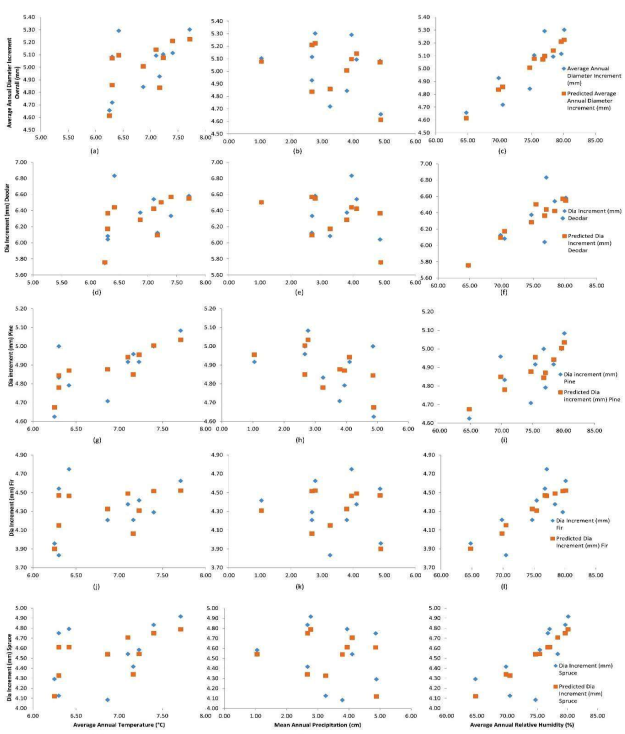


Fig. 2. Diameter increment (mm) relation with climatic variables

Table 2. Regression models developed with increment as dependent variable and climatic factors as independent variables

Species	Model	R^2
Overall	$Y=2.266-0.008\beta_0-0.017\beta_1+0.038\beta_2$	0.75
Deodar	$Y=3.526-0.109\beta_0-0.081\beta_1+0.051\beta_2$	0.62
Pine	$Y=3.342+0.081\beta_0-0.014\beta_1+0.014\beta_2$	0.56
Fir	$Y=1.135-0.059\beta_0+0.011\beta_1+0.048\beta_2$	0.57
Spruce	$Y=1.060+0.056\beta_0+0.017\beta_1+0.041\beta_2$	0.51
D1	$Y=1.281+0.038\beta_0+0.069\beta_1+0.1041\beta_2$	0.50
D2	$Y=2.912-0.224\beta_0-0.087\beta_1-0.060\beta_2$	0.92
D3	$Y=0.435+0.179\beta_0-0.041\beta_1+0.044\beta_2$	0.53
D4	$Y=4.680-0.132\beta_0-0.034\beta_1+0.016\beta_2$	0.18

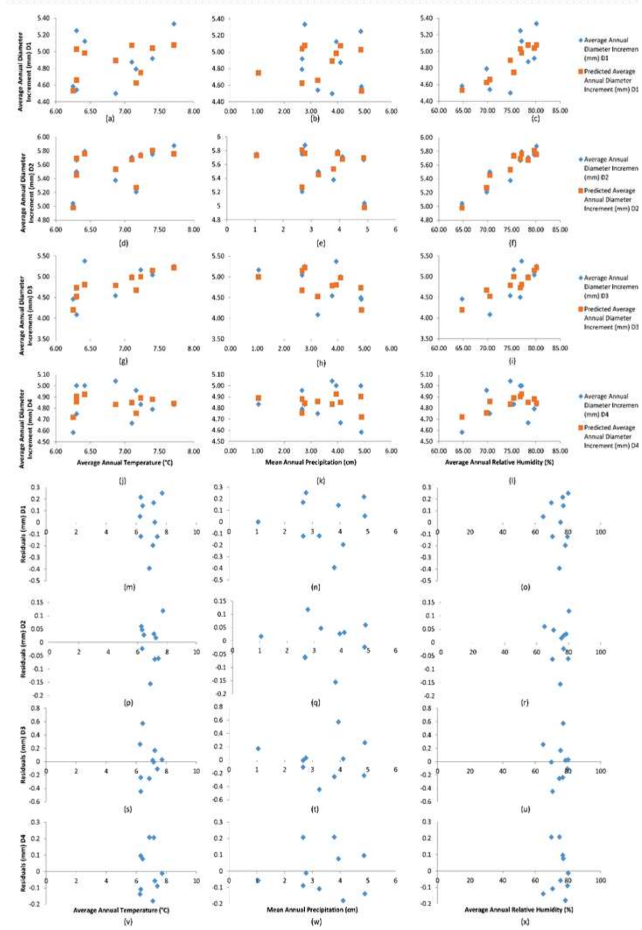


Fig. 3. Regression analysis of diameter increment (mm) with climatic variables (a-l) and residuals (mm) (m-x)

CONCLUSION

Among species *Cedrus deodara* showed a highest average growth rate of 6.32mm/year over a period of 10 years while *Abies pindrow* showed the lowest over a period of 10 years. Average diameter increment of diameter class 2 was found highest irrespective of species and sites while the lowest average diameter increment was observed for diameter class 3 and 4.Diameter class 1, 2 and 3 registered higher growth rates when the climatic variables were on higher side while only diameter class 4 showed lowest growth rate when the climatic variables were on lower side. Prediction models (species wise and diameter class wise) developed for increment relation with climatic variables fairly predict increment (growth) under said climatic variables. The hypothesis that different conifer species in the geographical region put up similar growth has been proven otherwise. Further the average increment put on by conifers in general over the time showed significantly different results for different diameter classes refuting the hypothesis. Among the species *Cedrus deodara* (Deodar) distinctly exhibited highest growth among the species and diameter class (2) expressed the highest growth among all diameter classes in general.

Diameter increment relation with the climatic variables (precipitation, temperature and relative humidity) was best explained for *Cedrus deodara* followed by *Abies pindrow* while as this relation in terms of diameter classes was best explained for diameter class 2. Higher coefficient of determination expresses the strength of relations with the given set of variables. In general, the relation of species and diameter with climatic variables had a proximity with an acceptable R^2expressing coherence between predicted and observed values.

The study could have incorporated other climatic factors as well into the study to have a more comprehensive study. However, due to lack of past data on other factors including atmospheric CO_2, nitrogen deposition, sunshine hours etc. which have the capability to influence the growth and increment. However, the study indicates that response of temperature and precipitation continue to be the driving force for positively influencing the growth of conifers in the region. Although the study doesn't use very high-quality measurement technique for ring measurement, there might have introduced some unavoidable errors in measurements, but findings of the study give reasonably good results. The study addresses the data gap that existed in the region in terms of increment studies conducted on conifer species and diameter classes. Moreover, the study demonstrates the potential of tree ring analysis to assess the relationship of past growth with climatic variables which would help predict future growth under the changing climate scenario.

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AUTHOR's CONTRIBUTION

AAW and UM initiated and conceptualized the study. All authors (UM, AAW, AAG, SM, MAI, AF, SF, IAS) contributed to field data collection and lab work. UF, AAW and IAS contributed to data evaluation. All authors (UM, AAW, AAG, SM, MAI, AF, SF, IAS) contributed to writing and reviewing the manuscript.

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Indigenous Technological Knowledge (ITK) Applied to Specific Herbal Medicinal Plants for Common Ailments: Study From Assam, Northeast India

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Abstract: The present study was undertaken to explore the knowledge and existing practices of indigenous technological knowledge (ITK) by rural women of Assam, North-east India on herbal medicinal plants for the treatment of common ailments. Simple random sampling design was followed for selection of two blocks . One hundred (100) numbers of female respondents were selected from four villages of the two blocks. First-hand information was gathered by personal interview method through structured interview schedule. The majority (67.00%) of the respondents was from medium socio-economic status and 68.00 percent of the respondent had medium level of knowledge. The highest percentage of respondents (98.00%) had used different indigenous technological knowledge on herbal medicinal plants for treatment of common ailments such as cold and cough, digestive problems, diabetes, urinary disorder, depression, urinary problem, cuts and wounds and skin disease. The different parts (leaf, fruit, root and whole plant) of herbal medicinal plants were use for curing above mentioned ailments in the form of paste, extract, chutney or curry.

Keywords: Indigenous Technological Knowledge (ITK), Herbal medicinal plants, Rural women

India is inhabited by a large number of people having diverse ethnic group. There are over 400 different tribes and other ethnic groups residing mostly in rural areas in India. Most of them are still living in the remote forest areas and depend to a great extent on the indigenous system of medicines. The knowledge on herbal medicinal plants has been continuing for years and has been transmitted orally from generation to generation. Plants and their parts used by the different tribal and non-tribal people has some or the other relevance with the plants that used by these traditional healers residing in this remote part of India. However, recently it seems that this type of knowledge on traditional medicine is vanishing from the modern society since younger generations are not interested to carry on this tradition. In India, folk medicine plays an important role in rural areas. It is estimated that traditional medicine use 8,000 plant species and more than 25,000 herbal formulas (Sen et al 2016).

Health status of Indian women especially in rural area is in poor state. Women and girl child are sometimes not allowed to health centres or local dispensaries even if these are available in their village. Therefore with all the limited means and resources at her disposal, tries to r health through traditional medicinal knowledge and plant resources available in rural areas. But this forced responsibility has also helped her to acquire basic knowledge about local plants and use of different plant parts as therapeutics. With the help of self-acquired traditional knowledge are well mastered in identify the plant at right development stage of use through visual markers which sometimes are not even known to the scientific world the elderly women play a pivotal role in retaining and passing on traditional knowledge to the next generation. Women share and practiced herbal medicinal plants for both in daily diet as well as curing different ailments.

The people of Assam have good thriving knowledge on several common diseases as well as their remedial therapies with the traditional use of different parts of naturally available herbal medicinal plants like root, leaves and shoots since time immemorial. Scientific documentation of indigenous traditional knowledge through survey of medicinal properties of herbal medicinal plant specimens are important for the conservation and sustainable utilization of natural resources extensively used in human welfare. Therefore, it is the need of the hour to preserving the rich indigenous traditional knowledge of tribal women of Assam, North East India. Thus, the present study was undertaken to understand the ITKs applied to specific herbal medicinal plants for treatment of common ailments.

MATERIAL AND METHODS

The study was carried out in Jorhat District of Assam, North-East India. The descriptive research design, especially survey method was used in this study. A simple random sampling design was followed for selection of two blocks namely Dhekorgarah and Titabor from respective subdivision i.e. Jorhat and Titabor. A list of villages from each of the selected blocks was collected from Block Development Officers. While collecting data special care was taken to select such villages, which were most inaccessible to medical institute, poor transportation facilities and where record of use of such traditional practices of herbal medicine has been continuing till date. The four villages, two villages from each block were purposively selected. For the selection of respondents, a list of total household was prepared from each of the selected villages with the help of village leader. Adopting the methods of the ethnomedicinal information was collected through general conversation with the informants such as village headman (gaon buhas), traditional healers (Bejs), local men and women (Barbhuiya 2022). There was a formal discussion with these informants. The women folk were given a significant role in the discussion since they possess more information about the use of local herbs in primary curing of various diseases. Among those 25 numbers of married women from different age and classes were selected from each village by using simple random sampling method. Thus altogether, 100 numbers of respondents were selected for the present study. The data regarding plant name, plants parts used, form or mode of preparation and treatment for common ailments were collected through personal interview method with the help of the interview schedule developed and analysed.

RESULTS AND DISCUSSION

Socio economic status of the respondents: The majority (68.00%) of the respondents had medium level of knowledge followed by 17.00 percent of the respondents had high level of knowledge and only 15.00 percent had low level of knowledge. It is assumed that rural women might require more information regarding herbal medicinal plants and might be due to the fact that respondents had less exposure to attain the training on herbal medicinal plants.

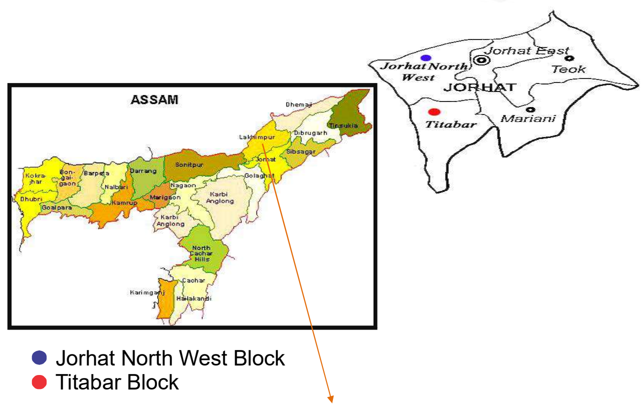

Fig. 1. Location of site of study area

All the respondents have full knowledge on some statement (Table 2). These are herbal medicinal plants are to be included it in our daily diet. Herbal medicinal plants can be used in different forms such as juice, paste, solid, liquid, semi liquid, ointment, powder. Holy basil (tulsi) is good for cough relief and thyme leaf gratiola (brahmi) for brain. Mint (podina) can stop vomiting. Indian patchouli (Hukloti) helps to heeling, colocasia (kosu) is a rich source of iron and curry leaves (norohinho) can increase hunger. *Trigonella foenum* graecum (methi) is very bad for pain and swelling.Second and third highest percentage of the respondents have knowledge on henna (Jetuka) as beneficial for hair and skin and "Assam is very rich in herbal medicinal plants (97.00 and 94.00 percent , respectively).

Practices of ITK among rural women on herbal medicinal plants for treatment of common ailments: The data on existing practices of ITK on herbal medicinal plants according to parts use, purpose of use and form of use of the selected 30 numbers of herbal medicinal plants (Table 3). The data on existing practices o herbal medicinal plants by rural women for treatment of five common ailments i.e. cough and cold, digestive problems, urinary disorder, diabetes and skin disease are presented in Table 3. Majority of the respondent (98.00%) use leaf of nine numbers of herbal medicinal plants

Table 1. Description of different forms of herbal medicinal plants commonly used

	Description
Paste	Herbal paste is called kalka in Ayurveda. It is one of the basic dosage forms. In this study paste is defined as the fresh herb ground as it is or by adding water. Paste is used for external application and oral administration.
Extract	In Ayurveda pharmaceutics and therapeutics, extract is described as primary and most potent dosage form. In this study extract is defined as immediately after collection of herbs, it is washed, crushed and by applying pressure the liquid or juice obtained. It can be used directly or diluted with water.
Curry	In this study curry means first make a smooth paste of herbs, than heat oil, put the paste, boil the paste with a little more water and adjust seasoning with little salt and turmeric powder.
Oil	Herbal oil is pure, whole, organic oil from natural source like herbs. In this study oil is defined as base oil infused with one or more herbs- combining the nourishing and soothing qualities of the oil with the healing properties of the herbs.
Raw	In this study raw form is define as directly eating or chewing fresh parts of the herbs.

namely holy basil, curry leaf, mint, Chinese flower, Indian sorrel, heart leaf, acid plant, henna and amaranth. Maximum percent of respondents (88.00%) use fruit of black pepper. The 76.00 percent of respondents use stem of amaranth and 98.00 percent of respondents use root of three herbal medicinal plants i.e. turmeric, garlic and onion. Maximum (98.00%) use the whole plant of four numbers of herbal medicinal plants i.e. Asiatic pennywort, theme leaved gratiola, prickly amaranth and green amaranth.

Highest percentage of respondents (98%) use holy basil

Table 2. Distribution of respondents according to responded percentage of the knowledge statements regarding herbal medicinal plants

N=100

Statements	Percentage (%)	
	Yes	No
Herbal medicinal plants are to be included in our daily diet.	100	0
Herbal medicinal plants can be used in different forms such as juice, paste, solid, liquid, semi liquid, ointment, powder etc.	100	0
Holy basil (tulsi) is good for cough relief.	100	0
Thyme leaved gratiola (brahmi) is good medicine for brain.	100	0
Trigonella foenum graecum (methi) is very bad for pain and swelling.	100	0
Mint (podina) can stop vomiting.	100	0
Indian Patchouli (Hukloti) helps to heeling.	100	0
Colocasia (kosu) is a rich source of iron.	100	0
Curry leaves (noroxinho) can increase hunger.	100	0
Henna (Jetuka) is beneficial for hair and skin.	97	3
Assam is very rich in herbal medicinal plants.	94	6
Ginger (adda) cures pain.	90	10
Herbal medicine has contributed to primary health care.	82	18
Garlic (nohoru) can prevent bacterial infection.	71	29
Henna (Jetuka) is beneficial for hair and skin.	70	30
Thumba (Durun) is very bad for low blood pressure.	67	33
Root of Shame Plant (Nilaji Bon) is use for curing piles.	57	43
Herbal medicinal plants cannot be used by pregnant women.	57	43
Herbal medicinal plants have less side affect.	54	46
Acid plant (dupor tenga) helps to cure urinal infections.	47	53
Asiatic Pennywort (Manimuni) cures fever.	45	55
Black nightshade (Bhekuri tita) helps to relieves pain.	43	57
Thyme leaved gratiola (Brahmi) leaves is bad for pain and blood.	43	57
Herbal medicinal plants are not very expensive.	42	58
High doses of herbal medicines are dangerous to health.	42	58
Amaranth (Moricha) is good for blood.	39	61
Aloevera (saalkuori) helps to kill worms.	36	64
Aloevera (saalkuori) helps in purifies the blood.	35	65
Rosy Periwinkle leaf (nayantora) is good for diabetic patient.	33	67
Stone Breaker (mati amlokhi) cures viral infections.	28	72
Prickly amaranth (Hati khutura) is beneficial in skin care.	27	73
Turmeric leaf (Halodhi paat) helps to reduce depression.	21	79
Chiretta (Sirota) is good for stomach trouble.	12	88
Onion (ponoru) helps to relieve irritation.	10	90
Eclipta prostrata(Bhringraj) is good for tooth ache.	8	92

Table 3. Distribution of respondents according to parts use, purpose of use and form of use of selected 30 herbal medicinal plants

N=100 (Multiple respond table)

Herbs			Part use (%)					Purpose of use (%)										Form of use (%)		
Scientific name	English name	Local name	Leaf	Fruit	Stem	Root	Whole plant	Cough	Stomach trouble	Skin care	Depression	Urinary problem	Increase appetite	Hair care	Diabetes	Increase memory	Cuts and wounds	Paste	Extract	Chutny/ Curry
Ocimum tenuiflorum	Holy basil	Tulsi	98					98		58									98	
Aloevera	Aloevera	Saalkuwori	42							36				34					42	
Murraya koenigii	Curry leaf	Norohinho	98						98				98	51						98
Centella asiatica	Asiatic pennywort	Manimuni					98		98										54	44
Mentha arvensis	Mint	Podina	98		30					98			98					62		98
Paederia foetida	Chinese flower	Bhedailota	98						98											98
Curcuma longa	Turmeric	Halodi	58			98		32	55	70	20						48	20	70	63
Oxalis Corniculata Linn	Indian sorrel	Tengesi	98							51			70					51		98
Houttuynia cordata Thunb	Heart leaf	Mosondori	98						98				98							98
Bacopa monnieri	Thyme leaved gratiola	Brahmi					98									98				98
Bryophyllum pinnatum	Acid plant	Dupor tenga	98									98							98	
Pogostemon Heyneanus	Indian patchouli	Hukloti	83						47								78			83
Colocasia esculenta	Colocasia	Kosu	52							52										52
Solanum nigrum	Black nightshade	Bhekuri tita		66				44							28					66
Catharanthus roseus	Rosy periwinkle	Noyontora	31												31				31	
Allium sativum	Garlic	Nohoru				98			59				40							98
Coriandrum sativum	Coriander	Dhaniya	82		70								82							82
Leucus aspera	Thumba	Durun	90						70						20				41	58

Cont...

Table 3. Distribution of respondents according to parts use, purpose of use and form of use of selected 30 herbal medicinal plants

N=100 (Multiple respond table)

Herbs			Part use (%)					Purpose of use (%)										Form of use (%)		
Scientific name	English name	Local name	Leaf	Fruit	Stem	Root	Whole plant	Cough	Stomach trouble	Skin care	Depression	Urinary problem	Increase appetite	Hair care	Diabetes	Increase memory	Cuts and wounds	Paste	Extract	Chutny/ Curry
Allium cepa	Onion	Piyaj	76			98		24		47				75				66		98
Zingiber officinale	Ginger	Adda	70			92		74					52						74	92
Phyllanthus amarus	Stonebreaker	Mati amlokhi		22		38			38									38		
Lawsonia inermis L.	Henna	Jetuka	98							98				98				98		
Acorus calamus	Sweet flag	Boch				76		76	24									70	24	
Swertia Chirata Ham.	Chiretta	Sirota	45						45						18				45	
Piper nigrum	Black pepper	Jaluk		88	24			88			12									88
Mimosa Pudica L.	Shameplant	Nilaji bon				10			10										10	
Trailing eclipta	Eclipta prostrate	Bhringraj	66						58					66				58	66	
Amaranthus spinosus	Prickly amaranth	Hati khutura				70	98			47		21							70	98
Amaranthus viridis	Green amaranth	Khutura					98			48			34							98
Amaranthus caudatus	Amaranth	Moricha	98		76					21				18	56					98

for cough relief followed by Black pepper (88.00%) and sweet flag (76.00%). The 98.00 percent of respondents use curry leaf, Asiatic pennywort, Chinese flower and heart leaf for curing stomach trouble. The 98.00 percent of respondents used mint followed by turmeric (90.00%) and aloe vera 84.00 percent for skin care. 20percent of the respondents use turmeric leaf followed by black pepper 12.00 percent use for relief from depression. The 98.00 percent of respondents use Curry leaf, Mint and Heart leaf for increase appetite. The 56.00 percent of respondents use amaranth for curing diabetes followed by Rosy Periwinkle (31.00%). For increase memory 98.00 percent of respondents use thyme leaved gratiola. The 78.00 percent of respondents use Indian Patchouli for cuts and wounds followed by Turmeric (48.00%).

CONCLUSION

The herbs are natural products they are free from side effects, they are comparatively safe, eco-friendly and locally available. Traditionally there are lots of herbs used for the ailments related to different seasons. There is a need to promote them to save the human lives. North-East region in India is one of the important parts of mega bio-diverse region as it has been bestowed with diverse specific endemic plants. The present study on knowledge on the ethno-medicinal uses of most of the plant species used by rural women of Jorhat district of Assam, North-East India indicate revealed that 67 percent of respondents have medium level of knowledge on herbal medicinal plants. The 80% of respondents from the study shows that medicinal plants play an important role in proving primary health care to the rural people. The majority of the respondents practiced selected 30 herbs for medicinal purpose. They had use leaf, fruit, stem, root of the plant and the whole plant in the form of paste, extract, Chutney/curry for curing cough, stomach trouble, skin care, depression, urinary problems, increase appetite, hair care, diabetes, increase memory and cuts wounds. Mostly the respondents use holy basil, curry leaf, heart leaf, mint, henna, Indian sorrel etc. for primary care of ailments. These herbal products are today the symbol of safety in contrast to the synthetic drugs, that are regarded as unsafe to human being and environment. Folk medicine is the result of decades of accumulated knowledge and practices by people who live in rural communities based on their needs and provides an important source of information to assist the search for new pharmaceuticals. It can be recommend that the knowledge of respondents encourage to continue for use of herbal medicinal plants for the treatment of common ailments. More comprehensive scientific explanation and research needs to be carried out to draw the complete picture of traditional use of ingenious plants of this area.

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Comprehensive Review of Maple Trees: Evolution, Biogeographical Distribution, Ecology, and Economic Significance with Emphasis on Canada

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***Abstract*:** Maple trees, iconic symbols of Canada's temperate forests, epitomize a profound evolutionary heritage, intricate biogeographical distribution, and remarkable ecological adaptations. This comprehensive review synthesizes existing research on maple trees, exploring their evolutionary origins, historical migration patterns, soil preferences, climate requirements, and adaptive strategies. Through an interdisciplinary approach, we examine the interplay of geological phenomena, environmental dynamics, and human influences that shape the distribution and diversity of maple species worldwide. Insights gleaned from this review enhance our understanding of maple trees' ecological significance and inform conservation efforts to preserve their habitats and genetic diversity amidst global environmental changes.

***Keywords*:** Maple trees, Evolutionary history, Biogeographical distribution, Ecological adaptations, Economic significance

This review aims to synthesize existing research on the evolutionary history, biogeographical distribution, ecological adaptations, and economic significance of maple trees, with a focus on Canada. Maple trees, belonging to the genus *Acer*, are iconic components of temperate forests across the Northern Hemisphere. Their evolutionary journey spans millions of years, with fossil evidence tracing their origins to the Paleogene period. Early *Aceraceae* species established themselves in the temperate forests of North America and Eurasia, setting the stage for the diverse array of maple species seen today (Manchester 1999). Geological processes such as continental drift and climatic fluctuations have been crucial in shaping the evolutionary trajectory of maples, highlighting their deep-rooted history in the Northern Hemisphere (Wolfe 1997, Manchester 1999).

Maple trees comprise over 100 species globally, with 10 natives to Canada, including sugar maple (*Acer saccharum*), black maple (*Acer nigrum*), and red maple (*Acer rubrum*) (Abrams 1998). Each species has unique characteristics and ecological roles: sugar maple is renowned for its vibrant autumn foliage and economic value (Abrams 1998), red maple is noted for its adaptability to varied soil and moisture conditions (Hutchinson and Vankat 1997), Norway maple (*Acer platanoides*) is valued for its ornamental qualities and shade tolerance (Nowak et al. 2008), and Japanese maple (*Acer palmatum*) is prized for its decorative foliage and compact size (Kozlowski and Pallardy 1997). Extensive research has examined structural features of sugar maples, such as root systems, trunk morphology, and leaf characteristics (Nave et al 2011, Vanderklein et al 2018). These trees thrive in temperate climates, adapting to diverse soil types and elevations (Peel et al 2007, Davis et al 2000). Seasonal changes and the ecological importance of Canadian sugar maples in deciduous forests are well-documented (Smith 2005, Jones and Davis 2009, Brown et al 2012, White 2017).

Despite extensive research on maple biology, gaps remain in understanding their biogeographical distribution and ecological adaptations (Millar et al 2007, Foster et al 2018). This review synthesizes existing literature on maple species' distribution patterns, soil preferences, climate requirements, and adaptive strategies. Understanding the evolutionary history, distribution, and ecology of maple trees is crucial for informed conservation and management strategies. Sustainable forest management practices, including habitat protection, restoration, and climate-resilient silviculture, are essential for preserving maple ecosystems and their benefits amidst environmental challenges. Collaboration among scientists, policymakers, and land managers is vital for integrating scientific knowledge into decision-making and ensuring the long-term sustainability and resilience of maple forests.

The primary objectives of this review are to explore the evolutionary history and biogeographical distribution of maple trees, examining how these factors have shaped their diversity and spread across different regions. It aims to investigate the ecological adaptations and interactions of maple trees within their environments, shedding light on their role in various ecosystems. Additionally, this review will analyze the economic significance of maple trees, with a particular focus on their impact on Canadian forestry and agriculture. Finally, the review will discuss the conservation implications of the findings and outline future research directions to address existing gaps and challenges in the field.

Evolutionary History and Biogeographical Distribution

Evolutionary origins: The evolutionary lineage of maple trees traces back to the ancient landscapes of the Paleogene period. Fossil evidence reveals the presence of early *Aceraceae* species in temperate forests of North America and Eurasia, indicating their deep-rooted origins in the Northern Hemisphere. There are 10 native species in Canada, including sugar maple (*Acer saccharum*), black maple (*Acer nigrum*), and red maple (*Acer rubrum*) (Abrams 1998) (Fig. 1). Geological events, including continental drift and climatic fluctuations, played pivotal roles in shaping the evolutionary trajectory of maple trees, fostering speciation and diversification over geological time scales (Wolfe 1997, Manchester 1999).

Effect of continental drift and climate change: The distribution and diversity of maple species have been significantly influenced by continental drift and climate change. The breakup of the supercontinent Pangaea during the Mesozoic era (~200 million years ago) led to the separation of landmasses into distinct continents, facilitating the migration of maple species across land bridges such as the Bering land bridge and corridors (Fig. 2). This allowed them to colonize new habitats and establish diverse populations (Scotese 2001, Sanmartín and Ronquist 2004, Tiffney 1985, Graham 1999, Maddison and Maddison 1992, Tiffney 2008). Subsequent tectonic activity, including the opening of the North Atlantic Ocean, created geographic barriers that restricted gene flow between populations (Wolfe 1997, Manos and Stanford 2001). Today, maple trees are found across North America, Europe, Asia, and parts of Africa, reflecting their adaptability to diverse climates and habitats. Contemporary factors such as climate change, habitat fragmentation, and human activity continue to shape their geographic range and genetic diversity (Rajora et al. 2016, Harmer et al. 2018).

Centers of diversity: North America and eastern Asia are key centers of diversity for maple species, reflecting their adaptability to various temperate climates. In North America, regions like the eastern U.S. and Canada host numerous species such as sugar maple (*Acer saccharum*) and red maple (*Acer rubrum*), shaped by historical geological events like the breakup of supercontinents and climatic shifts (Abrams 1998). Eastern Asia, including China, Japan, and Korea, similarly supports a rich variety of maples, such as Japanese maple (*Acer palmatum*) and Chinese maple (*Acer buergerianum*), influenced by glacial and interglacial periods. These centers of diversity illustrate how historical and environmental factors have fostered a broad array of maple species adapted to diverse habitats (Fig. 3).

Ecological Adaptations and Environmental Interactions

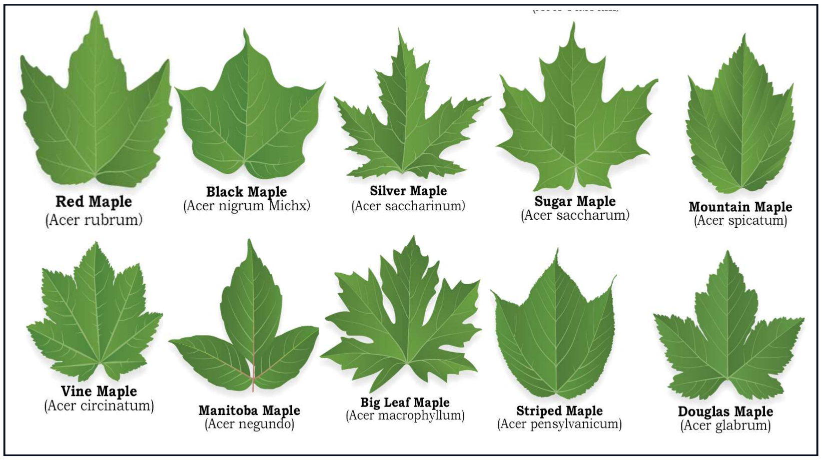
Soil preferences and growth conditions: Maple trees exhibit remarkable ecological versatility, thriving in various soil types from well-drained loamy soils to moisture-rich wetland habitats (Bradford and Johnson 1999). They are known for their adaptability to a range of soil pH levels, from slightly acidic to neutral, which further contributes to their wide distribution. Their robust root systems enable them to stabilize and enhance soil quality, which in turn supports their growth in diverse environments. This adaptability is a key factor in their ability to colonize different habitats and contribute to ecosystem stability. Brief description and habitat of sugar maple species of North America and Canada is mentioned in Table 1.

Seasonal transformations: The seasonal changes in maple trees, particularly their vibrant fall foliage, are adaptive strategies to temperate climates with distinct seasonal variations. This colorful display is not only a visual spectacle but also serves to optimize photosynthesis before the winter dormancy period (Jones and Davis 2009). Figure 4 illustrate Transition of Maple Tree Leaves from summer to fall (Prakash 2023). Additionally, the timing of leaf drop and the associated nutrient recycling play a crucial role in forest nutrient dynamics, benefiting soil health and fostering subsequent plant growth. These transformations enhance the aesthetic and ecological value of temperate forests, attracting both wildlife and human observers.

Interactions with other species: Maple trees play a crucial role in forest ecosystems, providing habitat and food for various plant and animal species. Their interactions with other species, including symbiotic relationships with mycorrhizal fungi, are essential for maintaining forest biodiversity and health (Simard and Durall 2004). For instance, the association with mycorrhizal fungi enhances nutrient uptake, particularly phosphorus, which supports the growth of both maples and surrounding vegetation. Furthermore, maples serve as key food sources for herbivores and provide shelter for various wildlife, contributing to the overall ecological balance and resilience of forest ecosystems.

Economic Significance

Maple syrup production: Maple trees, especially sugar maple (*Acer saccharum*), hold significant economic importance, particularly in North America and Canada. The maple syrup industry, primarily centered in eastern Canada, contributes millions of dollars annually to the national economy and supports rural communities. The production process involves tapping sugar maple trees during the spring



(Adopted from https://mapleleavesforever.ca/what-is-a-native-canadian-maple/)

Fig. 1. Leaves of native species of Canada Maples

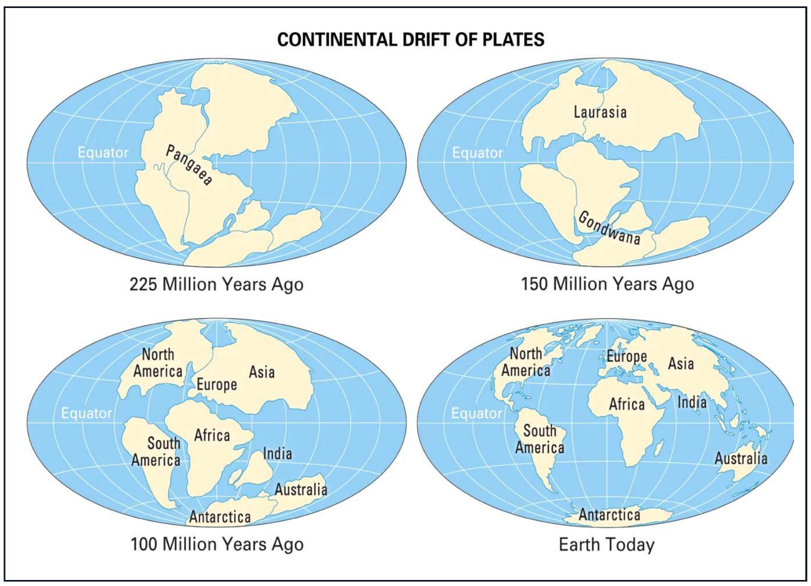


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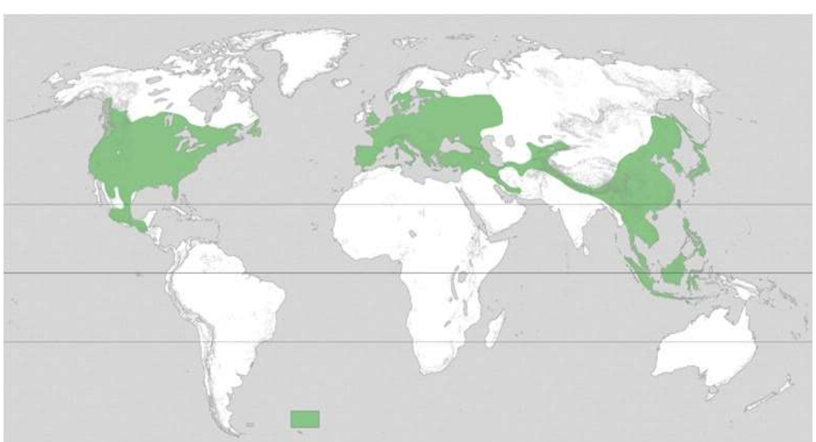
Fig. 2. Locations of continents in different periods



https://arboretum.harvard.edu/stories/model-maples

Fig. 3. Word map of Maple Trees

thaw to collect sap, which is then processed into syrup through evaporation and concentration. This traditional practice has substantial economic benefits and continues to be a vital industry in the region (Gabriel et al 2012).

Timber and wood products: Maple wood, known for its quality and versatility, is highly valued in the timber industry. Its dense, hard characteristics make it ideal for products such as furniture, flooring, cabinetry, and musical instruments, which require durability and fine grain. The wood's natural luster and ease of finishing further enhance its desirability, supporting various industries and sustainable forestry practices (USDA Forest Service 2012). Additionally, the economic value of maple timber promotes responsible forest management practices, ensuring a balance between utilization and conservation.

Horticulture and landscaping: Maple trees are popular in horticulture and landscaping due to their aesthetic appeal. Species like Japanese maple (*Acer palmatum*) and red maple (*Acer rubrum*) are cultivated for their vibrant foliage and decorative features, enhancing garden designs and urban green spaces (Nowak et al 2008). Their varying sizes, shapes, and colors make them suitable for diverse landscaping needs, from ornamental plantings to shade trees. Additionally, the adaptability of these species to different soil types and climates makes them a practical choice for enhancing both residential and commercial landscapes.

Conservation and Future Research Directions

Conservation implications: Understanding the evolutionary history, distribution patterns, and ecological dynamics of maple trees is crucial for informed conservation and management strategies. Sustainable forest management practices, including habitat protection, restoration efforts, and climate-resilient silvicultural practices, are essential for preserving maple ecosystems and their ecological and economic benefits in the face of ongoing environmental challenges. Collaboration among scientists, policymakers, and land managers is vital for integrating scientific knowledge into decision-making processes and ensuring the long-term sustainability and resilience of maple forests (Millar et al 2007, Foster et al 2018).

Knowledge gaps: Future research should address existing knowledge gaps in the study of maple trees. Critical areas include examining the long-term impacts of climate change on maple populations, such as shifts in distribution and phenological changes, as well as understanding the genetic basis of their adaptability to varying environmental conditions (Williams and Dumroese 2013). Additionally, investigating the role of maples in forest ecosystem services, such as carbon sequestration and habitat provision, can provide insights into their ecological value and inform conservation strategies.

Advancing sustainable management: Advancements in sustainable management practices are necessary to support the conservation of maple trees. Research should focus on developing strategies to mitigate the impacts of environmental stressors, such as invasive species and soil

Adapted from Prakash 2023

Fig. 4. Transition of maple tree leaves from summer to fall, Winnipeg, Canada

Table 1. Description, habitat and range of sugar maple species (*Acer saccharum*)

Sugar maple species	Description	Habitat and range
Acer saccharum	Large branching, straight-trunked tree; leaves have five pointed lobes, and turn brilliant red in the fall; flowers are tiny, bell-shaped, and long-stalked; clustered maple keys have U-shaped wings that grow slightly apart.	Common in hardwood forests in deep, well-drained soils, from Ontario to Maritimes and south in the US to Georgia and Kansas.

https://www.thecanadianencyclopedia.ca/en/article/maple degradation, on maple forests (Nave et al 2011). Additionally, efforts should be directed towards promoting the sustainable use of maple resources by integrating ecological and economic considerations into forest management practices. This includes enhancing reforestation techniques and exploring methods for reducing the ecological footprint of maple-related industries.

CONCLUSION

Maple trees, with their rich evolutionary heritage, complex biogeographical distribution, and remarkable ecological adaptations, are essential components of temperate forests and hold significant value for human societies. This comprehensive review has highlighted their evolutionary origins, historical migration patterns, soil preferences, climate requirements, and adaptive strategies. By exploring the interplay of geological processes, environmental dynamics, and human influences, we have gained a deeper understanding of the resilience and adaptability of maple trees within terrestrial ecosystems. The economic significance of maple trees, particularly in maple syrup production, timber, and horticulture, underscores their multifaceted contributions to local economies and cultural heritage. To ensure the continued availability and resilience of maple trees in the face of global changes, sustainable management and conservation efforts are crucial. Future research should focus on addressing existing knowledge gaps, such as the long-term impacts of climate change on maple populations and the genetic basis of their adaptability. Advancements in sustainable management practices, including habitat protection, restoration, and climate-resilient silviculture, are essential for preserving the ecological and economic benefits of maple trees. Collaboration among scientists, policymakers, and land managers is vital to integrating scientific knowledge into decision-making processes and ensuring the long-term sustainability of maple forests. This review offers an extensive overview of different aspects of maple trees but acknowledges the reliance on existing literature.

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Rhododendron arboreum Sm. in the Indian Himalayan region: Ecology, Uses Exploitation and Threats

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Abstract: *Rhododendron arboreum* is one of the dominant species of the Indian Himalayan region. The species is well known for its beautiful flowers and ecological importance. The flowers of *R. arboreum* is used by the local people to make juice, jam, syrup, chutney, honey, squash, etc. But, increasing demand of its products and uncontrolled collection of flowers by local inhabitants for ethno-botanical purposes and fulfillment of their basic livelihood, the species is facing threat in its natural habitat. Additionally, reduced regeneration capability due to various ecological and anthropogenic factors has led to drastic reduction in natural population of *R. arboreum* in forest. Sustainable harvesting practices and community awareness can help balance human needs with ecological preservation ensuring the survival of this vulnerable species for future generations. Present review focuses on ecological behavior of the species, its socio-economic importance, regeneration, threats and an immediate conservation measure to combat it's over exploitation.

Keywords: Indian Himalayan region, Medicinal importance, Distribution, Exploitation and Conservation

The Indian Himalayas are one of the most diverse geo-climatic zones and biodiversity rich area. It is extended for more than 2400 km in length, and shows the tremendous variation in prevalent climatic conditions from subtropical to boreal forest type (Rawal et al 2018, White et al 2019). It covers 12 states of India of India from Jammu & Kashmir, Himachal Pradesh, Uttarakhand, West Bengal, Assam, Tripura, Mizoram, Manipur, Nagaland, Meghalaya and Arunachal Pradesh. Its forest cover is vital to maintain environment and ecological balance (Wani et al 2022) and provide numerous ecological and environmental services. Indian Himalayan Region is mostly dominated with different types of *Rhododendron*s species. The word *Rhododendron* originates from a Greek words 'rhodon' (rose) and 'dendron' (tree), which means rose tree (Iqbal and Negi 2017). *Rhododendron*s are dominant and primitive group of flowering plants belonging to family Ericaceae (Singh et al 2009, Menon et al 2012). Linnaeus was the first to name the genus, *Rhododendron* (Purohit 2014). There are about 1025 species in the world (Chamberlain et al 1996), among which 87 species, 12 subspecies and 8 varieties are recorded in Indian Himalayan Region (Basnett and Ganesan 2022). In Western Himalayas 6 species of *Rhododendron* are recorded as *viz Rododendron arboreum*, *R. anthopogon, R. barbatum, R. campanulatum*, *R. lepidotum* and *R. nivale* (Sekar and Srivastava 2010). *Rhododendron*s are either evergreen or deciduous, shrubs or trees, found mainly in Asia. They are widely distributed throughout the southern high-lands of Appalachian Mountains of North America (Paul et al 2010), extending across Europe, Asia to Japan, from extreme north of the Equator (Rawat et al 2017), southern and north-eastern China, Myanmar, Thailand, Malaysia, Indonesia, Philippines and New Guinea.

Habit and Habitat

R. arboreum is one of the splendid, valuable and impressive species of genus *Rhododendron*. The name arboreum comes from the Latin word arboreum, which is "tree-like" (Orwa et al 2009, Srivastava 2012). It is an evergreen shrub or small tree with beautiful red blossoms that is also known as Burans in India and Gurans in Nepal. The species holds a high socio-cultural veneration and has been entitled as the 'National flower' of Nepal (*de* Milleville 2002, Tewari et al 2018), 'State tree' of Uttarakhand and Sikkim and State flower of Nagaland (Srivastava 2012, Gaira et al 2014, Tewari et al 2018). The species is widely distributed from Western to Eastern Himalayan region and various other neighboring countries (Giriraj et al 2008). *R. arboreum* forests of Milke Danda in the eastern Nepal are possibly the largest *Rhododendron* forest in the world (de Milleville, 2002). It strives under the canopy of oak forests such as *Quercus leucotrichophora* and *Q. floribunda* forests in the low to mid hills and *Q. semecarpifolia* forests in the high hills (Chauhan 1999). *R. arboreum* holds the Guinness World Record for the World's largest *Rhododendron* species and is well-known for its therapeutic and commercial significance.

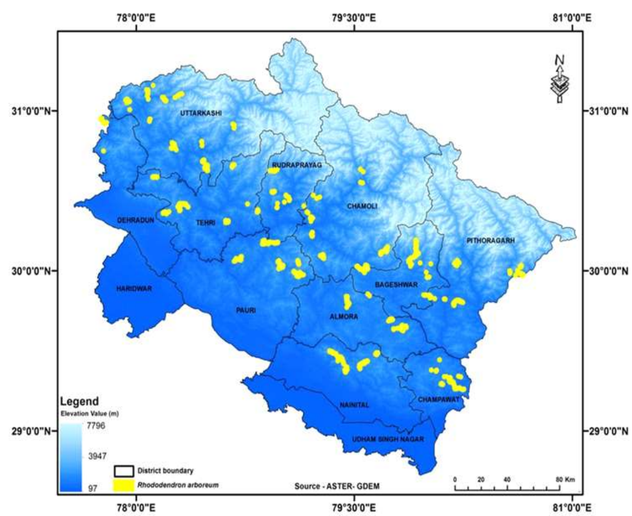
Indian Himalayan region provides an ideal habitat for *R.*

arboreum in India. The origin of discovery of the plant is north India from Kashmir to Bhutan, as well as in the hills of North-eastern states between 800-3000m amsl (Srivastava 2012). Forest type includes from subtropical and temperate to subalpine and alpine ecosystems in the range of 3000-3500m amsl (Bhattacharyya and Sanjappa 2008). The species also dwells in Bhutan, China, Myanmar, Nepal, Sri Lanka, Thailand, Pakistan and Tibet (Sekar and Srivastava 2010). *R. arboreum* has the widest elevation range in comparison to any other species in India (Naithani 1984). In Western Himalayas the species is found as an associated species of *Quercus*, *Myrica nagi*, *Neolitsea pallens*, *Alnus nepalensis*, *Viburnum mullaha*, and *Pinus roxburghii* (Negi et al 2013). This keystone species of Indian Himalayan region flourishes well in fair light, moist and acidic soils (Srivastava 2012). 12-17^{O}C of mean annual temperature and 200-1800m mean annual rainfall is favored by *R. arboreum* with well drained sandy and loamy soil along with light woodland (semi-shade) or no shade conditions (Orwa et al 2009). *R. arboreum* is distributed in 11 different district of Uttarakhand (Bhandari et al 2020, and Chauhan et al 2021) (Table 1 and Fig. 1).

Morphological Characteristics

The trunk of *R. arboreum* is often heavily branched (Fig. 2a), twisted, or gnarled (Orwa et al 2009) (Fig. 2b and c). Exfoliating in thin flakes, the bark is reddish brown, soft, and rough (Chauhan, 1999). The oblong-lanceolate leaves are 10-20 cm long and 3.6 cm broad. When young, the petiole is covered in white scales and crowded towards the ends of branches (Orwa et al 2009). It has shiny green with strongly imprinted veins from above, while the underside is cinnamon or reddish brown. *R. arboreum* has a wide range of flower colours, from deep scarlet to red with white lines, pink to white. When in full bloom, the species can have up to twenty flowers on a single truss, making it a magnificent spectacle. The vivid red variants of this *Rhododendron* are often found at lower elevations. The flowers are bright red and arranged in thick globose cymes (Chauhan 1999) (Fig. 3, 4). Filaments filiform, anthers-ovate, style-capitate, calyx-fine cleft, corolla-tube spotted funnel shaped, stamens-hypogynous decreasing, anthers-ovate (Paxton 1849). Fine lobes form the capsule-curved centre, which can be up to 3.8 cm long and 1.25 cm. Seeds are small, dark brown, compressed, thin, and linear, with an obvolute membrane (Orwa et al 2009).

Floral biology and phenology of *R. arboreum:* The genus *Rhododendron* has two distinct flowering seasons depending upon the altitudinal gradient, ranging from the month of February to April in the lower altitude and May to



Source: Bhandari et al 2020

Fig. 1. Distribution map of *R. arboreum* in Uttarakhand

Table 1. Distribution of *Rhododendron arboreum* in Uttarakhand

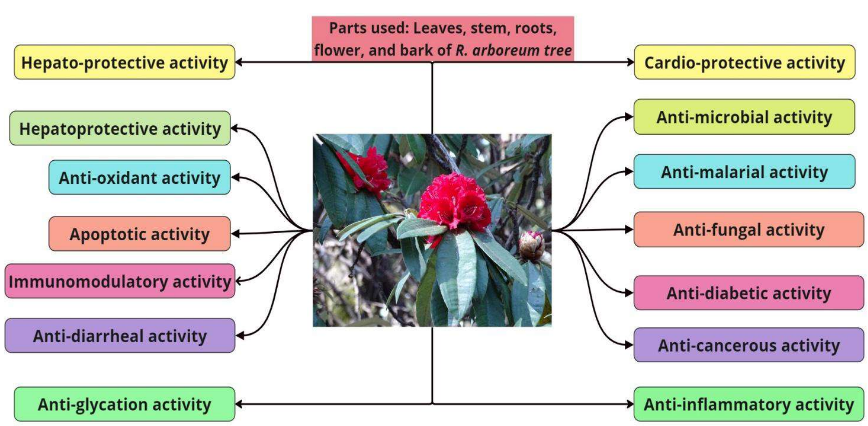
District	Places of distribution	References
Almora	Ranikhet, Manila, Kosi katarmal, Almora, Dunagiri, Binsar	Bhandari et al (2020)
Bageshwar	Dharamghar, Kaushani (Border), Laubanj, Kapkot	Bhandari et al (2020)
Chamoli	Nandasain, Nauti, Mohankhal, Gairsain, Gwaldam,	Chauhan et al (2021), Bhandari et al (2020)
Champawat	Chirapani, Siutal,	Bhandari et al (2020)
Dehradun	Mussoorie, Chakrata, Deoban, Tiuni, Nagtibba	Bhandari et al (2020)
Nainital	Kalona, Manora/ Takula, Tippen Top, Pangot, Vinayak, Bhanwali, Maheshkhan, Mukteswar	Bhandari et al (2020)
Pauri Garhwal	Phadkhal, Khirsu, Dhdhatoli, Peethsen, Chorikhal, Adwani,	Chauhan et al (2021), Bhandari et al (2020)
Pithoragarh	Munsiyari, Didihat, Pithoragarh, Kunj kharak, Sandev,	Bhandari et al (2020)
Rudraprayag	Khadpatiya, Ghimtoli, Chopta, Badhanital,	Chauhan et al (2021), Bhandari et al (2020)
Tehri Garhwal	Jhadipani, Ranichauri, Dhanauti, Chamba, Chirbatiya, Chandrabadani temple, Narendra nagar,	Chauhan et al (2021), Bhandari et al (2020)
Uttarkashi	Radi top, Dharsu, Bhokki & Sukki top, Jarmola top, Chaurangi khal, Ghes	Bhandari et al (2020)

June at higher altitudes (Ziello et al 2009). *R. arboreum* first flowering occurs in March-May while second time flowering takes place in June-July. Sometime, early flowering may also take place in January and February (Iqbal and Negi 2017) which brings the scientific attention to study the phenology and reproductive behavior of this species. *Rhododendron* inflorescence varies from three to more than twenty blooms

Fig. 2. *Rhododendron arboreum* plant parts: a) Tree, b) Flowers on branches, c) Flower

(Bhattacharyya 2011). Racemes or corymbs yield few to clusters of flowers reduced to one and in a variety of colors, typically with a contrasting throat blotch or spot (Mao et al 2001, Bhattacharyya 2011). In general *Rhododendron* flowers are bell-shaped, tubular, funnel-shaped, or saucer-shaped. Each flower has spots and blotches on it, and the inflorescence is usually a cluster of 20-25 flowers. Spots and blotches can be observed on the inner surfaces of the petals, and blotches can be seen at the base of the petals (de Milleville 2002). The hermaphrodite flowers are insect-pollinated (Orwa et al 2009). Leaves appear to be small, ranging from 10 to 20 cm on an average (Kondratovics and Kondratovics 2017). Visitors are drawn to the aesthetic splendor of fully developed flowers of *R. arboreum* throughout its blossoming time (Srivastava, 2012). During the summer (March to June), they mostly flower and provide an aesthetic look with a variety of enchanting colours of petals, including red (Mao et al 2001, Bhattacharyya 2011).

Regeneration status of *R. arboreum:* *Rhododendron* in Himalayan region is already facing serious issues of natural regeneration.Germination is a complicated process influenced by a variety of biological (species, seed viability, seed dormancy, seed size) and environmental factors (moisture availability, temperature, relative humidity, light intensity and duration) (Singh et al 2010). *Rhododendron* can be propagated through seed, stem cuttings, layering, grafting (Wells, 1985), micro-propagation (tissue culture) (Anderson, 1984), and even leaf bud cuttings (Blazich et al 1991, Hartmann et al 2002). Since *R. arboreum* is a naturally regenerating species, the forest floor is where 95% of its regeneration occurs. The forest floor is usually susceptible during the flower-harvesting season, and a great deal of regrowth is lost during this time leading to decline rates of regeneration (Chauhan et al 2021). *R. arboreum* show signs



(Srivastava 2012, Kaur et al 2023)

Fig. 3. Medicinal properties of *R. arboreum*

of J-shaped distribution where density of saplings are found to be very less than that of seedlings and adult trees. Density of the tree is found to be decreased with increasing girth (Paul et al 2019). Additionally, with the growth of seedlings, decrease in resource availability as seedlings get larger, the transition of producing tissues to structural tissues, the self-shedding of leaves, and various other related processes cause the ratio of relative growth of the species to decrease (Iqbal et al 2023). It is therefore clear that *R. arboreum* is vulnerable and is facing poor regeneration issues along with anthropogenic pressure. Thus, study of seed germination is of utmost importance for conservation.

Utilization and socio-economic values of *R. arboreum:* *R. arboreum* is mostly used by local inhabitants in the Indian Himalayan Region for ethno-botanical purposes and fulfillment of their basic livelihood (Menon et al 2012) and also used as a medication to treat diarrhoea, dysentery and dyspepsia. Consumption of dried flowers of *Rhododendron* after frying in ghee can even help in checking dysentery. Young leaves of *Rhododendron*s are very good astringent and poultice. Their fine paste can be applied to forehead to mitigate severe headaches. Coughs, diarrhoea, and dysentery are all treated with the bark's juice. The flower's petals are also consumed to aid in the removal of any animal bone that has lodged in the throat (Srivastava, 2012). The sweet and sour blooms of *R. arboreum* are used in the making of squash, jams, jellies, and local beer in hilly areas. It is a popular and enjoyable drink that is consumed once a day as a refreshing appetiser and also to prevent high altitude sickness. Chutney is made using freshly picked petals (paste). To get rid of bed lice, the leaves' juice is sprayed on cots and beds. Its wood is used in making charcoal and fuel. 'Khukri' (knife) handles, packsaddles, gift-boxes, gunstocks, and posts are all made from the grained wood of *R. arboreum* (Paul et al 2005).

Medicinal properties of *R. arboreum:* *R. arboreum* is a species of the genus *Rhododendron* that is significant for its aesthetic, medicinal, and economic qualities. The tree's bark, leaves, and flowers all have significant therapeutic benefit (Swamidasan et al 2020). Numerous secondary metabolites, including alkaloids, flavonoids, glycosides, saponins, tannins, steroids, and phlobatannins, are present in significant amounts in the plant (Srivastava 2012 and Swamidasan et al 2020). This plant possesses anti-malarial, anti-microbial, anti-oxidant, immunomodulatory, ant-diabetic, anti-inflammatory, anti-diahhreal, and anti-fungal properties in its leaf, flower, and bark extracts (Verma et al 2010, Srivastava 2012, Sonar et al 2013, Swamidasan et al 2020) (Fig. 3). The species is overexploited and is under a lot of anthropogenic strain due to its great medicinal and aesthetic properties.

Threats to *R. arboreum:* Edible flowers that are used for making syrups and beverages, and being an excellent fire wood that burns early under damped conditions due to presence of polyphenols and flavonoids make *R. arboreum* a very vulnerable species (Sekar and Srivastava 2010). This has

Table 2. Medicinal properties of *R. arboreum*

Medicinal properties of *R. arboreum*	Plant parts used	References
Anti-microbial activity	Flower, leaves, bark,	Verma et al 2011, Srivastava 2012, Saklani and Chandra 2015, Chauhan et al, 2016, Iqbal and Negi 2017, Lal et al 2017, Kashyap et al 2017 Kaur et al 2023
Anti-diabetic activity	Flower, stem, root,	Bhandary and Kawabata 2008, Srivastava 2012, Raza et al 2015, Prakssh et al 2016, Gautam and Chaudhary 2020, Kaur et al 2023
Anti-inflammatory activity	Flower, leaves, bark, roots,	Verma et al 2011, Srivastava 2012, Nisar 2016, Swamidasan et al 2020, Kaur et al 2023
Anti-malarial activity	Flower	Verma et al 2011, Srivastava 2012, Kaur et al 2023
Anti-diarrheal activity	Flower	Srivastava 2012, Prakssh et al 2016, Kaur et al 2023
Anti- fungal activity	Flower, leaves,	Nisar 2013, Srivastava 2012, Kaur et al 2023
Hepato-protective activity	Flower, leaves,	Prakash 2007, Verma et al 2011, Srivastava 2012, Kaur et al 2023
Anti-oxidant activity	Flower, leaves, bark,	Anpin et al 2010, Prakssh et al 2016, Kashyap et al 2017, Gautam et al 2020, Kaur et al 2023
Apoptogenic activity	Flower, leaves,	Kashyap et al 2017, Gautam et al 2020, Kaur et al 2023
Immunomodulatory activity	Flower, leaves,	Srivastava 2012, Rawat et al 2018, Kaur et al 2023
Anticancer activity	Flower, leaves, roots, bark, stem	Srivastava 2012,Gautam et al 2018, Gautam and Chaudhary 2020, Kaur et al 2023
Cardio-protective activity	Leaves, stem, roots	Manjunatha et al 2011, Nisar et al 2011, Srivastava 2012, Cheng et al 2017, Parcha et al 2017, Kaur et al 2023
Anti-glycation	Flower	Verma et al 2011, Raza et al 2015, Prakssh et al 2016

already led to scarcity of few species and few are at the verge of extinction. Therefore, conservation of *Rhododendron*s by means of *in situ* and *ex situ* conservation strategies is the need of hour. *R. arboreum* is a wild plant species with considerable ecological importance and its flowers have a unique therapeutic and nutritional value. The flowers are edible and are used to make a pleasant drink in the Central Himalayan mountain region. Despite its high medicinal value and enormous bioprospecting potential, the species has received little attention in the western Himalayas for conservation and management (Negi et al 2013). The Central Himalaya, notably Uttarakhand, is a major religious and tourist destination, with millions of pilgrims and visitors passing through each year (Maikhuri et al 2004), resulting in a significant market demand for the commodity. However, unemployment is a major issue in Uttarakhand right now. There are very little opportunities available for young people, both educated and uneducated in the public sector. The fear of unemployment somehow reduced significantly when few unemployed of the region immersed themselves in the preparation of high-quality wild food items (Negi et al 2011). As *R. arboreum* grows abundantly in the wild and requires no further inputs other than gathering the flowers, the total output and net return for its products are extremely high. Many businesses have linked this venture to eco-tourism and have reaped significant financial rewards by marketing their products during the peak tourist season. Collecting such wild edibles and related value added goods which have high long-term prospects as a source of income for the locals increases the risk of exploitation of the species leading to its extinction. The climate change is also showing adverse effect on *Rhododendron*s. Gaira et al (2014) investigated the effects of climate change on *R. arboreum* flowering in India's Central Himalayas. Studies from all across the world have produced evidence of the effects of climate change on phenology and species persistence. However, for the vast majority of locations and species, including the climate-sensitive Himalayan biodiversity hotspot, datasets or evidence is unavailable.

Deforestation and unsustainable extraction for firewood and incense by local people are the two biggest risks to *Rhododendron*s. If suitable conservation efforts are not taken, time is not so far for a group of rare/endangered *Rhododendron*s to be wiped out from the biota in near future (Singh et al 2003, 2009, Bhandari et al 2020). Climate change has a major impact in occurrence of *R. arboreum* position. Its habitat has been directly suffered with change in altitudes, elevations of 11m as per the evidence. Additionally, rise in temperature has also lead to early blooming of flowers and foliar damage (Ranjitkar et al 2014, Joshi et al 2024, Veera et al 2019, 2023). This shift in temperature and altitude is a major threat to survival and distribution of *R. arboreum*. Veera et al (2023) has thrown light on this elevation shift which is an issue to be highlighted as even if the elevation has rose, the species survival is in danger beyond 3500m amsl.

Aforesaid description and review elaborated the use of *Rhododendron* as one of the primitive and widely distributed plant genera in the mountains. Inspite of such multifarious uses of *Rhododendron*, the genus remained one of the most ignored groups of plants when comes to scientific enquiry. Among *Rhododendron, R. arboreum* and its varied subspecies are majorly concentrated from Western to Eastern Himalayas within the Indian Himalayan Region (IHR). About 90 per cent population of the species is found in this region. This wide distribution is possible only because of the species' endurance to tolerate extreme temperature range and to grow in diverse habitats. Combination of light and small seeds of *R. arboreum* privileges the species to strongly get disseminated by wind and animals, perhaps leading to long dispersal and wider degree of adaptation and distribution. Such pollen dispersal capability of the species shapes its spatial genetic structure and hence estimates the extent of gene flow between individuals and populations (Hahn et al 2016). The degree of gene flow can be influenced by deciding whether an individual is either out crossing or self crossing, often called as mating system patterns of the population (Whitehead et al 2018). Although distribution patterns and regeneration status of *R. arboreum* has been studied by various researchers in the past, but seed germination and survivability of the seedlings has not been studied in much detail. Moreover, mating system analysis and gene flow of the species remained untouched which will greatly help in its conservation and related breeding programme.

It is reported that natural populations of *R. arboreum* in the Himalayas are steadily dwindling due to human influences and anthropogenic disturbances associated with deforestation, unsustainable extraction, over-exploitation and agricultural practices. All these activities have collectively put pressure on *Rhododendron* forests and many of its species have now become endangered, rare, or threatened (Singh et al 2003). Due to high economic, medicinal value and high ecological significance *R. arboreum* species is overexploited by local inhabitant in the Himalayas. Therefore, urgent needs of conservation strategies are in demand to restore *Rhododendron* populations in the wild. Time is not so far when human land use patterns and climate change will put negative pressure on *R. arboreum* diversity and distribution (Sala et al 2000). Singh et al (2009) surveyed the problem of deforestation and unsustainable firewood and incense extraction by local people and focused on incorporating biotechnological and traditional ways to combat the threat to the existence of these plants. Major approach was to identify ways to conserve *Rhododendron* populations in the wild by mass propagating them *in vitro* and *ex situ* and restoring them in the wild. It was therefore concluded that thorough understanding of the protocols is required to conserve and monitor natural populations of *Rhododendron*s including *R. arboreum*. Furthermore, government has initiated strategies to conserve floral diversity of *R. arboreum* by only using 60% of the flowers from a tree which will help in sustainable management of the species for regeneration naturally as the remaining bloom will mature into seeds. However, lack of this knowledge to local communities, NTFP collectors and distributors has created an issue of over-exploitation (Negi et al 2013). Therefore, more awareness programs and capacity building involving Van Panchayats, gram sabhas and NGOs is a major necessity to educate locals.

CONCLUSION

The review highlights *Rhododendron* as a vital yet scientifically neglected genus in the mountains, particularly *R. arboreum*, which thrives in the Indian Himalayan Region (IHR). Despite its wide distribution due to adaptability and effective seed dispersal, studies on its seed germination and mating system remain limited. Human activities and climate change threaten its populations, necessitating urgent conservation strategies. Efforts should include *in vitro* and *ex situ* propagation, sustainable management, and education for local communities to prevent over-exploitation. Government initiatives and awareness programs are crucial for conserving the ecological and economic value of the species.

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Exploring the Nutritional Value of Different Tree Leaf Meal Combinations in Himachal Pradesh

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***Abstract*:** Most farmers in Himachal Pradesh use the foliage from important plant species such as *Leucaena leucocephala*, *Acacia catechu* and *Albizia chinensis* to feed their livestock. The current study was conducted at Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan to assess the nutrient composition of various combinations of tree leaf meals. In September 2020, leaf samples from three different species: *Leucaena leucocephala*, *Acacia catechu* and *Albizia chinensis* were collected, sun-dried and various combinations of tree leaf meals were prepared by blending them in varying proportions. The proximate analysis of the tree leaf combinations indicated notable differences. The contents of crude fibre (CF), neutral detergent fibre (NDF) and acid detergent fibre (ADF) were significantly higher in the combination of *L. leucocephala* (1): *A. catechu* (1): *A. chinensis* (3). Conversely, in *L. leucocephala* (3): *A. catechu* (1): *A. chinensis* (1) compositions the content of Crude protein (CP), acid insoluble ash (AIA) and phosphorus (P).were significantly higher, mainly attributed to the higher proportion of *L. leucocephala*. The nutritional value of the tree leaf meal combination which consisted of a mixture of *L. leucocephala, A. catechu* and *A. chinensis* in a ratio of 3:1:1, had the highest nutritional value. This leaf meal combination has a higher concentration of crude protein and phosphorus and a minimum concentration of crude fibre, neutral detergent fibre and acid detergent fibre.

***Keywords*:** Tree leaf meals, *L. leucocephala*, *A. catechu*, *A. chinensis*, Livestock feeding

Livestock farming constitutes a primary source of livelihood in India's rural economy. Livestock production stands as a crucial pillar of rural livelihoods, contributing substantially to the socioeconomic landscape of the nation. Approximately 20.5 million individuals in India depend on livestock for their sustenance, highlighting its significance in supporting livelihoods. Moreover, the livestock sector contributes about 16 per cent of the overall income of small agricultural households, presenting its substantial economic contribution (Dash 2017). The livestock sector holds considerable importance in generating cash revenue through the processing of various products such as milk, butter, eggs, wool and others. However, livestock production is hampered mostly by the restricted availability of green fodder, especially during the dry season. In India, there is a significant deficit in concentrated feed ingredients, amounting to 44%, green fodder 35.6% and dry fodder, with a shortage of 10.95% and the projected demand for dry fodder by 2050 is expected to reach 1012 million tonnes, while the demand for green fodder is estimated to be 631 million tonnes (IFGRI Vision, 2050). Providing enough fodder to cattle during the lean period can be quite challenging. One strategy to overcome this shortage is to utilize underutilized feed resources like tree leaves. Fodder trees serve as vital sources of both protein and energy, crucial for maintaining animal health, promoting growth rates and enhancing milk and wool production. Tree stands out as one of the most dependable sources of fodder due to their extended rotation period. They play a crucial role in producing nutrient-rich fodder, especially during lean periods and can provide green fodder with a nutritional value equivalent to that of leguminous crops (Dhillon et al 2023). Tree leaves can also be processed into tree leaf meals, offering a concentrated form of nutrition that can be effectively utilized in animal feeding. In the Northwest Himalayas, the leaves of Leucaena, Acacia and Albizia are commonly utilized as tree fodders. However, there are limited studies available on their utilization in the form of tree leaf meals. Consequently, the purpose of this research was to assess the nutritional value of various combinations of tree leaf meals for their effective utilization in animal feeding.

MATERIAL AND METHODS

Study area: The present study was conducted at Dr Y.S. Parmar University of Horticulture and Forestry, located in Nauni, Solan-173230, Himachal Pradesh, in 2020-21. The sampling site is situated at an elevation of 1275 m above mean sea level in the mid-Himalayan zone of Himachal Pradesh. It falls within the coordinates of 30°50' 30'' to 30°52' 0'' N latitude and 77°08' 30'' to 77°11' 30'' E longitude, as indicated by Survey of Indian Toposheet number 53F/1.

Methodology adopted: To prepare tree leaf meal, 15 trees from each species (*Leucaena leucocephala*, *Acacia catechu* and *Albizia chinensis*) were randomly chosen and pruned in September 2020. The leaves were then harvested from these trees and composite samples were gathered for evaluating the nutritional content of each species. The harvested foliage biomass was spread out on plastic sheets and exposed to sunlight for a period of four to five days to facilitate drying. The dry leaves were extracted from the plastic sheet on either the fifth or sixth day, placed in bags and stored in a dry and well-ventilated area, protected from direct sunlight. The leaf meal was prepared by making the different ratios of three species (*Leucaena leucocephala*, *Acacia catechu* and *Albizia chinensis*) in a total of ten different combinations. After mixing the leaf meals in various proportions, the samples were gathered for the assessment of proximate, Van Soest principles and mineral contents. The proximate principles i.e. dry matter (DM) (%), crude protein (CP) (%), ether extract (EE) (%), crude fibre (CF) (%), total ash (%), nitrogen free extract (NFE) (%) were estimated using the standard methods of AOAC (2000). The neutral detergent fibre (NDF%) and acid detergent fibre (ADF%) were determined by the standard method (Van Soest et al 1991). The ash content was assessed by incinerating samples at 560° C for 8 h in a muffle furnace. Following this, the ash from each sample underwent additional analysis for calcium and phosphorus using calorimetric and spectrophotometric procedures. The calcium (Ca) content was determined by using the flame photometer method, while phosphorus (P) content was analysed using the atomic absorption spectrophotometer method. The data was analysed by using OPSTAT statistical software, as described by Sheoran (2010).

RESULTS AND DISCUSSION

Nutritional evaluation of the species: The highest DM, EE, NFE, TA, and Ca were in *Acacia catechu*. The highest CF, NDF and ADF were in *Albizzia chinensis* and the highest CP, AIA, and P in *Leuceana leucocephala*. The lowest CP, NDF, ADF and P in *Acacia catechu*, lowest EE, NFE, TA, and AIA in *Albizzia chinensis* and the lowest DM, CF, and Ca in *Leuceana leucocephala* leaves (Table 1).

Nutritive value of different tree leaf meal combinations: The mean dry matter content of leaf meal combinations prepared from *L. leucocephala, A. catechu* and *A. chinensis* leaves in different ratios was 90.55 per cent. The DM per cent was statistically at par in all treatments. The DM decreased in the following order T10 > T4 > T1 > T7 > T5 > T3 > T9 > T6 > T2 > T8. Patra et al (2002) also observed a dry matter percentage of 90.33 per cent in leaf meal prepared from a mixture of *L. leucocephala, M. alba* and *A. indica* in a 2:1:1 ratio. Bairagi et al (2004) reported a dry matter content of 92.65 per cent in the nutritive evaluation of *L. leucocephala* leaf meal. The lower amounts of moisture required for the preservation of the leaf meal were evidenced by the larger DM values in the tree leaf meal. The higher moisture level could otherwise cause meals to deteriorate while being stored.

The mean CP content of leaf meal combinations prepared from *L. leucocephala, A. catechu* and *A. chinensis* leaves in various ratios was 16.11 per cent. The CP content in different tree leaf meals was in the following order i.e., T8 > T2 > T6 > T5 > T1 > T10 > T4 > T7 > T3 >T9, respectively. The mean CP was maximum in T8 (18.19 %) and minimum in T9 (14.48%). The highest CP content in T8 could be attributed to the higher proportions of *L. leucocephala* leaves in the leaf meal mixture. The decrease in the CP content in the treatment T9 prepared by mixing *L. leucocephala, A. catechu* and *A. chinensis* in the ratio of 1:3:1 is because the leaves of *A. catechu* contained lower CP content as compared to the other fodder tree species. Patra et al (2002) also recorded 23.13 per cent CP in the leaf meal prepared from a mixture of *L. leucocephala, Morus alba* and *Azadirachta indica* in a ratio of 2:1:1 and 15.9 per cent CP in leaf meal of *Acacia nilotica* as reported by Rubanza et al (2007).

The mean EE content of leaf meal combinations prepared from *L. leucocephala, A. catechu* and *A. chinensis* leaves in various ratios was 4.13 per cent. The mean EE content of the leaf meal sample was highest in T9 (4.52%) and lowest in T10 (3.57%). The EE content of the treatments decreased in the following order i.e. T9 > T5 > T2 > T3 > T8 > T1 > T7 > T4 > T6 > T10. Anbarasu et al (2004) reported 4.25 per cent EE content in leaf meal mixture was prepared by using *L. leucocephala, Morus alba* and *Tectona grandis* in a ratio of 2:1:1.

The crude fibre content in various leaf meal combinations ranged between 18.01 and 25.87 per cent with the highest CF in T10 (25.87%) and lowest in T8 (18.01). Adedeji et al

Table 1. Nutritional composition of fodder tree leaves used for making leaf meal

Tree species	DM (%)	CP (%)	EE (%)	CF (%)	NDF (%)	ADF (%)	NFE (%)	TA (%)	AIA (%)	Ca (%)	P (%)
Leuceana leucocephala	33.73^c	21.19^a	4.76^b	16.49^c	30.23^b	20.20^b	49.64^b	7.90^a	0.95^a	0.96^c	0.28^a
Acacia catechu	64.19^a	10.87^c	5.11^a	19.67^b	29.29^c	19.52^c	56.38^a	7.96^a	0.81^b	3.59^a	0.05^c
Albizia chinensis	47.09^b	13.56^b	2.35^c	35.25^a	39.08^a	23.44^a	43.16^c	5.66^b	0.62^c	2.07^b	0.13^b

Means with different superscripts within the column differ significantly ($p<0.05$)

(2013) and Ncube et al (2018) reported 13.85 and 13.00 per cent CF in the *L. leucocephala* and *Acacia angustissima* leaf meal, respectively. The mean NDF content of leaf meal combinations prepared from *L. leucocephala, A. catechu* and *A. chinensis* leaves in various ratios was 31.00 per cent. The highest NDF content was in T10 (33.49%) and the lowest NDF content was in T9 (29.72%). The lower NDF content in T9 is mainly due to the low NDF content in *A. catechu* leaf samples. Rubanza et al (2007) recorded 18.90 per cent NDF content in *Acacia nilotica* leaf meal and Anbarasu et al (2004) observed 29.1 per cent NDF in the leaf meal mixture prepared by mixing *L. leucocephala*, *M. alba* and *T. grandis* in a ratio of 2:1:1. The overall means of ADF content of leaf meal combination prepared from *L. leucocephala, A. catechu* and *A. chinensis* leaves in various ratios was 19.19 per cent. The highest ADF content was in T10 (20.42%) and the lowest ADF content was in T9 (18.66%). Rubanza et al (2007) and Safwat et al (2014) reported 19.7, 9.5 and 25.90 per cent ADF in the leaf meals of *L. leucocephala*, *Acacia nilotica* and *L. leucocephala*, respectively.

The mean NFE content of leaf meal combinations prepared from *L. leucocephala, A. catechu* and *A. chinensis* leaves in various ratios was 50.93 per cent. NFE content among treatments was found highest in T9 (53.23%) and the lowest content was recorded in T10 (47.98%). The findings of Barman and Rai (2003), and Hassan and Abd El-Dayem (2019) reported NFE content between 46.22 to 52.35 per cent in leucaena leaf meal.

Among treatments, the highest total ash content (7.72%) was in T9 and minimum TA content was observed in T10 (6.77%). Anbarasu et al (2004) recorded 11.9 per cent ash content in the leaf meal mixture which was prepared from *Leucaena leucocephala*, *Morus alba* and *Tectona grandis* in 2:1:1 ratio and Acacia leaf meal mixture contained 7.65 per cent ash content as reported by Hassan and Abd El-Dayem (2019). The overall means of AIA content of leaf meal combination prepared from *L. leucocephala, A. catechu* and *A. chinensis* leaves in various ratios was 0.79 per cent. Among treatments, the highest mean acid insoluble ash content (0.86%) was in T8 and minimum AIA content was in T10 (0.72%). The trend of AIA in leaf meal is as T8 > T2 > T5 > T3 ≥ T6 > T1 ≥ T9 > T7 > T4 > T10. Reddy and Elanchezhian (2008) reported that 1.94 per cent AIA was observed in subabul leaf and 0.93 per cent AIA in *Acacia auriculiformis*.

The mean Ca content of leaf meal combinations prepared from *L. leucocephala, A. catechu* and *A. chinensis* leaves in various ratios was 2.19 per cent. The highest value for Ca content was recorded for treatment T9 and lower value for was in T8. The decreasing trend for Ca is as follows T9 > T3 > T7 > T5 > T1 > T10 > T4 > T2 > T6 > T8. Patra et al (2002) reported 1.77 per cent Ca content in leaf meal prepared from the mixture of *L. leucocephala*, *M. alba* and *A. indica* in a 2:1:1 ratio.

Table 2. Nutritive value of different tree leaf meal combinations

Tree leaf meal combination	DM (%)	CP (%)	EE (%)	CF (%)	NDF (%)	ADF (%)	NFE (%)	TA (%)	AIA (%)	Ca (%)	P (%)
T1 (*L. leucocephala: A. catechu: A. chinensis* - 1:1:1)	90.87	16.12^{cdef}	4.14^{abcd}	21.70^{bc}	30.90^{cd}	19.18^{bc}	50.69^{cd}	7.35^{ab}	0.79^{bcd}	2.22^{cd}	0.14^{cde}
T2 (*L. leucocephala: A. catechu: A. chinensis* - 2:1:1)	90.22	17.26^{ab}	4.41^{ab}	19.77^{d}	30.22^{de}	18.92^{bc}	51.03^{cd}	7.53^{a}	0.83^{ab}	1.89^{e}	0.18^{ab}
T3 (*L. leucocephala: A. catechu: A. chinensis* - 1:2:1)	90.42	15.13^{fg}	4.33^{abc}	20.53^{cd}	30.20^{de}	18.78^{c}	52.41^{ab}	7.59^{a}	0.80^{bc}	2.56^{ab}	0.12^{de}
T4 (*L. leucocephala: A. catechu: A. chinensis* - 1:1:2)	90.94	15.78^{ef}	3.89^{bcd}	24.65^{a}	32.55^{ab}	19.70^{ab}	48.69^{ef}	6.99^{bc}	0.74^{de}	2.11^{d}	0.14^{cde}
T5 (*L. leucocephala: A. catechu: A. chinensis* - 2:2:1)	90.54	16.30^{bcde}	4.43^{ab}	19.67^{d}	29.77^{e}	18.71^{c}	51.89^{bc}	7.71^{a}	0.82^{ab}	2.29^{cd}	0.15^{bcd}
T6 (*L. leucocephala: A. catechu: A. chinensis* - 2:1:2)	90.26	16.86^{bcd}	3.78^{cd}	22.17^{b}	31.69^{bc}	19.45^{bc}	49.98^{de}	7.20^{abc}	0.80^{bc}	1.84^{e}	0.17^{abc}
T7 (*L. leucocephala: A. catechu: A. chinensis* - 1:2:2)	90.63	15.16^{fg}	3.99^{abcd}	22.10^{b}	31.56^{bc}	19.35^{bc}	51.53^{bc}	7.22^{abc}	0.76^{cde}	2.42^{bc}	0.12^{de}
T8 (*L. leucocephala: A. catechu: A. chinensis* - 3:1:1)	90.2	18.19^{a}	4.28^{abc}	18.01^{e}	29.90^{de}	18.73^{c}	51.84^{bc}	7.68^{a}	0.86^{a}	1.71^{e}	0.19^{a}
T9 (*L. leucocephala: A. catechu: A. chinensis* - 1:3:1)	90.31	14.48^{g}	4.52^{a}	20.07^{d}	29.72^{e}	18.66^{c}	53.23^{a}	7.72^{a}	0.79^{bcd}	2.74^{a}	0.11^{e}
T10 (*L. leucocephala: A. catechu: A. chinensis* - 1:1:3)	91.07	15.81^{ef}	3.57^{d}	25.87^{a}	33.49^{a}	20.42^{a}	47.98^{f}	6.77^{c}	0.72^{e}	2.12^{d}	0.13^{de}
Mean	90.55	16.11	4.13	21.45	31.00	19.19	50.93	7.38	0.79	2.19	0.15

Means with different superscripts within the column differ significantly ($p<0.05$)
DM (Dry matter), CP (Crude protein), EE (Ether extract), CF (Crude fibre), NDF (Neutral detergent fibre), ADF (Acid detergent fibre), NFE (Nitrogen free extract), TA (Total ash), AIA (Acid insoluble ash), Ca (Calcium) and P (Phosphorus)

Phosphorus plays a crucial role in animal nutrition as it is essential for the development of bones, teeth and nerve cells. The overall means of P content of leaf meal combination prepared from *L. leucocephala, A. catechu* and *A. chinensis* leaves in various ratios was 0.15 per cent. The highest value of P was in T8 (0.19%) and the lowest in T9 (0.11%). Abou-Elezz et al (2011), Brown et al (2016) and Ncube et al (2018) reported 0.24, 0.14 and 0.17 per cent phosphorus content in *L. leucocephala* leaf meal, *Acacia karroo* leaf meal and *Acacia angustissima* leaf meal, respectively.

CONCLUSION

The nutritive value of the tree leaf meal prepared by mixing *L. leucocephala, A. catechu* and *A. chinensis* in the proportion of 3:1:1 was considered as the better tree leaf meal characterized by higher CP and P content with minimum CF, NDF and ADF content. The surplus tree leaves in the monsoon season can be efficiently conserved in the form of tree leaf meal and can be incorporated as a concentrate during the lean period. However, to assess the impact of tree leaf meal on animal health and production, additional animal trials are needed to be carried out.

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Effect of Processing on Nutrient Content of Karanda (*Carissa carandas*) Fruit at different Stages of Maturity

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Abstract: The rural India's nutritionally rich fruit Karanda (*Carissa carandas*) is a versatile but under-exploited plant found throughout the dry regions of India. This study was aimed to evaluate the effect of blanching and gamma irradiation at 0.25, 0.5 and 1.00 kGy on nutritional composition of karanda fruit at three maturity stages (raw, ripe and dried). Ripe karanda fruits reported high moisture content and significant reduction of moisture content was from raw to dried fruit. The carbohydrate and fat content were highest in dried fruits. There was a significant decrease from raw to ripe. The dried karanda fruits had highest protein content, crude fiber, ash, ascorbic acid and β- carotene followed ripe and raw. The moisture content was highest in ripe fruits at 0.25kGy followed by 1.0kGy. The carbohydrate content increased significantly from raw to dried stage in fresh blanched and irradiated fruits. The protein content also increased with the advancement of maturity stage i.e. highest at dried stage. Blanched dried karanda fruit had highest fat content. Significant difference was not observed in the fibre content of raw karanda fruit in blanching. Highest fibre content was in irradiated raw karanda fruit at 0.5 kGy. Significantly higher ash content were observed in blanched fruit. Processing treatments had no impact on ascorbic content in ripe fruit. During processing, the β- carotene content decreased significantly, except in raw fruit where, blanching and irradiation at 0.25kGy has higher content.

Keywords: Karanda Fruit, Blanching, Maturity stages, Nutrient content

Tropical fruits, which are at present underutilized, have an important role to play in satisfying the demand for nutritious, delicately flavoured and attractive natural foods of high therapeutic value. The tendency is to avoid chemicals and synthetic foods and preference for nutrition through natural resources. The underutilized fruits like karonda, Indian gooseberry, Aegle marmelos, *Malabar plum*, passion fruit, phalsa, pomegranate, pumpkin, tamarind, wood apple etc. are the main sources of livelihood for the poor and play an important role in overcoming the problem of malnutrition (Gajanana et al 2010). *Carissa carandas* commonly known as karanda belongs to family Apocynaceae and c*arandas* is large dichotomously branched evergreen shrub with short stem and strong thorn in pairs. This species is a rank-growing, straggly, woody, climbing shrub, usually growing to 10 or 15 ft (3–5m) high, sometimes ascending to the tops of tall trees. The plant is native and common throughout India (Kirtikar and Basu 2003).

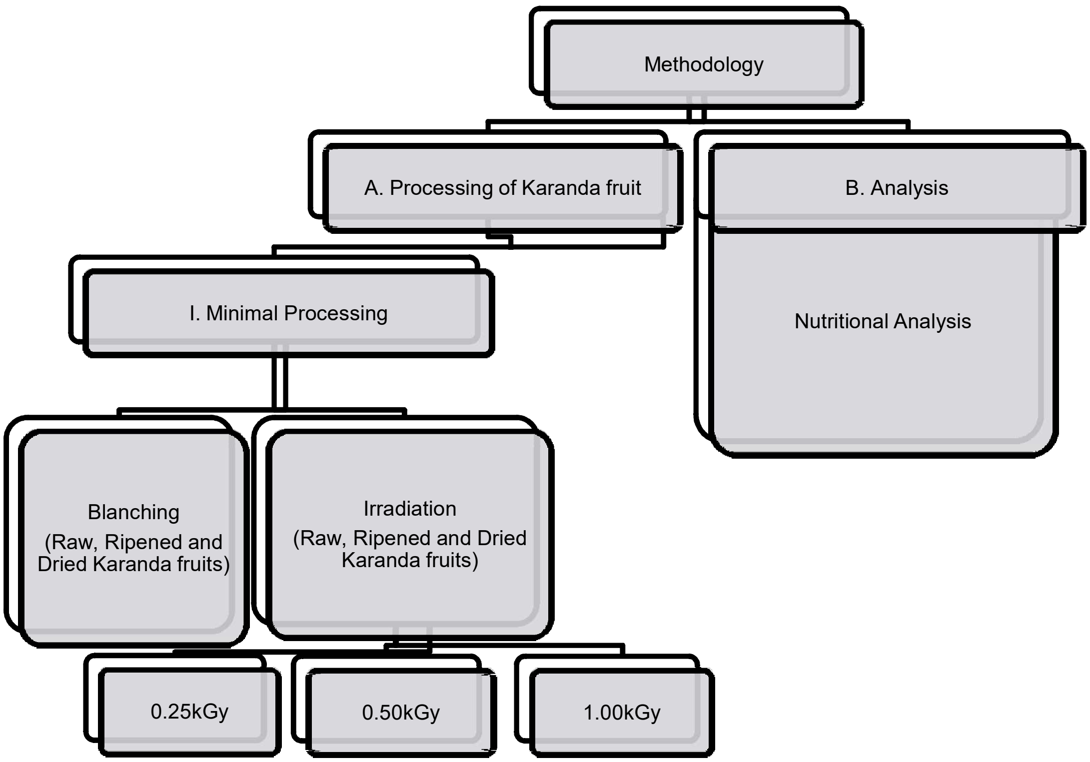
MATERIAL AND METHODS

Karanda fruits were procured from Agriculture Research Institute, Rajendranagar and irradiation was done at Gama Radiation Unit, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad. Moisture, protein, carbohydrate, fat and ascorbic acid (AOAC 2000) and crude fibre (AOAC 2005) were analysed. β - Carotene content was estimated by the method given by Srivastava & Kumar (2003).

RESULTS AND DISCUSSION

Moisture: The moisture content of the karanda fruit was estimated in various stages of maturity of the fruit from raw, ripe and dried stage to study the variation in moisture content. The highest moisture was in ripe fruits (84.76 %). Significant reduction of the moisture content from raw (81.07 %) to dried stage (10.00 %) was observed. Patel and Rao (2013) observed similar trend in the moisture content of *C. carandas L*. fruit which was higher in young stage and a gradual decrease slightly from mature to ripened stage (79.96, 76.63 and 73.23%).

The moisture was also estimated in different minimal processing i.e. in blanching and irradiation at 0.25 kGy, 0.5 kGy and 1.0 kGy. There was a significant increase in moisture content in blanching in raw (83.25%) and ripe (85.20%) stages and reduction in the dried stage (9.54 %). During irradiation process the moisture content was highest in ripe stage at 0.25kGy (85.7%) followed by 1.0kGy (85.25%). In raw karanda fruit the moisture content was higher in irradiation at 0.5kGy followed by 1.00kGy and at 0.25kGy. In contrast in the dried karanda fruit the moisture content was significantly lower in all the treatments: blanching (9.54%) and irradiation (9.22 to 9.54 %) than fresh dried fruit



(10.00%). The moisture content was lowest in irradiation at a dose of 0.5kGy (8.71 %). The reduction in the moisture content on different stages of maturity was also observed by Gopalan et al (2009) in fruits and vegetables and Vendramini and Trugo (2000) in acerola fruit at three different maturity stages.

Carbohydrates: The carbohydrate content was found higher in dried fruits compared to raw and ripe fruits could be attributed to lesser amount of moisture which exhibits the concentration of total solids of the fruit. The carbohydrate content of all the three maturity stages of karanda fruit increased significantly from raw (1.88 g) to dried (61.32 g) stage. The similar trend was also observed in blanching and irradiation at different stages. Anand and Deborah (2016) on nutritional value of a selected fifteen wild edible fruits collected from Boda and Kolli hills of Tamil Nadu reported that the carbohydrate content of 50.41 and 55.8% in *C, carandas* and *C, spinarum* respectively. When the carbohydrate content was compared in different processing in raw and ripe fruit with irradiation at 0.25kGy recorded highest carbohydrate content of 1.92 and 2.06, respectively. Blanched dried fruit recorded highest carbohydrate content of 62.59 g.

Protein: The protein content also increased with the advancement of maturity stage i.e. highest at dried stage, but there was no significant difference between raw and ripe fruits in fresh, blanching and irradiation treatments. Patel and Rao (2013) reported that the proteins in the fruit of *C, carandas* increased from its young stage (0.28 g) until the fruit ripens (3.37 g).

In the dried fruits, significant difference was observed between fresh, irradiation at 0.25kGy and 1.00 kGy. The protein content during blanching (9.58g) and irradiation at 0.5 kGy (9.38g) of dried fruit showed significantly lower than unprocessed (9.78 g), irradiation at 0.25 kGy (9.88g) and 1.00 kGy (9.94 g). The processing treatments had no influence on protein content of the fruit. Deep red stage of maturity showed highest amount of protein and significant change in later maturity stage due to transition in protein biosynthesis (Opara et al 2011).

Fat: In fresh stage, dried karanda fruit had highest fat content (5.24 g) followed by ripe and raw fruit. Among the maturity stages and processing, blanched dried karanda fruit had highest fat content (5.50 g) followed by dried irradiated fruit at 1.00kGy with significant difference. However, fresh dried (5.24 g) and in irradiated fruit at 0.5kGy (5.29 g) showed no significant difference. Lowest fat content was in dried irradiated fruit at 0.25kGy (5.12 g). Both the fresh raw and ripe fruits recorded highest fat content (2.23 g, 2.31 g respectively), followed by irradiation at 0.25kGy and 0.5kGy with no significant difference, followed by blanching (2.11 g in raw, 2.10 g in ripe) and irradiation at 1.00kGy (1.98 g in raw and 1.90g in ripe fruit). The increase of fat content during maturity and processing can be explained as accumulation of fat in maturity stage, yields from cuticle and suberin in maturity. Reduced fatty acid yield in blanching due to fatty alcohols (Lenucci et al 2006).

Crude fiber: The crude fibre content increased significantly with the maturity of the fruit i.e. dried fruit with highest fiber content followed by ripe and raw fruit. Same trend was also observed in the processed fruit. In dried fruit the irradiation treatment at 1.00 kGy showed highest fibre content followed by blanching and irradiation at 0.25 kGy with no significant difference. This was followed by irradiated fruit at 0.5 kGy and fresh unprocessed dried karanda fruit which was significant. The ripe fruit irradiated at 0.5 kGy recorded highest fiber content (2.13 g) followed by irradiated fruit at 0.25kGy then blanched (1.85 g) and irradiated fruit at 1.00kGy (1.83 g) with no significant difference. The lowest fibre content was in unprocessed fruit (1.80 g). Significant difference was not observed in the fibre content of raw karanda fruit in blanching (1.82 g), irradiation at 0.25kGy and at 1.00 kGy. Highest fibre

Table 1. Proximate analysis of the karanda fruit under different treatments (per 100g)

Parameters	Stage of ripening	Fresh	Blanching	Irradiation			CD value
				0.25 kGy	0.5 kGy	1.0 kGy	
Moisture (%)	Raw	81.070.06^{b2}	83.250.91^{b1}	81.120.02^{b2}	81.570.11^{b2}	81.170.02^{b2}	0.06
	Ripe	84.76 0.03^{a3}	85.200.01^{a2}	85.770.02^{a1}	84.440.05^{a4}	85.250.02^{a2}	
	Dried	10.000.10 ^{1c}	9.54±0.05 ^{c2}	9.570.01^{2c}	8.710.05^{c4}	9.220.05^{c3}	
CD value				0.04			
Carbohydrates (g)	Raw	1.88±0.01 ^{b2}	1.72±0.01 ^{c4}	1.92±0.02 ^{c1}	1.77±0.01 ^{c3}	1.77±0.02 ^{c3}	0.03
	Ripe	1.89±0.01 ^{b2}	1.87±0.02 ^{b2}	2.06±0.06 ^{b1}	1.82±0.01 ^{b3}	1.87±0.01 ^{b2}	
	Dried	61.32±0.05^{a3}	62.59±0.01^{a1}	61.01±0.02^{a4}	62.09±0.09 ^{a2}	62.06±0.02 ^{a2}	
CD value				0.01			
Protein (g)	Raw	1.59±0.04 ^{b1}	1.61±0.01 ^{b1}	1.60±0.02 ^{c1}	1.70±0.02^{b1}	1.71±0.02 ^{b1}	0.12
	Ripe	1.61±0.02 ^{b1}	1.59±0.01 ^{b1}	1.80±0.02 ^{b1}	1.74±0.05^{b1}	1.66±0.01 ^{b1}	
	Dried	9.78±0.08 ^{a1}	9.58±0.49 ^{a2}	9.88±0.04 ^{a1}	9.38±0.04^{a3}	9.94±0.08 ^{a1}	
CD value				0.07			
Fat (g)	Raw	2.23±0.03 ^{c1}	2.11±0.08 ^{b3}	2.15±0.01 ^{b2}	2.14±0.09^{b2}	1.98±0.02 ^{b4}	0.05
	Ripe	2.31±0.06 ^{b1}	2.10±0.07 ^{b3}	2.16±0.12 ^{b2}	2.16±0.04^{b2}	1.90±0.08 ^{c4}	
	Dried	5.24±0.05 ^{a3}	5.50±0.02 ^{a1}	5.12±0.03 ^{a4}	5.29±0.01^{a3}	5.42±0.01 ^{a2}	
CD value				0.03			
Crude fiber (g)	Raw	1.75±0.01 ^{c3}	1.82±0.01 ^{c2}	1.82±0.03 ^{c2}	1.96±0.02^{c1}	1.81±0.02 ^{c2}	0.02
	Ripe	1.80±0.02 ^{b4}	1.85±0.02 ^{b3}	1.91±0.01 ^{b2}	2.13±0.01^{b1}	1.83±0.02 ^{b3}	
	Dried	6.95±0.05 ^{a4}	7.06±0.01 ^{a2}	7.04±0.01 ^{a2}	7.01±0.02^{a3}	7.18±0.05 ^{a1}	
CD value				0.01			
Ash (%)	Raw	2.95±0.03 ^{c3}	2.85±0.02 ^{c4}	2.93±0.04 ^{c3}	3.03±0.01^{c2}	3.06±0.01 ^{c1}	0.02
	Ripe	3.18±0.01 ^{b1}	3.07±0.01 ^{b4}	3.02±0.01 ^{b5}	3.11±0.01^{b3}	3.15±0.02 ^{b2}	
	Dried	3.76±0.04 ^{a3}	3.99±0.02 ^{a1}	3.76±0.01 ^{a3}	3.76±0.01^{a3}	3.88±0.02 ^{a2}	
CD value				0.01			
Ascorbic acid (mg)	Raw	9.92±0.06 ^{c2}	9.61±0.45 ^{c1}	10.04±0.11^{c1}	9.54±0.44 ^{c2}	9.96±0.06 ^{c1}	0.22
	Ripe	10.84±0.16^{b1}	11.02±0.09^{b1}	11.02±0.24^{b1}	10.87±0.30 ^{b1}	10.89±0.18 ^{b1}	
	Dried	9.17±0.06^{a3}	9.48±0.22^{a1}	9.00±0.33^{a2}	9.05±0.03 ^{a2}	9.93±0.16 ^{a1}	
CD value				0.13			
β - carotene (µg)	Raw	9.73±0.39 ^{c3}	10.83±0.23^{c2}	11.64±0.39^{c1}	8.62±0.17 ^{c5}	9.16±0.31 ^{c4}	0.22
	Ripe	15.65±0.08^{b1}	14.39±0.18^{b2}	15.53±0.33^{b1}	14.09±0.07 ^{b3}	13.49±0.23 ^{b4}	
	Dried	16.33±0.12^{a2}	16.59±0.14^{a1}	16.62±0.07^{a1}	15.28±0.10 ^{a3}	14.54±0.34 ^{a4}	
CD (p=0.05)				0.13			

content was reported in irradiated raw karanda fruit at 0.5 kGy (1.96 g) and lowest in unprocessed raw fruit (1.75 g). Guerrero and Fuentes (2009) found that breaker stage of tomato (cherry pera variety) had higher fibre content.

Ash: The analysis of ash content in different maturity stages and processing showed significant increase in three maturity stages i.e. from raw (2.95 %) to ripe (3.18 %) and dried karanda fruit (3.76 %). Similar trend was also observed in blanching and irradiation at different dosages. The highest ash content during processing and unprocessed conditions was recorded in dried karanda. Significantly higher values were reported in blanched fruit (3.99 %) followed by irradiated fruit at 1.00kGy. Significant difference was not observed in the ash content of irradiated fruit at 0.25 and 0.5kGy when compared with raw and unprocessed fruit.

Ascorbic acid: The ascorbic acid content ranged from 9.61 to 10.84 mg in different maturity stages studied. There was a significant increase in ascorbic acid content from raw to maturity stage and the same trend was also found in blanched and irradiated fruits. The ascorbic acid content in raw fruit when subjected to blanching and irradiation showed significant difference between raw unprocessed (9.92 mg) and irradiated fruit at 0.5kGy (9.54 mg). In the blanched and irradiated fruits at 0.25 and 1.00kGy no significant difference was observed. The processing treatments had no impact on ascorbic content in ripe fruit, as there was no significant difference among the treatments. In dried karanda fruit, the ascorbic acid content was maximum in irradiation at 1.00kGy 9.93 mg followed by blanching and had no significant difference in the treatments between irradiation at 0.25 and 0.5kGy. The blanching and irradiation at 1.00 kGy showed increase in ascorbic acid content whereas the irradiation at 0.25 and 0.5 kGy showed minimal decrease in ascorbic acid compare to unprocessed dried fruit. But in other two maturity stages *i.e.* raw and ripe karanda fruits, the treatments had no significant impact on the ascorbic acid content. Among the maturity stages ascorbic acid was increased from raw (9.92 mg) to ripe stage (10.84 mg) and decline in dried stage (9.17 mg). The reduction in ascorbic acid with maturity may be due to oxidative destruction by enzymes, mainly ascorbic acid oxidase or due to conversion in acid to sugar (Rahman et al., 2010). Atefeh et al., (2013), observed that several factors including: cultivar, row spacing and different stages of maturity can influence ascorbic acid, soluble solids, *β*-carotene and lycopene in tomato fruits.

β-Carotene: The β-carotene content was highest in dried karanda fruit (16.33µg) compared to raw (9.73 µg) and ripe stages (15.65 µg) and the difference was significant in unprocessed fruit. The increase in β - carotene during maturity may be due to conversion of chlorophyll to carotenoids. During processing in three maturity stages, the β- carotene content decreased significantly, except in raw fruit where, blanching (10.83µg) and irradiation at 0.25kGy (11.64 µg) has higher content than the unprocessed raw fruit (19.73 µg). Guarte et al (2005) reported that blanching at 80°C will inactivate carotenoid oxidizing enzymes without showing significant carotene degradation. In ripe fruit the β carotene content was significantly higher in unprocessed (15.65 µg) > irradiated at 0.25kGy > blanched > irradiated at 0.5kGy > and 1.00kGy. In dried fruit, blanching (16.59 µg) and irradiation at 0.25kGy (16.62 µg) showed higher content of β carotene than unprocessed dried fruit (16.33 µg). However, irradiation at 0.5kGy and 1.00kGy showed significantly lower β- carotene when compared with the unprocessed dried fruit. This trend can be attributed to the degradation of carotenoids during processing. The most of the wild fruits were comparable to cultivated fruits in nutritive value and were suggested for commercial cultivation.

CONCLUSION

The karanda (*Carissa carandas*) is important traditional and underutilized fruit crop for arid and semi-arid regions in tropical and sub-tropical regions. It is known for its ability to withstand adverse conditions. It is good source of vitamin C and rich in carotenes. When the fruit was subjected to blanching and irradiation treatments at raw, ripe and dried stages the carbohydrate, fat and ash content was found highest in dried blanched fruits, protein, crude fibre and ascorbic acid in dried irradiated fruit at 1.00kGy, and β-carotene in dried irradiated fruit at 0.25 kGy.

AUTHOR's CONTRIBUTION

Supraja. T conducted the experiment, did statistical analysis and wrote the first draft of manuscript. K. Uma Maheswari, K. Uma Devi, M. Prasuna and D. Srinivasa Chary guided throughout the study, helped in designing the work, proof reading and critical correction of manuscript. Sujatha. M assisted in analysis and writing the drafts.

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Evaluation of Different Sowing Methods and Varieties on Performance and Agro-Meteorological Indices of Wheat

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Abstract: The field experiment was conducted at Punjab Agricultural University, Ludhiana during the *rabi* season of 2022-23 to study the effect of different sowing methods on phenological development, agro-meteorological indices and wheat yield. Two wheat varieties *viz.* PBW-725 and PBW-869 were sown under three different sowing methods *viz.* conventional sowing, happy seeder sowing and super seeder sowing. Different phenological stages of wheat crop were recorded by visual observations. Crop sown with happy seeder required more number of days to progress through various growth stages (emergence to maturity) followed by those sown with the super seeder and conventional method. The happy seeder sown crop exhibited higher helio-thermal use efficiency, photo-thermal use efficiency, heat use efficiency and radiation use efficiency compared to crop sown with super seeder and conventional methods which contributed to more yield under happy seeder sowing. Linear relationships were established between various agro-meteorological indices and grain yield. The analysis revealed a positive correlation of growing degree days, helio-thermal units, photo-thermal units, and heat use efficiency with grain yield, indicating that increases in these agro-meteorological indices are associated with higher grain yields.

Keywords: Agro-meteorological indices, Microclimate, conventional sowing, Happy seeder sowing, Super seeder sowing

Wheat is widely adapted crop, thriving under diverse conditions ranging from temperate irrigated to dry and high rainfall areas, as well as warm humid to cold dry environments. Cultivating wheat following a rice crop presents a valuable opportunity to enhance productivity and effectively handle rice stubble management. The rice-wheat cropping system is widely adopted globally, particularly in Asia, covering an extensive area of 240 million hectares (Nawaz et al 2019). Asia contributes 826 million tons (Mt) to the total global rice residue production which is difficult to manage leading to challenges like residue burning, rising greenhouse gas emissions, soil health degradation, declining productivity and decreasing groundwater levels (Goswami et al 2019). In India, it covers an area of about 31.6 million hectares with a production of 106.41 million tonnes. The three most important wheat-growing states are Uttar Pradesh, Punjab and Haryana making up about 60% of the nation's total area and the issue of rice residue burning is particularly severe in these regions (Anonymous 2022). The combustion of crop residues leads to the emission of black carbon, a significant contributor to the warming of the lower atmosphere (Chaudhary et al 2021). Hence, retaining crop residues is crucial as it mitigates the release of black carbon, thereby providing environmental benefits and promoting the well-being of wheat crop. Currently, lot of efforts are going into the cultivation of wheat in retained residues of rice crop in the field. This approach aims to address the challenges arising from the uncontrolled burning of paddy residues, particularly in the North-Western region of India which involves the sowing of wheat under resource conservation methods (happy seeder and super seeder). These resource conservation methods have many benefits to the crop as the addition of residues to the soil provides vital nutrients that aid in the growth of crops. In particular, it supplies roughly 31-42 kg N/ha (nitrogen), 8 kg P/ha (phosphorus), 34-61 kg K/ha (potassium), and 2.1-2.2 tonnes C/ha (carbon) every crop cycle (Hung et al 2019). Rani et al (2019) stated that the adoption of resource conservation practices is considered as a helpful tool for improving soil properties and mitigating the adverse effects of climatic changes.

Wheat is heat sensitive crop. Grain production in India was considerably reduced (25% in Punjab) by heat stress, which was exhibited by the occurrence of much higher than average temperatures for 15-25 days during the reproductive phase of wheat (Bal et al 2022). Hence, to eliminate the effects of higher temperature, there is a need for sowing the wheat under resource conservation methods as these methods influence the heat and water balance of the soil during the growing season (Sidhu et al 2020). Crop growth and yield are closely linked to temperature-based agro-meteorological indices like growing degree days (GDDs), helio-thermal units (HTUs), and photo-thermal units (PTUs). These indices, along with thermal efficiencies, are essential for understanding phenology and yield. Accumulated GDD can estimate the timing of different crop developmental stages (Sidhu et al 2020). While extensive research exists on these indices for conventionally sown wheat crop, there is limited knowledge for crops sown with happy seeder and super seeder. Evaluating these methods can help to identify the practices to enhance heat units and manage the impacts of climate change on crop growth and productivity. Keeping this in view, the present study was planned to evaluate the agro-meteorological indices of wheat sown with happy seeder and super seeder (residues retained and incorporated).

MATERIAL AND METHODS

Experimental details: The field trial was conducted at Punjab Agricultural University, Ludhiana. It is situated at latitude of 30°54'N, longitude of 75°48'E and at an altitude of 247 m above mean sea level. Two wheat varieties (PBW-725 and PBW-869) were sown under three different sowing methods (conventional sowing, happy seeder sowing and super seeder sowing) during the *rabi* season of 2022-23. The experiment was laid out in a strip plot design with three replications. During the experiment, phenological stages such as tillering, booting, flowering, milk, dough, and physiological maturity were observed visually. Growing degree days, helio-thermal units and photo-thermal units were computed for the crop at various phenological stages. The helio-thermal use efficiency, photo-thermal use efficiency, heat use efficiency and radiation use efficiency were computed as for biomass and grain yield.

Computation of Agro-Meteorological Indices

Growing degree days (^{o}C day): Growing degree days are used to predict plant growth and development during the growing season. Growing degree days (GDDs) were computed from complete emergence to physiological maturity, revealing an increasing GDD requirement throughout this period. GDDs were calculated (Nuttonson 1955):

$$GDD\ (^{o}C\ day) = \sum (T_{max} + T_{min})/2 - T_b$$

Where; T_{max} = Maximum temperature (^{o}C), T_{min} = Minimum temperature (^{o}C)

T_b = Base temperature (5^{o}C for wheat crop) (Slafer 1995)

Helio-thermal units (^{o}C day hour): It is the product of GDD and actual sunshine hours for a given day (Rajput 1980).

$$HTU\ (^{o}C\ day\ hour) = \sum (GDD \times \text{actual bright sunshine hours})$$

Photo-thermal units (^{o}C day hour): Photo-thermal units are represented by the product of GDD and the day length of that particular day (Rajput 1980).

$$PTU\ (^{o}C\ day\ hour) = \sum (GDD \times \text{day length})$$

Helio-thermal use efficiency (kg/ha/^{o}C/day hour): The quantity of dry matter or grain yield produced per unit of accumulated helio-thermal units is known as helio-thermal use efficiency (Dar et al 2018):

$$\text{Helio-thermal use efficiency (kg/ha/°C/day hour)} = \frac{\text{Grain or dry matter yield}}{\text{Accumulated helio-thermal units}}$$

Photo thermal use efficiency (kg/ha/^{o}C/day hour): It is the ratio of grain yield to photo thermal units (PTU). It was computed by dividing the grain yield or total dry matter by the total photo thermal units (Major et al 1975):

$$\text{PTUE (kg/ha/°C/day hour)} = \frac{\text{Grain or dry matter yield}}{\text{Accumulated photo-thermal units}}$$

Heat use efficiency (kg/ha/^{o}C/days): It denotes the amount of dry matter produced per growing degree day and is calculated (Sastry et al 1985):

$$\text{HUE (kg/ha/°C/days)} = \frac{\text{Grain or dry matter yield}}{\text{Accumulated growing degree days}}$$

Radiation use efficiency (kg/ha/MJ): Radiation use efficiency (RUE) is determined by biomass accumulation and the canopy's ability to intercept photosynthetically active radiation (IPAR) (Monteith 1977):

$$\text{RUE (kg/ha/MJ)} = \frac{\text{Grain or dry matter yield}}{\text{Accumulated intercepted PAR}}$$

RESULTS AND DISCUSSION

Crop phenology: Different residue management practices affect the phenology of wheat sown with happy seeder, super seeder and conventional method. The temporal progression to physiological maturity varied among wheat crop sown using different techniques. Notably, crop sown with the happy seeder exhibited an extended period to attain physiological maturity, while the conventional sown crop exhibited the shortest duration. Specifically, for variety PBW-869, the physiological maturity phase required 161 days when sown with happy seeder, 157 days with super seeder and 154 days with conventional sowing. Similarly, variety PBW-725 took 158 days with happy seeder, 155 days with super seeder and 150 days with conventional sowing to reach physiological maturity (Table 1). The observed prolongation in the time required for physiological maturity in crop sown with the happy seeder and super seeder methods can be attributed to an elongated vegetative growth phase as compared to conventional sowing. Sidhu et al (2020) documented an extended duration for wheat crop sown with happy seeder method to attain physiological maturity in comparison to those sown conventionally. Singh et al (2023) also reported an increased temporal requirement for physiological maturity in wheat crop sown with the happy seeder method compared to conventional sowing.

Growing degree days (GDDs): Wheat sown with the happy seeder exhibited the highest accumulation of growing degree days (GDDs) at various phenophases. Specifically, variety PBW-869 demonstrated the highest GDD accumulation (1971.8°C days), followed by variety PBW-725 (1902.2°C days) when sown with happy seeder. In contrast, GDD accumulated by super seeder sown varieties PBW-869 and PBW-725 were 1861.6 and 1842.8°C days, respectively. The increased time taken by crops sown with the happy seeder to reach physiological maturity implies that these crops experience more days with temperatures conducive to GDD accumulation, contributing to the observed higher GDD values. Under conventional sowing, variety PBW-869 and variety PBW-725 accumulated 1825.1 and 1756.7°C days of GDD, respectively, which are lower as compared to happy seeder and super seeder (Table 2). Kaur et al (2016) also reported a positive relation between the accumulated growing degree days and the time taken to reach maturity in wheat varieties. The varieties with an extended duration to maturity, as observed by Kaur (2022), demonstrated higher GDD accumulation.

Helio-thermal units (HTUs): The highest accumulation of HTU (°C day hours) occurred in wheat sown with the happy seeder (Table 3). This increased accumulation of HTU can be attributed to extended duration which exposes the crop to a greater cumulative amount of heat, contributing to the higher

Table 1. Phenological behaviour of wheat varieties under different methods of sowing during *rabi* 2022-23

Phenological stages	Conventional		Happy seeder		Super seeder	
	PBW 725	PBW 869	PBW 725	PBW 869	PBW 725	PBW 869
Complete emergence	6	7	8	9	7	8
CRI	19	20	22	23	19	21
Maximum tillering	41	43	47	49	43	45
Jointing (Start)	56	58	65	68	59	62
Flag leaf initiation	70	71	79	83	73	77
Booting (Start)	81	84	92	96	85	89
Heading (Start)	95	97	106	111	99	103
Anthesis (Start)	102	104	114	120	107	110
Milking (Start)	113	115	125	130	117	121
Soft dough (Start)	123	126	136	141	129	132
Hard dough (Start)	137	140	146	151	142	145
Physiological maturity	150	154	158	161	155	157

Table 2. Effect of sowing methods and varieties on accumulated growing degree days (°C day hour) of wheat under different irrigation treatments during *rabi (*2022-23)

Phenological stages	Conventional		Happy seeder		Super seeder	
	PBW 725	PBW 869	PBW 725	PBW 869	PBW 725	PBW 869
Complete emergence	125.1	139.0	153.9	167.2	139.0	153.9
CRI	297.3	309.1	334.3	345.9	297.3	322.3
Maximum tillering	547.9	582.4	598.4	609.6	566.2	598.4
Jointing (Start)	658.7	665.2	694.7	710.4	671.4	682.0
Flag leaf (Start)	727.0	734.5	786.2	821.7	747.8	770.1
Booting (Start)	803.1	813.4	903.9	947.4	837.4	873.3
Heading (Start)	947.4	957.7	1068.0	1143.8	984.5	1022.9
Anthesis (Start)	1010.8	1037.4	1186.7	1278.5	1083.5	1130.4
Milking (Start)	1186.7	1202.9	1356.9	1443.3	1231.5	1263.8
Soft dough (Start)	1324.4	1373.4	1539.8	1616.0	1425.7	1481.6
Hard dough (Start)	1553.9	1616.0	1698.6	1773.2	1630.8	1679.3
Physiological maturity	1756.7	1825.1	1902.2	1971.8	1842.8	1861.6

HTU accumulation. The slower progression through growth stages allows for more days with temperatures conducive to HTU accumulation, resulting in the observed higher values of HTU in happy seeder sown crop followed by super seeder sown crop and conventional sown crop. Variety PBW-869 accumulated the highest helio-thermal units accumulation (14222.5°C day hours) followed by variety PBW-725 (13461.1°C day hours) sown with happy seeder. In case of super seeder sowing, variety PBW-725 accumulated 12780.0°C day hours helio-thermal units, while variety PBW-869 accumulated 13009.4°C day hours helio-thermal units. On the contrary, conventional sowing resulted in a lower accumulation of HTU because conventional sown crop required less number of days to complete various phenophases. Variety PBW-869 accumulated 12564.1°Cday hours and variety PBW-725 accumulated 11769.9°C day hours. Singh (2019) observed that HTU requirement was highest in wheat sown with happy seeder as compared to conventional sowing. Singh et al (2023) also reported similar results and observed more accumulation of HTU in wheat sown with happy seeder due to delayed physiological maturity.

Photo-thermal units (PTUs): Under happy seeder sowing, variety PBW-869 exhibited the highest photo-thermal units (21801.3°C day hours) followed by variety PBW-725 (20922.3°C day hours) as it showed delayed maturity. In happy seeder sowing, the crop undergoes a prolonged vegetative period and takes more days to reach maturity. This extended duration exposes the crops to a greater cumulative amount of heat and light, contributing to higher PTU accumulation. The combination of increased thermal time (due to more days) and enhanced light exposure under happy seeder sowing conditions results in higher PTU. In super seeder sowing, variety PBW-869 and variety PBW-725 accumulated 20416.0°C day hours and 20182.2°C day hours, respectively. Conventional sowing, which required less number of days to reach maturity, resulted in the lowest accumulation of PTU. Variety PBW-869 and variety PBW-725 accumulated 19962.3 and 19115.8°Cday hours, respectively, under conventional sowing (Table 4). Singh (2019) observed similar findings of high PTU requirement in happy seeder sown wheat as compared to conventional sowing.

Heat use efficiency for total biomass: HUE reveals a progressive increase from conventional sowing to super seeder sowing, with the highest HUE observed in happy seeder sowing (Table 5). Specifically, the happy seeder sown crop exhibited a notable HUE of 8.9 kg/ha/°C/day, surpassing the HUE values of 8.8 kg/ha/°C/day for super seeder sowing and 8.6 kg/ha/°C/day for conventional sowing. The higher efficiency in converting heat units into biomass under the happy seeder sowing method may be attributed to the positive influence of residues which is responsible for increased biomass production, leading to the observed higher HUE. Gupta et al (2020) also reported that crops with extended growth duration tend to produce higher biomass.

Helio-thermal use efficiency for total biomass: The happy seeder sowing method exhibited the highest HTUE for total biomass (1.3 kg/ha/°C/day hours), followed by super seeder sowing and conventional sowing (Table 5). This observed pattern in HTUE values suggests that the crop sown with the happy seeder exhibits higher efficiency in utilizing both solar

Table 3. Effect of sowing methods and varieties on accumulated helio-thermal units (°C day hours) of wheat during *rabi* (2022-23)

Phenological stages	Conventional		Happy seeder		Super seeder	
	PBW 725	PBW 869	PBW 725	PBW 869	PBW 725	PBW 869
Complete emergence	186.0	218.0	352.1	462.5	86.0	352.1
CRI	1399.4	1493.8	1699.0	1795.3	1399.4	1599.4
Maximum tillering	3364.0	3586.2	3589.1	3605.2	3502.7	3589.1
Jointing (Start)	3768.5	3773.7	3822.4	3822.4	3788.0	3808.4
Flag leaf (Start)	3843.9	3896.4	4244.5	4350.6	4014.1	4108.0
Booting (Start)	4307.5	4307.5	4965.5	5355.8	4501.6	4703.5
Heading (Start)	5355.8	5364.1	6133.8	6693.5	5516.2	5886.5
Anthesis (Start)	5771.6	5989.5	7130.0	7885.2	6259.4	6549.4
Milking (Start)	7130.0	7287.1	8701.3	9546.5	7450.1	7727.9
Soft dough (Start)	8376.3	8866.3	10113.6	10721.5	9351.1	9929.7
Hard dough (start)	10247.5	10721.5	11468.3	11959.7	10885.8	11339.0
Physiological maturity	11769.9	12564.1	13461.1	14222.5	12780.0	13009.4

radiation and thermal time for biomass production compared to crops sown using super seeder and conventional methods. The likely contributing factor to this higher HTUE in the happy seeder sown crop is the delayed maturation compared to super seeder and conventional sowing. Dar et al (2018) also reported higher helio-thermal use efficiency in crops with delayed maturity.

Photo-thermal use efficiency for total biomass: Photo-thermal use efficiency for total biomass indicated that wheat sown with the happy seeder had the highest value (0.8 kg/ha/°C/day hours) followed by super seeder and conventionally sown crops. The highest PTUE observed under happy seeder sowing can be attributed to the extended duration required to reach maturity, leading to increased biomass compared to super seeder and conventional sowing methods. The prolonged growth period under happy seeder sowing enables the crop to capture more solar radiation and accumulate thermal time, leading to a more efficient conversion of these resources into biomass. Sidhu et al (2020) also observed higher PTUE in wheat sown with the happy seeder due to the longer duration required for growth.

Radiation use efficiency for total biomass: RUE exhibited a distinct pattern, with happy seeder sown crop recording the highest value (4.0 kg/ha/MJ) followed by super seeder sown crop and conventional sowing method (Table 5). This observed trend in RUE values can be attributed to the superior performance of wheat sown with the happy seeder characterized by enhanced photosynthetically active radiation (PAR) interception and leaf area index (LAI), followed by the super seeder and conventional sowing methods. The greater interception of PAR and higher LAI in the happy seeder sown crop contributed to higher RUE. Priadkina et al (2020) also reported a positive correlation between higher PAR and LAI values and increased radiation use efficiency in wheat.

Heat use efficiency for grain yield: The crop sown using the happy seeder method exhibited the highest HUE (3.1 kg/ha/°C/day) followed by super seeder sown crop and conventional sowing method (Table 5). The higher HUE observed in the happy seeder sown crop may be attributed to

Table 4. Effect of sowing methods and varieties on accumulated photo-thermal units (°C day hours) of wheat under different irrigation treatments during *rabi* 2022-23

Phenological stages	Conventional		Happy seeder		Super seeder	
	PBW 725	PBW 869	PBW 725	PBW 869	PBW 725	PBW 869
Complete emergence	1309.6	1464.0	1609.4	1747.6	1464.0	1609.4
CRI	3089.3	3210.3	3468.2	3586.8	3210.0	3345.5
Maximum tillering	5635.8	5983.7	6144.5	6257.4	6056.0	6144.5
Jointing (Start)	6752.7	6818.4	7117.0	7276.7	6907.0	6988.2
Flag leaf (Start)	7445.9	7522.4	8052.9	8419.4	7887.0	7887.1
Booting (Start)	8227.2	8333.6	9276.3	9734.5	8956.0	8956.2
Heading (Start)	9734.5	9843.3	11056.3	11901.6	10310.0	10555.8
Anthesis (Start)	10422.0	10716.4	12384.4	13424.1	11597.0	11752.1
Milking (Start)	12384.4	12567.2	14320.3	15315.0	13072.0	13256.9
Soft dough (Start)	13947.8	14509.7	16467.7	17391.4	15111.6	15766.8
Hard dough (Start)	16638.1	17391.4	18401.6	19319.6	17756.0	18165.0
Physiological maturity	19115.8	19962.3	20922.3	21801.3	20182.2	20416.0

Table 5. Heat use efficiency, helio-thermal use efficiency, photo-thermal use efficiency and radiation use efficiency of wheat varieties under different sowing methods during *rabi* (2022-23)

Treatments	Heat use efficiency (kg/ha/°C/day)		Helio-thermal use efficiency (kg/ha/°C/day hours)		Photo-thermal use efficiency (kg/ha/°C/day hours)		Radiation use efficiency (kg/ha/MJ)	
	Total biomass	Grain yield	Total biomass	Grain yield	Total biomass	Grain yield	Total biomass	Grain yield
Conventional sown wheat	8.6	3.0	1.2	0.4	0.7	0.2	3.2	1.2
Happy seeder sown wheat	8.9	3.1	1.3	0.5	0.8	0.3	4.0	1.4
Super seeder sown wheat	8.8	3.0	1.3	0.4	0.7	0.2	3.9	1.3

the presence of remaining residues in the field, contributing to a higher grain yield. The residues left in the field under happy seeder sowing are likely to enhance the efficiency of heat energy utilization, leading to higher HUE. Singh (2019) also observed that treatments involving the retention or incorporation of straw in the field tend to use heat energy more effectively compared to treatments without residue retention.

Helio-thermal use efficiency for grain yield: HTUE was highest in the crop sown with the happy seeder (0.5 kg/ha/°C/day hours) followed by super seeder and conventional method (Table 5). This may be due to the longer growth duration associated with wheat sown using the happy seeder sowing followed by super seeder and conventional sowing. Attri and Sandhu (2023) also noted higher helio-thermal use efficiency in crops exhibiting a longer growth duration.

Photo-thermal use efficiency for grain yield: The crop sown with happy seeder exhibited the highest PTUE (0.3 kg/ha/°C/day hours) followed by super seeder sowing crop and conventional sowing (Table 5). The relatively lower grain yield observed in the conventional sowing method compared to the happy seeder and super seeder methods which contributed to the decreased photo-thermal use efficiency in conventional sowing. The positive correlation between delayed maturity and higher PTUE, leading to increased grain yield, was also observed by Gupta et al (2020). This indicates that the prolonged growth duration associated with happy seeder and super seeder methods enhances the efficient utilization of solar radiation and thermal time, leading to elevated PTUE and consequently, augmented grain yield.

Radiation use efficiency for grain yield: The happy seeder exhibited the highest RUE (1.4 kg/ha/MJ) followed by super seeder and conventional sowing (Table 5). This may be due to the reason of more favourable micro-environment under happy seeder sown wheat followed by super seeder and conventional sowing. The improved RUE in the happy seeder sown crop could be attributed to factors such as enhanced soil moisture retention likely facilitated by residue retention. Zhou et al (2021) reported increased RUE in plots with higher moisture retention due to residue retention. The presence of residues in the field, particularly under happy seeder sowing may contribute to improved soil conditions, fostering a micro environment conducive to higher RUE.

Relationship between different agro-meteorological indices and grain yield: Linear regression equation was developed to establish the relationship of GDD, HTU, PTU and HUE with grain yield across different sowing methods (pooled data). The analysis revealed a positive correlation of

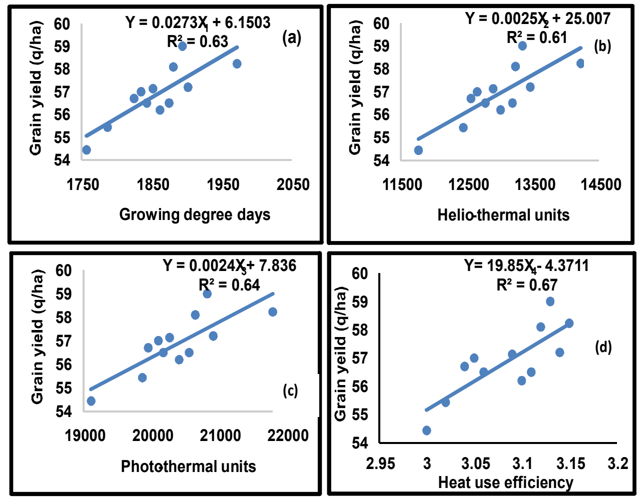

Fig. 1. Relationship between different agro-meteorological indices (GDD (a), HTU (b), PTU (c) and HUE (d)) with grain yield under different sowing methods during *rabi* 2022-23

GDD, HTU, PTU, and HUE with grain yield. This positive association suggests that an increase in growing degree days, helio-thermal units, photo-thermal units, and heat use efficiency is associated with higher grain yields (Fig. 1). The coefficient of determination (R^2) values indicated that 63, 61, 64, and 67% of the variation in grain yield can be attributed to growing degree days, helio-thermal units, photo-thermal units, and heat use efficiency, respectively. The linear regression equations derived for different treatments further demonstrated that improvements in accumulated growing degree days (AGDD) and heat use efficiency during various growth stages corresponded to an increase in wheat crop grain yield. Kaur et al (2016) reported a highly significant and linear relationship between grain yield and AGDD. Gupta et al (2020) identified a linear relationship between AGDD and heat use efficiency with grain yield, emphasizing the positive impact of a longer duration taken by wheat to complete phenophases on grain yield. Kaur (2022) also observed a positive relationship between growing degree days and grain yield in wheat. The regression equation developed between different agro-meteorological indices and grain yield is as under:

$Y=0.0273X_1+6.1503$ ($R^2 = 0.63$)

$Y=0.0025X_2+25.007$ ($R^2 = 0.61$)

$Y=0.0024X_3+7.836$ ($R^2 = 0.64$)

$Y=19.85X_4-4.3711$ ($R^2 = 0.67$)

Where;

Y – Grain yield (q/ha)

X_1 – Growing degree days (^oC days), X_2 – helio-thermal units (^oC day hour), X_3 – photo-thermal units (^oC day hour), X_4 – heat use efficiency (kg/ha/^oC/days)

CONCLUSION

Wheat sown with happy seeder took more number of days to reach physiological maturity followed by super seeder and conventional sowing. Happy seeder sown crop accumulated the highest GDD, HTU, PTU, HTUE, PTUE, HUE, RUE followed by super seeder and conventional sowing which might be attributable to more number of days taken to reach maturity under happy seeder sown wheat. Variety PBW-869 is a longer duration variety as compared to variety PBW-725, hence it accumulated more agro-meteorological indices. The relationship between different agro-meteorological indices and grain yield were found to be positive which showed that with an increase in GDD, HTU, PTU and HUE, the grain yield also increases. Based on this study, farmers are recommended to adopt the happy seeder sowing method and consider using the PBW-869 variety for higher yields, as this method accumulates more agro-meteorological indices positively correlated with grain yield. Regular monitoring of these indices and integrating this practice with other crop management strategies can enhance wheat productivity and mitigate climate change effects.

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Classification of Rice Genotypes based on NUE under Changing Climate

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Abstract: In the event of high input agriculture, more emphasis on fertilizer use efficiency, especially nitrogen use efficiency (NUE) has to be given to safeguard the economic as well as environmental resources under rice production system. Though the nutrient use efficiency mainly depends on the efficient fertilizer management practices, the existing N use efficiency pattern under varied doses and the factors responsible for N use efficiency in existing fertilization application in various soil and crop varieties need to be studied for further improvement in N use efficiency. With this view, field experiment was initiated to determine various classes of NUE at different N levels under the promising rice varieties/genotypes by following split plot design. The genotypes were grouped as efficient & responsive (9), non-efficient & responsive (4), efficient & non-responsive (6) and non-efficient & non-responsive (13) genotypes. The efficient and responsive genotypes can be used for high input Agriculture. The lowest agronomic nitrogen use efficiency was observed under higher dose of 150 % recommended dose of nitrogen than other doses. The physiological nitrogen use efficiency of the genotypes decreased with increasing nitrogen fertilizer.

Keywords: Rice, NUE, ANUE, PNUE, Efficient and responsiveness

The rapid rate of climate change and its magnitude is a great concern globally nowadays. Variation in climatic events and the increase in extreme weather have a significant serious threat to socioeconomic and livelihood (Zhang et al 2014). Soltani et al (2016) reported that alteration in the frequency of temperature and rainfall leads to increases in extreme events like heat waves (extreme temperature), flood and cyclones (extreme rainfall), drought (an increase of dry spell, evapotranspiration and failure of monsoon). Highest number of extreme weather events (>15%) have occurred in Maharashtra in the past 50 years followed by West Bengal (9%), Kerala (7.5%), Karnataka (7.5%), Uttar Pradesh (7.1%), and Rajasthan (7.1%). The states most vulnerable to cyclones are Andhra Pradesh (32%), Odisha (20%), West Bengal (15%), Tamil Nadu (15%) and Gujarat (5%) (Annals 2024).

Recently, the cyclone Michaung ravaged Tamil Nadu's capital city during the first week of December, 2023 and several localities received the season's total rainfall in just 36 hours. In the January, 2024, southern districts of Tamil Nadu experienced a record of 110 cm rainfall in a day. This heavy rain lashed the southern districts of Tamil Nadu and completely wiped out farmland in Thoothukudi district, leaving farmers high and dry. Thousands of acres of farmland had been entirely inundated owing to the heavy-rain induced flood. Further, a considerable increase in the count, intensity and duration of heat waves and warm night episodes across Tamil Nadu between the periods 1951-1983 and 1984-2016 was observed. During flooding lot of fertile soil along with nutrients are washed out. It is estimated that over 5.3 billion tonnes of soil has been lost annually through water erosion with a loss of ~8 Mt of NPK. Therefore, the nutrient deficiency is appearing on the crop plants during these extreme climatic events. It is observed that in India the nutrient deficiency in the order of: 95, 94, 48, 25, 36.5, 23.4, 12.8, 7.1 and 4.2% for N, P, K, S, Zn, B, Fe, Mn and Cu, respectively (Annals 2024). The limiting nutrients do not allow the full expression of other nutrients, thereby, lowering the fertilizer responses and crop productivity. Nitrogen being a basic component of many organic molecules *viz*., nucleic acids and proteins (Lea and Miflin 2018), it is a major limiting mineral source for most of the plant species in its acquisition and assimilation. In the event of climatic impact, more emphasis on nutrient use efficiency, especially nitrogen use efficiency (NUE) has to be given to safeguard the economic as well as environmental resources in Agriculture.

Though the nutrient use efficiency mainly depends on the efficient fertilizer management practices, the existing N use efficiency pattern under varied doses and the factors responsible for N use efficiency in existing fertilization methods in various soil and crop varieties need to be studied for further improvement in N use efficiency, grouping and classification of genotypes.

MATERIAL AND METHODS

Field experiment was conducted during *pishanam* season, 2017 at Agricultural Research Farm, Rice Research station, Tamil Nadu Agricultural University, Ambasamudram, Tirunelveli, with split plot design comprising of thirty two rice genotypes asmain plots and four N levels (N_0 (control), N_1 (50 %RDN), N_2 (100 % RDN) and N_3 (150 % RDN) as subplots with three replications**.** All there commended package of practices was followed to raise a good crop (CPG, 2020). The nursery was raised separately for 32 different rice genotypes under SRI methods (raised beds with dimension of 120 cm wide, 15 cm height with buffer channel of half meter wide all round to facilitate easy drainage).The N fertiliser (urea) was applied as per the treatment schedule. The urea was applied in four equal split doses during before planting (basal 25 %) and top dressing at tillering at 30 DAT (25 %), panicle initiation at 60DAT (25 %) and flowering stage at 75 DAT (25 %).The grain and straw yields was recorded plot wise on harvest and converted in to kg ha^{-1} with 14% moisture. Soil samples before and after the crops was analysed for various physical, chemical and biological properties. The following nitrogen use efficiencies were derived from the parameters such as quantity of N applied quantity of N taken up and grain yield of N applied and control treatment etc. under various rice genotypes at different levels of nitrogen application.

a) Agronomic efficiency = grain yield in fertilized plot – grain yield in unfertilized plot / quantity of N applied
b) Physiological N use efficiency = Gain yield in fertilized plot – grain yield in unfertilized plot / uptake in fertilized plot – uptake in unfertilized plot
c) Apparent N recovery efficiency = Difference between the uptake/quantity of N applied x 100
d) Partial factor Productivity = grain yield at N levels / N application dose

The rice genotypes were classified as Efficient and Responsive (ER), Efficient and Non- responsive (ENR), Non-efficient and Responsive (NER) and Non- efficient and Non-responsive to nitrogen fertilizer based on the NUE. The various methods and the parameters used to classify the genotype in to various classes using normal scatter diagram are listed below.

X axis	Y axis	Author
Grain yield at low level nitrogen	Nitrogen use efficiency	Fageria (2003)
Grain yield at low level nitrogen	Physiological N use efficiency	Kosar et al (2003)
Dry matter yield at low level of nitrogen	Efficiency index	Siddiqi and Glass (1981)
Dry matter yield at low level of nitrogen	Dry matter yield at high level of nitrogen	Gill et al (2011)
Grain yield at low level of nitrogen	Total uptake of nitrogen at high level nitrogen application	
Efficiency Index	N utilization efficiency	Fageria (2007)

RESULTS AND DISCUSSION

Grain and straw yields of rice: Grain and straw yields increased in a linear model with the addition of nitrogen at different levels from 60 to 180 kg ha^{-1} (Table 1). Grain yield varied from 1543 kg ha^{-1} at control (CB14533) to 8150 kg ha^{-1} at 150% N (ASD 16) with an average value of 5155 kg ha^{-1}. Among four N levels highest grain and straw yields were recorded at N_3 (180 kg ha^{-1}) by the most of the rice cultures, except the AS 12051, ACK 14004, CB08702, CB 13539 and PM 12009 which did not respond to higher dose nitrogen (180 kg ha^{-1}). ASD 16 recorded highest mean yield of 6698 kg ha^{-1} followed by MDU5 (6014 kg ha^{-1}), ADT 45 (5875 kg ha^{-1}) responded to higher dose of N applied. In cultivars, the highest mean yield was observed in TR 13083 (6695 kg ha^{-1}) followed by TM 12077 (6162 kg ha^{-1}). The percent increase of grain yield was maximum (57.55%) in CB 14533 though it gave lowest yield among all the genotypes. The straw yield varied from 3011 kg ha^{-1} (CB14533) to 10292 kg ha^{-1} (ASD16) with an average of 7505 kg ha^{-1}. The variation in yield among different rice varieties was due to the differential efficiency in converting dry matter into grain. Similar findings were also reported in rice varieties under different nitrogen levels by Priyadarsini and Prasad (2003).The significant and positive correlation existed between grain yield and other yield attributes such as number of tillers leaf area index clearly showed the genotypic characters influenced the growth parameters, which in turn contributed more canopy structure i.e. leaf area index by canopy photosynthetic efficiency of the particular variety which resulted higher dry matter production (Amanullah et al 2007). The higher level of nitrogen application influenced the growth parameters such as root length, root volume, leaf area index, plant height, number of tillers hill^{-1} resulted increased dry matter production which is evidenced from the positive correlation associated between the grain yield and other growth and yield attributing parameters such as root length, root volume, leaf area index, plant height, number of tiller hill^{-1} and dry matter production.

For grain yield, the same trend was followed as straw yield. The overall highest mean yield was recorded by TR13083 (9388 kg ha^{-1}) which was on par with ASD 16 (8884 kg ha^{-1}). The lowest yield of 4798 kg ha^{-1} was in CB 14533 but the percentage increase in both grain and straw yields by computed to control by highest level of N was more in this cultivar CB14533 which indicate the response level was high in cultivar.

Nitrogen use efficiency: The nitrogen use efficiency has been considered in three different perspectives as:

1. Production efficiency (ANUE and PFP_N)
2. Absorption efficiency (N uptake and ANRE) and
3. Utilization efficiency (PNUE, NHI and NP)

Production efficiency: The production efficiency of applied N is reflected in two ways since the crop uses native and applied N. The combined effect of applied and native N on grain yield production is termed as partial factor productivity (PFP_N) and the effect of applied N alone for the production of grain yield is termed as agronomic nitrogen use efficiency (ANUE). The efficiency of applied N on production of grain yield, biomass, protein yield and number of filled grains with respect to genotypes and levels of nitrogen application are discussed below.

Partial factor productivity: PFP_{GY} is an aggregate efficiency index of uptake of both indigenous soil N, fertilizer N, and the efficiency with which acquired N converted to grain yield (Cassman et al 2003). In general, the partial factor

Table 1. Grain and straw yields (Kg ha^{-1}) of rice influenced by genotypes and levels of nitrogen application

Genotypes/N level	Grain yield (Kg ha^{-1})					Straw yield (Kg ha^{-1})				
	N_0	N_{50}	N_{100}	N_{150}	Mean	N_0	N_{50}	N_{100}	N_{150}	Mean
ASD 16	5284	6175	7183	8150	6698^{a}	7333	8235	9675	10292	8884^{b}
ADT 39	3682	4921	5778	6814	5299^{h}	6031	7484	7906	8478	7474^{k}
ADT 43	4259	4691	5500	6723	5293^{h}	7405	7909	8051	8739	8026^{g}
ADT 45	4606	5339	6299	7256	5875^{d}	7239	7932	8267	9251	8172^{f}
CO 51	4587	4940	5576	6371	5368^{g}	7163	7399	8091	8414	7767^{i}
TPS 5	3643	4660	5550	5924	4944^{ij}	5124	6723	7268	7528	6660^{m}
MDU 5	5549	5660	6188	6659	6014^{c}	7823	7754	8584	8713	8218^{ef}
ANNA 4	5289	5355	5512	5577	5433^{f}	7061	7445	7405	7751	7415^{k}
AS 12051	3889	4410	4754	4681	4433no	5778	6663	6798	6174	6353^{n}
AS 12104	4556	5493	6226	6428	5676^{e}	5292	7833	8288	8411	7456^{k}
AD 09206	3254	4374	4969	5372	4492^{n}	6759	7072	7304	7903	7259^{l}
AD 10034	4968	5317	5390	5497	5293^{h}	7333	7961	8000	8351	7911^{h}
ACK 14001	4837	5844	6678	6929	6072^{c}	6757	7888	9290	9753	8422^{c}
ACK 14004	4510	5549	5864	5771	5423^{fg}	6333	7288	7857	7298	7194^{l}
CB 06803	3536	4775	5542	6012	4966^{i}	6661	7055	7577	8557	7462^{k}
CB 08702	4335	4811	5287	5078	4878^{k}	7612	7984	8900	8453	8237^{f}
CB 13539	3029	3401	3750	3429	3402^{r}	6113	6198	6831	6424	6391^{n}
CB 14508	4350	5156	6144	7051	5675^{e}	6777	7949	8655	9724	8276de
CB 14533	1543	2030	2526	4420	2433^{s}	3011	3701	5000	7479	4798^{p}
TR 09027	2878	3291	4294	5107	3892^{q}	4173	6400	6926	7992	6373^{n}
TR 05031	4632	5895	6275	6717	5880^{d}	7209	7811	8500	9621	8285de
TR 13069	3811	4204	4795	5873	4671^{m}	7013	7373	7500	8507	7598^{j}
TR 13083	5778	6479	7188	7333	6695^{a}	8540	8979	9773	10262	9388^{a}
TR 13007	5056	5762	6220	6627	5916^{d}	7724	7999	8557	8972	8313^{d}
TM 07335	4947	5495	6209	6862	5878^{d}	6000	7225	8253	8739	7554^{j}
TM 09135	3660	4594	4890	5502	4661^{m}	5889	7310	8111	8310	7405^{k}
TM 10085	3673	5015	6051	7157	5474^{f}	4944	7407	8273	9823	7612^{j}
TM 12059	3868	4587	5085	5512	4763^{l}	6552	6989	7367	7873	7195^{l}
TM 12061	2911	3322	4542	5438	4053^{p}	4000	5703	7013	7513	6057^{l}
TM 12077	4304	6020	7119	7206	6162^{b}	6190	8639	8957	9233	8255de
PM 12009	3372	5222	5536	5418	4887^{k}	6070	7823	8017	7845	7438^{k}
EC 725224	2956	4517	4611	5419	4376^{o}	5051	6359	6501	7383	6323^{n}
Mean	4111^{d}	4916^{c}	5548^{b}	6047^{a}	5155	6342^{d}	7328^{c}	7922^{b}	8430^{a}	7505
		G	N	G × N	N× G		G	N	G × N	N× G
CD (p=0.05)		61.84	22.15	124.92	125.31		70.81	26.12	146.27	147.79

productivity rice genotypes varied from 35.7 to 69.3 kg (kg N)$^{-1}$ (Fig. 1a). The efficient rice varieties such as ASD16, ADT39, ADT45, MDU5, AS12104, AD10034, ACK14001, ACK14004, CB14508, TR05031, TR13083, TM13007, TM10085, TM12077 and PM12009 recorded the PFP_{GY} with more than the average PFP_{GY} of rice genotypes, which is almost equal to the PFP_{GY} of irrigated rice in well-managed systems (60 kg kg^{-1}). The genotypic potential and differences of partial factor productivity in rice were significantly changed with cultivar types. The partial factor productivity of grain yield decreased with increased dose of nitrogen from 50 to 150 % recommended dose of nitrogen in all the rice genotypes (Fig. 1b). The cultivars recorded more PFP of grain yield under low level of 50 % recommended dose of nitrogen level than 100 and 150 % recommended dose of nitrogen and it ranged from 19.05 to 108 kg (kg N)$^{-1}$. The highest partial factor productivity of grain yield produced by the genotype TR13083, ASD16 and TM12077 under 50 % recommended dose of nitrogen application (Fig. 1c) mainly because of the higher yield supported by native nitrogen ratio of Y_0/N_t (native nitrogen efficiency) observed in this experiment. This is evidenced by Janaki (2000). Similar trends were observed in the partial factor productivity from the reported work of biomass yield, protein yield and number of filled grains.

Agronomic N use efficiency: The ANUE for rice genotypes varied from 1.52 to 22.73 kg (kg N)$^{-1}$ with an average value of 12.09 kg of grain produced per kg of N applied (Fig. 2a). The genotypes namely ASD16, ADT39, ADT45, TPS5, AD09206, ACK14001, CB06803, TR05031, TM10085, PM12009 and EC725224 had agronomic N use efficiency of more than 15 kg (kg N)$^{-1}$ and other varieties / culture showed less than 15 kg (kg N)$^{-1}$. The variation in agronomic N use efficiency among the genotypes indicates difference in biochemical and physiological characteristics, nutrient uptake by roots, remobilization and translocation of absorbed N to different plant organs Samonte et al (2006) also stated that the large genotypic variation in agronomic nitrogen use efficiency was probably due to low yield potential. The lowest agronomic nitrogen use efficiency was observed under higher dose of 150 % recommended dose of nitrogen than other doses (Fig.

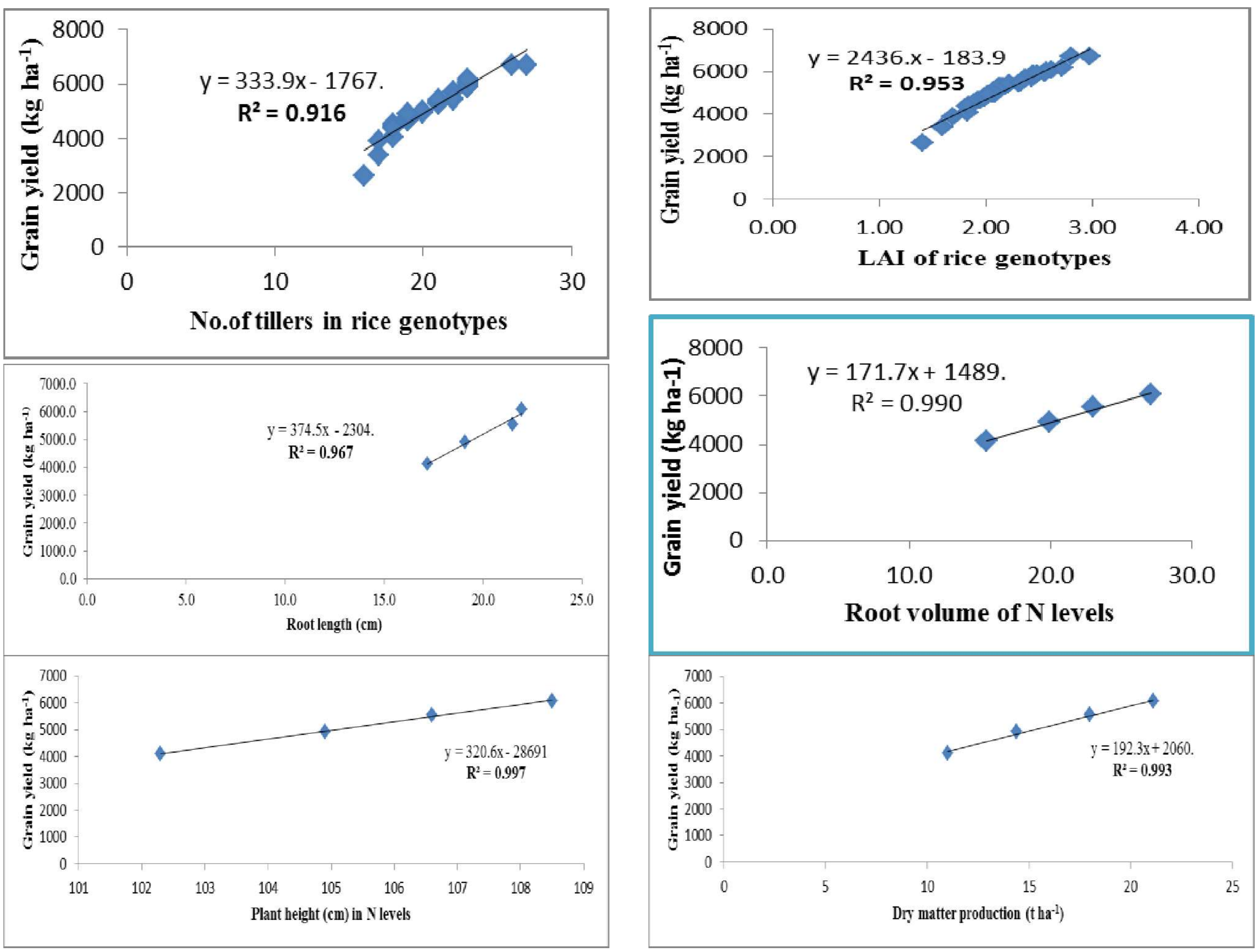

Fig. 1. Correlation between rice grain yield (kg ha^{-1}) and growth parameters

2b). This is in agreement with Peng *et al*. (2007). The agronomic nitrogen use efficiency showed a decreased linear response to applied N because there was no increase in utilization efficiency but increase in production of grain yield with enhanced N fertilization in rice can only be achieved by higher N uptake. The reason for decreased nitrogen use efficiency with N application is not clear (Artacho et al 2009). Among the interaction between rice genotypes and nitrogen levels (Fig. 2c), the decreasing trend of ANUE with increasing N level noticed in the rice genotypes of ADT39, TPS5, AS12051, AS12104, AD09206, AD10034, ACK14001, ACK14004, CB06803, CB08702, CB13539, TR05031, TM13007, TM09135, TM10085, TM12059, TM12077, PM12009 and EC725224 was due to non-response of variety to higher level nitrogen application as observed by Noureldin et al (2013) who reported that agronomic efficiency increased up to optimum levels of nitrogen application and decreased beyond. Low agronomic N use efficiency reflect limited yield response to fertilizer N application because of high indigenous soil N levels (Peng et al 2006). The rice genotypes namely ASD16, ADT45, CO51, MDU5, CB14533, CB14508, TR13069, TM07335 and TM12061 showed increasing trend of ANUE with increasing N levels from 50% recommended dose of nitrogen (60 kg N ha^{-1}) to 150% recommended dose of nitrogen (180 kg N ha^{-1}) which indicated the yield response of genotypes to high level of fertilizer N addition high with less utilization efficiency. This trend indicated the larger variation between nitrogen uptake and N utilization for the particular genotypes. Therefore, it is necessary to develop cultivars that have more efficient in absorption of applied N, in order to minimize loss of N from soil to nearby water bodies and make more economic use of applied fertilizer with higher utilization efficiency, which not only increase rice grain yield but also present environmental pollution

Absorption efficiency: Large genotypic variations also exist in many varieties under this experiment. The efficiency of the crop in absorbing native N from the no fertilizer N added is different from fertilizer N added, due to the indirect effect of applied N on the availability or acquisition of native N and called “added N interactions” (Jenkinson et al 1985).

Recovery or apparent recovery efficiency: The recovery efficiency ranged from 11.90 % by the genotypes CB13539 to 65.10 % by the genotype CB14508 due to greater variation (Fig. 3a) in physiological or morphological characteristics of the genotype might result in this kind of phenomenon (Roy et. al, 2004). The study on genotypic variations for grain yield and N use efficiency were prevalent. Wang et al (2015) also supported the result of this experiment. In general, the apparent N recovery efficiency decreases with increasing fertilizer N rates (Fig. 3b). The excess N supply is susceptible to loss through runoff, leaching and gaseous emissions (Fageria and Baligar 2001). The interaction of genotypes with levels of nitrogen application (Fig. 3c), grouped the varieties into two groups. In one group the apparent N recovery efficiency generally decreases as the nitrogen doses increased in the genotypes viz., ASD16, ADT39, TPS5, AD09206, AD10034, ACK14001, CB06803, CB08702, CB14508, TR09027, TR05031, TM12077, PM12009 and EC725224. These varieties doesn't have the capacity to absorb excess N supply whereas in another group of genotypes with ADT43, CO51, ANNA 4, AS12104, AS12051, ACK14004, CB13539, TR13083, TM13007, TM07335, TM09135, TM10085, TM12059 and TM12061 showed increasing trend of ANRE up to 100 % recommended dose of nitrogen. The nitrogen use efficiency at higher N rate pointed out that rice plant are unable to absorb or utilize N at higher rates or the rate of N uptake by plant cannot keep pace with the loss of nitrogen. The similar result of negative correlation of recovery efficiency with N application rate was obtained by Dong et al (2012). The relative amount of N that the crop can recover from the available N pool depends on the relative sink strength of physiological or morphological character of the variety (Inthapanya et al 2000, Shi 2002). Apparent N recovery efficiency reflect the percentage of fertilizer N recovered in above ground plant biomass (Dobermann 2007).

Physiological N use efficiency: The physiological nitrogen use efficiency varied from 6.90 to 74.71 kg $(kg\ N)^{-1}$ with a mean of 31.59 kg$(kg\ N)^{-1}$. the genotypes CB14533 had maximum physiological efficiency of 71.71 kg $(kg\ N)^{-1}$ followed by PM12099 (62.39 kg $(kg\ N)^{-1}$) and the minimum efficiency of 8.82 kg $(kg\ N)^{-1}$ under AD10034 (Fig. 4a). This is due to the difference among the varieties in PNUE. Among the various nitrogen level, 50 % RDN enhanced physiological N use efficiency of 32.50 kg $(kg\ N)^{-1}$ followed by 100 % of RDN which was on par with 150 % RDN (30.98 kg $(kg\ N)^{-1}$) which were statistically equal (Fig. 4b).In general, the physiological nitrogen use efficiency of the genotypes decreased with increasing nitrogen fertilizer. The higher physiological N use efficiency of 81.93 kg $(kg\ N)^{-1}$ by the genotype CB14533 with 100 % recommended dose of nitrogen might be due to genotypic character of the variety under the sufficient level of N application. However, the 16genotypes showed decreasing trend with increased nitrogen application (Table 1). The uptake of N by different rice genotypes increases with increasing the rates of N application, but it reduces the N utilization efficiency (Fig. 4c). The capability of increase in yield per kg nitrogen declined remarkably with increasing nitrogen application (Devika et al 2018).PNUE increased with enhancement of nitrogen application under the genotypes ASD 16, ADT43, CO51, MDU 5, ANNA 4, CB08702, CB14508, TR0927, TR13069, TM07335, and TM12061 due to responsiveness of these varieties to the applied N. López-Bellido and López-Bellido (2001) suggested that physiological N use efficiency increased with nitrogen application and reflected the utilization of absorbed nitrogen efficiently by rice plant.

The genotype CB14533 at higher level of 150 % recommended dose of nitrogen application showed the diminishing trend of PNUE at higher N rates pointed out that rice plants indicated the inability to absorb or utilize nitrogen at higher rates or the rate of nitrogen uptake by plant cannot keep pace with the loss of nitrogen (Feng et al 2011).

Classification of rice genotypes: The various methods and the parameters are used to classify the rice genotypes as efficient and responsive (ER), efficient and non- responsive (ENR), non-efficient and responsive (NER) and non- efficient and non- responsive to applied nitrogen fertilizer. The low N use, high cost of N fertilizers, various N losses through leaching and NH_3 volatilization and other geopolitical issues has compelled the scientists to identify more N efficient genotypes and their mechanisms to increase N use efficiency in agriculture is a first pre-requisite for future production and productivity of rice. A number of methods and parameters have been proposed for classifying genotypes for their N use efficiency. Most of the above mentioned methods classify genotypes at low N conditions, hence this categorization may not classify the genotypes responded under high N level (Jothimani 2021).

However, the genotypes were classified based on absorption and utilization capacities such as agronomic N

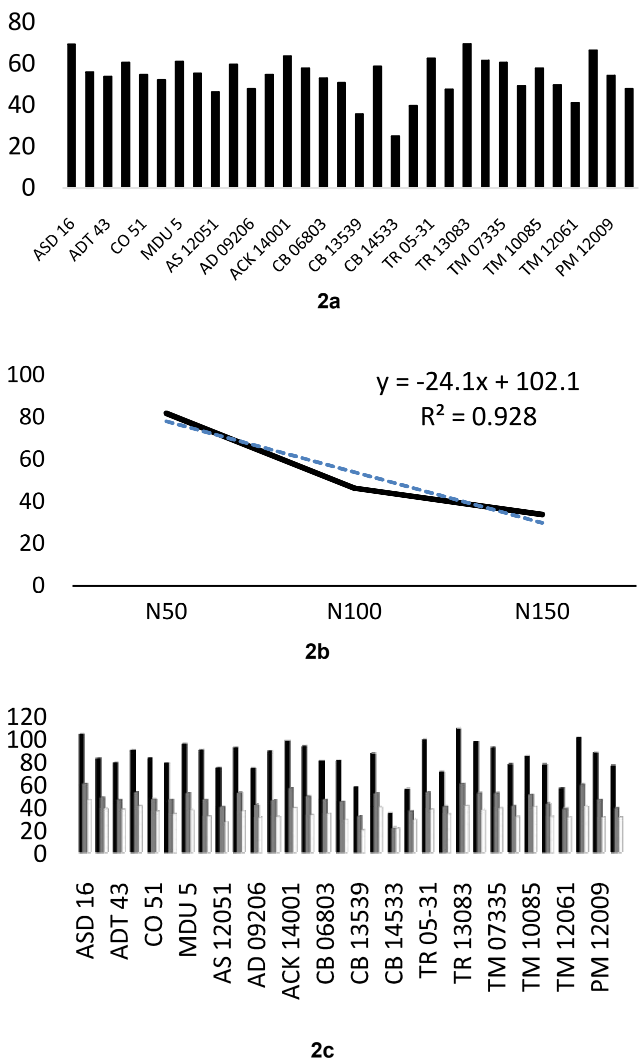

Fig. 2. PEP (Kg (kg (N)$^{-1}$) as influenced by genotypes (a), N level (b) and their interaction (c)

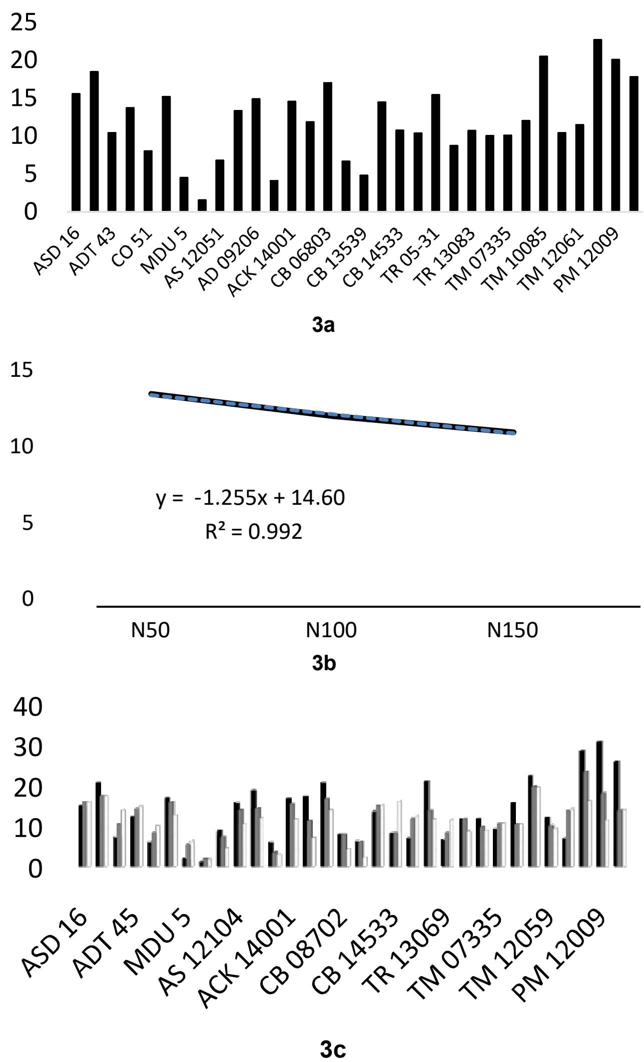

Fig. 3. ANUE PEP (Kg (kg (N)$^{-1}$) as influenced by genotypes (a), N level (b) and their interaction (c)

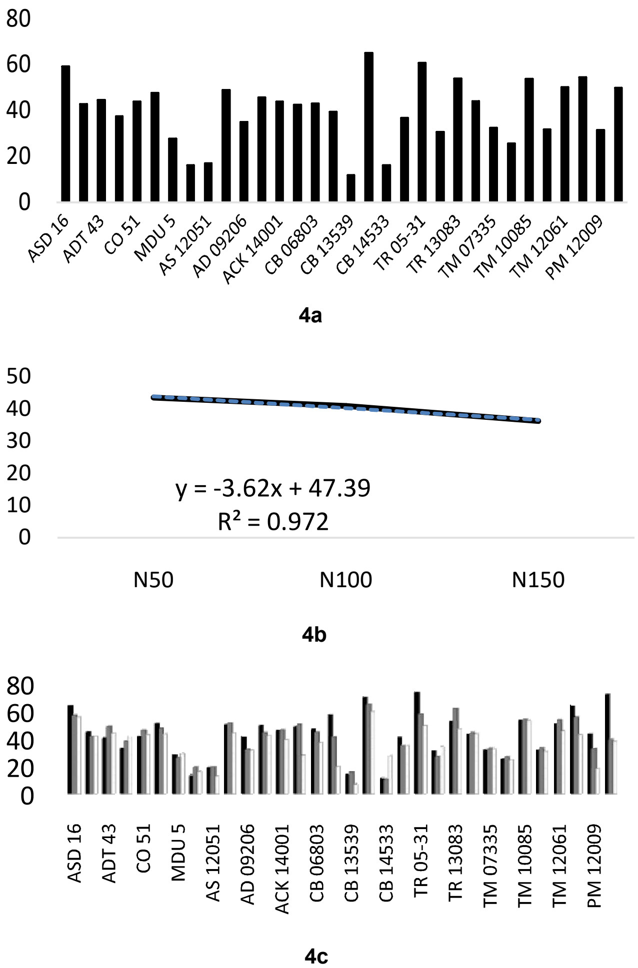

Fig. 4. ANRE as influenced by genotypes (a), N level (b) and their interaction (c)

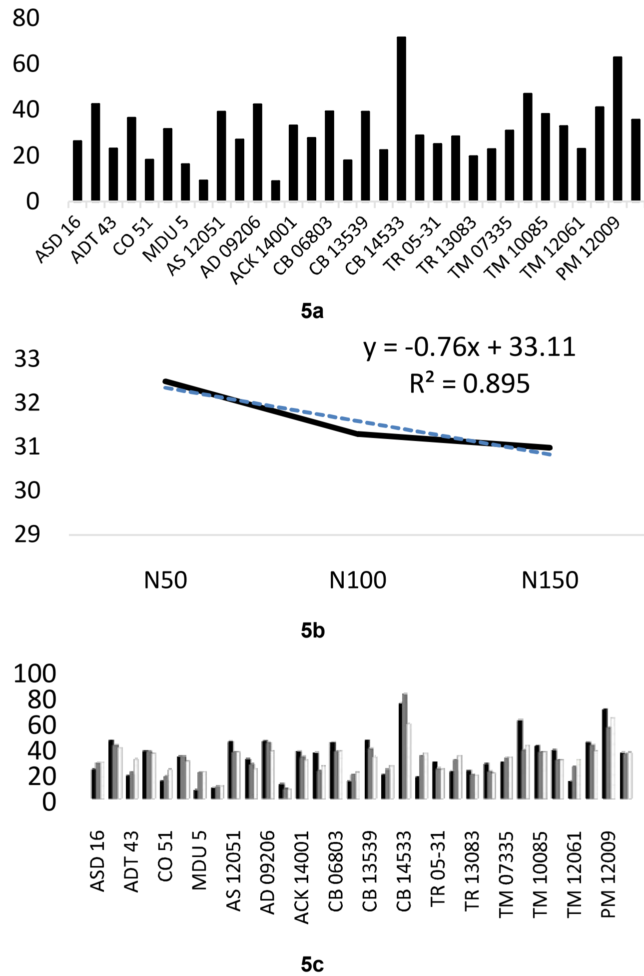

Fig. 5. PNUE as influenced by genotypes (a), N level (b) and their interaction (c)

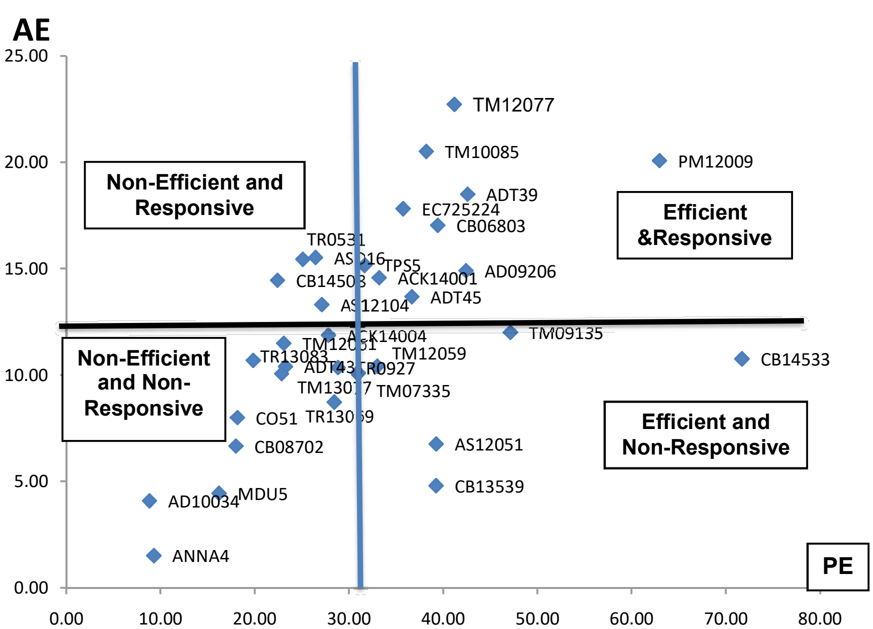

Fig. 6. AE and PE model to classify rice genotypes based on NUE

use efficiency and physiological N use efficiency at six different combinations of N levels (kg ha^{-1}) used in this experiment viz., 0x60, 0x120, 0x180, 60x120, 60x180, 120x180 (Table 2). The symbol x shows that the genotypes were efficient and responsive at the combinations of low and high rate of N application. Some genotypes showed their

Table 2. Efficiency of genotypes at different combinations of N doses

Efficient	0x60	0x120	0x180	60x120	60x180	120x180
ASD 16	x	x	x	x	x	x
ADT 39	x	x	x	x	x	x
ADT 43	0	0	0	0	0	0
ADT 45	x	x	x	x	x	x
CO 51	0	0	0	0	0	0
TPS 5	x	x	x	x	x	x
MDU 5	0	0	0	0	0	0
ANNA 4	0	0	0	0	0	0
AS 12051	0	0	0	0	0	0
AS 12104	x	x	0	x	0	x
AD 09206	x	x	x	x	x	x
AD 10034	0	0	0	0	0	0
ACK 14001	x	x	x	x	x	x
ACK 14004	x	0	0	0	0	0
CB 06803	x	x	x	x	x	0
CB 08702	0	0	0	0	0	0
CB 13539	0	0	0	0	0	0
CB 14508	x	x	x	0	0	x
CB 14533	0	0	0	0	0	x
TR 0927	0	0	x	0	x	x
TR 05-31	x	x	x	x	x	0
TR 13069	0	0	x	0	0	x
TR 13083	0	0	x	x	0	0
TM 13007	0	0	0	0	0	0
TM 07335	0	0	x	0	x	0
TM 09135	x	0	0	0	0	0
TM 10085	x	x	x	x	x	x
TM 12059	0	0	0	0	0	0
TM 12061	0	x	0	0	0	x
TM 12077	x	x	x	x	x	x
PM 12009	x	x	x	x	x	x
EC 725224	x	x	x	x	x	x

X – Efficient and Responsive

Table 3. Grouping and classification of rice genotypes based on NUE

Group	Cultivars	Number
Efficient and responsive	ADT 45, ADT 39, TPS 5, PM 12009, TM 10085, CB 06803, AD 09206, ACK 14001, EC 725224	9
Efficient and non-responsive	ASD 16, TR 0531, CB 14508, AS 12104	4
Non-efficient and responsive	CB 14533, TM 09135, CB 13539, AS 12051, TM 07335, TM 12059	6
Non-efficient and non-responsive	Anna 4, MDU 5, Co 51, ADT 37, TR 0927, AD 10034, CB 08702, TM 13077, TR 13069, TR 13083, TM 12061, ACK 14004, TR 0927	13

efficiency and responsive to the all the combination of applied N rates. Some genotypes were efficiency and responsive in nature at the limited combination of applied N. However, the symbol 0 shows that the genotypes were not either responsive or efficient to the application of N in combinations.

Further, a scattered diagram was drawn by plotting agronomic N efficiency in X axis and physiological N efficiency in Y axis. An intercept line was drawn at the mean agronomic and physiological efficiencies with perpendicular and parallel line on the scattered diagram which divided the graph into four equal quadrants. The top left quadrant had non efficient and responsive varieties, the top right quadrant represented the efficient and responsive group of rice varieties, the bottom left quadrant had non-efficient and non-responsive varieties and the bottom right quadrant represented non efficient and responsive varieties (Fig. 5).

The9 efficient and responsive (ER), 5 efficient and non-responsive (ENR),6 Non-efficient and responsive (NER) and 13 Non – efficient and non-responsive (NENR) genotypes were classified.

CONCLUSION

This AE vs PE model classified rice genotypes for varied types and practices of rice farming. The efficient and responsive genotypes can be used for high input Agriculture whereas the efficient and non-responsive cultures can be used for low input Agriculture (organic farming).

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Climate Resilient Practices for Augmenting Foxtail millet *Melia dubia* System Productivity and Carbon Sequestration

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***Abstract*:** Investigation to know the effect of climate resilient practices on carbon sequestration and productivity of foxtail millet-*Melia dubia* agroforestry was carried out during 2018-2019 to 2019-20 under UAS, Raichur, Karnataka. The foxtail millet was cultivated in five years old *Melia dubia* spaced at 9 m x 3 m. The experiment comprising of eleven treatments with three replications, laid out in randomized block design in agroforestry system involving various combination of FYM + poultry manure + panchagavya alternated with vermiwash imposed treatment showed its significant superiority in grain yield of foxtail millet (1487 kg ha^{-1}) over all other treatments. Significantly higher carbon stock in biomass was observed with the application of FYM + poultry manure **+** panchagavya (22.03 t ha^{-1}) followed by FYM + poultry manure + panchagavya + vermiwash spray (21.97 t ha^{-1}) while lower biomass was recorded with no organic manurial treatment (17.34 t ha^{-1}) in agroforestry system. Significantly higher carbon sequestration (80.87 t ha^{-1}) was observed with the application of FYM + poultry manure **+** panchagavya while lowest was with no organic manures treated plot (63.64 t ha^{-1}) with tree association. Significantly higher net returns of the system of Rs. 3,21,056 ha^{-1} were obtained with FYM+ poultry manure + panchagavya + vermiwash spray except FYM + poultry manure + foliar spray of 3% panchagavya (Rs. 3,20,715 ha^{-1}) over all other treatments. No organic manurial treatment recorded significantly lower net returns from the system (Rs. 2, 40, 937 ha^{-1}). For higher and sustainable system productivity and income in agroforestry application of FYM (50 %) + poultry manure (50 %) equivalent to 100 % recommended 'N' along with foliar spray of 3% panchagavya at 30 and 45 DAS or foliar spray of 3% panchagavya at 30 DAS alternated with 5 % vermiwash at 45 DAS could be advised under organic production system.

Keywords: Agroforestry, Organics, Carbon, Sequestration, Productivity

Tree component in agroforestry systems is significant sink of atmospheric carbon (C) due to their fast growth and high productivity. By including trees in agricultural production systems, agroforestry can, arguably increase the amount of C stored in lands devoted to agriculture, while still allowing for the growing of food crops[2]. In agroforestry system, tree components are managed, often intensively by pruning of minimizing competition and maximize complementarity. The pruned materials are mostly non-timber products. Such materials are often returned to soil. Besides, the amount of biomass and therefore C that is harvested and exported from the system is relatively low in relation to the productivity of the tree. Therefore, unlike in tree plantations and other mono culture systems, agroforestry seems to have unique advantage in terms of C sequestration (Kulkarni 2017).

Recently *Melia dubia* tree species has been introduced in north eastern part of the state which is popularly known as Kalyana Karnataka region. Though it has multiuse, farmers are reluctant in adopting forestry system because of lack of knowledge on scientific cultivation. There is a need to take up studies on these research gaps for economic growth of *Melia* species under given agro-eco-system (Banyal et al 2018).

Millets are comparable to that of super cereals like rice and wheat due to their capacity to withstand drought, adaptable to poor environment and input management. They are suitable for inclusion in multiple/intercropping systems because of its short duration and adjustable to mid season correction. Thus, millets deserve a greater importance than the major cereal crops. Being eco-friendly, these crops are suitable for fragile and vulnerable eco-systems and regarded as preferred crop for sustainable and green agriculture. Hence promotion of millets can lead to efficient management of natural resources and holistic approach in sustaining precious agro-biodiversity. Among the eight millets, foxtail millet (*Setaria italica*) is extensively cultivated in Kalyana Karnataka region and it is an indigenous crop known for its rich nutritive value and fairly drought tolerant (Anon. 2018). In this context, either under sole cropping or with agroforestry system, it is worth to mention that nutrient management through organics plays a major role in exploiting the potential crop yields apart from maintaining soil health as a results of buildup of soil organic matter, beneficial microbes and enzymes thus improving soil physical and chemical properties under organic production system. In a farming system approach, the nutrient needs are met out through recycling process (Aarti et al 2023). Climate smart practices like use of organics, millet crop and tree plantation were tested for productivity and carbon sequestration potentiality in *Melia dubia* tree plantation.

MATERIAL AND METHODS

The experiment was conducted for two years (2018-19 and 2019-20) at Santhekallur under UAS, Raichur which represents Northern Dry Zone of Karnataka (Zone 3), situated between latitude of 15° 99N and longitude of 76° 66 E with a mean sea level of 499 m. There were eleven treatments with three replications, laid out in completely randomized block design. The treatments consisted of application of no organic manure (control), FYM equivalent to 100 per cent RDN, FYM (50%) + Vermicompost (50 %) and FYM (50 %) + Poultry manure (50 %) equivalent to 100 per cent RDN alone and in combination with foliar spray of 3.0 per cent panchagavya and 5.0 per cent vermiwash at 30 and 45 DAS and foliar spray of 3.0 per cent panchagavya at 30 DAS alternated with 5.0 per cent vermiwash at 45 DAS. Grain and straw yield of foxtail millet was recorded at physiological maturity and used for total biomass production from agroforestry system. Following observations were made for calculation of total biomass and carbon sequestration and stock.

Tree Observations

Bole height (m): It was measured using a measuring tape fixed on a straight wooden stick from the ground level to the crown point, which was expressed in metre (m).

Diameter at breast height – DBH (cm): It was measured with measuring tape at 1.37 m above the base of the plant and it was expressed in centimeter (cm).

Total wood volume (m^3): The standing volume of trees was calculated (Kulkarni 2017)

$$\text{Volume } (m^3) = \pi \times (D/2)^2 \times H$$

Where, D is the diameter at breast height (DBH in m) H is the bole height of the tree (m).

Tree biomass (t ha^{-1}): Biomass estimation was carried out using volume (tree bole height, DBH) and wood density. Wood density of 6 years old *Melia dubia* tree is 500.2 kg m^{-3}.

Above ground biomass (AGB) (t ha^{-1})

$$\text{Above ground biomass} = \text{Volume } (m^3) \times \text{Wood density } (kg\ m^{-3}).$$

Then biomass was converted into t ha^{-1}.

Below ground biomass (BGB) (t ha^{-1}): Below ground biomass of the tree was calculated using 0.26 factor of root: shoot ratio (Naguven 2012).

$$\text{BGB } (kg\ tree^{-1}) = \text{AGB } (kg\ tree^{-1}) \times 0.26$$

Then biomass was converted into t ha^{-1}.

Total tree biomass (t ha^{-1}): Sum of above ground and below ground biomass gave total biomass (TB) of the tree (Pandya et al 2014).

TB (kg $tree^{-1}$) = AGB (kg $tree^{-1}$) + BGB (kg $tree^{-1}$). Then biomass was converted into t ha^{-1}.

Carbon Stocks and Sequestration

Carbon stocks (t ha^{-1}): Both above and below ground biomass was converted into above and below ground carbon stocks was calculated (Naguven 2012).

$$\text{Carbon stocks } (t\ ha^{-1}) = 0.50 \times \text{TB } (t\ ha^{-1})$$

The total carbon storage was calculated by adding carbon stocks in above and below ground biomass.

Carbon sequestration (t ha^{-1}): The CO_2 equivalents (quantity of C x 44/12) were arrived from carbon stocks for calculating CO_2 sequestration (t ha^{-1}) by biomass of *Melia dubia* trees in agroforestry system (Naguven 2012).

$$\text{Carbon sequestration } (t\ ha^{-1}) = \text{C stock} \times 44/12$$

RESULTS AND DISCUSSION

Effect of climate smart practices on grain and stalk yield : The foxtail millet cultivation with recommended organic nutrient practices without tree component recorded significantly higher grain yield (1656 kg ha^{-1}) when compared to all other organic manurial treatments with *Melia dubia* plantation system (801 to 1487 kg ha^{-1}) (Table 1). In agroforestry system, application of FYM + poultry manure + panchagavya alternated with vermiwash spray (T_{10}) resulted in significantly higher grain yield (1487 kg ha^{-1}) and it was found on par with FYM + vermicompost + panchagavya alternated with vermiwash spray- T_9 (1440 kg ha^{-1}), FYM + poultry manure + panchagavya (1412 kg ha^{-1}), FYM + vermicompost + panchagavya spray (1406 kg ha^{-1}) and FYM + poultry manure + vermiwash (1403 kg ha^{-1}) which were on par with each other. The T_{10} recorded significantly higher straw yield (2611 kg ha^{-1}) over all other treatments except T_6, T_9, T_8, and T_5.

The mean grain yield of foxtail millet cultivated along with organic nutrient management schedule without tree component was 360 kg ha^{-1} higher than in association with tree component, indicating 28 per cent reduction with agroforestry system. This might be due to better utilization of solar energy without any shade effect of trees in open condition. Yield reduction in foxtail millet when intercropped with *Melia dubia* compared to sole crop without trees as an intercrop was due to reduced photosynthetic active radiation on crop canopy. These results were in conformity with the findings of Ashalatha et al (2015)in blackgram, Bhusara et al (2018) in greengram and Chandana et al (2020) in pearl millet when these crops were grown with *Melia dubia* species in agroforestry system.

The negative effect of tree on crop growth and yield of foxtail millet was reduced by application of organic nutrient management practices over a long period of time. As clearly indicated in the investigation *i.e.*, application of organic manures *i.e.*, FYM with poultry manure/vermicompost along with foliar spray of panchagavya and vermiwash alone or in alternate application (T_5 to T_{10}) resulted significantly higher yield than with no organic manurial treatment. These results were in line with findings of Bhat (2015) in *capsicum*, tomato, garden pea and cauliflower with *Melia composita* with application of vermicompost, Khan and Krishna (2016) in finger millet with *Melia azedaracha* by application of poultry manure, Pallavi et al (2016) in finger millet with *Melia* species with application of poultry manure. Use of balanced levels of nitrogen through organic sources has optimized the availability of nutrients and helped in inducing good vegetative growth. Increased grain yield might also be due to the increased photosynthetic activity which resulted in higher accumulation of photosynthates and translocation to sink due to better source and sink channel which resulted in higher grain yield. Similar results were also reported by Upendranaik et al (2018) and Krupashree (2019).

Effect of Climate Smart Practices Properties

Tree growth: At the end of second year of experimentation (2019) the highest bole height of *Melia dubia* was observed with treatment T_6 (10.45 m) followed by T_{10} (10.42 m) while lower was recorded with no organic manurial treatment over all other treatments (Table 2, 3). The higher tree diameter at breast height (DBH) was with T_7(66.8 cm) followed by T_6 .The lower DBH was registered with no organic manurial treatment (63.4 cm). *Melia dubia* tree wood volume was ranged from 49.99 (no organic manurial treatment) to 61.48 t ha^{-1} in T_6. Total biomass production was calculated by adding biomass production in below and above grounds, which was ranged from 6.23 to 44.07 t ha^{-1}. Treatment T_6 showed its significant superiority in total biomass production (44.07 t ha^{-1}) over T_2, T_1, T_3, T_{11} (6.23 t ha^{-1}) .

Total carbon stock in biomass (t ha^{-1}): Total carbon stock ranged from 3.11 to 22.03 t ha^{-1}. In agroforestry system, significantly higher carbon stock was observed in treatment as compared with T_1 (17.34 t ha^{-1}).

Carbon sequestration (t ha^{-1}) (Fig. 1): Total carbon sequestration in biomass was the sum total of carbon sequestration in above and below ground in both agroforestry and non-agroforestry system which ranged from 11.42 to 80.87 t ha^{-1}. Significantly higher carbon sequestration was observed with tree association than the without tree. Among the organic manurial treatments with *Melia dubia*, significantly higher carbon sequestration was observed with T_6 (80.87 t ha^{-1}) when compared with T_1, FYM and FYM + vermicompost. Next best treatment was T_{10} (80.64 t ha^{-1}), which in turn showed its significant superiority over rest of other treatments. Other treatments were intermediary in their effect. The significantly lower total carbon sequestration was observed with sole foxtail millet with recommended organic nutrient practices without tree component (11.42 t ha^{-1}).

The present study highlights that *Melia dubia* + foxtail millet agroforestry system is a better option than the sole agricultural cropping in respect of climate mitigation and sustainable productivity and doubling farmer's income. Hence, it is required to proceed with the system; otherwise the profit gained in-terms of carbon sequestration in the system would revert to the original state. Higher carbon sequestration with various agroforestry systems was also reported by Rahul Arya et al (2021).

System Economic analysis

Gross returns (Rs. ha^{-1}): At the end of sixth yea plantation (2019) significant variation in gross returns was observed between cultivation of foxtail millet with and without tree

Table 1. Grain yield and straw of foxtail millet as influenced by organic nutrient management practices in *Melia dubia* based agroforestry system (Pooled data)

Treatments	Grain yield (Kg ha^{-1})	Straw yield (Kg ha^{-1})
T_1 : No organic manure	801	1637
T_2: FYM equivalent to 100 % RDN	1104	2072
T_3 : FYM (50%) + Vermicompost (50%) equivalent to 100 % RDN	1227	2198
T_4: FYM (50%) + Poultry manure (50%) equivalent to 100 % RDN	1291	2270
T_5 : T_3 + Foliar spray of Panchagavya @ 3 % at 30 and 45 DAS	1406	2461
T_6: T_4 + Foliar spray of Panchagavya @ 3 % at 30 and 45 DAS	1412	2545
T_7 : T_3 + Foliar spray of Vermiwash @ 5 % at 30 and 45 DAS	1389	2402
T_8: T_4 + Foliar spray of Vermiwash @ 5 % at 30 and 45 DAS	1403	2484
T_9 : T_3 + Foliar spray of Panchagavya @ 3 % at 30 DAS and Vermiwash @ 5 % at 45 DAS	1440	2511
T_{10}: T_4 + Foliar spray of Panchagavya @ 3 % at 30 DAS and Vermiwash @ 5 % at 45 DAS	1487	2611
T_{11}: Sole foxtail millet without tree component	1656	3127
CD at 5%	90	188

NS: Not significant

components. Significantly lower gross returns were observed in sole foxtail millet with recommended organic nutrient practice without tree component (Rs 59,813 ha^{-1}). The significantly higher gross returns were with T_6 (Rs. 4, 05, 577 ha^{-1}) and T_{10}(Rs. 4, 05, 368 ha^{-1}), which were significantly superior over all other treatments. No organic manurial treatment recorded significantly lower gross returns (Rs. 3, 17, 189 ha^{-1}) in agroforestry system.

Net returns (Rs. ha^{-1}): At the end of sixth year of plantation (2019), net returns were significantly influenced by cultivation of foxtail millet with and without tree component. Significantly higher system net returns were obtained in all organic manurial treatments with agroforestry system from Rs. 2,40,937 to 3,21,056 ha^{-1} with an average of Rs. 289920 ha^{-1} over the treatment foxtail millet cultivation with recommended organic nutrient schedule in non agroforestry system (Rs. 37,111 ha^{-1}). In agroforestry system, significantly higher net returns of the system of Rs. 3 ,21, 056 ha^{-1} were obtained with T_{10}.

Benefit cost ratio: The significantly higher benefit cost ratio from the whole system was realized with application of T_{10} (4.81) followed by application of T_6 and T_7 which were significantly superior over all other treatments. Treatment T_1 recorded significantly lower benefit cost ratio in agroforestry system (4.16) compared to all the treatments. Foxtail millet cultivated organically with nutrient management schedule without tree component recorded significantly lower benefit cost ratio (2.63) over all other treatments.

Table 2. *Melia dubia* tree growth properties under organic nutrient management practices with foxtail millet inter cropping system

Treatments	Bole height (m)				DBH (cm)			
	Initial	2018	2019	Increment (%)	Initial	2018	2019	Increment (%)
T_1	10.00	10.10	10.20	1.99	62.1	63.2	63.4	2.09
T_2	9.85	10.05	10.21	3.62	61.8	62.3	64.1	3.70
T_3	9.82	10.05	10.23	4.13	62.2	63.2	64.6	3.82
T_4	9.78	10.02	10.35	5.75	63.1	64.10	65.6	3.92
T_5	9.65	09.88	10.18	5.42	63.4	64.8		4.14
T_6	9.85	10.25	10.45	6.01	63.1	64.1	66.5	5.33
T_7	9.62	09.98	10.25	6.45	64.2	65.3	66.8	4.01
T_8	9.65	09.89	10.28	6.43	59.4	60.8	65.5	5.07
T_9	9.85	10.20	10.45	6.00	62.3	63.8	65.8	5.54
T_{10}	9.7	10.15	10.42	7.30	62.0	63.8	65.9	6.04
T_{11}	-	-	-	-	-	-	-	-

See Table 1 for treatment details

Table 3. *Melia dubia* tree properties and carbon sequestration under nutrient management practices

Treatments	Wood volume (t ha^{-1})	Total biomass (t ha^{-1})	C stock above ground (t ha^{-1})	C stock below ground (t ha^{-1})	Total C stock (t ha^{-1})	C sequestration above ground (t ha^{-1})	C sequestration below ground (t ha^{-1})	Total C sequestration (t ha^{-1})
T_1	49.99	34.68	13.76	3.58	17.34	50.50	13.13	63.64
T_2	54.13	38.29	15.19	3.95	19.14	55.76	14.50	70.26
T_3	55.45	39.53	15.69	4.08	19.77	57.57	14.97	72.54
T_4	58.46	41.59	16.50	4.29	20.79	60.57	15.75	76.31
T_5	55.67	40.28	15.98	4.16	20.14	58.66	15.25	73.91
T_6	61.48	44.07	17.49	4.55	22.03	64.18	16.69	80.87
T_7	60.44	43.15	17.12	4.45	21.58	62.85	16.34	79.19
T_8	54.55	38.25	15.18	3.95	20.13	57.71	15.49	74.20
T_9	57.04	41.17	16.34	4.25	20.59	59.96	15.59	75.55
T_{10}	61.04	43.95	17.44	4.53	21.97	64.00	16.64	80.64
T_{11}	-	6.23	2.47	0.64	3.11	9.07	2.36	11.42
CD at 5%	-	4.05	1.61	0.42	2.02	5.89	1.53	7.42

It is evident that the intercropping of foxtail millet with *Melia dubia* showed maximum gross and net monetary returns when compared to sole cropping without tree component. Improved monetary returns from the system (tree + crop) are mainly due to higher biomass production from the tree in the form of timber with better performance of foxtail millet under organic nutrient management practices. This clearly shows that arable crops like foxtail millet when grown as an intercrop with the trees exhibit compatibility with the trees in mutual sharing of the natural resources available. Agroforestry practices fetched higher returns when compared to sole crop. These results are in accordance with results obtained by Chandana et al (2020) in pearl millet with *Melia dubia* based agroforestry systems.

CONCLUSION

By practicing climate smart practices in *Melia dubia* based agroforestry system, foxtail millet could able to show their potential yield even under shade stress condition which is mainly attributed to the application of organic manures like FYM, poultry manure and vermicompost in combination with foliar spray of panchagavya and vermicompost. The present study highlights, *Melia dubia* + foxtail millet agroforestry system as a better option than sole agricultural cropping. The combination of crop with tree in the study led to higher

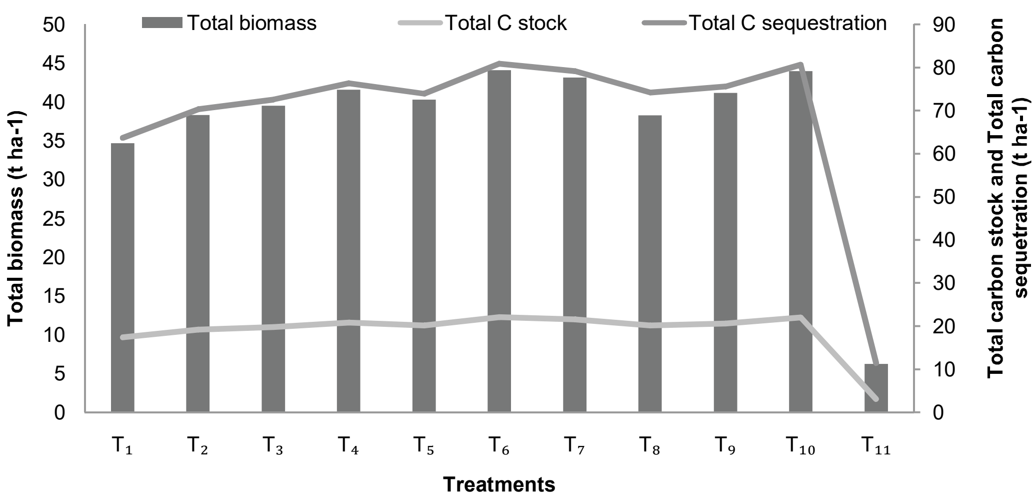

Fig. 1. *Melia dubia* tree total biomass, total c stock and total C sequestration under organic nutrient management practices foxtail millet- *Melia dubia* agroforestry system

Table 4. System economic analysis

Treatments	Agroforestry system (Crop + *Melia dubia*) at the end of 6th year of plantation (2019)			
	Gross returns (Rs. ha^{-1})	Cost of cultivation (Rs. ha^{-1})	Net returns (Rs. ha^{-1})	B : C ratio
T_1	317189	76252	240937	4.16
T_2	352032	83952	268080	4.19
T_3	364226	83857	280369	4.34
T_4	384215	83062	301153	4.63
T_5	371593	85657	285937	4.34
T_6	405577	84862	320715	4.78
T_7	399735	84557	315179	4.73
T_8	353976	83762	270214	4.23
T_9	380662	85107	295555	4.47
T_{10}	405368	84312	321056	4.81
T_{11}	59813	22702	37111	2.63
CD at 5%	3833	-	3833	0.09

biomass and carbon sequestration which is of positive benefit in mitigating climate change and maintaining ecological balance.

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Microbial Influence on Climate Change: Drivers, Mediators, and Mitigators of Global Environmental Shifts

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Abstract: Microorganisms are fundamental to the climate system, influencing global environmental changes in profound and often underappreciated ways. These microscopic organisms play crucial roles as both drivers and mediators of climate change, particularly through their involvement in key biogeochemical cycles, including the carbon, nitrogen, and sulfur cycles. Microbial activities contribute to the production and consumption of greenhouse gases such as carbon dioxide, methane, and nitrous oxide, thereby directly impacting atmospheric composition and climate dynamics. This abstract explores the dual role of microorganisms in climate change-both as contributors to global warming and as potential agents of climate change mitigation. It reveals how microbial processes in diverse ecosystems, such as soils, oceans, and wetlands, contribute to the release and sequestration of greenhouse gases. The paper also highlights the role of greenhouse microbes in carbon cycling, including the decomposition of organic matter, soil respiration, and the formation of carbon sinks, which are critical in regulating atmospheric CO_2 levels. Furthermore, the potential of microorganisms to mitigate climate change isfocusing on emerging biotechnological approaches. These include the enhancement of microbial processes for carbon capture and storage, the development of biofuels, and the use of microbes in bioremediation to reduce greenhouse gas emissions from agricultural and industrial activities. Additionally, the impact of climate change on microbial communities is, how shifts in temperature, moisture, and pH levels influence microbial diversity and ecosystem functions. This paper emphasizes the importance of integrating microbial science into climate models and environmental policies to effectively address the challenges posed by global climate change.

Keywords: Climate change, Microbial communities, Organic matter, Ecosystem, Greenhouse gases, Bioremediation, Greenhouse microbes, Carbon cycling

Climate change stands as one of the most critical global challenges of the 21st century, exerting far-reaching effects on ecosystems, biodiversity, and human societies.(Bardgett and Van Der Putten 2014). While considerable focus has been placed on the contribution of greenhouse gases, deforestation, and industrial activities to the acceleration of climate change, there is growing recognition of the significant role that microorganisms play in shaping the Earth's climate. Microbes including bacteria, archaea, fungi, and viruses are omnipresent in the environment and possess the remarkable ability to influence climate through diverse mechanisms. This essay delves into the intricate roles of microorganisms as drivers, mediators, and mitigators of global environmental changes, underscoring their essential contributions to the dynamics of climate chang. The Intergovernmental Panel on Climate Change (IPCC) Report (2022) highlights the interconnectedness of climate change with biodiversity and its far-reaching effects on ecosystems and human well-being. It stresses the critical need for conservation to mitigate climate change impacts.

Microbes influence climate processes in several ways. As drivers, they contribute to the production and consumption of key greenhouse gases such as carbon dioxide, methane, and nitrous oxide. Methanogenic archaea, for example, produce methane, a potent greenhouse gas, during the decomposition of organic matter in anaerobic environments such as wetlands and rice paddies. In contrast, methanotrophic bacteria mitigate methane emissions by consuming this gas before it can enter the atmosphere. Similarly, nitrifying and denitrifying bacteria play crucial roles in the nitrogen cycle, influencing the production and release of nitrous oxide, another potent greenhouse gas.The Convention on Biological Diversity's (CBD) 2023 statement emphasizes the crucial role biodiversity plays in both climate change mitigation and adaptation. The document stresses that conserving biodiversity is essential in addressing the challenges posed by climate change.

As mediators of climate change, microbes regulate the biogeochemical cycles of carbon, nitrogen, sulfur, and phosphorus, which are critical for the stability of ecosystems (Kang 2021).The microbial decomposition of organic matter is a fundamental process that controls the release of carbon dioxide into the atmosphere, while microbial activity in soil and ocean environments determines the sequestration of carbon, effectively influencing carbon sinks (Hutchins and Fu 2017). Moreover, microbial interactions with plants, through symbiotic relationships such as nitrogen fixation, also contribute to the regulation of atmospheric carbon and nitrogen levels (Tellerson 2024).

In the context of climate change mitigation, microbes offer promising avenues for carbon capture and storage. Microbial communities in the ocean, for instance, contribute to the biological pump, where carbon is transferred from the atmosphere to the deep ocean through photosynthesis and subsequent sinking of organic matter. Additionally, engineered microbes are being explored for their potential to enhance carbon sequestration in soils and promote bioenergy production, offering sustainable solutions for reducing greenhouse gas emissions. The roles of microbes in climate change are complex and multifaceted, encompassing their capacities as both contributors and potential mitigators of global environmental shifts (Timmis et al 2019, Rillig et al 2019). Understanding and harnessing microbial processes hold immense promise for addressing the challenges of climate change, providing innovative approaches to mitigate its impacts on ecosystems and human societies. As research in microbial ecology and climate science continues to evolve, it is crucial to integrate microbial processes into global climate models to develop more comprehensive strategies for climate adaptation and mitigation. (Bond-Lamberty and Thomson 2010).

Microbes as Drivers on Climate Change

Microorganisms are integral components of the Earth's biogeochemical cycles, playing a pivotal role in the cycling of carbon, nitrogen, sulfur, and other elements. These cycles are tightly linked to the regulation of greenhouse gases, such as carbon dioxide (CO_2), methane (CH+), and nitrous oxide (N+O), all of which are significant contributors to climate change. While microbes are essential for the functioning of these cycles, their activities can both mitigate and exacerbate climate change, positioning them as key drivers in the climate system (Singh et al 2010, 2019).

Carbon Cycle and Microbial Activity

Microbes play a central role in the global carbon cycle through processes such as carbon fixation, decomposition, and respiration. Photosynthetic microorganisms, including cyanobacteria and microalgae, significantly contribute to carbon sequestration by converting atmospheric CO2 into organic matter via photosynthesis (Iglesias-Rodriguez et al 2008). This natural process is a vital mechanism for reducing atmospheric CO2 levels, as it captures carbon and incorporates it into biomass. These microbial processes are particularly critical in marine environments, where large-scale carbon fixation occurs, contributing to long-term carbon storage in deep-sea sediments (Grossart and Schlingloff 2021a&b, Wang et al 2021). However, the balance between carbon sequestration and carbon release is influenced by microbial respiration, which converts organic carbon back into CO_2. In ecosystems like tropical forests and wetlands, where organic matter is rapidly decomposed, microbial respiration can offset the carbon sequestered by photosynthesis (Yang et al 2023). The release of CO_2 through this pathway illustrates the complexity of microbial contributions to the carbon cycle (Tao et al 2023). Li et al (2024) reviewed the microbial mechanisms behind carbon storage and decomposition, particularly focusing on the genetic basis of microbial communities involved in soil carbon dynamics and their role in global carbon cycles. Moreover, methanogenic archaea, which thrive in anaerobic environments such as wetlands, rice paddies, and the digestive tracts of ruminants, produce methane (CH_4), a greenhouse gas with a global warming potential far greater than CO_2 (Thauer et al 2008). The substantial release of methane into the atmosphere from these environments highlights the dual role of microbes in both mitigating and exacerbating greenhouse gas emissions. As a potent greenhouse gas, methane contributes to climate change, underscoring the need to better understand microbial processes in these ecosystems to manage their impact on global warming (Awala et al 2024). Recent studies highlight the vital roles of microbes in climate change mitigation. Microbial communities, particularly in ocean ecosystems, contribute significantly to carbon sequestration through processes like the microbial carbon pump (MCP). This mechanism transforms labile organic carbon into refractory dissolved organic carbon (RDOC), allowing for long-term carbon storage in the ocean's deep waters. MCP plays a complementary role to the biological pump, which transports particulate organic carbon from surface waters to the seafloor (Zhonghe Zhou and Zhengtang Guo 2023). This study explores how microbial processes in the ocean contribute to carbon sequestration, with a focus on the biological pump and microbial carbon pump. In addition to oceanic processes, soil microbes are critical for carbon storage on land. Researchers have recently integrated microbial DNA data into climate models, enhancing the understanding of how soil microbes store carbon from plant roots. This could lead to improved strategies for increasing soil carbon sequestration, contributing to both sustainable agriculture and climate change mitigation (Brodie et al 2024). This research integrates genetic information from soil microbes into climate models, providing insights into their role in carbon sequestration and climate change mitigation. Trivedi et al (2022) explained the microbial involvement in the carbon cycle, highlighting the potential of microbial communities to mitigate greenhouse gas emissions under climate change.

Nitrogen cycle and greenhouse gas emissions: Microbial activity is also at the heart of the nitrogen cycle, a crucial process for ecosystem health and productivity. Nitrogen-fixing bacteria, such as those in the genera *Rhizobium* and *Azotobacter*, convert atmospheric nitrogen (N_2) into ammonia (NH_3), a form of nitrogen that plants can utilize (Falkowski et al 2008). This microbial process is essential for the growth of terrestrial and aquatic ecosystems, driving primary productivity and supporting the food web. However, microbial processes like nitrification and denitrification can result in the production of nitrous oxide (N_2O), a greenhouse gas with a global warming potential nearly 300 times that of CO_2. Nitrifying bacteria, such as *Nitrosomonas*, oxidize ammonia to nitrate, while denitrifying bacteria, such as *Pseudomonas*, reduce nitrate to N_2O and N_2 under anaerobic conditions (Forster et al 2021, Stein and Klotz 2016).The release of N_2O from agricultural soils, driven by microbial activity, is a significant source of this potent greenhouse gas, particularly in regions with intensive farming practices (Thompson et al 2017, Jin et al 2022). This highlights the importance of managing agricultural systems to mitigate microbial-driven N_2O emissions and reduce their contribution to climate change (Zehr and Kudela 2011, Butterbach-Bahl et al 2020).

Sulfur Cycle and Climate Regulation

Microbes also influence the sulfur cycle, with significant implications for climate regulation. Marine bacteria, such as those in the genus *Thiomicrospira*, are involved in the oxidation of sulfide to sulfate, a process that can lead to the formation of sulfur aerosols in the atmosphere (Hutchins and Fu 2017, Moran and Durham 2023). These sulfur aerosols reflect sunlight away from the Earth's surface, contributing to a cooling effect on the climate. This microbialdriven process illustrates how sulfur cycling can influence the Earth's energy balance and moderate global temperatures. In addition, sulfur compounds such as dimethyl sulfide (DMS), produced by marine phytoplankton, serve as precursors to sulfate aerosols. These aerosols play a critical role in cloud formation, potentially altering weather patterns and influencing climate dynamics (Wang et al 2021). The complex interactions between sulfur-metabolizing microbes and atmospheric processes underscore the intricate ways in which microbial activity can drive climate regulation (Charlson et al 1987, Zhou et al 2021). The cooling effect associated with sulfur aerosols, though localized, demonstrates how microbial processes can have both warming and cooling effects on the climate, depending on the context.

Microbial mediation of plant-climate interactions: Microbes also mediate critical plant-climate interactions, influencing ecosystem responses to environmental changes. Mycorrhizal fungi, for example, form symbiotic relationships with plants, enhancing nutrient and water uptake. This interaction can significantly affect plant growth and carbon sequestration, particularly in forests and grasslands (Wagg et al 2021). Climate change-induced shifts in soil temperature and moisture can alter microbial community composition, which in turn impacts plant health and resilience to climate stressors such as drought and heat (Leifheit et al. 2020). In addition, rhizosphere microbes influence soil carbon cycling by modulating root exudates and organic matter decomposition (Bahram et al 2021, Terrer et al 2021, Delgado-Baquerizo et al 2020). Climate change can disrupt these microbial-plant interactions, potentially reducing ecosystem carbon storage capacity and altering the overall carbon balance. The interplay between microbial communities, plant responses, and climate stressors underscores the importance of microbial dynamics in shaping ecosystem resilience (Rastogi and Sani 2011).

Microbes as mitigators of climate change: Microbes can contribute to climate change through the production of greenhouse gases like methane and nitrous oxide, they also hold great potential as key players in climate change mitigation. Cavicchioli et al (2020) Harnessing their metabolic processes for carbon sequestration, bioremediation, and sustainable agriculture presents a promising path forward (O'Malley 2021). This area of research is rapidly advancing as scientists explore how microbial communities can be managed or engineered to reduce greenhouse gas emissions and enhance carbon storage.

Carbon sequestration by microbial communities: Microbial communities in soils and oceans are critical for carbon sequestration, and strategies to enhance this natural process are being explored as a way to mitigate climate change.

Soil carbon sequestration: Soil microbes, including bacteria and fungi, play a key role in converting plant-derived organic carbon into stable forms of soil organic carbon (SOC). The process occurs through microbial decomposition and transformation of organic material, resulting in the formation of humus-a stable form of organic matter that can persist for centuries (Delgado-Baquerizo et al 2020, Jansson and Hofmockel 2020). This microbial-driven process is critical for long-term carbon storage. Microbial degradation of plant residues involves pathways such as cellulose degradation (cellulases) and lignin breakdown (laccases, peroxidases). Soil microbial respiration and carbon stabilization pathways are linked to the production of humic substances that bind to soil minerals, creating long-term carbon sinks. Enhancing SOC storage can be achieved through sustainable agricultural practices, such as no-till farming, which minimizes soil disturbance, and the use of cover crops, which promotes microbial activity and carbon storage. These practices also improve soil health and reduce carbon losses from the soil (Jansson and Hofmockel 2020).

Marine carbon sequestration: In marine ecosystems, microbes contribute to the "biological pump" a process where phytoplankton capture atmospheric CO_2 via photosynthesis and then transfer this carbon to deeper ocean layers when they die and sink (Lal 2004). Heterotrophic bacteria then contribute to the degradation and mineralization of organic matter in the deep ocean, sequestering carbon in sediments (Paterson et al 2023). Photosynthetic carbon fixation (via the Calvin cycle) by phytoplankton. Microbial degradation and remineralization of organic carbon by bacteria, archaea, and other microorganisms (Lehmann and Kleber 2020). Enhancing the biological pump through nutrient fertilization, such as iron fertilization in iron-limited ocean regions, has been proposed as a geoengineering strategy (Louca et al 2016, Moran 2015). However, potential ecological impacts, such as oxygen depletion and shifts in marine food webs, require careful evaluation.

Bioremediation of greenhouse gases: Microbial bioremediation offers the potential to mitigate climate change by reducing atmospheric concentrations of potent greenhouse gases such as methane (CH_4) and nitrous oxide (N_2O).

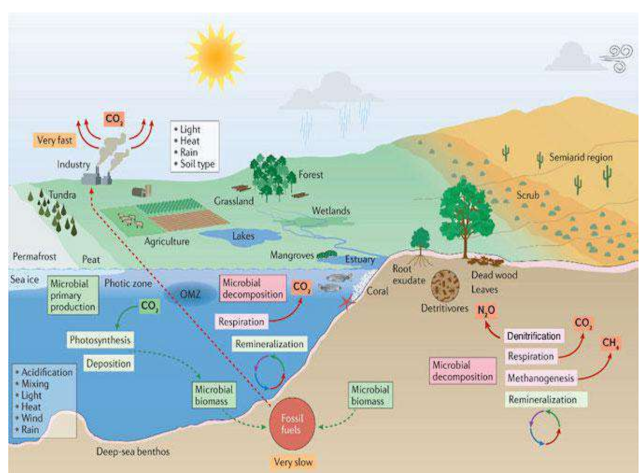
Methane mitigation: Methanotrophic bacteria, which metabolize methane as their carbon and energy source, can oxidize CH_4 into less potent CO_2. These bacteria are found in various environments such as wetlands, rice paddies, and landfill covers. Strategies to enhance methanotrophic activity, such as biofiltration in landfills and wetlands, are being explored to reduce methane emissions (Knief 2015, Conrad 2021). Methane oxidation via the methane monooxygenase (MMO) enzyme system in methanotrophic bacteria, converting CH_4 to CO_2 and water (Alkhatiband Del Giorgio 2021).

Nitrous oxide mitigation: Certain denitrifying bacteria can reduce N_2O emissions from soils through the denitrification process, converting N_2O into N_2, an inert gas. Denitrifying bacteria such as *Pseudomonas* and *Paracoccus* play critical roles in this process, which occurs under anaerobic conditions in soils. Denitrification pathway, involving enzymes like nitrite reductase (Nir), nitric oxide reductase (Nor), and nitrous oxide reductase (Nos), converting nitrate to dinitrogen gas (N_2). Optimizing soil management practices, such as improving soil aeration and balancing nitrogen inputs, can enhance microbial processes that reduce N_2O emissions from agriculture.

Microbial biotechnology for climate mitigation: Advances in microbial biotechnology present exciting opportunities for climate change mitigation, particularly through synthetic biology and bioengineering approaches. Synthetic biology is being used to engineer microbes with enhanced abilities to fix atmospheric CO_2 or convert it into valuable products (Krause et al 2022). For example, bioengineered strains of *Escherichia coli* or cyanobacteria can be optimized for more efficient CO_2 fixation or for producing biofuels and bioplastics (Trivedi et al 2020). Pathways Involved: Calvin-Benson cycle in autotrophic microbes, responsible for carbon fixation. Genetic engineering to enhance key enzymes like ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO).

Microbial fuel cells (MFCs): MFCs leverage electrogenic bacteria such as *Geobacter* and *Shewanella* to generate electricity by oxidizing organic matter (Lovley 2008). MFCs provide a dual benefit of producing renewable energy and treating wastewater, reducing both emissions and environmental pollution (Yassaa et al 2008). Electron transport chains in electrogenic bacteria that facilitate extracellular electron transfer to electrodes, generating electricity.

Microbial contributions to sustainable agriculture: Microbes can contribute significantly to sustainable agricultural practices, which, in turn, play a role in mitigating climate change. Biofertilizers: The use of biofertilizers containing beneficial microbes, such as nitrogen-fixing bacteria (*Rhizobium, Azospirillum*) and mycorrhizal fungi, reduces the need for synthetic fertilizers. This, in turn, decreases N_2O emissions associated with fertilizer



Source: National Library of Medicine

Microbes in aquatic and terrestrial environments produce and consume the greenhouse gases CO_2, CH_4 and N_2O. Soil and aquatic microbes produce these gases when decomposing organic matter to provide nutrients for plants and marine life, respectively.

application. Nitrogen fixation pathway, involving nitrogenase enzymes in nitrogen-fixing bacteria. Symbiotic associations between mycorrhizal fungi and plant roots, improving nutrient uptake and soil structure (Tedersoo et al 2020). Promoting microbial diversity through practices like crop rotation and the use of cover crops improves soil health, enhances carbon sequestration, and increases crop resilience to climate stresses.

Microbial influence on cloud formation: Microbial communities also play a critical yet underappreciated role in cloud formation and, consequently, in regulating Earth's climate. Certain microbes, particularly those found in marine and terrestrial environments, produce volatile organic compounds (VOCs) such as isoprene and terpenes (Rosenfeld et al 2022, Yuan et al 2021, Böck et al 2022). These VOCs contribute to the formation of secondary organic aerosols (SOAs), which act as cloud condensation nuclei (CCN). The presence of CCN is essential for cloud formation, which can influence local and global climate by affecting the Earth's albedo, or the reflectivity of the planet's surface (Yuan et al 2021, Lehmann and Kleber 2020).

The microbial production of VOCs and their impact on atmospheric processes highlight an important feedback mechanism that is not yet fully understood. This area of research is critical for better understanding how microbial activities influence cloud properties, precipitation patterns, and overall climate dynamics (Yuan et al 2021, Banerjee et al 2021, Klein et al 2023). Microbial inoculants, designed to promote the growth of specific beneficial microbes, are also being developed to enhance themicrobes as key drivers: Microorganisms play a critical role in driving climate change through processes like carbon cycling, methane production, and nitrogen fixation (Banerjee et al 2021). Their activities significantly influence the balance of greenhouse gases, which directly impacts global temperature and climate patterns.

Mediators of environmental shifts: Microbial communities act as mediators in ecosystems, modulating the effects of climate change on various biogeochemical cycles (Lehmann and Kleber 2020). Their adaptive responses to environmental changes can either exacerbate or mitigate climate change impacts, depending on the conditions.

Potential as mitigators: Certain microbial processes offer potential mitigation strategies for climate change. For example, microbes involved in carbon sequestration, bioremediation, and bioenergy production can reduce greenhouse gas emissions and stabilize atmospheric carbon levels.

Interconnectedness of microbial functions: The complex interplay between microbial functions and climate systems underscores the need for a comprehensive understanding of microbial ecology. This knowledge is crucial for developing effective strategies to harness microbial potential in combating climate change.

CONCLUSIONS

Microorganisms are pivotal in influencing climate change through their roles as drivers, mediators, and potential mitigators of global environmental shifts. As drivers, microbes significantly contribute to the carbon and nitrogen cycles, influencing the concentration of greenhouse gases like carbon dioxide, methane, and nitrous oxide in the atmosphere. Their metabolic activities can accelerate climate change, particularly through the production of potent greenhouse gases. As mediators, microbial communities adapt to and modulate environmental changes. Their responses to temperature fluctuations, nutrient availability, and other climate-related factors can either amplify or dampen the effects of climate change. This mediation is complex and context-dependent, with microbial interactions often dictating the resilience or vulnerability of ecosystems under stress. Importantly, microbes also hold potential as mitigators of climate change. Processes like microbial carbon sequestration, methane oxidation, and bioenergy production can reduce atmospheric greenhouse gas levels, offering sustainable solutions to environmental challenges. Harnessing these microbial processes requires a deeper understanding of microbial ecology and its integration with climate science. Future research should focus on unravelling the intricate mechanisms by which microbes influence climate systems and exploring their potential in mitigating climate change. By advancing our knowledge in this area, we can develop innovative strategies to manage microbial processes for environmental stability. Microbes are integral to the Earth's climate system, acting as both contributors to and potential solutions for climate change. Their influence underscores the need for continued research and the integration of microbial science into climate change mitigation strategies.

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Identifying Drought Tolerant Genotypes in Okra with Physiological and Molecular Approaches

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***Abstract*:** Okra (*Abelomoschus esculentus* L.) is an annual herbaceous plant and belongs to family Malvaceae. Drought stress affects okra growth and productivity, disrupting physiological functions and the photosynthetic rate, resulting in yield losses. By understanding the physiological response of okra genotypes under drought stress conditions is critical to the selection of drought-tolerant accessions. Hence, to identify drought tolerance lines, initially selected thirty genotypes for preliminary drought screening, later advanced with six relatively promising genotypes *viz.* P-8, White velvet, Arka abhay, Parbani kranti and Arka anamika and Bagalkot local were considered for further study. The plants were exposed different water potential levels stresses by induced slanting glass plate technique in the laboratory. The genotypes performance was analysed at different Poly ethylene glycol-6000 osmotic stress concentrations for germination, RWC, SPAD value, and root traits up to 22 days at seedling stage revealed Arka abhay and Parbani kranti lines as relatively drought tolerant. These genotypes further exposed to water stress (65% Field capacity) with control under field evaluation. The genotypes Arka abhay and Parbani kranti were drought tolerant genotypes with higher RWC, root length, lateral roots, chlorophyll and pod yield under induced stress conditions. The genotypes were analyzed for genetic diversity with ISSR DNA molecular markers. The dendrogram revealed tolerant lines were grouped in one cluster and the rest in other clusters. Arka abhay and Parbani kranti were relatively drought tolerant and could be suitable to grow under mild water stress condition.

***Keywords*:** Chlorophyll, Drought, ISSR, Okra, PEG-6000, Roots, SLW, Water potential

Okra is a commercial vegetable crop belongs to family Malvaceae which can be grown on wide range of soils, but well drained fertile soils with adequate organic matter result to high yield (Akinyele and Temikotan 2007). The crop is widely cultivated throughout the year in the tropics. Drought is one of the major ecological factors limiting crop production and food quality globally, especially in the arid and semi-arid areas of the world. Drought alone accounts for yield losses ranging between 30 and 100% in okra, primarily when the stress occurs during the flowering and pod-filling stages (Mkhabela et al 2021). Okra plant plants subjected to a low level of stress (watered once a week) performed better than those moderately stressed. Water stress is one of the limiting factors in crop growth and yield which reduces dry matter production, yield and yield components through decreasing leaf area and accelerating leaf senescence (Emam and Seghatoleslami 2005). One of the most common methods used to determine the tolerance of plants to abiotic stresses is the evaluation of the germination capacity of seeds under abiotic stress conditions (Larcher 2000). Hence, in this direction conducted a study with an objective of screening of the okra genotypes by physiological drought tolerant traits under both laboratory and field ultimately to find out relatively drought tolerate okra lines.

MATERIAL AND METHODS

The experiment was conducted during the years 2014-15 and 2015-16 at University of Horticultural Science, Udyanagiri, Bagalkot, Karnataka, India. It is situated at 14˚47' Northern latitude, 75˚59' East longitude and at an altitude of 612.05 meters above the mean sea level. Initially thirty okra genotypes were screened for water stress basal study. Out of which we chose six relatively promising genotypes for further extensive drought tolerance study.

Laboratory study by PEG-6000: The experiment has been laid out in factorial randomized block design with main factor (Factor 1) includes four PEG-6000 concentrations whereas, sub factor (Factor 2) includes six genotypes (P-8, White velvet, Arka Abhay, Parbhani Kranti, Arka Anamika and Bagalkot local) with three replications. The experiment was carried out in the laboratory condition under ambient temperature. To induce water stress, 0%, 5%, 10% and 15% Poly ethylene glycol 6000 (PEG-6000) concentrations solutions were used for the study. It represents the decrease in water potential as the concentration of the PEG-6000 solutions increases. The seeds were allowed to grow in above PEG-6000 solutions by slanting glass plate technique. The observations *viz.,* seed germination (%), SPAD value, shoot length(cm), root length(cm), number of lateral roots and relative water content were recorded with 14 and 22 days after sowing.

Field evaluation by induced water stress: Under field study, six okra genotypes were grown under two water regimes conditions *viz.* well irrigated condition and water stressed condition (65% field capacity). The field capacity was maintained by controlled intervals water supply to plots. The experiment was laid out in split plot design with main factor (Factor 1) includes well irrigated and water stress condition whereas the six okra genotypes as sub factor (Factor 2) with three replications. Under induced water stress level, the genotypes were evaluated for plant height, number of leaves per plant, relative water content (RWC), root length, number of lateral roots, SPAD value, pod length and pod yield were recorded at 60 days after sowing(DAS).

Genetic diversity analysis by ISSR molecular markers: To know the genetic diversity of the among the okra genotypes the genotypic variations was analysed by using DNA based ISSR molecular markers. Fresh Leaf samples were harvested and homogenized to fine powder in liquid nitrogen (-80°C) using mortar and pestle. Total DNA was extracted from fresh leaves of individual lines using cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1990). Annealing temperature for all the twenty ISSR primers was standardized by polymerase chain reaction.

PCR conditions: The test solutions were made up to a final volume of 20µl containing 50 ng of template DNA, 10 pM decamer primer, 1x reaction buffer, 0.1 mM dNTP mix and 1.0 U Taq DNA Polymerase. The amplification was performed using Eppendorf Thermocycler with a hot start for 2 minutes at 94ºC; followed by 35 cycles of denaturing at 94°C for 30 seconds; annealing for 30 seconds; and product extension for 5 minutes at 72°C. The PCR products after ISSR amplification were analyzed in 1.5% agarose gel containing Ethidium bromide electrophoresis system to resolve the different molecular configuration of a DNA molecule as well as to separate DNA fragments of different weights. DNA was stained by soaking the gel in a 0.5-mg/mL ethidium bromide solution and visualized under Gel documentation system. The genetic polymorphism was analyzed and the Dendrogram obtained from ntysis software for genetic similarity study of six okra lines.

RESULTS AND DISCUSSION

Laboratory Evaluation by PEG-6000

Germination (%): The seed germination percentage decreased with the decrease in water potential or by increasing PEG concentrations. The highest mean of germination percentage at all the PEG concentrations recorded in the genotypes Arka abhay (95.83) and P-8(95.83), whereas, least in Arka anamika (87.5%). However, interaction effect was non-significant (Table 1). Similar trend was observed by Larcher (2000).

SPAD values: The SPAD values was recorded at 22 days after sowing was decreased with increase of the water PEG-6000 stress levels. The mean SPAD value at PEG solutions with 0% and 15% PEG concentrations were 27.40 and 14.90 respectively. Significantly highest mean SPAD value at all the PEG concentrations recorded in Parbani kranti (24.17) followed by Arka abhay (22.79), whereas, least in Bagalkot local (19.08), however their interaction effect was non-significant .Decrease in chlorophyll content under drought

Table 1. Effect of PEG-6000 water stress on seed germination and SPAD values of okra

Genotype	Seed germination (%)					SPAD values at 22DAS				
	PEG 6000 concentration (%)				Mean	PEG 6000 concentration (%)				Mean
	0	5	10	15		0	5	10	15	
P-8	96.67	96.67	96.67	93.33	95.83	27.73	24.47	20.60	15.17	21.99
White velvet	96.67	96.67	93.33	90.00	94.17	26.37	22.40	18.20	13.70	20.17
Arka Abhay	96.67	96.67	96.67	93.33	95.83	28.47	25.43	21.13	16.13	22.79
Parbhani Kranti	96.67	93.33	93.33	90.00	93.33	30.13	26.27	23.10	17.17	24.17
Arka Anamika	96.67	86.67	86.67	80.00	87.50	27.23	23.13	19.40	14.23	21.00
Bagalkot Local	96.67	96.67	93.33	86.67	93.33	24.47	21.60	17.27	13.00	19.08
Mean	96.67	94.44	93.33	88.89	93.33	27.40	23.88	19.95	14.90	21.53
For comparing	CD at 1%					CD at 1%				
Factor1	3.760					1.329				
Factor 2	NS					1.049				
Interaction	NS					NS				

stress conditions could be related to photo-oxidation resulting from oxidative stress which reduces the photosynthetic process in plants, results were in accordance with earlier findings (Ashraf 2009, Altaf et al 2015).

Shoot length (cm): The shoot length was decreased with increasing PEG concentration percentage. At 14 DAS the mean seed shoot length at PEG solutions with 0% and 15% PEG concentrations were 8.85cm and 7.46cm respectively. The significantly highest mean of shoot length at all the PEG concentrations in Parbani kranti (9.13) whereas, least in Bagalkot local (7.73). In their interaction effect the genotype Parbani kranti recorded significantly highest shoot length (9.60) among all the genotypes and in all the PEG concentrations. At 22DAS, significantly highest mean of shoot length at all the PEG concentrations was in the genotype Parbani kranti (11.73) whereas, least in Bagalkot local (9.50). The genotype Parbani kranti recorded significantly highest shoot length (13.40) in all the PEG concentrations (Table 2). Bhatt and Rao, (2005 observed that reduction in shoot length and plant height was associated with a decline in the cell enlargement and more leaf senescence in *A. esculentus* under water stress.

Root length (cm): The root length was increased with the decrease in water potential (or by increasing PEG concentration). At 14DAS, significantly highest mean of root length at all the PEG concentrations recorded in the genotype Parbani kranti (12.82) whereas, least in Bagalkot local (11.45). In their interaction effect the genotype Parbani kranti recorded significantly highest root length (14.70). At 22DAS, there was significantly highest root length was recorded in Arka abhay (22.85) whereas, least in Arka anamika (19.63). In their interaction, Arka abhay recorded significantly highest root length (25.10) (Table 3). Such increase in linear growth of roots is possessed by either increase of gibberlins or cytokinins or to the ability of roots to branch and elongate quickly in try to acquire underground water to tolerate the stress conditions, which thus enable plants to survive properly irrespective of water stress. Similar results were also reported by Kader et al (2014) and Hasan et al (2018).

Number of lateral roots: The number of lateral roots was increased with the increasing PEG concentration. At 14DAS, significantly highest mean of number of lateral roots at all the PEG concentrations recorded in Parbani kranti (12.82) whereas, least in Bagalkot local (11.45). In their interaction effect also Parbani kranti recorded significantly highest number of lateral roots (27.90) among all the genotypes and in all the PEG concentrations. At 22DAS, significantly highest mean of number of lateral roots at all the PEG concentrations recorded in Arka abhay (33.48) whereas, least in white velvet local (29.88). In their interaction effect also Arka abhay (37.00) was significantly more number of lateral roots (Table 4).

Relative water content: The leaf relative water content (RWC %) was decreased with the increasing PEG concentrations. At 14DAS, significantly highest RWC % recorded in Arka abhay (81.58) whereas, least in Bagalkot local (76.05). In their interaction effect Arka abhay recorded significantly highest RWC % (90.50) among all the genotypes and in all the PEG concentrations compared to others. At 22DAS, significantly highest mean RWC % at all the PEG concentrations recorded in P-8 (73.30) followed by Parbani kranti (72.45), whereas, least in Bagalkot local (71.03). In their interaction effect P-8(87.6) recorded significantly

Table 2. Effect of PEG-6000 water stress on shoot length of okra at 14 and 22 days after sowing

Genotype	Shoot length at 14 DAS					Shoot length at 22 DAS				
	PEG 6000 concentration (%)				Mean	PEG 6000 concentration (%)				Mean
	0	5	10	15		0	5	10	15	
P-8	9.20	9.00	9.00	8.00	8.80	12.20	11.80	10.90	9.50	11.10
White velvet	8.90	8.70	8.60	7.80	8.50	11.77	11.60	10.50	9.20	10.77
Arka Abhay	8.50	8.20	8.00	6.90	9.13	11.00	10.83	9.90	8.60	10.08
Parbhani Kranti	9.60	9.40	9.20	8.33	9.13	13.40	12.10	11.30	10.10	11.73
Arka Anamika	8.70	8.40	8.17	7.40	8.17	11.27	11.07	10.20	8.87	10.35
Bagalkot Local	8.20	8.20	8.20	6.30	7.73	10.20	10.50	9.60	7.70	9.50
Mean	8.85	8.65	8.53	7.46	8.58	11.64	11.32	10.40	8.99	10.6
For comparing	CD at 1%					CD at 1%				
Factor1	0.207					0.123				
Factor 2	0.159					0.289				
Interaction	0.326					0.536				

highest RWC % compared to others genotypes (Table 5). This decline of relative water content is consistent with earlier studies (Zhang et al 2010, Prabhakar et al 2018).

Field evaluation by induced water stress: Six okra genotypes were evaluated for drought tolerance under water stress condition (65% field capacity) and well irrigated condition as control.

Plant height (cm): At 60 DAS there was a significance difference for genotypes and water levels and their interaction effect. There was significantly higher mean plant height in well irrigated condition (63.0) compared to water stress condition (54.7). In well irrigated regime among the genotypes Arka anamika (64.4) showed significantly higher plant height whereas, least recorded in Bagalkot local (59.6). In water stress regime among the genotypes White velvet (57.8) showed significantly higher plant height whereas, least in Bagalkot local (51.8). In their interaction effect, Arka anamika (64.4) recorded significantly highest plant height among all genotypes and in both the water regime levels (Table 6). Altaf et al (2015) observed that drought reduced the plant height and maximum reduction in plant height was observed in highest levels of drought (50%) than the lower levels and concluded that drought up to 50% could be fatal for okra but plant can survive at low level of drought. Reduction in plant height under severe moisture stress, could be due to decrease in cell elongation and cell division so it gradually reduces leaf area. The results are in agreement with the results obtained by Kader et al (2010).

Table 3. Effect of PEG-6000 water stress on root length(cm) in okra at 14 and 22 days after sowing

Genotype	Root length(cm) at 14 DAS					Root length(cm) at 22 DAS				
	PEG 6000 concentration (%)				Mean	PEG 6000 concentration (%)				Mean
	0	5	10	15		0	5	10	15	
P-8	11.00	11.17	13.20	12.37	11.93	19.00	20.40	22.00	24.00	21.35
White velvet	10.30	10.20	12.27	13.80	11.64	18.20	19.90	21.20	22.70	20.50
Arka Abhay	11.90	12.80	12.00	13.40	12.53	21.60	21.30	23.40	25.10	22.85
Parbhani Kranti	11.40	11.50	13.67	14.70	12.82	19.60	20.70	22.50	24.20	21.75
Arka Anamika	9.70	10.00	12.80	14.20	11.68	17.00	18.60	20.60	22.30	19.63
Bagalkot Local	10.60	10.70	11.50	13.00	11.45	18.70	20.20	21.60	23.23	20.93
Mean	10.82	11.06	12.57	13.58	12.0	19.02	20.18	21.88	23.59	21.2
For comparing					CD at 1%					CD at 1%
Factor1					0.297					0.675
Factor 2					0.221					0.416
Interaction					0.458					0.905

Table 4. Effect of PEG-6000 water stress on number of lateral roots at 14 and 22 days after sowing

Genotype	Number of lateral roots at 14 DAS					Number of lateral roots at 22 DAS				
	PEG 6000 concentration (%)				Mean	PEG 6000 concentration (%)				Mean
	0	5	10	15		0	5	10	15	
P-8	20.60	23.00	24.50	26.70	11.93	27.80	30.50	32.00	35.57	31.47
White velvet	18.93	22.23	23.60	25.70	11.64	26.20	28.73	31.00	33.60	29.88
Arka Abhay	22.40	24.83	25.40	27.90	12.53	30.60	32.30	34.00	37.00	33.48
Parbhani Kranti	20.00	23.40	24.80	27.23	12.82	28.63	31.00	32.80	36.10	32.13
Arka Anamika	18.33	21.67	23.10	25.00	11.68	25.10	28.00	29.70	32.00	28.70
Bagalkot Local	19.33	22.60	23.97	26.10	11.45	27.00	29.77	31.53	34.73	30.76
Mean	19.93	22.96	24.23	26.44	12.0	27.56	30.05	31.84	34.83	31.07
For comparing					CD at 1%					CD at 1%
Factor1					0.629					0.237
Factor 2					0.384					0.364
Interaction					0.769					0.687

Number of Leaves per plant: There was significantly higher mean number of leaves per plant at in well irrigated condition (13.5) compared to water stress condition (12.4). In well irrigated regime among the genotypes White velvet (14.4) showed significantly higher number of leaves per plant whereas, least recorded in Bagalkot local (12.4). In water stress regime also among the genotypes White velvet (13.1) showed significantly higher number of leaves per plant whereas, least recorded in Bagalkot local (11.4). In their interaction effect the genotype White velvet (14.4) recorded significantly highest number of leaves per plant among all genotypes and in both the water regime levels (Table 6.).

Leaf Relative water content (RWC%): There was significantly higher mean RWC % in well irrigated condition (78.4) compared to water stress condition (71.1). In well irrigated regime among the genotypes Arka abhay (85.0) showed significantly higher RWC % whereas, least was in Aarka anamika (72.3). In water stress regime also among the genotypes, Arka abhay (75.3) showed significantly higher RWC % whereas, least recorded in Arka anamika (67.1). In their interaction effect the genotype Arka abhay (85.0) recorded significantly highest RWC % among all genotypes and in both the water regime levels compared to others (Table 7). The reduction of relative water content under moderate and severe stress is probably an oxidative injury at the cellular level under water stress has high lipid peroxidation which decrease the stability of cell membrane and led to lose more water from cells.

Root length (cm): There was significantly higher mean root length in water stressed regime condition (30.7) compared to

Table 5. Effect of PEG-6000 water stress on Leaf RWC (%) in okra at 14 and 22 days after sowing

Genotype	Relative water content (%) at 14 DAS					Relative water content (%) at 22 DAS				
	PEG 6000 concentration (%)				Mean	PEG 6000 concentration (%)				Mean
	0	5	10	15		0	5	10	15	
P-8	88.17	82.00	76.40	71.90	79.62	87.60	76.60	67.40	61.60	73.30
White velvet	87.40	80.80	74.80	66.90	77.48	84.70	74.70	66.40	60.60	71.60
Arka Abhay	90.50	84.20	78.10	73.50	81.58	85.33	75.60	66.80	60.90	72.16
Parbhani Kranti	89.40	82.87	77.20	72.60	80.52	85.70	75.90	67.00	61.20	72.45
Arka Anamika	85.00	80.40	73.10	65.70	76.05	85.10	75.10	66.60	60.70	71.88
Bagalkot Local	86.60	81.47	75.50	68.20	77.94	84.40	73.60	65.80	60.30	71.03
Mean	87.84	81.96	75.85	69.80	78.9	85.47	75.25	66.67	60.88	72.1
For comparing				CD at 1%					CD at 1%	
Factor1				0.249					0.295	
Factor 2				0.391					0.449	
Interaction				0.736					0.847	

Table 6. Effect of water stress on plant height and number of leaves per plant in okra

Genotype	Plant height (cm) at 60 DAS			Number of leaves per plant at 60 DAS		
	Well irrigated	Water stressed	Mean	Well irrigated	Water stressed	Mean
P-8	63.8	55.3	59.5	13.4	12.6	13.0
White velvet	66.0	57.8	61.9	14.4	13.1	13.8
Arka Abha	62.8	53.8	58.3	14.3	12.2	13.2
Parbhani Kranti	61.5	52.6	57.1	13.0	12.2	12.6
Arka Anamika	64.4	56.9	60.7	13.8	12.8	13.3
Bagalkot Local	59.6	51.8	55.7	12.4	11.4	11.9
Mean	63.0	54.7	58.9	13.5	12.4	13.0
For comparing			CD at 5%			CD at 5%
Main			0.688			0.352
Sub			0.388			0.205
M × S			0.602			0.405

well irrigated condition (27.9). In well irrigated regime among the genotypes Arka abhay (31.8) showed significantly higher root length whereas, least recorded in Aarka anamika (24.6). In water stress regime, Arka abhay (33.2) showed significantly higher root length compared to others whereas, least recorded in Arka anamika (28.1). In their interaction effect also Arka abhay (31.8) recorded significantly higher root length (Table 7). Hasan et al (2018) observed, increase in root length under drought treatment. Generally, the root length of plants increases during a water stress conditions because the plants try to acquire underground water to tolerate the stress condition, in line with this, the root length is greater in drought tolerant species compared to sensitive species.

Number of lateral roots:. There was significantly higher mean number of lateral roots in water stressed condition (30.2) compared to well irrigated condition (27.5). In well irrigated regime among the genotypes Arka abhay (30.4) showed significantly higher number of lateral roots whereas, least recorded in Aarka anamika (25.1). In water stress regime, Arka abhay (34.3) showed significantly higher number of lateral roots whereas, least recorded in Arka anamika (27.0). In their interaction effect also Arka abhay (34.3) recorded significantly more number of lateral roots (Table 7). Reduction in roots, root diameter under severe moisture stress, could be due to decrease in cell elongation. The results of our study are in accordance with the findings of Hasan et al (2018).

SPAD Value: There was significantly highest mean SPAD value in well irrigated condition (26.1) compared to water stressed condition (22.1). In well irrigated regime among the genotypes Arka abhay (30.2) showed significantly higher SPAD value whereas, least recorded in Aarka anamika (23.0). In water stress regime, Arka abhay (25.8) showed significantly higher SPAD value whereas, least recorded in Arka anamika (17.2). In their interaction effect also significant (Table 7).

Pod length (cm): There was significantly highest mean pod length in well irrigated condition (10.7) compared to water stressed condition (9.9). In well irrigated regime among the genotypes Parbani kranthi (11.4) showed significantly higher pod length whereas, least recorded in Bagalkot local (9.6). In water stress regime, P-8 (11.5) showed significantly higher pod length whereas, least recorded in Bagalkot local (8.4). In their interaction effect also P-8 (11.5) significantly recorder higher pod length (Table 8). When okra plant exposed to 50 % level of drought reduces the growth and photosynthetic pigment which resulting in reduction of pod yield gradually. In the same line, length and number of pods per plant showed positive direct effect on pod yield.

Pod Yield (t/ha): There was significantly highest mean pod yield in well irrigated condition (18.19) compared to water stressed condition (14.81). In well irrigated regime among the genotypes P-8 (19.46) showed significantly higher pod yield whereas, least recorded in Bagalkot local (16.72). In water stress regime also, P-8 (15.54) showed significantly higher pod yield whereas, least recorded in Bagalkot local (14.02). In their interaction effect also P-8 (19.46) significantly recorded higher pod yield (Table 8).

Genetic diversity analysis by ISSR markers: To genetic diversity was analysed for six okra genotypes by using twenty ISSR genetic markers (Table 9). Initially DNA from leaf was

Table 7. Effect of water stress on Leaf Relative water content(%), Root length(cm) and Number of lateral roots in okra at 60 DAS

Genotype	Leaf Relative water content (%)			Root length (cm)			Number of lateral roots		
	Well irrigated	Water stressed	Mean	Well irrigated	Water stressed	Mean	Well irrigated	Water stressed	Mean
P-8	79.4	73.0	76.2	28.2	31.2	29.7	27.9	30.4	29.2
White velvet	74.5	68.8	71.7	26.4	29.2	27.8	26.2	28.7	27.5
Arka Abhay	85.0	75.3	80.2	31.8	33.2	32.5	30.4	34.3	32.4
Parbhani Kranti	82.5	72.0	77.3	29.2	32.1	30.7	28.7	31.8	30.3
Arka Anamika	72.3	67.1	69.7	24.6	28.1	26.4	25.1	27.0	26.1
Bagalkot Local	76.4	70.3	73.4	27.1	30.2	28.6	26.8	29.2	28.0
Mean	78.4	71.1	74.7	27.9	30.7	29.3	27.5	30.2	28.9
For comparing			CD at 5%			CD at 5%			CD at 5%
Main			1.327			0.717			0.225
Sub			1.034			0.585			0.235
M × S			1.48			0.831			0.325

Table 8. Effect of water stress on SPAD value, pod length and pod yield in okra at 60 DAS

Genotype	SPAD value			Pod length (cm)			Pod yield (t/ha)		
	Well irrigated	Water stressed	Mean	Well irrigated	Water stressed	Mean	Well irrigated	Water stressed	Mean
P-8	26.2	23.4	24.8	11.9	11.5	11.7	19.46	15.54	17.75
White velvet	25.4	20.1	22.7	11.0	10.1	10.6	18.46	14.93	17.65
Arka Abhay	30.2	25.8	28.0	10.4	9.8	10.1	18.19	14.70	17.61
Parbhani Kranti	27.0	24.5	25.8	11.4	10.6	11.0	18.73	15.22	17.62
Arka Anamika	23.0	17.2	20.1	10.0	9.2	9.6	17.58	14.44	17.53
Bagalkot Local	24.5	21.7	23.1	9.6	8.4	9.0	16.72	14.02	17.36
Mean	26.1	22.1	24.1	10.7	9.9	10.3	18.19	14.81	17.59
For comparing			CD at 5%			CD at 5%			CD at 5%
Main			1.14			0.225			0.036
Sub			0.928			0.235			0.049
M × S			1.319			0.325			0.065

Table 9. List of ISSR primers used for genetic diversity study with their sequence

Primer name	Sequence (5'-3')	Sl. No	Primer name	Sequence (5'-3')
UBC811	GAG AGA GAG AGA GAG AC	11	SPS7	$(GTG)_5$
UBC841	GAG AGA GAG AGA GAG AYC	12	SPS1	$(GAC)_5$
UBC826	ACA CAC ACA CAC ACA CC	13	I3	$(GA)_9A$
UBC818	CAC ACA CAC ACA CAC AG	14	ISSR1	$(CT)_8$ TG
UBC834	AGA GAG AGA GAG AGA GYT	15	ISSR2	$(CT)_8$ AC
UBC835	AGA GAG AGA GAG AGA GYC	16	ISSR4	$(AGC)_4$ GT
UBC850	GTG TGT GTG TGT GTG TYC	17	ISSR5	$(CAC)_3GC$
I2	$(GA)_9T$	18	ISSR6	$(CTC)_3$ GC
SPS8	$(GGA)_4$	19	ISSR7	$(GACA)_3$
SPS4	$(AGG)_6$	20	ISSR8	$(GACA)_3$ GC

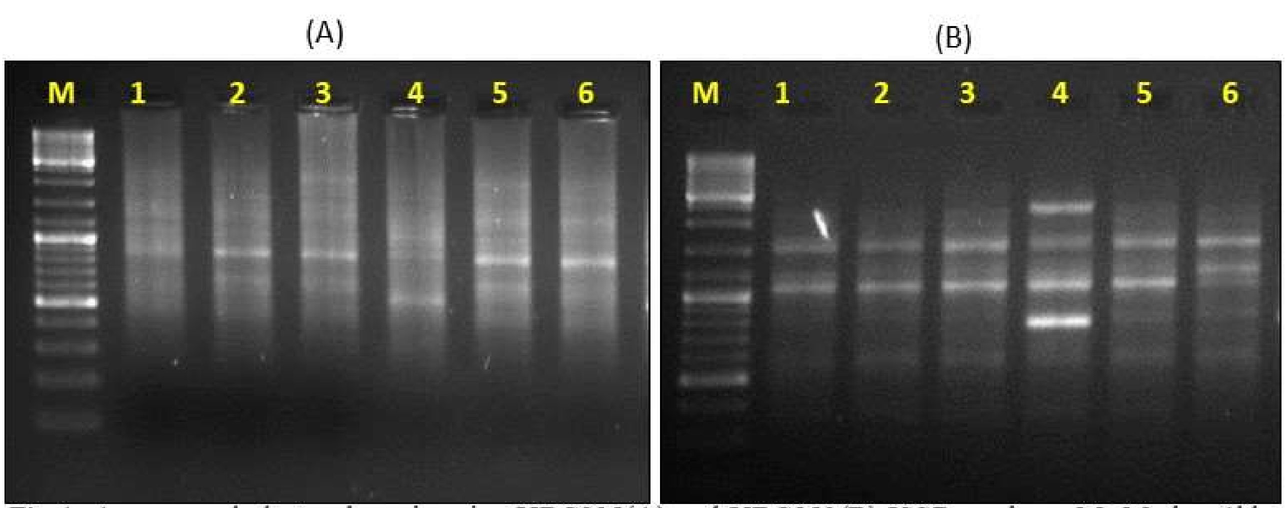

Fig 1. Agarose gel electrophoresis using UBC835(A) and UBC850(B) ISSR markers. M- Marker 1kb ladder, Lane 1-6 Okra lines (1- P-8, 2-White velvet, 3-Arka abhay, 4-Parbani kranti, 5-Arka anamika and 6- Bagalkot local)

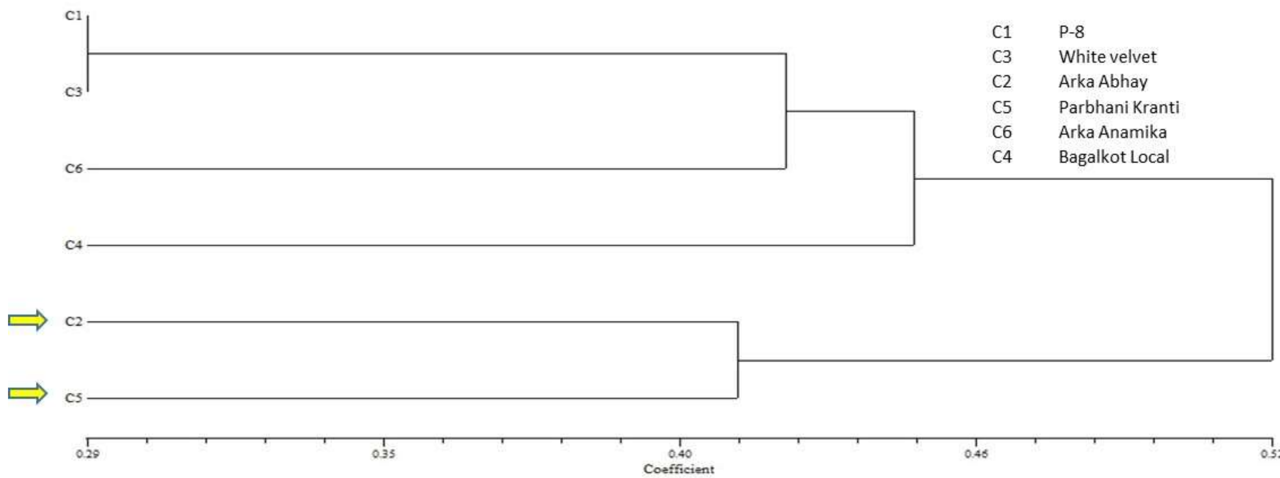

Fig 2. Dendrogram depicting the six Okra lines based on the genetic similarity generated by ISSR markers

isolated from using CTAB method and quantified using Nanadrop. Annealing temperature for all the ISSR primers was standardized by PCR (polymerase chain reaction). PCR performed for all the 20 primers to amplify to arrive allelic variations. Out of twenty primers eleven primers showed polymorphisms (Fig 1). The results showed that moderate genetic polymorphism (29.0%) among okra genotypes. Dendrogram obtained from ntysis software grouped the six okra lines into different clusters. The relatively water stress tolerant lines (Arka abhay and Parbani kranti) were clustered in one group and the rest were grouped in other cluster (Fig. 2).

CONCLUSION

Based on laboratory evaluation PEG-6000 induced water stress and field stress it is revealed that among six okra genotypes Arka abhay followed by Parbani kranti were relatively drought tolerant by exhibiting physiological drought adoptive traits like high relative water content, RWC, SPAD, root traits with better plant height.

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Prevalence of Plastic Debris in Beach Sediments of Sutrapada, Saurashtra Coast of Gujarat, India

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***Abstract*:** This study evaluates plastic debris occurrence and physical characteristics from selected stations in beach sediments at Sutrapada, Saurashtra coast of Gujarat, India, from November 2020 to April 2021. Sutrapada Chowpatty (Station 1) and Sutrapada Bandar (Station 2) were selected for beach sediment sample collection. The abundance of macro-plastics, meso-plastics, and microplastics in stations 1 and 2 ranged between 2.9 to 8.5 and 3 to 10.1 items/m^2, 5.5 to 10.1 and 3.55 to 8.72 items/m^2, 4.33 to 20.33 and 6.83 to 16.33 items/100g, respectively. At Stations 1 & 2, the dominant macro-plastic shape, size and colour were fragment and fibre, >2.5 cm and 5 cm and white and blue, respectively. At Stations 1 and 2, the dominant meso-plastic shape, size and colour were fragment and thermocoel, 1 cm – 1.5 cm and 1.5 – 2 cm (both stations) and white and yellow, respectively. Stations 1 and 2, dominated by microplastic shapes, size and colour, were fibre, 1-2 mm & 2-4 mm and black for both stations, respectively. These results could be attributed to fishing, small-scale fisheries business, and tourism activities in a coastal town. The present study provides the origins of plastic waste in coastal areas, laying the groundwork for formulating sustainable, enduring plans and strategies to manage and control plastic pollution in the coastal environment.

***Keywords*:** Plastic pollution, Beach sediment, Microplastic, Physical characteristics, Sutrapada

Plastic is manufactured by synthetic or semi-synthetic organic polymer and is cost-effective, lightweight, robust, long-lasting, and corrosion-resistant (Thompson et al 2009). Ultimately, every plastic waste finds its way into marine environments as debris (Hopewell et al 2009). Plastic debris is classified according to size: macro-plastics, meso-plastics and microplastics (Cheshire et al 2009). Macro-plastics refer to plastic fragments larger than 2.5 cm, representing the most prominent and easily detectable type of debris along shorelines and readily removable. Meso-plastics consist of particles ranging from 5 mm to 2.5 cm. Unlike larger macro debris, meso-plastic particles are frequently buried and evade removal during cleanup efforts. Plastic particles smaller than 5 mm are called microplastic (Arthur et al 2009). Microplastics in the environment can be divided into two categories: primary microplastics and secondary microplastics. Primary microplastics may be deliberately manufactured to be that size (GESAMP 2019), whereas secondary microplastics are generated from weathering or breakdown of macro-plastic and meso-plastic items. Primary data and additional information regarding coastal litter in India can be accessed from the different regions across the country, such as the Nicobar Islands (Sen 2003), Gulf of Cambay (Reddy et al 2006), Karnataka coast (Sridhar et al 2009), Northern Gulf of Mannar (Ganesapandian et al 2011), Tamil Nadu (Kaladharan et al 2012), urban beaches in Mumbai (Jayasiri et al 2013), Mangalore coast (Sulochanan et al 2014), Kerala, Karnataka and Chennai coast (Kumar et al 2016) and Tamil Nadu (Sathish et al 2019). Plastic items are also found in the digestive systems of deceased fishes (James et al 2020), zooplankton (Collignon et al 2012), crustaceans (Daniel et al 2020), mussel (Karlsson 2017), bivalves (Li et al 2015), seabirds and marine mammals (Lusher 2015).

Sutrapada is a coastal town near Veraval on Gujarat's Saurashtra coast and has Gujarat Heavy Chemical Pvt. Ltd. (GHCL), a major chemical industry. The town also has a fishing harbor that supports local livelihoods. Sutrapada Chowpatty supports local tourism activities, especially during holidays. This led to the widespread use of plastics, including fishing nets, packaging, and fishing debris. Studies are needed to assess the occurrence and abundance of plastic debris in Sutrapada. This study aims to evaluate the occurrence of plastic debris, its distribution and the physical characteristics of the beach sediment samples along the Sutrapada coast.

MATERIAL AND METHODS

Study area: The two stations were selected for sampling, namely Sutrapada Chowpatty (Station 1) and Sutrapada Bandar (Station 2), situated at 20.838429° 70.477588° and 20.833949° 70.487397° located on the Saurashtra Coast of Gujarat, India. These stations exhibit a combination of rocky and sandy substrates and are characterized by a rich assortment of molluscan shells, seaweeds and coastal fish species. Sutrapada scenic chowpatty draws many visitors daily and hosts religious fairs, especially on weekends, showing anthropogenic stresses and their impact on natural ecosystems.

Sampling procedures and collection: The study was conducted for six months, from November 2020 to April 2021. At each chosen location, sampling was carried out using a 1 m^2 quadrate at monthly intervals. The sampling was conducted along two-line transects, each spanning 100 m from the high tide line and fixed during the sampling period. Triplicate samples were collected from random areas along the shoreline within pre-decided line transects. Plastic debris was classified into three groups based on particle size given by Cheshire et al (2009): macro-plastics (> 2.5 cm), meso-plastics (between 5 mm-2.5 cm) and microplastics (< 5 mm), respectively. Due to their larger dimensions, macro- and meso-sized plastic fragments were manually gathered from the quadrats and filtered through a 5 mm sieve. The retained macro-plastic and meso-plastic debris samples were analyzed using the visual identification method (GESAMP 2019). To prevent contamination, all collected samples were promptly packed before transportation to the laboratory to quantify and analyses physical characteristics such as shape, type, size, and colour.

Sampling and extraction of microplastic: Within a quadrate, one kg surface sediment sample is collected at 2 cm depth and transferred to the laboratory for further processing. The method proposed by Qiu et al (2016) was used to extract microplastic from sediment samples. To identify the presence of microplastics in the sample, the filter paper underwent examination using a stereo-zoom microscope (40x) equipped with a digital camera. The identified microplastics were separated and photographed individually according to their shape, size and colour, and all relevant information was recorded and documented.

RESULTS AND DISCUSSION

Macro-plastics abundance: The abundance of macro-sized plastics in the beach sediment varied between 2.9 to 8.5 and 3.0 to 10.1 items/m^2 at station 1 and station 2, respectively. The average abundance of macro-plastics at station 1 and station 2 was 6.5 and 6.9 items/m^2 was attributed to fishing activities, small-scale fisheries business, and waste disposal proximity, indicating that both stations significantly contributed to land-based sources of pollution. Jeyasanta et al (2020) also observed that fishing and recreational activities majorly cause macro-plastic distribution.

Physical characteristics of macro-plastics: The shape of macro-plastics identified from beach sediments at Station 1 was dominated by fragment-shaped followed by fibre, filmed plastic, thermocol, pellet, plastic pouches, bottles, food wrappers and cutleries, which might be due to tourism activity, ocean currents and land-based sources (Table 1). Allsopp et al (2006) reported the plastic waste on the shoreline likely came from external sources and may have been carried by ocean gyres, winds, and sea currents. Station 2 was fibre-shaped macro-plastic dominant, followed by fragments, foamed plastic, and plastic pouches, which might be due to fishing-related activities and small-scale business proximity to coastal areas. Lee et al (2015) observed that fibre-shaped macro-plastics were dominant, comprising 54.7% of the plastic content across twelve South Korean beaches. Station 1 dominant sizes ranged between >2.5 cm and 5 cm, followed by 10-20 cm, >40 cm, 5-10 cm, and 20-40 cm. Furthermore, Station 2's dominant sizes ranged between >2.5 cm and 5 cm, followed by 20-40 cm, 10-20 cm, 5-10 cm, and >40 cm, respectively. The most common sizes were due to the fragmentation of large plastic fragments into small macro-sized plastics.

In station 1, white-coloured particles were dominant, followed by transparent, red, green, blue, brown, yellow, black, and grey. In station 2, blue-coloured particles were dominant, followed by white, transparent, yellow, brown, and black. The variety of colours could be attributed to the diverse origins of the plastic, stemming from different sources. The smaller and multiple colours macro-plastic particles might appeal to marine organisms as feed, potentially elevating their chances of being ingested (Behera et al 2021). Blaskovic et al (2017) observed transparent and white colours were the dominant plastic litter reported in sediments from the Croatian marine protected at Telascica Bay.

Meso-plastics abundance: Meso-sized plastic items were found in samples collected from beach sediment stations at Sutrapada. The abundance of meso-sized plastics varied between 5.5 to 10.1 items/m^2 and 3.5 to 8.7 items/m^2 at stations 1 and 2, respectively. The average abundance of meso-plastics was 7.1 and 6.1 items/m^2 at stations 1 and 2, respectively. Jeyasanta et al (2020) reported an abundance of 9.3 items/m^2 of meso-plastics from the beaches of the Tuticorin, Southeast coast of India.

Physical characteristics of meso-plastics: Fragment-shaped meso-plastics were dominantly identified at station 1, followed by fibre, thermocoel and plastic pouch. At station 2, thermocoel was the dominantly identified meso-plastic, followed by plastic pouch, fragments and fibre, which might be the due breakdown of larger plastic through mechanical forces, photolysis, thermo-oxidation, thermo-degradation and possibly via biodegradation processes (Zhao et al 2015). Disposal waste and damaged fishing nets are also believed to be important sources of meso-plastic fibres (Browne et al 2011). Meso-plastic sizes ranging from 5 mm to 2.5 cm were reported from sediment at station 1 with dominant sizes ranging between 1 - 1.5 cm, followed by 1.5 cm - 2.0 cm, >5 mm - 1.0 cm and 2 cm - 2.5 cm. At station 2, dominant sizes were reported to range between 1.5 cm - 2 cm, followed by 1 cm - 1.5 cm, >5 mm - 1 cm, and 2 cm - 2.5 cm. The total of eight colours comprised of white, yellow, red, green, transparent, black, blue, and brown were reported from both stations. At station 1, white-coloured particles were dominant, while at station 2, yellow-coloured particles were dominant. Jeyasanta et al (2020) reported similar coloured meso-plastics from Tuticorin beaches on the southeast coast of India. Blettler et al (2017) described varied colours as being reported due to the origin of plastics from different sources and the intensive weathering process of macro-plastics.

Microplastics Abundance and Physical Properties

Microplastics abundance: The abundance of microplastics in the beach sediment varied between 4.3 to 20.3 items/100 g and 6.8 to 16.3 items/100 g at stations 1 and 2, respectively. The average abundance of microplastics was 10.5 and 10.5 items/100 g, possibly sourced due to fishing activity generated waste, dense fisher population, and regular influx of beach visitors. Fishing is a primary contributor of microplastics to beaches, given that they serve as primary fishing hubs for nearby fishing villages (Dowarah and Devipriya 2019). Dekiff et al (2014) reported that the presence of microplastics in beach sediment is attributed to anthropogenic activities.

Physical characteristics of microplastics Fibre-shaped microplastics were dominated in station 1 and station 2, respectively. At station 1, other shapes were fragments, films, foam, and pellets. In contrast, at station 2, fragments, films, foam, and pellets were reported, possibly due to the segregation and repair of fishing nets and small-scale fisheries business proximity to the beach. Fibre and fragment-shaped microplastic emerged from fisheries activities and small-scale businesses (Lusher et al. 2017 and Sathish et al. 2019). The sizes of microplastics reported from beach sediment ranged between <0.5 mm to 5 mm. The major size range at station 1 was 1 mm - 2 mm while station 2 was 2 mm - 4 mm. Other size compositions at station 1 were 2 mm - 4 mm, 0.5 mm - 1 mm, >0.5 mm, 4 mm - 5 mm, while at station 2, was 1 mm - 2 mm, 0.5 mm - 1 mm, 4 mm - 5 mm and >0.5 mm, respectively. Young and Elliot (2016) reported that most particle sizes ranged from 2-4 mm at Kamilo Beach and Kahuku Beach in Hawaii during the entire study period.

In total, seven different coloured microplastics were

Table 1. Composition of physical characteristics of macro-plastic from beach sediment

Shape composition (%)			Colour composition (%)			Size composition (%)		
Shape	Station 1	Station 2	Colour	Station 1	Station 2	Size(cm)	Station 1	Station 2
Fibre	18	43.2	White	40.3	20.1	> 2.5-5	38.5	39.1
Fragments	48.5	22.2	Transparent	16.0	15.5	5-10	12.1	8.0
Film	9.0	18.5	Red	11.5	0.0	10-20	25.0	19.0
Thermocoel	7.0	0.0	Green	10.6	0.0	20-40	4.2	32.3
Pellet	6.0	0.0	Blue	9.2	40.0	> 40	20.1	1.5
Plastic pouch	4.5	16.5	Brown	5.2	7.8			
Bottles	3.0	0.0	Yellow	3.50	10.8			
Food wrappers	2.5	0.0	Black	1.82	6.0			
Cutleries	1.3	0.0	Grey	1.7	0.0			

Table 2. Composition of physical characteristics of meso-plastic from beach sediment

Shape composition (%)			Colour composition (%)			Size composition (%)		
Shape	Station 1	Station 2	Colour	Station 1	Station 2	Size(cm)	Station 1	Station 2
Fibre	22.1	10.5	White	29.7	23.5	0.5-1.0	21.5	19.8
Fragments	30.5	11.0	Transparent	8.4	9.5	1.0-1.5	34.5	30.0
Thermocoel	21.5	43.2	Red	18.8	17.7	1.5-2.0	28.9	33.4
Plastic pouch	16.7	25.6	Green	11.9	11.9	2.0-2.5	15.1	16.6
Bottles cap	2.0	3.0	Blue	3.3	4.9			
Straw	0.5	2.0	Yellow	20.8	25.5			
Irregular item	6.4	4.5	Black	6.9	6.7			

Table 3. Composition of physical characteristics of micro-plastic from beach sediment at station 1 & 2

Shape composition (%)			Size composition (%)			Colour composition (%)		
Shape	Station 1	Station 2	Colour	Station 1	Station 2	Size(cm)	Station 1	Station 2
Fibre	59.3	51.0	>0.5	13.4	9.0	White	23.1	18.5
Fragments	14.2	24.3	0.5-1.0	22.7	18.3	Transparent	10.3	14.3
Film	12.0	10.7	1.0-2.0	31.3	27.3	Red	14.4	12.5
Foam	10.2	8.2	2.0-4.0	24.1	32.0	Green	6.8	12.5
Pellet	4.0	5.5	4.0-5.0	8.9	13.2	Blue	11.4	16.0
						Yellow	3.7	2.2
						Black	30.0	23.9

recorded from beach sediments. The black-coloured microplastics dominated at station 1 and station 2, respectively. At station 1, other colour compositions are white, red, transparent, blue, green, and yellow. In station 2, other colour compositions are white, blue, transparent, red, green, and yellow. The colour variation among microplastic particles may be due to their origin from different sources. Furthermore, the small size and multicolour of the microplastic particles may favour their intake by marine organisms (Sathish et al 2019), eventually leading to accumulation and transfer to higher tropic levels. Retama et al (2016) suggested that coloured microplastics attract predatory fishes since they resemble their prey and pose severe threats to marine ecosystems.

CONCLUSION

The plastic debris contamination of the beach sediment of Sutrapada town could be attributed to fishing activities, small-scale fisheries business, and anthropogenic activities. Further, ocean currents are responsible for plastic debris' widespread contamination through transportation across large distances. Meanwhile, continuous monitoring of plastic debris sources and contamination along the beaches is much needed, and awareness among the locals is required through regular beach cleaning activities. These studies highlight the significant risks posed by introducing plastic debris into aquatic environments, the need for public awareness, and the importance of its impact on marine ecosystems and human health.

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Optimizing the Production of Biodegradable Containers from Corn Husks Using Hot Mold Forming: Study on Temperature, Forming Time and Husk Arrangement

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***Abstract*:** Corn husks are an abundant agricultural byproduct left over from the processing of animal feed. This byproduct holds the potential to create value-added products for farmers. The objective of this research is to utilize discarded corn husks by converting them into containers through hot mold forming. The variables studied include temperature, time, husk arrangement, and binder ratio. The testing process involved the following steps: setting the temperature at three levels-130°C, 140°C, and 150°C; forming time at three levels-1 minute 30 seconds, 2 minutes, and 2 minutes 30 seconds; and arranging the corn husks in two ways: 1) using 3 mature husks with 6 young husks, and 2) using 6 mature husks. The binder ratio (water: starch) was set at 1:1 and 1:2. The mold used for forming had dimensions of 12x12x2.5 cm (W x L x H). After forming, the containers were tested for storage over a period of 1 month and were evaluated according to the community product standards 1557/2563. The optimal conditions for producing containers from corn husks through hot mold forming were a temperature of 140°C, a forming time of 2 minutes, and the first husk arrangement method. The containers produced under these conditions were strong, durable, free from mold, well-formed, and stable. After a 1-month storage period, no mold was observed, and the containers remained strong and intact. However, after 3 months, mold was found on all containers made from corn husks under all conditions.

***Keywords*:** Corn husk, Agricultural by product, Biodegradable containers

Thailand is currently grappling with a significant waste management issue, particularly with the disposal of single-use containers, a problem largely driven by the growing preference for convenience among consumers. Foam food containers, which are widely popular due to their low cost, lightweight, and ease of availability, are particularly problematic. These containers, however, take an alarming 450 years to decompose, posing serious environmental challenges. The widespread use of foam containers, especially by vendors of ready-to-eat meals, exacerbates this issue as they are discarded immediately after use, contributing significantly to the country's waste problem. Moreover, the disposal of foam is expensive, requiring considerable resources for proper waste management.

In response to this pressing issue, researchers have proposed a solution that not only mitigates environmental concerns but also provides economic benefits to farmers. By utilizing the abundant agricultural byproducts from animal feed processing, specifically corn husks, this research aims to add value to these materials while reducing environmental waste. Corn husks are one of the most prevalent agricultural waste products. In 2023,Thailand's corn production is estimated to reach 5.4 million metric tons, which marks an increase from previous years. This growth is attributed to an expansion in planting areas and favorable farm-gate prices. The higher production also aligns with the country's efforts to manage agricultural byproducts, such as corn husks, more sustainably. Farmers often dispose of these waste materials through burning, which is a major contributor to air pollution and smog.

Given Thailand's extensive corn cultivation, the country faces a significant challenge each year in managing the agricultural waste generated post-harvest and processing. To address this, researchers propose converting corn husks into biodegradable containers that can naturally decompose within a short period, offering a sustainable alternative to traditional, non-biodegradable packaging materials. Corn is a key agricultural product in Thailand, ranking among the country's primary crops. It is predominantly cultivated in the northern and central regions, where favorable climatic conditions support its growth. The production of corn has been steadily increasing due to its economic importance, particularly in the animal feed industry, where it serves as a crucial ingredient. However, the processing of corn for feed generates substantial agricultural byproducts, with corn husks being one of the most abundant. While traditionally considered waste, corn husks have the potential to be transformed into valuable products, enhancing both the sustainability and profitability of corn cultivation.

Recent studies have highlighted the utility of agricultural byproducts like corn husks in creating biodegradable materials (Castrillón et al 2021), aligning with global efforts to reduce plastic waste and promote environmentally friendly alternatives. The conversion of these byproducts into biodegradable containers addresses waste management issues and provides an additional revenue stream for farmers.(Maraveas 2020, Enawgaw et al 2023)

This study aims to optimize the production of biodegradable containers from corn husks using hot mold forming, focusing on the effects of temperature, forming time, and husk arrangement on the quality and durability of the final product. By exploring these variables, the research seeks to contribute to the sustainable use of agricultural byproducts in Thailand, offering a viable solution for reducing environmental impact and enhancing the economic value of corn cultivation (Enawgaw et al 2023).

MATERIAL AND METHODS

The equipment used for the molding process includes a BAMBOO II thermal press machine with a mold size of 12 x 12 x 2.5 cm. Sweet corn husks are used as the primary material, with tapioca flour acting as the binder to facilitate adhesion. Wax paper is placed between the corn husks and the mold to prevent sticking. The testing process begins with the selection of corn husks, avoiding any husks with mold. The selection must also ensure that the husks are appropriate for the specific arrangement required and that overly small husks are not used, as they can complicate the arrangement process. The fresh corn husks are then cleaned and air-dried for 1-2 days to reduce moisture and prevent mold growth. After drying, the husks are sorted, removing any moldy or excessively small pieces. On average, each ear of corn has about 14-15 husks, comprising 8 young husks and 6 mature husks, accounting for 58% and 42% of the total, respectively (Fig. 1). Before performing the thermal molding, spray plain water evenly on the corn husks to help them unfold, making it easier to arrange them on the molding machine. Then, cut parchment paper to fit the molding blocks for both the upper and lower molds. This will prevent the corn husks from sticking to the molds and make cleaning easier.

The testing begins with arranging the corn husks in two methods. In Method 1, use 9 corn husks-3 mature husks and 6 young husks-arranged in 3 layers. In Method 2, use 6 mature corn husks arranged in 2 layers. In both methods, the husks are arranged in alternating layers, perpendicular to each other. It is crucial to arrange the corn husks meticulously during molding, as improper arrangement may cause gaps or misshapen containers. Molding is then performed at set temperatures of 130, 140, and 150 degrees Celsius, with molding times of 1:30, 2:00, and 2:30 minutes. Tapioca flour is used as a binder at ratios of water to flour of 1:1 and 1:2. Each condition is tested in three repetitions. After molding, the excess edges are trimmed off (Fig. 2).

After one month of storage at room temperature, the container samples are evaluated according to the Thai Community Product Standards 1557/2563. The evaluations include measuring moisture content using a hot air oven at 105 degrees Celsius for 3 hours until the weight remains constant. Microbial testing is also conducted with the following criteria: (a) Mold must be less than 100 colonies per sample, (b) No presence of Salmonella spp., (c) No presence of Staphylococcus aureus, and (d) Total plate count must be less than 1x10^3 colonies per sample.

The containers are then assessed using a Rubric score of 1 to 5 (Fig. 3).

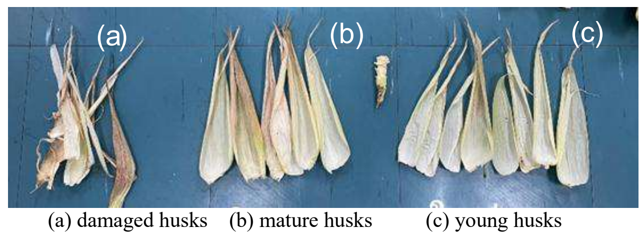

Fig. 1. Corn husk

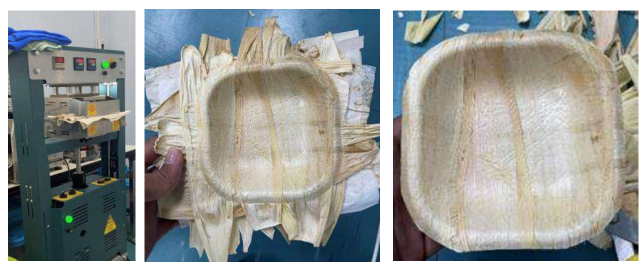

Fig. 2. Thermal molding of containers from corn husks

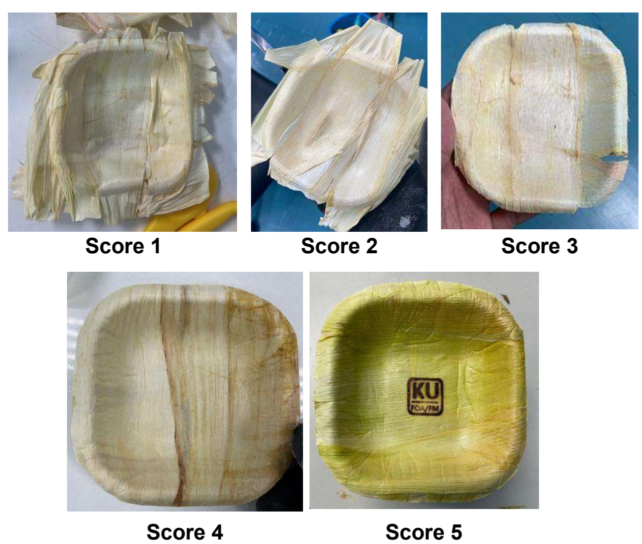

Fig. 3. Rubric score assessment (1-5 point)

Score 1: The container lacks form, is too thin to maintain shape, or has mold.

Score 2: The container forms but is not very strong; no mold is present. There may be visible starch residue, and some parts are incomplete, such as loose or perforated corn husks.

Score 3: The container forms with moderate strength but has thin spots. Some areas may be swollen or not tightly bonded.

Score 4: The container is strong and has sufficient thickness, with corn husks firmly attached and no mold present.

Score 5: The container is very strong, with substantial thickness and all corn husks firmly attached. There are no gaps, holes, or burn marks, and the container has an appealing color with no mold or dirt.

RESULTS AND DISCUSSION

Thermal molding of containers from corn husks: The molding temperature, molding time, the arrangement of corn husks, and the binder ratio are significant factors influencing the quality of the molded containers (Tables 1, 2). The binder ratio 1:1 for Arrangement Method 1, at a molding time of 1.30 minutes and temperatures of 130, 140, and 150 degrees Celsius, the score was consistently 2 under all conditions. At molding times of 2 and 2.30 minutes, the scores were maximum at140 and 150 degrees Celsius was 5. For arrangement method 2, at a molding time of 1.30 minutes, the maximum score was 3 at temperatures of 150 degrees Celsius. At molding times of 2 and 2.30 minutes, the maximum scores were 3.5 and 4 at 140 and 150^{o}C, respectively.

The optimal condition for molding containers from corn husks, achieving the highest Rubric score of 5, was at a temperature of 140 degrees Celsius and a molding time of 2 minutes using Arrangement Method 1. This method involved using 3 mature corn husks and 6 young corn husks with a binder ratio of 1:1, resulting in the best outcomes in terms of strength, durability, and overall quality of the container. However, at 150 degrees Celsius and a molding time of 2.30 minutes with arrangement method 1 and a binder ratio of 1:1, a score of 5 was also achieved. Despite this, the higher temperature and longer time led to increased energy consumption, making it less favorable. Additionally, containers produced under the optimal conditions maintained their integrity without mold growth for up to 1 month. However, when stored for up to 3 months, mold was observed in containers under all conditions, highlighting the limitations of the material for long-term storage.

Binder ratio 1:2 for Arrangement Method 1, at molding times of 1.30, 2.00, and 2.30 minutes the maximum scores at 150, 140, and 150 degrees Celsius were 3, 4, and 3, respectively. For Arrangement Method 2, at a molding time of 1.30 minutes and temperatures of 150 degrees Celsius, the maximum scores were2. At molding times of 2.00 and 2.30 minutes, the maximum scores were consistently 2 across all temperature ranges.Using a binder ratio of 1:2, the optimal condition remains Arrangement Method 1 at 140 degrees Celsius. However, scores decreased when molding time exceeded 2 minutes or when Arrangement Method 2 was used. The results show that adjustments in temperature and molding time directly impact the strength and integrity of the containers. Temperatures that are too high or too low, as well as inappropriate molding times, can lead to containers with incomplete or insufficient structural strength. This experiment highlights the importance of controlling these variables when producing biodegradable containers from corn husks. A critical aspect of molding containers from corn husks is the

Table 1. Thermal molding of containers with a binder ratio of 1:1

Temperature	Molding time (minutes)					
	Arrangement method 1			Arrangement method 2		
	1.30	2.00	2.30	1.30	2.00	2.30
130	2	2.5	3	1	2.5	3
140	2	5	3.5	2	3.5	3
150	2	3	5	3	2	4

Table 2. Thermal molding of containers with a binder ratio of 1:2

Temperature	Molding time (minutes)					
	Arrangement method 1			Arrangement method 2		
	1.30	2.00	2.30	1.30	2.00	2.30
130	2	3.5	1	1	2	2
140	2	4	2.5	1	2	2
150	3	3	3	2	2	1

Table 3. Inspection of containers according to Thai community product standards 1557/2563

Item	Test results	Unit	Reference testing methods
Moisture	8.2	%	-
Mold	<10est.	cfu/piece	In-house method TE-Mi-017 based on AOAC (2019) 997.02
Salmonella spp.	Not Detected	per piece	ISO 6579-1:2017/Amd.1:2020.
Staphylococcus Aureus	Not Detected	per piece	ISO 6888-3:2003/Cor 1:2004.
Total plate count	1.5 x 10^2est.	cfu/piece	Compendium of Methods for the Microbiological Examination of Foods (APHA), 5^{th} Edition, 2015, Chapter 3

arrangement of the husks during the molding process. Since the molding machine moves during operation, the corn husks can shift, leading to misalignment, gaps, or arrangements that differ from the intended setup. This issue can be mitigated by carefully adjusting and observing the husks until the press reaches the molding point and positioning them correctly. This requires meticulous handling, which extends the molding time. Molding containers from corn husks differs from using other natural materials like banana leaves, teak leaves, or lotus leaves, which are larger and typically used as a single piece. Corn husks are smaller, requiring multiple husks to form one container, making the arrangement process more challenging. Thus, selecting a mold size appropriate for corn husks is crucial. Corn husk containers are not suitable for holding liquids or wet foods as they tend to swell and disintegrate. However, they are well-suited for dry items, such as snacks or dry foods.

Inspection of containers according to Thai community product standards 1557/2563: The results of the container Inspection according to the Thai Community Product Standards 1557/2563 (Table 3). The results show that the moisture content is 8.2%, and mold count is less than 10 est. No *Salmonella* spp. or *Staphylococcus aureus* were detected. The total plate count is 1.5 x 10^2 est. cfu/piece, complies with the Thai Community Product Standards 1557/2563 for containers. This shows that containers made from heat-molded corn husks can be used safely.

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CONCLUSION

The container production from corn husks using hot molding demonstrates the efficiency of transforming agricultural waste into value-added products. The optimal conditions for molding were temperature of 140°C, using a Type 1 arrangement with 6 mature corn husks layered in two levels, a 1:1 binding starch ratio, and molding time of 2 minutes. Utilizing corn husks for container production presents a promising option for reducing agricultural waste and creating biodegradable containers. However, further process development is needed to enhance the durability and longevity of the products.

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Revolutionizing Biofuel: A Novel Approach to Sustainable Briquette and Pellet Production

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Abstract: This study introduces a novel approach to producing high-efficiency briquettes and pellets using a combination of agricultural residues such as coconut husk, arecanut shell, and sawdust along with tamarind kernel powder (TKP) as a natural binding agent. The aim is to create sustainable biofuels that serve as eco-friendly alternatives to fossil fuels, addressing both environmental and energy security challenges. Prototypes were developed and tested for bulk density, calorific value, moisture content, and ash content. The results indicate that the pellets achieved a superior bulk density of 860 kg/m³ and a calorific value of 16,703 kJ/kg, surpassing many existing biofuel options. Additionally, the briquettes exhibited low ash content (4.1%) and competitive moisture levels (9.1% for pellets), making them efficient in combustion. This research highlights the potential of using inexpensive and abundant agricultural waste, enhanced with TKP, to produce biofuels that are both energy-efficient and environmentally sustainable. With localized production in resource-abundant regions, this approach can contribute to enhancing national energy security and reducing dependence on fossil fuels.

Keywords: Biofuels, Briquettes, Pellets, Renewable energy, Energy security

Energy security has become an increasingly critical issue in the context of global economic stability and development and growing global demand for energy, driven by population growth, urbanization, and industrial expansion, has intensified concerns about the sustainability of traditional energy systems, particularly those reliant on fossil fuels.

Fossil fuels, such as coal, oil, and natural gas, have been the dominant sources of energy worldwide, meeting approximately 80 percentage of global energy needs (Kpalo et al 2020). However, their rapid depletion and associated environmental pollution have become pressing global concerns. The gradual depletion of fossil fuel reserves has created significant challenges for energy security. Moreover, the environmental ramifications of fossil fuel extraction and consumption including greenhouse gas emissions and climate change are of growing concern. The need to transition to more sustainable and cleaner forms of energy is becoming ever more urgent.

In this scenario, biofuels have emerged as a viable solution for addressing both the sustainability of energy supplies and environmental concerns. Biofuels are renewable energy sources derived from biological materials, including agricultural residues, forestry waste, and other biomass resources. By harnessing these materials, biofuels offer the potential to reduce dependency on finite fossil fuels while promoting a more sustainable energy future. Biomass and biofuels are emerging industries, driven by a growing demand for sustainable fuel alternatives (Obi et al 2013). Among the various forms of biofuels, solid biofuels-specifically briquettes and pellets-stand out as efficient and versatile alternatives. Briquettes and pellets are produced by compressing biomass into compact, energy-dense forms that can be easily transported, stored, and used for a range of applications, from residential heating to industrial energy production. Their utilization helps mitigate waste from agricultural and forestry processes while providing a renewable and low-carbon energy source.

Briquetting leverages a natural process, utilizing a natural binder found in all biomass. Lignin, a solid component of biomass, transforms into a liquid under high pressure and heat, binding waste materials together to create high-density biofuel. A briquette is a block of combustible material used as fuel for starting and maintaining fires (Surajo and Mustapha 2017). The relevance of briquettes and pellets in modern energy systems is particularly notable in regions that rely heavily on traditional biomass for cooking and heating. Additionally, the localized production of briquettes and pellets can contribute to rural economic development, creating jobs and fostering energy independence.

Biomass briquettes and pellets have emerged as some of the most efficient energy sources. Variety of waste materials have been employed to produce these fuels. Municipal solid waste has been used to replicate biomass briquettes for on-site energy production (Romallosa and Kraft 2017). Sawdust, date palm trunks, and various crop residues have been used to create biomass briquettes without requiring a binding agent (Garrido et al 2017).

Biofuels like briquettes and pellets play a crucial role in addressing climate change due to their carbon-neutral nature. The carbon released during combustion is balanced by the carbon absorbed by plants, creating a closed cycle, unlike fossil fuels that release long-stored carbon. As fossil fuel reserves deplete, biofuels are emerging as essential renewable alternatives in global energy strategies (World Economic Forum 2023).

This study develops high-efficiency briquettes and pellets using agricultural waste and tamarind kernel powder (TKP) as a natural binder. The prototypes are designed to deliver improved calorific value and energy efficiency while supporting sustainable energy production, waste reduction, and energy security in the region. Tamarind kernel powder (TKP) is a polysaccharide extracted from the endosperm of tamarind seeds (*Tamarindus indica Linn*). This TKP-based gum is a valuable thickening and stabilizing agent with diverse applications. TKP is widely available and economically viable. It is used as an additive, preservative, gelling agent, solidifying agent, binding agent, and stabilizer (Bhavini et al 2018). The combination of these raw materials is expected to result in briquettes and pellets with a higher calorific value compared to those currently available in the market. To create both briquettes and pellets, separate machinery was utilized for each process. The collected raw materials were transported to specialized briquette and pellet production facilities located in the Kolar district of Karnataka, where prototypes were successfully produced for testing and analysis. The objectives of this study are to develop high-efficiency briquettes and pellets using agricultural waste and TKP to enhance calorific value and promote sustainable energy production and support regional energy security.

MATERIAL AND METHODS

Study location and resources: This study focused on the production of briquettes and pellets from agricultural wastes, specifically coconut husk, arecanut shell, and sawdust, mixed in equal proportions. To produce the prototype for testing, 3 kilograms of each raw materials were utilized. The raw materials were sourced from the Davangere (14.0° N to 14.6° N and 75.9° E to 76.4° E) and Tiptur (13.0° N to 13.3° N and 76.5° E to 76.8° E) regions of Karnataka, known for their high production of these agricultural by-products. In addition to these materials, tamarind kernel powder was incorporated as a natural binding agent to enhance both the binding capacity and density of the final products.

Methodology

Briquettes and pellets production: The following methods were employed to produce briquettes and pellets:

Drying: The raw material must be dried to a suitable moisture content before processing. The ideal moisture content varies depending on the type of raw material and the equipment used. Generally, agricultural waste and wood require a moisture content of 8-12%, while mechanical piston presses can handle up to 15% moisture, and hydraulic systems can handle up to 15-30%.

Pulverization: The raw material must be reduced to a suitable particle size before it enters the densification process. The particle size should not exceed 25% of the diameter of the final product for most densification equipment.

Pre-conditioning: To make the raw material softer and easier to work with, superheated steam is often added between the pulverization and densification stages. This conditioning process improves the binding properties of the material and helps prevent the briquettes from falling apart. For pelletizing, a small amount of water and TKP (about 10% and 8% of the raw material weight) is added to the mixture.

Compression of raw materials: The study used a hydraulic press process, which involves compacting the biomass in both the vertical and horizontal directions. The standard briquette weight is 4-6 kg, with dimensions of 450 mm x 160 mm x 80 mm. The power required for briquetting is 37 kW for 1800 kg/h. Pelletizing is a similar process but uses smaller dies to produce cylindrical briquettes between 5-10 mm in diameter and 50 mm in length. The pelletizer has a number of dies arranged as holes bored on a thick steel disk or ring, and the material is forced into the dies by means of two or three rollers. Pellets have good mechanical strength and combustion characteristics.

Cooling: To prevent briquettes from breaking apart, piston press systems often have a cooling track where the material can slowly cool down before being cut to the desired length.

Storing and Transporting: After cooling, the briquettes and pellets are typically stored before combustion. Storage can take place outdoors under a roof, indoors, in containers, or other methods.

Production of TKP: The following are the steps to produce TKP (Bhavini et al 2018)

Cleaning: The kernels are first cleaned to remove dirt and debris with vibrating cleaner machine is used to separate and discard small and large particles.

Roasting: The shell coating of the kernel is firmly attached and needs to be softened. Roasting is an important process that helps to separate the shell from the endosperm and also reduces the water content in the TKP.

Separation: Once the shell is softened, it can be removed from the kernel. The shell is more brittle than the kernel, making separation easier. This step is essential as the shell is not suitable for use in various applications.

Grinding: The separated kernels are then ground in ball mills to produce a fine, smooth powder.

Screening: The ground powder is screened through screens of less than 50 microns to ensure a uniform particle size. This is important to prevent clogging during application and ensure even spreading of the thickener.

Testing and analysis: For testing and analysis, the study conducted various proximate analyses to evaluate the quality and performance of the briquettes and pellets. This included the determination of moisture content, bulk density testing, and calorific value assessment (Bhujbal et al 2023). These tests were carried out in a systematic and scientific manner to obtain accurate and reliable results that would validate the efficiency and suitability of the produced briquettes and pellets for practical applications.

Moisture content: The moisture content of the briquettes was measured using the ASAE Standard S358.2 for forage. Two-grams of samples was oven dried for 24 hours at 105 ± 2°C until their mass remained constant. These tests were conducted at the Seed Testing Laboratory of the University of Agricultural Sciences, Bangalore.

Density test: The test is performed in accordance with the guidelines specified by ISO 18847:2016**,** which provides the standard procedures for determining the bulk density of solid biofuels. The bulk density testing was conducted at the laboratory of the National Centre for Biological Studies, Bangalore.

Calorific value: A bomb calorimeter was used to measure the calorific value of the ground material. The calorimeter consists of a bomb, a metal container, and a thermally insulated jacket. A temperature transducer inside the unit records temperature changes during fuel combustion with cooling system. The gross calorific value was determined experimentally by combusting 2 grams of sample under specific conditions in a bomb calorimeter according to the ASTM D2015-96 standard. The test was conducted at the Bioenergy Research and Quality Assurance Laboratory of the University of Agricultural Sciences, Bangalore.

RESULTS AND DISCUSSION

The proximate analysis results for the biomass residues and coal reveal significant insights into the efficiency and potential of the developed prototypes.

Bulk density performance: The bulk density measurements indicate that the pellets exhibit the highest density at 860 kg/m³, which signifies a high degree of compaction. This enhanced density is attributed to the pelletizing process and the incorporation of tamarind kernel powder (TKP) as a binding agent, demonstrating that the prototype briquettes and pellets are more compact compared to both the biomass residues and coal. Among the biomass residues, the ASC prototype, composed of arecanut shell, sawdust, and coconut husk, also shows a higher bulk density of 302 kg/m³ compared to the GSB formulation, which has a density of 282 kg/m³. This suggests that the ASC mixture benefits from improved compaction and binding properties, potentially due to the optimal combination of its constituent materials. Ideal briquettes possess low moisture content, high density, and high calorific value (Arewa et al 2016).

Moisture content: The ideal moisture content for briquetting depends on the specific feedstock. However, recommend general range of moisture content is 8-12% (FAO 1996). The moisture content of the prototypes reveals that the pellets have the lowest moisture content at 9.1%, which is advantageous for combustion efficiency, as lower moisture content generally results in higher energy output and better performance. In comparison, the ASC prototype has a moisture content of 12.1%, which is slightly higher than the GSB residue's moisture content of 10.7%. ASC's moisture content is somewhat higher, but remains within an

Table 2. Ash content of various biomass

Biomass	Ash content (%)
Saw dust	1.3
Arecanut shell	5.1
Coir pith	6.0
Groundnut shell	6.0
Eucalyptus biomass	6.2*
Coal	25.0-45.0**

Source: FAO -Field Document No.46 1996
*Muhdi et al (2019). **PIB -Ministry of Coal report (2018)

Table 1. Proximate analysis of biomass samples

Parameters	Units	Biomass samples			
		GSB	ASC	Pellets	Coal
Bulk density	kg/m³	282	302	860	492
Moisture content	%	10.7	12.1	9.1	7.9
Gross calorific value	kJ/kg	10536	13587	16703	28874
Ash content*	%	4.5	4.1	4.1	35

GSB: Groundnut shell, Saw dust and Biomass; ASC: Arecanut shell, Saw dust and Coconut husk are the various combination of raw materials used for making the briquettes; *Authors calculations, based on the average values in Table 2.

acceptable range for biomass fuels. Both prototypes have higher moisture content compared to coal, which has a moisture content of 7.9%. Despite this, the improved compaction and calorific value of the prototypes may mitigate the impact of their slightly higher moisture content.

Calorific value: The highest energy content at 28,874 kJ/kg, were observed in coal reflecting its high energy density. However, given the environmental concerns associated with coal, the focus shifts to the biomass-based prototypes. Among the prototypes, the pellets demonstrate the highest calorific value of 16,703 kJ/kg, indicating that the innovative use of agricultural residues and TKP results in a fuel with substantial energy content. This makes the pellets a competitive alternative in terms of energy output. The calorific value of the briquette is reported between 13000-16000 kJ/kg (Gill et al 2018). The ASC prototype also exhibits a significant calorific value of 13,587 kJ/kg, which is notably higher than that of the GSB residue, which has a calorific value of 10,536 kJ/kg. Although neither prototype matches coal's calorific value, their use of inexpensive agricultural residues and TKP presents a viable and sustainable alternative.

Ash content and combustion efficiency: Ash content refers to the non-combustible residue left after the material has been burned, and lower ash content is generally desirable as it indicates a cleaner, more efficient fuel with fewer emissions and waste. A low ash content is preferable in biomass, as levels exceeding 4% can lead to slagging (Kaliyan and Morey 2009). The ideal ash content of biomass briquettes is in range of 0.91 to 5.44% (Arulkumar et al 2019).

Both the ASC and pellet prototypes have relatively low ash content (4.1%). This suggests that these prototypes produce minimal residue during combustion. The low ash content also indicates that a larger proportion of the biomass material is converted into energy during combustion, enhancing the overall efficiency of these fuels. The GSB biomass residue also exhibits a slightly higher ash content at 4.5%, which, while marginally higher than the ASC and pellet samples, still represents a low ash output. This makes GSB a fairly efficient fuel, although the ASC and pellet prototypes demonstrate slight advantages in terms of cleaner combustion. In stark contrast, coal has an ash content of 30%, which is significantly higher than any of the biomass samples. This high ash content reflects one of the major drawbacks of coal as a fuel source, as it leads to higher emissions, more waste, and greater environmental harm. The ash generated from coal combustion also contributes to pollution and requires more frequent maintenance in industrial systems, adding to its overall environmental footprint. The substantially lower ash content of the ASC and pellet prototypes highlights their superiority over coal in terms of environmental impact and combustion cleanliness. These results further underscore the value of using agricultural residues as an alternative fuel source, offering a more sustainable and efficient option with far fewer negative environmental consequences. Overall, the ASC and pellets prototypes display promising characteristics in terms of bulk density, ash content and calorific value, highlighting their potential as effective alternatives to traditional fossil fuels. The research underscores the innovative approach of utilizing agricultural waste and TKP to enhance the efficiency and sustainability of biomass fuels.

Environmental sustainability and energy security: In addition to the proximate analysis results, the regional assessment of crop residues in Karnataka reveals a substantial opportunity for local briquette and pellet production. The state's diverse agricultural wastes offer a robust foundation for producing sustainable and efficient biofuels.

The widespread availability of agricultural by-products across Karnataka highlights the feasibility of utilizing these resources for biofuel production. The cost analysis of briquettes and pellets reveals a significant economic advantage over coal. Briquettes are priced at approximately ₹8-12 per kg, and pellets at ₹18-21 per kg, while coal ranges between ₹30-40 per kg. This cost difference highlights the affordability of biomass fuels, making them a competitive alternative to coal. Furthermore, the raw materials used for the prototypes-agricultural residues like arecanut husk, coconut husk, and sawdust are not only locally sourced but also sustainable, reducing dependence on non-renewable resources. By utilizing these inexpensive, widely available raw materials, biomass fuels offer both cost-effectiveness and environmental benefits. The presence of several start-ups in the state dedicated to this field further emphasizes the potential for innovation and local engagement. Combining various agricultural residues, with sawdust emerging as a crucial component, can enhance the quality and efficiency of the biofuels produced. Effective promotion and support for indigenous biofuel production can significantly impact the country's energy security (Patil 2020). By leveraging local resources and fostering a robust biofuel industry, Karnataka can contribute to a more sustainable energy future and reduce dependence on fossil fuels.

CONCLUSION

This study evaluated the efficiency of biomass-based prototypes such as ASC (Arecanut Husk, Sawdust, and Coconut Husk) and pellets developed from agricultural residues in comparison to traditional coal. The prototypes exhibited superior performance in terms of compaction,

Table 3. Region-wise potential crop residues for briquettes and pellets production in Karnataka

Region	Districts	Potential crop residue for briquettes and pellets	Approximate calorific value (~kcal/kg)
North Karnataka (15.0° N to 17.9° N and 74.1° E to 77.5° E)	Bagalkote, Ballari, Vijayanagara, Belagavi, Bidar, Gadaga, Koppala, Kalaburagi, Raichuru, Vijayapura, Yadgiri	Groundnut Shell, Paddy husk, Maize cobs and stalk, Chilli, Red gram and Cotton stalks and residues, Sugarcane Bagasse, Mesquite twigs	3100-4500
East Karnataka (12.9° N to 13.9° N and 77.4° E to 78.4° E)	Kolara, Chikkaballapura	Coconut husk, Eucalyptus, Acacia, Chilli stalk, Mesquite twigs	3500-4500
West Karnataka (14.0° N to 16.0° N and 74.3° E to 76.8° E)	Uttara Kannada, Dharwada, Belagavi, Haveri	Paddy husk, Eucalyptus, Acacia, Sugarcane Bagasse, Red gram stalk, Maize cobs and stalk, Chilli stalk	3200-4200
South Karnataka (12.0° N to 14.5° N and 76.5° E to 77.5° E)	Bengaluru Rural, Bengaluru Urban, Ramanagara, Tumakuru, Mandya, Mysuru, Chamarajanagara	Sugarcane Bagasse, Arecanut husk, Coconut husk, Paddy husk, Tamarind husk	3400-4500
Malnad Region (13.0° N to 15.5° N and 75.5° E to 76.8° E)	Chikkamagaluru, Kodagu, Hassan, Shivamogga	Bamboo, Eucalyptus, Acacia, Coffee husk, Coconut husk, Arecanut husk, Paddy husk, Maize cobs and stalk	3500-4400
Central Karnataka (14.0° N to 15.5° N and 75.5° E to 76.5° E)	Davanagere, Chitradurga	Arecanut husk, Maize cobs and stalk, Coconut husk, Paddy husk, Mesquite twigs	3500-4200
Coastal Karnataka (12.5° N to 14.0° N and 74.5° E to 75.5° E)	Udupi, Dakshina Kannada	Coconut husk, Paddy husk, Arecanut husk	3500-4400

combustion efficiency, and environmental impact. The use of tamarind kernel powder (TKP) as a binding agent contributed to improved density and structural integrity. The prototypes also showed competitive moisture content, supporting efficient combustion, and demonstrated significant advantages in terms of cleaner combustion with lower ash content compared to coal. These findings underscore the potential of these biofuels as sustainable alternatives to fossil fuels. Furthermore, the abundant availability of agricultural residues in Karnataka highlights the feasibility of local biofuel production, contributing to energy security and environmental sustainability.

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Assessment of Anti-oxidant and Photocatalytic Activity of *Pamburus missionis* Swingle Extracts through GC-MS and ICP-OES Analysis

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Abstract: In this current world of polluted environment, antioxidants play a vital role in mitigating oxidative damage and protecting against harmful effects on human health and the environment by neutralising free radicals. The photocatalytic activity can facilitate the degradation of pollutants and toxic substances. Therefore, the exploration of novel antioxidant and photocatalytic agents from natural resources such as plant extracts is integral for the development of effective therapeutic strategies and tenable solutions to combat these pressing issues. *Pamburus missionis,* a rutaceae member which is native to India and Southeast Asia, has been used in Ayurveda with the name "Kudangal" for different types of digestive, respiratory and skin problems. Phytochemical analysis revealed the presence of alkaloids, flavonoids, phenols, glycosides and steroids. GC-MS analysis unveiled totally 8 compounds from leaf, 5 and 8 from stem and bark extracts. ICP-OES analysis disclosed the different mineral elements such zinc (Zn), iron (Fe), copper (Cu), magnesium (Mg) and so on. In DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, bark extract showed highest antioxidant property (81.99%) followed by leaf(69.34%) and stem (48.42%). The leaf extract exhibited good photocatalytic activity (80.50%) followed by bark(72.88%) and stem, (55.72%). The present study demonstrated the antioxidant and photocatalytic potential of *Pamburus missionis*.

Keywords: Antioxidant, Photocatalytic, GC-MS, ICP-OES, Chemical compounds, Plant extracts

Free radicals (FRs) are generated due to cellular metabolism and exposure to environmental stressors like UV radiation, pollution, smoke. Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) shows positive effects on cellular responses and immune function at moderate levels. Excessive concentrations can rise oxidative stress, an adverse process that may lead to chronic diseases like cancer, atherosclerosis, neurodegenerative diseases (Baliyan et al 2022). Antioxidants are the compounds, alleviate oxidative damage by FRs through electron donation, radical scavenging and enzymatic activity. Their assistance in neutralizing FRs is vital for maintaining overall health and well-being. Through endogenous production and dietary intake, humans acquire a range of antioxidants, including both confirmed and putative compounds. Higher plants synthesize a diverse array of secondary metabolites, especially polyphenols and flavonoids represent potent source of antioxidants (Aryal et al 2019). These phytochemicals participate in various biochemical pathways and play a crucial role in combating oxidative stress, thereby protecting against various health issues (Pung Rozar et al 2024). For assessing antioxidant activity, DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay is widely employed and affordable technique. DPPH, stable purple colour free radical reacts with antioxidants in the sample and turns into yellow hue, resulting in decrease in absorbance at 517nm (Ramakrishna and Savithramma 2023). Photocatalytic activity of plant extracts refers to the ability of its various bioactive compounds to harness light energy for breaking down harmful pollutants such as methylene blue, paving the way for innovative environmental remediation strategies (Zhang et al 2019).

The increasing concern over environmental pollution and human health has sparked interest in exploring sustainable and ecofriendly solutions from natural resources. Medicinal plants, in, particular have garnered significant attention due to their potential antioxidant and photocatalytic properties. The integration of both these properties in a single material has significant implications for the development of multifunctional therapeutics and environmental technologies. Medicinal plants have been used for centuries as folk medicine, providing a foundation for exploring their antioxidant activity and photocatalytic potential and are widely available, making them a viable option for large scale applications and they also offer environmentally benign alternative for synthetic materials.

Pamburus missionis, a tropical plant species native to western ghats of India has been used in ancient traditional medicinal system for its benefits. In earlier studies, phytochemical analysis was performed using different solvents and observed the presence of alkaloids, phenols, tannins, flavonoids, steroids, glycosides and coumarins in different proportions in different parts (Yaswanthi et al 2024). However, it's antioxidant and photocatalytic activity remains largely unexplored. This study aims to investigate these properties of this plant extracts using Gas Chromatography-Mass Spectrometry (GC-MS) and Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) analysis. GC-MS will facilitate the identification and quantification of bioactive compounds responsible for antioxidant activity, while ICP-OES will enable the determination of elemental composition and photocatalytic potential. The findings of this research contribute to the understand *Pamburus missionis*'s therapeutic and environmental applications, providing valuable insights into its potential as a sustainable source of antioxidant and photocatalysts.

MATERIAL AND METHODS

Pamburus missionis, belongs to Rutaceae, with only one species and is evergreen tree, 10-15 tall, branched with stout-straight spines (Fig. 1). Leaves are elliptical, dark green, 6-10 cm long. Flowers are white, tetramerous and Fruits, globose berry highly glandular (Fig. 2). The parts were collected from Mamandur forest, beside Balupalle, Karakambadi Rural, Tirupati, Andhra Pradesh *GPS:* 13°46'02.6" N; 79°26'02.5" E. They are thoroughly washed and shade dried about 20 days and ground into fine powders, stored for further study.

Plant extracts preparation: One_ g of powdered plant materials were dissolved in 20mL of distilled water (DW) and subjected to thermal extraction. The mixtures were heated on a water bath at 60°C for 20 minutes and then allowed to stand overnight at room temperature. Following incubation, the mixtures were filtered to obtain crude extracts.

Methods

DPPH assay: DPPH stock solution was prepared by dissolving 10mg of DPPH in 100mL of Methanol, which yielded a solution mixture with an absorbance of around 1.305 at 517 nm. In the test tubes, 3 mL DPPH workable solutions (1mL of DPPH stock solution + 2mL of methanol) were combined with 100 µL of leaf, stem and bark extracts respectively. As a standard, 3mL of DPPH workable solution often mixed with 100µL of methanol. After 30 min incubation in complete darkness, the absorbance was therefore determined at 517 nm. The percentage of antioxidants was estimated (Ramakrishna and Savithramma 2019, Baliyan et al 2022).

Percentage of antioxidant activity= [(Ac−As) ÷Ac] × 100

where: Ac-Control reaction absorbance; As-Testing specimen absorbance.

Methylene blue dye degradation: Methylene blue (MB) is a commonly used model pollutant for photocatalytic degradation studies. 10mg of MB was combined with 1L of DW. To 100mL of this solution, 10 mg of Plant powders were added and kept under the sunlight. The absorbances were noted at 664nm using UV-VIS spectroscopy after 5 min, 15 min, 30 min and 60 min of incubation. The percentage of dye degradation was calculated by using below formula (Yugandhar et al 2012):

Percentage of dye degradation = $((A_i - A_f)/A_i) \times 100$

Where A_i = Absorbance initial; A_f = Absorbance final

GC-MS analysis: GC-MS analysis was used to identify and quantify the specific compounds in the plant's extracts. *P. missionis* was subjected to this test to identify the novel compounds that were aiding in antioxidant activity. Methanolic extracts were prepared by soaking 100mg of plant powders in 1mL of methanol for 24 h at room temperature. The mixture was filtered and performed analysis using GC-MS QP2010, SHIMADZU (Konappa et al 2020).

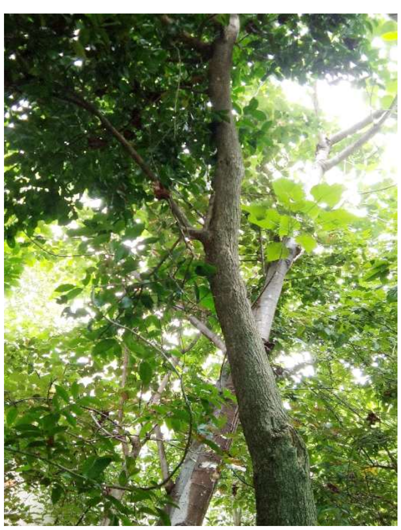

Fig. 1. *Pamburus missionis*

Fig. 2. Leaves and fruit

ICP-OES analysis: ICP-OES analysis is a Spectro analytical technique used to detect and quantify elemental concentrations. To identify elemental composition of Different parts of the *P. missionis*, it was subjected to ICP-OES analysis using Perkin Elmer 7000DV ICP-OES model. 100mg of plant powders were digested with 1ml of 30% of $H_2O_2$and 7mL of 70% $HNO_3$and kept in a muffle furnace for 10 min at 170°C. Then these were filtered and made upto 25mL and performed the analysis (Yugandhar and Savithramma 2017).

RESULTS AND DISCUSSION

The aqueous extracts of *Pamburus missionis* exhibited potent DPPH radical scavenging activity (Table 1). The results demonstrated that the bark had the high scavenging activity (81.99%), followed by the leaf (69.34 %) and the stem showed less activity comparably. Through GC-MS analysis, various bioactive compounds are identified in the leaf, stem and bark with their potential uses (Table 2-4). In leaf, total eight compounds were found. 2- butynoic acid which is a synthon in variety of reactions, including cycloacylation of phenols to flavones & chromones. Ethane,1-chloro-1-fluoro is an effective catalyst and Hydrogen halide scavenger for hydrogen fluoride and hydrogen chloride (NCBI 2024) 1,4-Dimethyl-5-oxabicyclo [2.1.0] pentane is also found in *Chara baltica, Dysphania ambrosioides*. (Tatipamula 2019). Cyclotrisiloxane, hexamethyl exhibit somewhat antibacterial and antioxidant activities (Momin and Thomas 2020). In stem, out of five compounds identified, three were same as in the leaf and the other two namely, 2- Butanone is sweet odour colourless liquid generally present in some foods like banana, cabbage, citrus fruits etc. (Api 2019) and Octane,1-iodo- no sufficient records. From bark, also eight compounds were discovered. 2-butanone, 3-methyl is commonly found to be present in all the three parts. Propiolic acid exhibit potent antioxidant activity due to presence of alkynyl group, allows it to effectively quench radicals and prevent oxidative damage (Kumar et al 2013). 2-chloroethyl methyl sulfoxide, an intermediate, particularly in the production of pesticides, herbicides and fungicides (Singh et al 2015). Arsenous acid, tris (trimethylsilyl) ester is generally a reagent in organic synthesis, like arsenic-containing compounds which may be utilised for treating cancer and infectious diseases (NCBI 2024). From these results, evidence for antioxidant property

Table 1. Antioxidant activity of *P. missionis* extracts with DPPH

Parameter	Control	Leaf	Stem	Bark
Absorbance at 517nm	1.305	0.400	0.673	0.235
% of antioxidant	--------	69.34%	48.42%	81.99%

Table 2. GC-MS of methanolic extract of leaves

Retention time	Name of the compound	Molecular formula	Molecular weight	Peak area (%)	Uses
1.040	2-Butynoic acid	$C_4H_4O_2$	84	1.027	Anti –inflammatory and anticancerous agent, Synthon
1.094	Ethane, 1-chloro-1-fluoro	C_2H_4ClF	82	93.852	Catalyst,
1.144	Ethanol	C_2H_6O	46	1.106	Disinfectant and Antiseptic
1.169	1,4-Dimethyl-5-oxabicyclo [2.1.0] pentane	$C_6H_{10}O$	98	0.079	-----------
1.200	Dimethyl sulfide	C_2H_6S	62	2.370	Gas odorant, catalyst, impregnator, food flavoring agent, anti-coking agent.
1.420	N-Nitroso-2-methyl-oxazolidine	$C_4H_8N_2O_2$	116	0.395	Liver Carcinogen
1.644	2-Butanone,3-methyl	$C_5H_{10}O$	86	0.158	Intermediate for production of herbicides and dye precursors
2.997	Cyclotrisiloxane, hexamethyl	$C_6H_{18}O_3Si_3$	222	0.316	Antibacterial and antioxidant, Softening agent in textile.

Table 3. GC-MS of methanolic extract of stem

Retention time	Name of the compound	Molecular formula	Molecular weight	Peak area (%)	Uses
1.091	Ethane, 1-chloro-1-fluoro	C2H4ClF	82	98.973	Catalyst,
1.394	2-Butanone	C4H8O	72	0.391	Cleaning agent
1.636	2-Butanone, 3- methyl-	C5H10O	86	0.195	Intermediate for production of herbicides and dye precursors
1.786	Octane 1-iodo-	C8H17I	240	0.097	----------
2.975	Cyclotrisiloxane, hexamethyl-	C6H18O3Si3	222	0.0195	Antibacterial and antioxidant, Softening agent in textile.

of this plant's parts, especially, Cyclotrisiloxane, hexamethyl in leaves and stem were responsible for their antioxidant nature while, propiolic acid in bark a dominant antioxidant, hence expressed better activity than other parts extracts.

Methylene blue dye degradation is a widely studied process in photocatalysis and environmental remediation. The experiment with plant powders demonstrated the leaf's excellent photocatalytic property (80.5%), followed by the bark and stem (Table 5). Flavonoids, polyphenols and chlorophylls play a vital role in this property, also, elemental composition contribute its part. To unveil the composition of different elements, the powders were analysed using ICP-OES technique. Through this test, total 11 elements and their quantities were determined (Table 6). Among those, mainly zinc, copper and iron are essential for photocatalytic activity. The leaf's photocatalytic efficiency was significantly boosted by its elevated levels of these micronutrients and also due to required amounts of phenols, flavonoids and tannins. Though the stem has good amounts of these elements, it has reduced amounts of secondary metabolites when compared to bark. Therefore, the bark expressed better photocatalytic activity than stem. The increase in demand for sustainable materials with antioxidant and photocatalytic properties had led to renewed interest in medicinal plants because of their easy availability and ecofriendly nature. *Pamburus* is one of such plant, has to be explore more to understand its potentials on various applications. The previous study on secondary metabolites of this plant, enhanced the research

Table 5. Photocatalytic activity through Methylene dye degradation by *Pamburus missionis*

Time	Dye degradation (%)		
	Leaf	Stem	Bark
5 min	41.31	15.89	21.18
15 min	47.46	22.88	29.66
30 min	65.46	49.57	54.87
60 min	80.50	55.72	72.88

Table 4. GC-MS of methanolic extract of bark

Retention time	Name of compound	Molecular formula	Molecular weight	Peak area (%)	Uses
1.007	Argon	Ar	40	1.913	Used in lasers, medical imaging, food packaging etc.
1.032	Propiolic acid	C3H2O2	70	1.177	Antioxidant, antimicrobal, corrosion inhibitor and UV stabilizers
1.075	2-Chloroethyl methyl sulfoxide	C3H7ClOS	126	93.472	Intermediate in the production of antibacterial, antifungal and antiviral agents
1.125	Ethanol	C2H6O	46	0.294	Disinfectant and Antiseptic
1.169	Di-isopropyl ether	C6H14O	102	1.030	Solvent in organic synthesis, pharmaceuticals.
1.377	2,4-Pentanedione, 3-methyl	C6H10O2	114	0.883	Flavor and fragrance, intermediate in organic synthesis, corrosion inhibitor
1.624	2-Butanone,3-methyl	C5H10O	86	0.588	intermediate for production of herbicides and dye precursors
2.055	Arsenous acid, tris(trimethylsilyl) ester	C9H27AsO3Si3	342	0.588	Used in the synthesis of arsenic based pharmaceuticals for treating cancer and infectious diseases.

Table 6. ICP-OES of *Pamburus missionis*

Name of the element	Units	Leaf	Stem	Bark
Nitrogen (N)	%	0.94	2.01	1.2
Phosphorous (P_2O_2)	%	0.1437	0.1028	0.1038
Potassium (K_2O)	%	2.254	1.832	1.009
Calcium (Ca)	%	2.556	2.116	0.7918
Magnesium (Mg)	%	0.4254	0.2613	0.0329
Zinc (Zn)	ppm	17.86	21.89	10.80
Iron (Fe)	ppm	235.3	211.5	132.1
Copper (Cu)	ppm	11.82	45.83	5.105
Manganese (Mn)	ppm	13.43	196.3	35.92
Boron (B)	ppm	55.34	28.45	6.203
Molybdenum (Mo)	ppm	4.200	52.28	3.900

interest to discover its biological activities which led to the present study. Furthermore, research is essential to uncover its capabilities.

CONCLUSION

The comprehensive evaluation of *Pamburus missionis* revealed its remarkable antioxidant and photocatalytic properties, underscoring its potential as a versatile natural resource. The identification of diverse bioactive compounds and essential elements highlights its therapeutic and environmental applications. The findings suggest that *Pamburus missionis* could be a valuable source of natural antioxidants and photocatalysts, warranting further investigation for its potential uses in environmental remediation like wastewater treatment and pollution control, and in medicinal applications as antioxidant supplements, antimicrobial agents etc.

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Studies on Air Pollution Tolerance Index of Indoor Plants for Interior Landscaping

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Abstract: The present investigation comprised of assessment of 20 indoor plants for air pollution tolerance index APTI were selected based on various biochemical parameters such as total chlorophyll content, leaf extract pH, relative water content and ascorbic acid content. Maximum relative water content was in *Zamioculcas zamiifolia*, ascorbic acid in *Sansevieria trifasciata*, total chlorophyll content in *Syngonium podophyllum* and leaf extract pH was in *Epipremnum aureum*. Based on APTI index *Zamioculcas zamiifolia, Sansevieria trifasciata*, *Epipremnum aureum*, *Diffenbachia camille* and *Aglaonema commutatum* were most effective for indoor air pollution tolerance and can be recommended to be used in indoor landscaping for urban areas.

Keywords: Air pollution tolerance index, Indoor plants, Urban landscape, Indoor pollution, Air quality

Plants have been linked with environment, health and happiness. The importance of indoor air quality to human health has become of increasing interest where people often spend over 85-90% of time in indoor environments, either at the workplace or residential (Marc et al 2018). Indoor plants have recently gained high popularity, especially in the post-Covid era (Han 2020 and Singh 2020). In urban areas, most citizens have long-term exposure to large amounts of harmful chemicals indoors, whether at home or working at the office (Shi et al 2015, Lukcso et al 2016). People are usually exposed to a higher intake or breathe in a greater concentration of air pollutants because these pollutants are more prevalent indoors than outdoors (Zhang et al 2017). According to WHO, indoor air pollution has been among the top 5 risks to public health (WHO 2022). As per a report by IHME, 2.6 million people died in 2016 owing to illnesses attributed to indoor air pollution. The sensitivity and tolerance to pollution in plants depend upon various biochemical parameters like ascorbic acid content, chlorophyll, relative water content and pH. The response of plants to air pollution can be assessed by the air pollution tolerance index which is being used by landscapers in selecting plant species for a particular area in order of their pollution tolerance. The effectiveness of plant species as bio-indicator or tolerance to air pollution depends upon the air pollution tolerance index . The tolerance and sensitivity to air pollutants depend on parameters like chlorophyll content, ascorbic acid content, leaf pH and relative water content. Chlorophyll content decreases due to the production of reactive oxygen species in the chloroplast under stress. Plants are also initial acceptors of air pollution and act as scavengers (Mahecha et al 2013). Hence, there is a need to screen plants based on APTI for their use as bio-indicators or to determine if they are tolerant to indoor air pollution.

MATERIAL AND METHODS

Area of study: The present investigation was carried out during the years 2021-22 and 2022-23 at the greenhouse complex, at Navsari Agricultural University, Navsari. Geographically, Navsari is situated at the coast of the Arabian Sea at 20^0 57' North latitude and 72^0 54' East longitude at an altitude of about 11.98 meters above the mean sea level.

Collection of samples: The study was conducted for the evaluation of different indoor plants based on their Air Pollution Tolerance Index. In this experiment, 20 indoor pot plants were grown in a 50 percent shade net house at the greenhouse complex, Department of Floriculture and Landscape Architecture, ASPEE College of Horticulture, during the years 2021 and 2022. Indoor plants selected for the present study are given in Table 1. The data on different biochemical parameters were recorded during June and September for two years *i.e.* 2021 and 2022 done. To study various biochemical parameters, leaf samples from the selected 20 indoor plant species were collected and analysed the different standard procedures. The leaf samples were brought to the laboratory in the ice box and washed with ordinary water, then with 0.1 N HCL, followed by distilled water for biochemical analysis.

Biochemical parameters: The ascorbic acid content (mg/g) in the leaves of different plants was estimated by the A.O.A.C. (1980) method. Total chlorophyll content (mg/g) in the leaves of different plants was estimated by using the method given by Hiscox and Israeistam (1979). Determination of pH was done by using a pH meter (Model – ESICO 1013) with a buffer solution of pH 4 and 9 (Barrs and Weatherly 1962). The relative water content (%) of the samples was estimated (Singh 1977). The air pollution tolerance index was determined by using biochemical parameters such as ascorbic acid t, total chlorophyll ct, relative water, and leaf extract of pH parameters through the following formula.

$$APTI = \frac{A\,(T+P) + R}{10}$$

Where,

A = Ascorbic acid content (mg/g), T = Total chlorophyll content (mg/g)

R = Relative water content (%). P = Leaf extract of pH

Statistical analysis: All the data were analysed statistically using the OPSTAT software.

RESULTS AND DISCUSSION

Significant variation in biochemical parameters and air pollution tolerance index of selected 20 indoor plant species was observed (Table 2).

Relative water content (%): The maximum relative water content (%) was in *Zamioculcas zamiifolia* followed by *Aglaonema commutatum*, *Sansevieria trifasciata* and *Diffenbachia camille*. There was a variation in relative water content in different plant species, which may be due to their different genetic makeup. Higher relative water content in *Zamioculcas zamiifolia, Aglaonema commutatum* and *Sansevieria trifasciata* may have favoured plants' resistance to stress conditions. Tsega and Deviprasad (2014) and Ogunkunle et al (2015) observed higher water content in plants under stress conditions such as air pollution.

Ascorbic acid (mg/g) content: *Sansevieria trifasciata* showed maximum ascorbic acid (mg/g) content followed by *Zamioculcas zamiifolia* and *Epipremnum aureum*. The variation in ascorbic acid content in different plant species may be due to their different genetic makeup (Chen et al 2004). Further, the increase in the level of ascorbic acid may result owing to the respective plant defence mechanism as suggested by (Cheng et al 2007). Stress conditions have been indicated to trigger plant defence mechanisms by increasing ascorbic acid levels in plants (Yannawar and Bhosle 2013).

Total chlorophyll (mg/g) content: S*yngonium podophyllum*

Table 1. Morphological characters of selected indoor plant species

Plant species	Common name	Family	Habit
Aglaonema commutatum	Chinese evergreens	Araceae	Evergreen herbaceous perennial shrub
Anthurium andraeanum	Flamingo flower plant	Araceae	Flowering potted plant
Begonia rex 'Cultorum'	Wax Begonia	Begoniaceae	Flowering potted plant
Chlorophytum comosum	Spider ivy	Asparagaceae	Succulents
Dieffenbachia camille	Dumb cane	Araceae	Evergreen herbaceous perennial pot plant
Dracaena reflexa	Song of india	Asparagaceae	Succulent shrub
Epipremnum aureum	Money plant	Araceae	Climber
Howarthia fasciata	Little zebra plant	Asphodelaceae	Succulents
Nephrolepis exaltata	Sword fern	Nephrolepidaceae	Fern
Peperomia obtusifolia	Baby rubber plant	Piperaceae	Succulents
Peperomia 'Scandens Green'	Cupid peperomia	Piperaceae	Succulents
Philodendron erubescens	Red leaf philodendron	Araceae	Evergreen herbaceous perennial climber
Philodendron 'Golden Goddess'	Lemon lime philodendron	Araceae	Evergreen herbaceous perennial climber
Portulacaria afra	Elephant bush	Didiereaceae	Succulents
Rhapis excelsa	Broad leaf lady palm	Arecaceae	Palm
Sansevieria trifasciata	Snake plant	Asparagaceae	Succulents
Sansevieria masoniana	Mason's congo	Asparagaceae	Succulents
Spathiphyllum wallisii	Peace lily	Araceae	Herbaceous perennial indoor plant
Syngonium podophyllum	Arrowhead plant	Araceae	Climber
Zamioculcas zamiifolia	ZZ plant	Araceae	Succulents

Table 2. Biochemical parameters and APTI index of selected indoor plant species (Pooled data of 2021-2022)

Genotype	RWC (%)	Ascorbic acid content (mg/g)	Total chlorophyll (mg/g) content	Leaf extract pH	Air pollution tolerance index (APTI)
Aglaonema commutatum	94.54	0.79	0.65	6.83	10.10
Anthurium andraeanum	86.57	0.21	0.23	6.64	8.78
Begonia rex 'Cultorum'	86.80	0.22	0.46	2.51	8.75
Chlorophytum comosum	91.58	0.22	0.76	7.78	9.32
Dieffenbachia Camille	94.24	0.79	0.59	7.41	10.12
Dracaena reflexa	90.12	0.20	0.65	7.33	9.16
Epipremnum aureum	92.39	0.80	0.69	7.83	10.14
Howarthia fasciata	85.91	0.22	0.24	5.72	8.72
Nephrolepis exaltata	90.09	0.29	0.59	7.06	9.23
Peperomia obtusifolia	86.14	0.28	0.41	7.33	8.84
Peperomia 'Scandens Green'	89.03	0.24	0.63	6.71	9.06
Philodendron erubescens	85.32	0.25	0.12	6.61	8.69
Philodendron 'Golden Goddess'	88.84	0.33	0.48	6.69	9.10
Portulacaria afra	87.96	0.28	0.11	4.46	8.91
Rhapis excelsa	86.93	0.57	0.78	5.11	9.03
Sansevieria trifasciata	94.53	0.83	0.64	6.63	10.18
Sansevieria masoniana	89.29	0.31	0.17	5.80	9.10
Spathiphyllum wallisii	87.16	0.24	0.73	6.34	8.87
Syngonium podophyllum	89.33	0.20	0.84	6.78	9.08
Zamioculcas zamiifolia	95.08	0.80	0.70	7.45	10.21
Mean	89.59	0.40	0.52	6.45	9.27
	G / Y / G×Y	G / Y / G×Y	G / Y / G×Y	G / Y / G×Y	G / Y / G×Y
CD (p=0.05)	0.58 / 0.16 / 1.10	0.034 / 0.014 / 0.041	0.02 / 0.01 / 0.03	0.11 / 0.04 / 0.16	0.10 / 0.04 / 0.17

showed maximum total chlorophyll content followed by *Rhapis excelsa*. These plants having more total chlorophyll content acts an indicator of tolerance against air pollution (Chandawat et al 2014). Begum and Harikrishna (2010) also reported that chlorophyll content varies from species to species, as well as other biotic and abiotic conditions.

Leaf extract pH: Among selected 20 indoor plant species, the maximum leaf extract pH was in *Epipremnum aureum* followed by *Chlorophytum comosum*. The pH of the leaf extract serves as an air pollution sensitivity indicator. Plants having low pH are known to be more sensitive while those having around 7 or more pH are more tolerant against air pollution (Chauhan *et al*., 2012, Kumar and Nandini 2013). High pH can increase conversion efficiency from hexose sugar to ascorbic acid, whereas low leaf extract pH has shown a strong correlation with air pollution sensitivity (Escobedo et al 2008, Pasqualini et al 2011).

Air pollution tolerance index (APTI): The maximum APTI was in *Zamioculcas zamiifolia,* which was statistically at par with *Sansevieria trifasciata* followed by *Epipremnum aureum*, *Diffenbachia camille* and *Aglaonema commutatum*. APTI is inter-relation of different plant species to different biochemical parameters *viz*. ascorbic acid content, chlorophyll content, water content and leaf extract pH. Thus, high APTI in *Zamioculcas zamiifolia, Sansevieria trifasciata, Epipremnum aureum*, *Diffenbachia camille* and *Aglaonema commutatum* could be a result of higher water content and ascorbic acid content. The effectiveness of plant species as bio-indicator or tolerance to air pollution depends upon the air pollution tolerance index (APTI). APTI index is the capability of plants to survive against air pollution and helps to determine the tolerance and sensitivity of plants against air pollution. APTI in plants among different species is influenced by chlorophyll content, ascorbic acid, relative water content and leaf extract pH as variation in air pollution tolerances varying from species to species and region earlier observed in indoor plants (Gholami et al 2016, Kumar et al 2022).

CONCLUSIONS

Indoor plants are capable of mitigating indoor air pollution is clearly identified in the study. The indoor plant's species *viz.,Zamioculcas zamiifolia, Sansevieria trifasciata, Epipremnum aureum*, *Diffenbachia camille* and *Aglaonema commutatum* have high APTI index and are found to be capable to combat against indoor air pollution. The indoor plant species having higher APTI index have more chances of survival and growth in the indoor polluted area. *Zamioculcas zamiifolia, Sansevieria trifasciata, Epipremnum aureum*, *Diffenbachia camille* and *Aglaonema commutatum* were the best indoor plants to tolerate indoor air pollution and can be suggested for the urban area having high pollution levels in indoor environmental conditions such as residential areas, offices, banks or shopping malls.

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Periphytic Algae of Achankovil River in Pandalam Municipality, Kerala

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Abstract: The present investigation focuses on the periphytic algae in the riparian microhabitats of the Achankovil River in Pandalam, Kerala, India. The study was conducted from December 2021 to December 2022 across six fixed sampling locations (PN1, PN2, PN3, PN4, PN5, and PN6). Monthly samples were collected from periphyton-colonized plants at each station, preserved, and identified according to standard procedures. The study identified 61 algal genera across five classes: Cyanophyceae, Euglenophyceae, Chlorophyceae, Charophyceae, and Bacillariophyceae. The number of taxa in each class was Cyanophyceae (5), Euglenophyceae (4), Chlorophyceae (11), Charophyceae (16), and Bacillariophyceae (25). The genus *Cosmarium* was the most frequently occurring with 7 species, followed by *Nitzschia* (6 species), *Pinnularia* (5 species), and *Navicula* (4 species). The highest number of algal genera was observed at PN2 (17), while the lowest was at PN5 (8).

Keywords: Achankovil river, Anthropogenic pollution, Phytoplankton diversity, Western Ghats forest

The world is experiencing drastic environmental effects from climate change, prompting researchers worldwide to investigate the consequences. Natural disasters not only endanger human lives but also cause irreversible changes and biodiversity loss, negatively affecting the quality of ecosystem services. Rich biodiversity indicates the safety and pristine nature of the Earth, but climate change, ecosystem degradation due to overuse and pollution, and the emergence of invasive species threaten biodiversity (Hannah and Lovejoy 2019). In August 2018, Kerala faced a major flood, resulting in significant loss of life and irreparable biodiversity loss, as well as the mixing of different water ecosystems (Pramanick et al 2021). The excess flow from three major rivers-the Pampa, Manimala, and Achankovil-severely affected the Pathanamthitta and Alappuzha districts. Enormous amounts of particulate matter and dissolved and undissolved solvents flowed through the rivers and adjacent streams, devastating micro- and macrohabitats. Riparian microhabitats along the rivers, including small streams, ponds, oxbow lakes, marshes, and wetlands, are crucial transitional regions between land and water (Rajbongshi and Das 2016). These areas are known for their rich algal biodiversity (Ramey and Richardson 2017). Aquatic macrophytes in such habitats can help improve water quality by removing excess nutrients and as substratum for the attachment of phytoplankton (Ngente and Mishra 2024). Micro-algae have the potential to effectively remove organic loadings from wastewater (Rasheed et al 2022).

The Achankovil River, one of the major west-flowing rivers in peninsular India, flows through the Kerala districts of Kollam, Pathanamthitta, and Alappuzha. This 128-kilometer river originates from the streams of Pasukidamedu in the southwestern ghats and flows through several important towns in the Pathanamthitta district, including Pandalam, and joins the Pampa River at Veeyapuram in the Alappuzha district. The Pandalam area is enriched with unique micro and macroflora, and many endemic plants of Kerala exist there (Krishnan and Harikrishnan 2017). Throughout its course, the river has developed numerous small and large water microhabitats, some of which are seasonal flood plains while others are ephemeral areas. The river and surrounding areas were severely affected by ecosystem changes, habitat loss, and species loss during the flood. The riverine bodies in the district are rich in fish and other biological species. Swapna (2009) recorded 52 fish species in the river. A new checklist with a record of 35 species of ichthyofauna in the Achankovil basin was prepared by Vishnu et al (2023). Phytoplankton in the water bodies are significant contributors of oxygen and play an essential role in maintaining the balance between living species. Previous algal enumerations in the river have focused only on its lotic systems with little attention given to the riparian phytoplankton and periphytic flora (Krishnan et al 2020, Krishnan and Dhar 2021). Therefore, present study was conducted on epiphytic in the flood-affected Achankovil River in Pandalam Municipality.

Table 1. Identified Periphyton of Achankovil River at Pandalam, Kerala

Name of class	Scientific name
Cyanophyceae	
	Anabaena cylindrica Lemmermann
	Arthrospira platensis (C.B.Rao) Desikachary
	Lyngbya sp.1
	Oscillatoria formosa Bory ex Gomont
	Rivularia sp.1
Euglenophyceae	
	Euglena caudata E. Hubner
	Euglena acus
	Phacus sp.1
	Phacus acuminatus
Chlorophyceae	
	Chlorococcum humicola (Nageli) Rabenhorst
	Coelastrum microporum Nageli
	Crucigeniella crucifera (Wolle) Komárek
	Dictyochloropsis sp.1
	Oedogonium sp.1
	Oocystis lacustris Chodat
	Radiococcus nimbatus (De Wildeman) Schmidle
	Scenedesmus denticulatus Lagerheim
	Scenedesmus ellipticus Corda
	Scenedesmus quadricauda (Turpin) Brébisson
	Spirogyra sp. 1
Charophyceae	
	Closterium navicula (Brebisson) Lütkemüller
	Closterium parvulum Nageli
	Cosmarium didymoprotupsum West & G.S.West
	Cosmarium hammeri Reinsch
	Cosmarium impressulum Elfving
	Cosmarium obsoletum (Hantzsch) Reinsch
	Cosmarium quadrum P.Lundell
	Cosmarium subprotumidum Nordstedt
	Cosmarium subtumidum Nordstedt
	Euastrum binale F. Crassum Joshua
	Euastrum denticulatum F.Gay
	Euastrum pulchellum Brébisson
	Micrasterias laticeps Nordstedt
	Pleurotaenium archeri Delponte
	Pleurotaenium ehrenbergii (Ralfs) De Bary
	Pleurotaenium trabecula Nageli
Bacillariophyceae	
	Achnanthidium minutissimum (Kutzing) Czarnecki

Cont....

Table 1. Identified Periphyton of Achankovil River at Pandalam, Kerala

Name of class	Scientific name
	Amphora inariensis Krammer
	Amphora sp.1
	Aulacoseira granulata (Ehrenberg) Simonsen
	Cyclotella meneghiniana Kutzing
	Cymbella sp.1
	Diadesmis confervacea Kutzing
	Frustulia rhomboides (Ehrenberg) De Toni
	Gomphonema affine Kutzing
	Gomphonema lagenula Kutzing
	Gomphonema olivaceum (Hornemann) Ehrenberg
	Gomphonema venusta Passy, Kociolek & Lowe
	Navicula lanceolata (C.Agardh) Kutzing, nom. illeg.
	Navicula sp.1
	Nitzschia agnita Hustedt
	Nitzschia clausii Hantzsch
	Nitzschia desertorum Hustedt
	Pinnularia divergens W.Smith
	Pinnularia gibba (Ehrenberg) Ehrenberg
	Pinnularia rectangularis Y.Liu, Kociolek & Q.-X.Wang
	Pinnularia sp.1
	Pinnularia viridis (Nitzsch) Ehrenberg
	Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot
	Sellaphora pupula (Kutzing) Mereschkovsky
	Synedra sp.1

*sp.-species

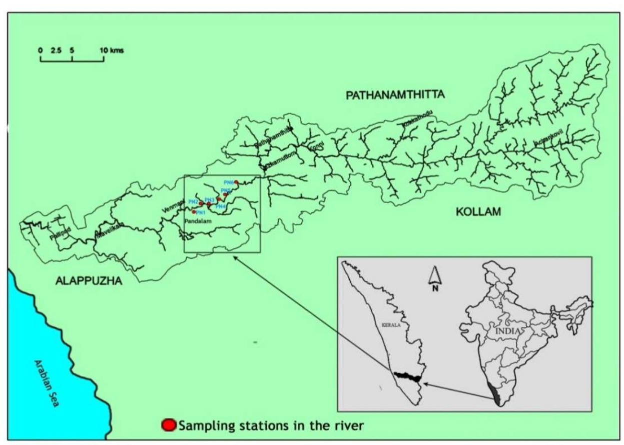

Fig. 1. Map of study area

MATERIAL AND METHODS

Pandalam Municipality covers a total area of 28.72 km² and, situated between 9.2250° N latitude and 76.670° E longitude. Selected six vulnerable wards for sampling, establishing a total of six sampling stations (one in each ward). The stations were designated as PN (Pandalam Station Number), specifically PN1 (9 ° 15'73"N and 76 °73'23"E), PN2 (9°13'28"N and 76°40'15"E), PN3 (9° 22'95"N and 76° 66'81"E), PN4 (9 °23'68"N and 76 °66'23"E), PN5 (9 ° 23'55"N and 76 °67'48"E), and PN6 (9 °22'44"N and 76° 69'09"E) (Figure 1). This study primarily aims to conduct a taxonomic analysis of different classes of periphytic microalgae in the river.

Sampling in the riverine lentic water bodies: Between December 2021 and December 2022, sample collections were conducted between 9 a.m. and 10 a.m. from the riverine water bodies of the Achankovil River at each of the fixed stations. Periphytons were collected from the leaves of colonization-supporting submerged plants such as *Hydrilla, Nymphaea*, and various grasses. The thin film of algae that developed on the surface of these plants was stripped and preserved in 100 ml of double-distilled water in pre-sterilized plastic bottles. All collected water samples were preserved in Lugol's iodine following standard procedures (Alan et al 2021). Periphytons were identified using a compound microscope (MX21i Clinical) at 100X magnification. Photographs were taken with an Olympus BX 40 camera attached to a stereomicroscope, obtained on a payment basis outside the institution. Identification was done using

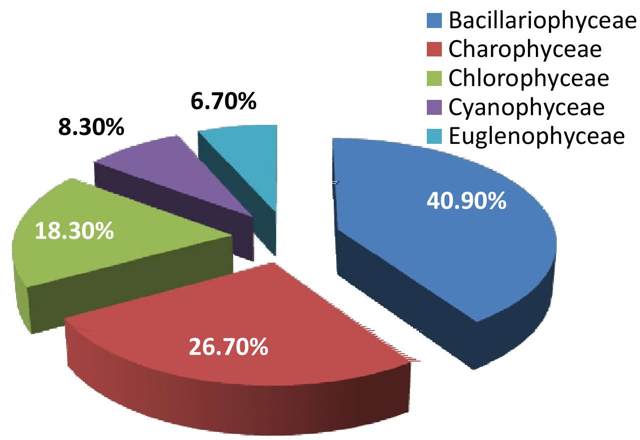

Fig. 2. Percentage wise distribution of different classes of Epiphytic algae

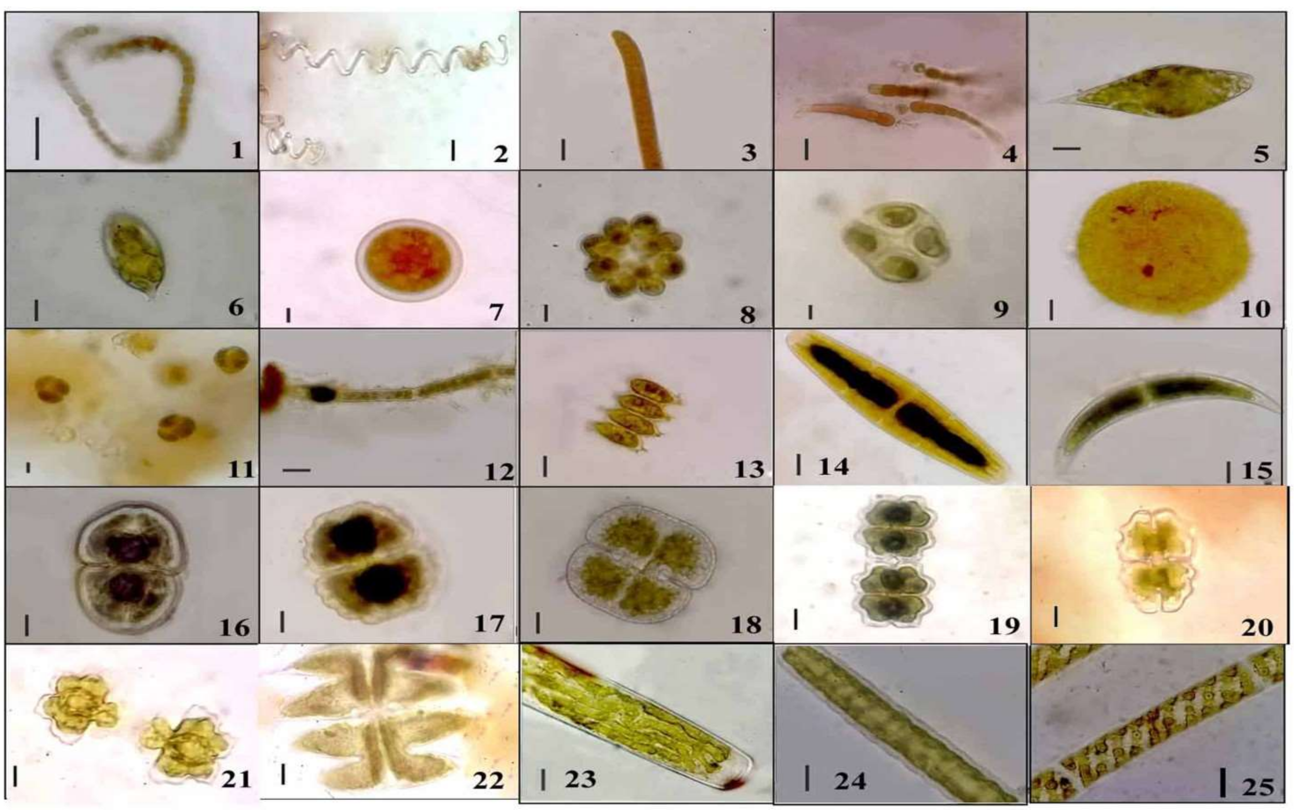

Plate 1. (1) *Anabaena cylindrica,* (2) *Arthrospira platensis,* (3) *Oscillatoria formosa,* (4) *Rivularia* sp., (5) *Euglena caudata,* (6) *Phacus sp,* (7) *Chlorococcum humicola,* (8) *Coelastrum microporum,* (9) *Crucigenia crucifera,* (10) *Dictyochloropsis* sp., (11) *Radiococcus nimbatus,* (12) *Oedogonium* sp., (13) *Scenedesmus denticulatus,* (14) *Closterium navicula* (15), *Closterium parvulum,* (16) *Cosmarium hammeri,* (17) *Cosmarium subprotumidum,* (18) *Cosmarium quadrum,* (19) *Euastrum binale,* (20) *Euastrum denticulatum,* (21) *Euastrum pulchellum,* (22) *Micrasterias laticeps,* (23) *Pleurotaenium archeri,* (24) *Pleurotaenium trabecula,* (25) *Spirogyra* sp. [Scale bars, Fig. 1-25: 10 μm]

standard keys for Cyanophyceae (Komarek and Anagnostidis 2014), Green algae (Karlson et al 2020, Guiry and Guiry 2023), and Diatoms (Bellinger and Sigee 2015, Spaulding et al 2021). The phytoplankton were separated into classes. They were classified according to the Round (1973) system. The samples were deposited in the Botany Laboratory at NSS College, Pandalam, Kerala.

RESULTS AND DISCUSSION

The flood-affected riverine areas of Pandalam exhibited rich algal diversity. This study documented 61 algal taxa from ephemeral to perennial lentic water habitats of the river, with 50 identified to the species level. The identified taxa belong to 61 genera under five classes: Cyanophyceae (5), Euglenophyceae (4), Chlorophyceae (11), Charophyceae (16), and Bacillariophyceae (25). Krishnan et al (2020) indicated the dominance of Chlorophyceae, while Charophyta and Bacillariophyta were dominant in the microalgae of rivers in Pathanamthitta (Harikrishnan 2010). The results corroborated these findings, with Bacillariophyceae being the dominant class (25 genera). The genus *Cosmarium* (Desmidaceae) was the most dominant, with seven species, followed by the diatoms *Pinnularia* and *Gomphonema*, each with four species. *Scenedesmus quadricauda* was present at all stations. The percentage distribution of different classes was 40.9% Bacillariophyceae followed by Chlorophyceae (26.7%) and Charophyceae (18.3%) (Figure 2). The highest number of algal genera was

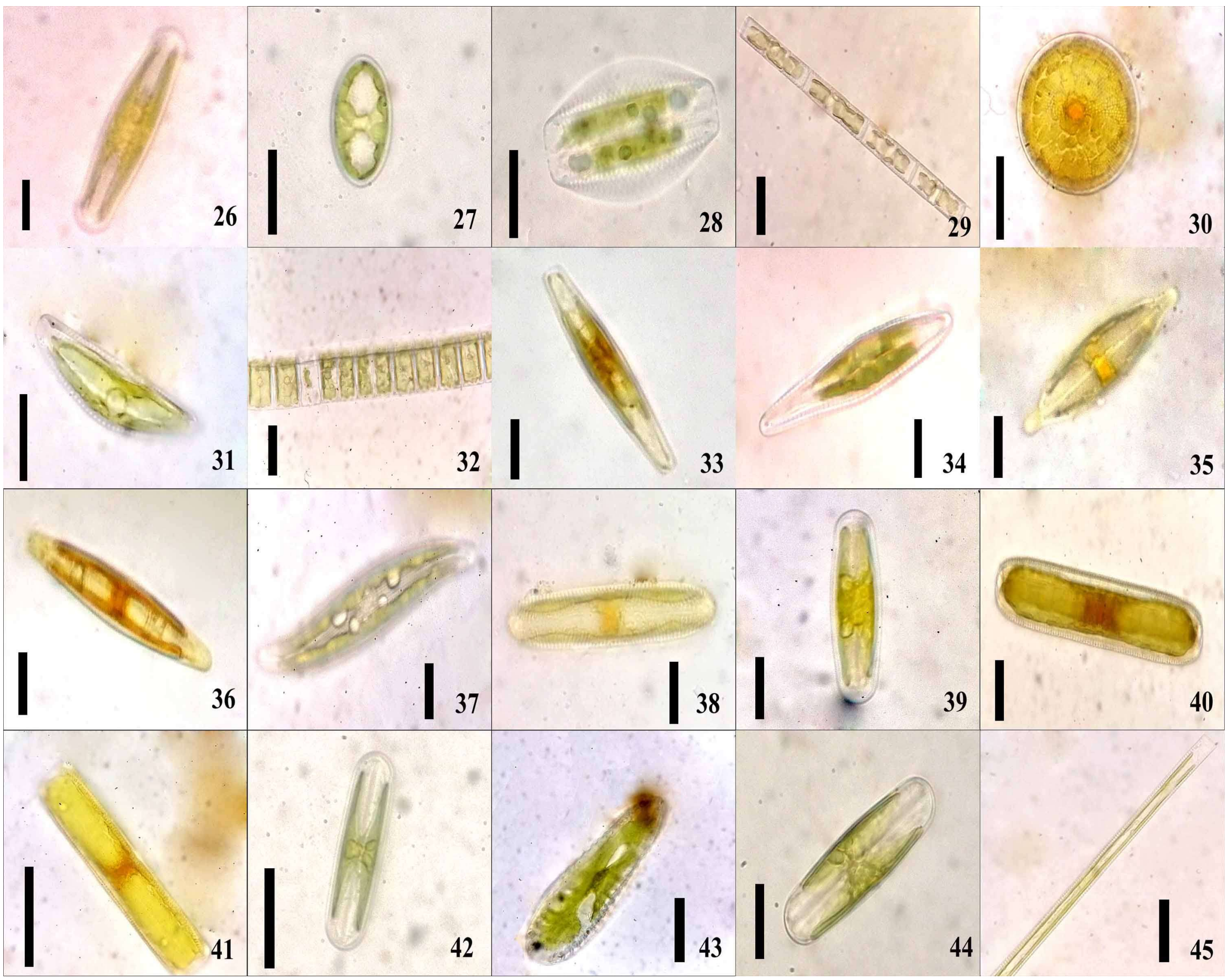

Plate 2. (26) *Achnanthidium minutissimum,* (27) *Amphora inariensis,* (28) *Amphora* sp., (29) *Aulacoseira granulata,* (30) *Cyclotella meneghiniana,* (31) *Cymbella* sp., (32) *Diadesmis confervacea,* (33) *Frustulia rhomboides,* (34) *Gomphonema affine,* (35) *Gomphonema lagenula,* (36) *Navicula lanceolata,* (37) *Nitzschia clausii,* (38) *Pinnularia divergens,* (39) *Pinnularia gibba,* (40) *Pinnularia rectangularis,* (41) *Pinnularia* sp., (42) *Pinnularia viridis,* (43) *Rhoicosphenia abbreviata,* (44) *Sellaphora pupula,* (45) *Synedra* sp. [Scale bars, Fig. 25-45: 10 μm]

at PN2 (17), and the lowest at PN5 (8 taxa). The high density of taxa at PN2 could be due to the presence of large riparian lentic water habitats, which allowed for multiple representative samples. In contrast, PN5 had fewer colonization-supporting submerged plants due to the presence of small rocks and mud-filled shores and was severely affected by landslides, leading to fewer periphytic algae samples.

In two pre-monsoon studies at Achankovil Pandalam, Chlorophyceae and Bacillariophyceae were dominant (Harikrishnan 2010, Krishnan et al 2020). The increased number of Euglenophytes at PN6 could be attributed to human contaminants increasing nitrate availability. This station is near the pilgrimage area of Pandalam Valiyakoikal Palace, heavily used by Sabarimala pilgrims for sanitary purposes. Kumar et al (2018) concluded that, higher number of Euglenophytes indicates decaying organic contaminants. Presence of pollution-tolerant *Scenedesmus* at this station indicates water degradation due to pollution (Paul and Sreekumar 2008).

Among the five classes of algae identified, most were dwellers in oligotrophic habitats, with desmids and diatoms being more numerous. Their predominance indicates good water quality (Thomas and Paul 2015). The dominance of diatoms and desmids in high-altitude oligotrophic lakes in Kerala has been documented by Krishnan (2012). Bacillariophyceae and Chlorophyceae were dominant in many rivers (Tas and Gonulol 2007).

CONCLUSION

This investigation reveals that the riparian lentic microhabitats of the Achankovil River in Pandalam Municipality are rich in periphyton biodiversity. Flood events have disturbed the community structure, leading to the mixing of waters and the presence of pollution indicators and flagellated forms at some stations. Additionally, anthropogenic influences at certain locations show a slight trend towards eutrophication, although the water is not highly contaminated. Strict measures should be implemented to protect the biodiversity-rich water bodies of Pandalam Municipality, especially the Achankovil River. The river is heavily used during the pilgrimage season, and care should be taken to prevent habitat and species loss during flood events, as Pandalam is flood-prone.

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Genetic Divergence Studies in Mungbean [*Vigna radiata* (L.) Wilzeck] Germplasm under Arid Environment

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Abstract: Field experiment with 79 genotypes of mungbean was conducted to study the genetic divergence in the mungbean genotypes at Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan during *kharif* 2017-18. Significance difference was observed among all 11 characters studied. These genotypes were grouped into fifteen clusters which indicate the existence of an ample amount of genetic diversity in the genotypes and therefore, signify the scope of selection for genetic improvement of mungbean. The cluster-III was largest with 26 genotypes followed by cluster-II with 20 genotypes, cluster-I with 12 genotypes, cluster-IV with 9 genotypes and cluster-XI with 2 genotypes. The remaining 10 clusters were mono-genotypic. The maximum intra-cluster distance was found in cluster-IV and the maximum inter-cluster distance was observed between cluster-III and cluster-IX. D^2 analysis exhibited that days to 50 per cent flowering, 100-seed weight, biological yield per plant, number of branches per plant and number of pods per plant contributed 91.79 per cent towards total divergence.

Keywords: Cluster analysis, Genetic diversity, Germplasm, Mahalanobis's D^2 Statistics, Mungbean, Tocher's method

Mungbean [*Vigna radiata* (L.) Wilczek, 2n=22, Fabaceae) is an important pulse crop which is cultivated throughout India. It is a short day, hot season crop, mainly grown in arid and semi-arid regions (Anita et al 2024). Mungbean has become an extremely valuable short-lived grain legume crop with many desirable characteristics, such as wide adaptability, low input requirements and the ability to improve soil fertility (Pooran and Can GM 2021). According to 3rd advance estimates for 2021-2022, the overall production of pulses in India to be 27.75 million tonnes. In India, a total of 2.85mt mungbean productions including 1.48mt in *Kharif* and 1.37mt in Rabi, accounting for 10% of all pulse production (Directorate of Economics and Statistics, Ministry of Agriculture and Farmers Welfare 2022).

India is the principle producer of mungbean in the world with an annual production of 3.17mt from an area of 5.50mha with the productivity of 570 kg per ha (Anonymous 2022-23). It is a drought hardy crop with ability to grow under harsh climate and medium to low rainfall conditions and grows on a variety of soils including black, red lateritic, gravelly and sandy soils. Well drained fertile sandy loam soil with a pH 6.3-7.5 is the best for mungbean cultivation (Sharma NK 2016). Genetic diversity is a dominant factor and also a precondition in any hybridization programme. Introduction of diverse parents in hybridization programme serves the purpose of combining advisable recombination. Multivariate analysis by means of Mahalanobis D^2 statistic is a dominant tool in quantifying the degree of divergence at genotypic level. Therefore, an attempt has been made in the present inspection with a view to approximate genetic divergence among a set of 79 genotypes of mungbean.

MATERIAL AND METHODS

The experiment was carried out at, Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan during *Kharif*, 2017-18. The experimental site is situated at 28.01^0N latitude and 73.22^0E longitude at an altitude of 234.70 meters. According to National Planning Commission, Bikaner falls under Agro-climatic Zone XIV (Western Dry Region) of India. The average rainfall of the zone is 265 mm. The experimental material consisted of 79 genotypes (Table 1) and was sown on July 6, 2017 in randomized block design with three replications accommodating 3 meters long two rows per replication at 30 cm spacing under sprinkler irrigated situation. All recommended agronomic practices were adopted for raising a healthy crop. The data were recorded for 11 characters viz. days to 50% flowering and days to maturity on a whole plot basis whereas, plant height (cm), number of branches per plant, number of pods per plant, pod length (cm), number of seeds per pod, 100 seed weight (g), biological yield per plant (g), seed yield per plant (g) and harvest index (%) were measured on five competitive plants in each replication.

The statistical analysis was performed using INDOSTAT

Table 1. Mungbean genotypes used for present investigation

Name of germplasm	Year of collection	Source of procurement
Germplasm procured from NBPGR, Regional Station, Jodhpur		
IC-39269	1993	Jodhpur, Rajasthan
IC-39275	1993	Kherapa, Jodhpur, Rajasthan
IC-39279	1993	*
IC-39288	1993	Nimbojhai, Nagour, Rajasthan
IC-39293	1993	Kadampura, Nagour, Rajasthan
IC-39298	1993	Bambor,Jodhpur, Rajasthan
IC-39300	1993	Jaswasar, Bikaner, Rajasthan
IC-39328	1993	Lalela, Barmer, Rajasthan
IC-39333	1993	Dhawa, Barmer, Rajasthan
IC-39352	1993	Manduwa, Barmer, Rajasthan
IC-39368	1993	Lunawas, Jodhpur, Rajasthan
IC-39375	1993	Nibali, Barmer, Rajasthan
IC-39383	1993	Godan, Jalore, Rajasthan
IC-39395	1993	Aburoad, Sirohi, Rajasthan
IC-39399	1993	Jaspura, Palanpur, Gujarat
IC-39409	1993	Kapara, Banaskantha, Gujarat
IC-39420	1993	Nearsami, Patan, Gujarat
IC-39427	1993	Harij, Patan, Gujarat
IC-39451	1988	Lakhtarar, Surendranagar, Gujarat
IC-39454	1988	Surendranagar, Gujarat
IC-39465	1988	Kalyana, Patan, Gujarat
IC-39483	1988	Kalapur, Surendranagar, Gujarat
IC-39492	1988	Dudhai, Mahesana, Gujarat
IC-39495	1988	Chandrani, Kachchh, Gujarat
IC-39500	1988	Kishangarh, Gujarat
IC-39515	1988	Kauth, Gujarat
IC-39580	1992	Bachau, Kutch, Gujarat
IC-39582	1992	Chilora, Kheda, Gujarat
IC-39591	1992	Sevelia, Kheda, Gujarat
IC-39604	1992	Bholi, Rajasmand, Rajasthan
IC-39608	1992	Nevra, Jodhpur, Rajasthan
IC-39610	1992	Osian, Jodhpur, Rajasthan
IC-52073	1992	*
IC-52076	1992	*
IC-52078	1992	*
IC-52081	1992	*
IC-52082	1992	*
IC-52087	1992	*
IC-55069	1992	*
IC-102792	1986	Banar, Jodhpur, Rajasthan
IC-102821	1986	Gidani, Jaipur, Rajasthan

Cont...

Table 1. Mungbean genotypes used for present investigation

Name of germplasm	Year of collection	Source of procurement
IC-102857	1986	Khasur, Dholpur, Rajasthan
IC-102963	1986	Avikanagar, Tonk, Rajasthan
IC-103014	1986	Alampur, Kheda, Gujarat
IC-103059	1986	Krakas, Amreli, Gujarat
IC-103204	1987	Gangawar, Chittorgarh, Raj.
IC-103207	1987	Dhinva, Chittorgarh, Rajasthan
IC-103244	1986	Bhrwasa, Didwana, Nagaur, Raj.
IC-103245	1987	Odda, Banswara, Rajasthan
IC-103785	1989	Khemlo, Vishsana, Rajasthan
IC-103821	1989	Nagdhan, Santrampur, Gujarat
IC-103973	1989	Barvalbhipor, Bhavnagar, Gujarat
IC-324012	-	*
IC-338868	1990	Sanari, Barmer, Rajasthan
Varieties procured from Agriculture University, Jodhpur		
Sweta		CSAVAT, Kanpur
IPM-02-3		ICAR-IIPR, Kanpur
IPM-02-14		ICAR-IIPR, Kanpur
Samrat (PDM-139)		ICAR-IIPR, Kanpur
GM-4		AAU, Pulse Res.Station,Vadodara
MH 2-15		CCSHAU, Hisar
MH-421		CCSHAU, Hisar
IPM-205-7		ICAR-IIPR, Kanpur
IPM 99-125 (Meha)		ICAR-IIPR, Kanpur
IPM-409-4		ICAR-IIPR, Kanpur
GAM-5		AAU,PulseRes.Station, Vadodara
COGG-912		TNAU, Coimbatore
Varieties procured from RARI, Durgapura, Jaipur		
RMG-62		SKRAU-ARS, Durgapura, Jaipur
RMG-268		SKRAU-ARS, Durgapura, Jaipur
RMG-344		SKRAU-ARS, Durgapura, Jaipur
RMG-492		SKRAU-ARS, Durgapura, Jaipur
Keshwanand Mung-1 (RMG-975)		SKNAU-RARI, Durgapura, Jaipur
Keshwanand Mung-2 (MSJ-118)		SKNAU-RARI, Durgapura, Jaipur
Varieties procured from ARS, Sriganganagar		
Ganga-1		SKRAU-ARS, Sriganganagar
Ganga-8		SKRAU-ARS, Sriganganagar
MUM-2		CCS Meerut University, Meerut
SML-668		PAU, Ludhiana
SML-832		PAU, Ludhiana
ML-683		PAU, Ludhiana
ML-818		PAU, Ludhiana

*Source was not mentioned by NBPGR, Regional Station, Jodhpur, Rajasthan, India

8.1 and XLSTAT 2021.2.2 software. Diversity analysis (D^2) was done by following the method of Mahalanobis (1936) and grouped into separate clusters following the Toucher's method as suggested by Rao (1952). Average intra and inter-cluster distances were determined using GENRES version 3.11, 1994 Pascal Intl. Software as suggested by Singh and Chaudhary (1985).

RESULTS AND DISCUSSION

There were significant differences among all the genotypes for all eleven traits studied that the material has sufficient genetic diversity to support the breeding programme for improving the seed yield of mungbean (Table 2). In this study, based on D^2 values using Tocher's method, 79 genotypes of mungbean were grouped into fifteen clusters (Table 3 & Fig. 1). The cluster-III contains maximum (26) genotypes followed by cluster-II with 20 genotypes, cluster-I with 12 genotypes and cluster-IV with 9 genotypes. Cluster-XI comprise only two genotypes; while the remaining ten clusters were mono genotypic indicating that these genotypes may be having completely different genetic makeup, thus leading to the formation of separate cluster.

Table 2. Analysis of variance for different characters of mungbean

Source of variation	d. f.	Days to 50% flowering	Days to maturity	Plant height (cm)	No. of branches per plant	No. of pods per plant	No. of seeds per pod	Pod length (cm)	100-seed weight (g)	Biological yield per plant (g)	Harvest index (%)	Seed yield per plant (g)
Replications	2	0.34	4.81	27.06	0.362**	0.76	0.46	0.48	0.001	2.04	0.98	0.63
Genotypes	78	250.90**	159.74**	1057.98**	0.635**	572.79**	2.84**	2.91**	0.580**	1252.01**	316.13**	162.12**
Error	156	0.18	1.79	12.36	0.006	1.99	0.20	0.16	0.003	6.87	3.07	0.37

*Significant at P = 0.05
** Highly significant at P = 0.01

Table 3. Composition of mungbean genotypes into fifteen different clusters by Mahalanobis's D^2 statistic and their salient features

Cluster	No. of genotypes	Composition of cluster / Name of genotypes	Salient features of the cluster
I	12	MH 421, GAM 5, SML 832, IPM 205-7, ML 683, COGG 912, RMG 268, MH 2-15, ML 818, Samrat, RMG 344, GM-4	Early flowering, early maturity, higher harvest index, long pod length and more number of pods
II	20	IC-39275, IC-103207, IC-103204, IC-39395, IC-39399, IC-39279, IC-103785, IC-39293, IC-39383, IC-103821, IC-39500, IC-55069, IC-103245, IC-102857, IC-39492, IC-39454, IC-39352, IC-39333, IC-39495, IC-102821	More plant height, late flowering and late maturity
III	26	IC-39610, IC-55069, IC-39483, IC-39300, IC-39298, IC-39580, IC-39465, IC-102963, IC-39368, IC-102792, IC-39591, IC-39451, IC-39515, IC-103244, IC-39427, IC-39375, IC-39420, IC-52076, IC-39604, IC-39582, IC-52073, IC-324012, IC-52082, IC-338868, IC-103973, IC-39608	More plant height, late flowering and late maturity
IV	9	RMG-62, Ganga-8, Keshwanand Mung-2, IC-39409, IPM 99-125, IPM 409-4, Sweta, IPM 2-14, IC-52087	Early flowering, early maturity and higher harvest index
V	1	RMG-492	Early flowering, early maturity, higher seed and biological yield
VI	1	Keshwanand Mung-1	Early flowering, early maturity, higher seed and biological yield
VII	1	IC-52081	Early flowering and early maturity
VIII	1	IC-103014	Late flowering and late maturity
IX	1	MUM-2	Early flowering, early maturity, higher seed and biological yield, more number of pods per plant
X	1	IC-39269	Late flowering and late maturity
XI	2	IC-39288, IPM 02-3	Early flowering, early maturity and higher harvest index
XII	1	SML-668	Early flowering, early maturity, higher harvest index and more number of pods per plant
XIII	1	IC-39328	Late flowering and late maturity
XIV	1	IC-103059	Late flowering and late maturity
XV	1	Ganga-1	

The genotype which belongs to the same cluster indicates to be more closely related than those belonging to different clusters. Similar findings were observed by Wesly et al (2020), Sridhar et al (2022), Kingsly et al (2023) and Srivastava et al (2024).

Improvement in yield and other related characters is the basic objective in any breeding programme. So, cluster diversity for seed yield and its contributing attributes should to be considered for selection of genotypes. In present investigation considerable differences were observed among the clusters for most of the characters studied (Table 4 and Fig. 2). The maximum intra-cluster D^2 value was observed for cluster-IV (145.87) followed by cluster-II (105.50), cluster-III (98.50) and cluster-XI (60.26) indicating that maximum differences exists among the genotypes that fall in these clusters. Therefore, such intra-cluster heterogeneity among the constituent genotypes obtained in the present experiment might serve as guideline to choose

Table 4. Average intra (in bold) and inter cluster (D^2) value for seventy-nine genotypes of mungbean

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
I	60.07	677.15	1606.57	227.82	115.92	127.23	684.92	856.99	220.64	438.28	502.11	140.82	899.04	988.44	280.05
II		105.50	393.13	474.87	775.82	927.18	341.21	149.43	1267.26	151.14	646.00	893.08	226.62	190.01	1020.94
III			98.50	1365.13	1731.46	1892.61	1030.80	463.01	2356.28	571.20	1497.69	1910.63	441.46	341.36	1779.50
IV				145.87	278.51	376.37	336.13	598.90	539.33	323.20	442.56	317.84	609.36	752.78	617.50
V					0.00	31.36	802.10	972.87	157.48	536.13	869.06	157.25	810.23	1121.39	256.62
VI						0.00	1056.73	1152.41	56.38	598.28	981.79	97.71	969.22	1287.33	130.86
VII							0.00	384.45	1424.19	466.79	392.85	1000.13	545.16	495.18	1442.52
VIII								0.00	1507.81	290.20	747.97	1037.36	346.82	117.43	1271.57
IX									0.00	810.00	1163.35	85.80	1336.46	1659.49	114.23
X										0.00	583.34	521.12	249.63	313.77	610.46
XI											60.26	825.85	1215.51	848.73	1191.46
XII												0.00	994.73	1221.79	163.11
XIII													0.00	397.50	1050.97
XIV														0.00	1321.38
XV															0.00

Table 5. Mean values for seed yield and component characters of mungbean

Cluster	Days to 50% flowering	Days to maturity	Plant height (cm)	No. of branches per plant	Number of pods per plant	Number of seeds per pod	Pod length (cm)	100-seed weight (gm)	Biological yield per plant	Harvest index	Seed yield per plant (gm)
I	37.00	67.00	57.32	1.93	37.46	10.75	7.88	1.91^{L}	72.31	28.57	20.46
II	47.00	74.00	87.35	2.37	17.51	10.30	7.69	3.58	33.31	18.13	5.92
III	59.00^{H}	83.00^{H}	89.70	2.54	9.58	9.50	6.86	3.51	35.70	9.10	3.21
IV	37.00	67.00	55.14	2.21	35.70	10.52	7.76	3.56	39.84	31.37	12.00
V	37.00	67.00	63.77	1.80	38.33	11.33^{H}	7.07	3.05	82.90	24.52	20.33
VI	38.00	67.00	64.87	2.10	44.60	11.33^{H}	7.31	3.27	92.13	25.27	23.27
VII	38.00	65.00	45.47^{L}	1.80	12.47	11.00	7.29	3.52	12.20^{L}	32.81	4.00
VIII	45.00	71.00	106.73^{H}	2.97	4.60	8.33^{L}	7.83	3.43	24.80	5.70	1.40
IX	36.00^{L}	67.00	62.83	2.50	55.60^{H}	10.67	7.56	3.56	96.70	26.14	25.27
X	47.00	74.00	92.53	2.60	35.67	11.00	6.88	3.84	39.67	23.55	9.33
XI	36.00^{L}	68.00	52.53	1.70^{L}	24.00	11.17	9.69^{H}	5.28^{H}	35.48	31.45	10.93
XII	36.00^{L}	64.00^{L}	66.03	3.00^{H}	46.80	11.00	7.93	3.65	66.43	33.33^{H}	22.17
XIII	49.00	82.00	85.53	2.70	23.60	11.33^{H}	7.35	2.45	24.93	29.18	7.20
XIV	49.00	75.00	90.40	3.00^{H}	1.53^{L}	9.00	2.46^{L}	3.64	43.20	1.10^{L}	0.47^{L}
XV	42.00	76.00	70.53	2.90	48.27	9.67	7.64	3.66	104.33^{H}	24.99	26.07^{H}

parents for the recombination breeding programme. The cluster-III and cluster-IX showed maximum inter-cluster distance of 2356.28 followed by cluster III and cluster XII (1910.63), cluster III and cluster VI (1892.61) and cluster III and cluster XV (1779.50) indicating that genotypes included in these clusters are genetically diverse. It indicated that these cluster pairs were most divergent and can be utilized in the hybridization programme for crop improvement as well as for studying the inheritance pattern of different characters in mungbean (Talukdar et al 2020, Goyal et al 2021, Sridhar et al 2022 , Gupta et al 2023 , Anita et al 2024).

The comparison of cluster mean values (Table 5) in mungbean genotypes indicated that cluster-XV had highest mean value for seed yield per plant (26.07) and biological yield per plant (104.33). Cluster-IX had highest value for number of pods per plant (55.60) and the lowest value for days to 50 per cent flowering (36), which is a desirable trait for arid zone. Cluster-VI had maximum mean value for number of seeds per pod (11.33). Cluster-XII had maximum value for harvest index (33.33), number of branches per plant (3.00) and the lowest value for days to 50 per cent flowering (36.00) and days to maturity (64.00); which is a desirable trait for arid zone. Cluster-III had highest value for days to 50 per cent flowering (59.00) and days to maturity (83.00). This comparison indicates that cluster XV, IX, VI, XII and III had better cluster means for most of the characters. Therefore, these clusters may be considered better for selecting genotypes with desirable characters. Similar findings were earlier reported by Goyal et al (2021), Sridhar et al (2022) and Gupta et al (2023) and Anita et al (2024). Amongst the characters, days to 50 per cent flowering contributed highest towards genetic divergence (61.60%) followed by 100-seed weight, biological yield per plant, number of branches per plant, number of pods per plant and seed yield per plant; while the remaining characters contributed little to genetic divergence (Table 3). Consequently, considering both cluster mean and per cent contribution of each character, genotypes belonging to cluster XV, IX, VI, XII and III found promising for use as breeding material in future hybridization programme. Similar results were also earlier reported by Mathankumar et al (2020), Tiwari et al (2022), Gupta et al (2023) and Srivastava et al (2024).

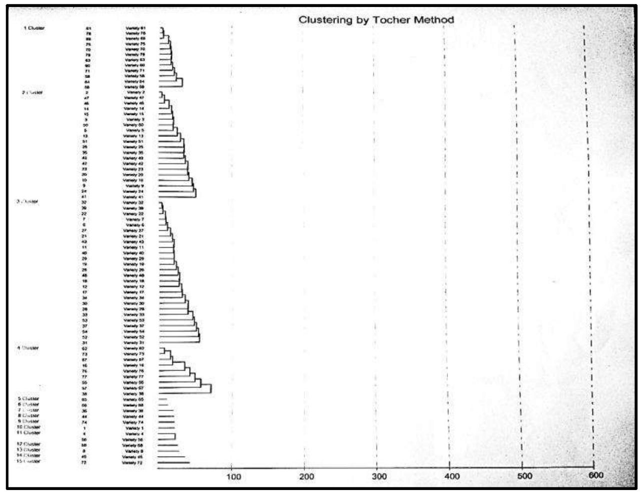

Fig. 1. Clustering pattern of seventy nine mungbean genotypes by Tocher's method

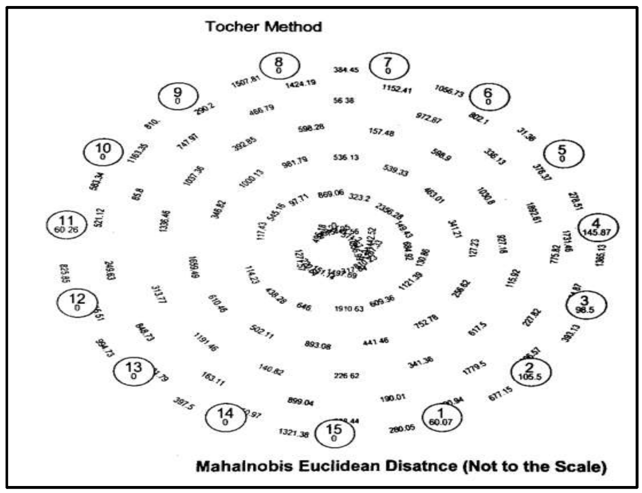

Fig. 2. Divergence average intra and inter cluster distance among grouped seventy nine mungbean genotypes

Table 6. Contribution of eleven characters towards total genetic divergence in mungbean

Name of characters	Per cent contribution of characters
Days to 50% flowering	61.6
Days to maturity	0.19
Plant height	1.59
Number of branches per plant	6.62
Number of pods per plant	4.45
Number of seeds per pod	0.16
Pod length	0.39
100-seed weight	10.00
Biological yield per plant	9.12
Harvest index	2.50
Seed yield per plant	3.38

CONCLUSON

The percentage contribution towards genetic divergence was found high for days to 50 per cent flowering followed 100 seed weight and biological yield per plant. The genotypes of cluster XV, IX, VI, XII and III had maximum inter-cluster distances as well as maximum cluster means for most of the yield component traits indicated that these genotypes were most diverse and good recombinants can be obtain by mating between these genotypes. Hence, these genotypes would be used as parental source for upcoming mungbean breeding programmes.

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Assessment of Soil Variation in Kaithal District using GIS and GPS

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Abstract: The current investigation was conducted in Kaithal district, focusing on Kalayat, Rajound, Pundri and Dhand block. The samples were collected from 106 villages across different blocks using GPS and maps were prepared subsequently using GIS. Soils in all four blocks was non saline, low to medium in organic carbon content and texture ranging from sandy loam to clay loam, loam and sandy clay loam. Bulk density varied between 1.32-1.59 Mg m^{-3}. Soils of all four blocks were low in available nitrogen (N) content, intermediate too high in phosphorus (P) and potassium (K) content and high in sulfur (S) content. All four blocks possess medium nutrient index in terms of phosphorus and potassium, whereas, these blocks have high values for sulphur nutrient index.The positive correlation was observed between the soil organic carbon and available N, P, K and S of soil. Inappropriate agricultural practices, intensive farming, monoculture cropping patterns and excessive irrigation contribute to soil fertility degradation. To mitigate these adverse effects, toned to employ a combination of biofertilizers, organic manures and appropriate use of chemical fertilizers.

Keywords: Fertility, GIS, GPS, Nutrient index

Soil is a crucial natural resource that supports the production of food, fodder, and fuel essential for the sustainability of humans and animals. As populations grow, the demand for food increases, placing greater pressure on soil resources. Throughout history, the success and survival of civilizations have been closely tied to the ability of their soils to provide necessary resources. This presents a significant challenge for scientists, planners, administrators, and farmers who must work to ensure food security for both current and future generations by managing soil resources efficiently. Evaluating the fertility status of the soil is essential for making well-informed decisions in agriculture. It enables farmers to grasp the nutrient composition of their soil, guiding choices on fertilization, crop selection and planting methods to optimize both crop yields and quality. Soil testing facilitates precise fertilizer application by analysing essential nutrients like nitrogen, phosphorus and potassium, as well as secondary and micronutrients to reducing costs and mitigating environmental impact. At global scale, about one-third of arable soils are deficient in micronutrients, particularly in zinc (Zn) (Cakmak et al 2017).

Haryana soils are among the most arable soil in northern India but still lack adequate nutrients for plant growth. Despite increased fertilizer use to cultivate high-yielding crop varieties, crops continue to extract more macro and micronutrients from the soil and ultimately leading to soil nutrient deficiency. In Haryana, the current status of Zn, Fe, Mn, Cu and B varied from 1.11 to 36.50, 0.0-55.00, 0.00-48.60, 0.00-13.00 and 0.00-13.70%, respectively with an average deficiency of 15.3, 21.6, 6.1 5.2 and 3.3 % (Shukla et al 2015). Kaithal is the northeastern district of Haryana, encompasses an area of 2317 square kilometres and is situated between 28° 31' and 30 ° 11' N latitudes and 76 ° 10' and 76 ° 41' E longitudes. The region is drained by the Yamuna, Ghaggar, Markanda and other seasonal streams that originate from the Siwalik range. It is predominantly covered by old and recent alluvial deposits of the Indo-Gangetic plain. The main crops grown in the region include wheat, rice, sugarcane, cotton and sorghum. Soil samples from Guhla block of Kaithal district indicate low levels of OC, N and P in 96, 16 and 16% of total samples, respectively (Sharma et al 2024).

Consequently, it is imperative to conduct regular evaluations of soil fertility to monitor alterations in both macro and micronutrient levels within the soil and to identify the specific nature and extent of any multi-nutrient deficiencies present. The present study has been designed with the following primary objectives to evaluate the soil fertility status of various blocks of Kaithal district and classify the soil according to its fertility characteristics.

MATERIAL AND METHODS

Study area: The Kalayat, Rajound, Pundri and Dhand blocks were selected for the study. The district lies between latitudes 29°31' and 30°12'N, and longitudes 76°10' and 76°42'E. There are 31 villages in Kalayat block, 24 villages in Rajound block, 25 villages in Pundri block and 26 villages in Dhand block. The district experiences a tropical steppe climate, which is semiarid and humid. Annual rainfall averages 511 mm, evenly spread across the area. The southwest monsoon usually arrives in late June. There are two main soil types: sierozem and desert soils. According to the soil testing and research laboratory in Kaithal, the soils in this district range from sandy to sandy loam in texture.

Soil sampling and analysis: For the current investigation, a total of 212 soil samples were collected at depth of 0-15 cm from 106 villages spanning various blocks. The number of soil samples across the various blocks, namely Kalayat, Rajound, Pundri and Dhand were 62, 48, 50 and 52, respectively. The soil samples were randomly collected from farmer's fields using a post hole auger and the longitude and latitude coordinates of each sampling site were recorded with a handheld GPS device. The collected samples were brought to the laboratory dried in the air, then crushed and sieved through a two mm sieve. Then soil samples underwent analysis for nutrient availability using standard analytical techniques mentioned below in Table 1.

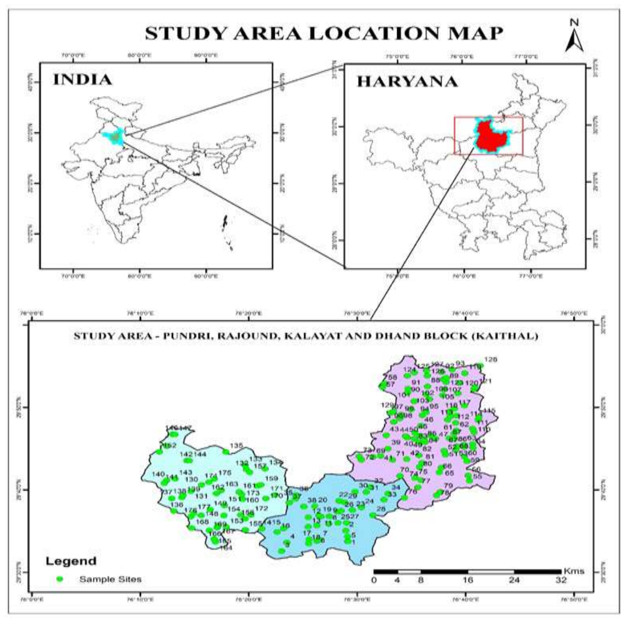
Statistical analysis: Correlation between soil properties and nutrients was worked out using the corplot package of R Software and graphs were prepared using R statistical program. Distribution maps for soil macronutrient status were created using ArcGIS 10.3 software.

RESULTS AND DISCUSSION

Soil pH: The mean value of pH of the soils was 8.09, 8.13, 8.22 and 8.04, in Kalayat, Rajound, Pundri and Dhand block, respectively (Table 3). Out of 212 soil samples, all the samples were alkaline in nature and none of the samples were acidic in these blocks. The main reason for the alkaline nature of these soils could be due to the presence of basic parent material and reaction between the soil colloids and applied fertilizers, this led to the creation of basic cations on the exchangeable sites of the soils (Sharma et al 2024). The alkaline nature might be high base saturation with uneven rainfall distribution, which resulted in accumulation of ions. Gyawali et al (2016) reported similar observations in the Kaithal district of Haryana.

Electrical Conductivity (EC): The mean EC of the soils was 0.45, 0.39, 0.38 and 0.45 dS m^{-1}, in Kalayat, Rajound, Pundri and Dhand block, respectively (Table 3). Out of total 212 soil samples, none of the samples were saline in these blocks. It indicated that soils in all four blocks were non-saline. Dabi (2011) mentioned that well-drained soil conditions resulting from intensive land use facilitate the removal of excess salts through percolating and drainage water. Sharma et al (2024) reported similar observations of leaching of base in Guhla block of Kaithal district.

Soil Organic Matter (OC): The mean OC of the soils was 0.46, 0.47, 0.44 and 0.49%, in Kalayat, Rajound, Pundri and Dhand block, respectively (Table 2). In general out of 212 soil samples, 42 % samples fell under low status, 53 % samples



Map 1. Location map of various blocks of Kaithal district

Table 1. Field parameters with their corresponding methods

Parameter	Methods/Instrument	Reference
pH	Potentiometric method	Jackson (1973)
Texture	International Pipette Method	Piper (1966)
EC(dS m^{-1})	Conductivity Meter	Jackson (1973)
Bulk density (Mg m^{-3})	Core Sampler	Bodman (1942)
Organic carbon (%)	Wet digestion method	Walkley and Black (1934)
Available N (kg ha^{-1})	Kjeldahl distillation	Subbiah and Asija (1956)
Available P (kg ha^{-1})	$NaHCO_3$ extraction and colorimetry	Olsen et al (1954)
Available K (kg ha^{-1})	NH_4OAc and Flame photometry	Jackson (1973)
Available S (kg ha^{-1})	-	Chensin and Yien (1950)
Nutrient Index	-	Parker et al (1951)

were medium and 5 % of the soil samples were high in OC category in these blocks. Majority of the samples in these blocks were with medium SOC and this could be due to the continuous rice-wheat system, which might have contributed more residues in soil (Sharma et al 2024). However, low SOC might be ascribed due to high rate of organic matter decomposition under hyperthermic temperature regime which results to extremely high oxidizing conditions reported by (Singh et al 2014).

Soil texture: The soil texture in the Kalayat block ranged from sandy loam to loam, in Rajound block from sandy loam to loam, in Dhand block from sandy clay loam to clay loam, in Pundri block from sandy clay loam to clay loam, and in Guhla block from sandy loam to loam (Table 3). These findings align with those of Gora (2013) and Gyawali et al (2016) in the Kaithal district of Haryana.

Bulk Density (BD): The average bulk density content of soils of Kalayat, Rajound, Dhand and Pundri block 1.50, 1.51, 1.37 and 1. (Table 3). Similar results were found by Singh et al (2014) and Gyawali et al (2016) in Kaithal district of Haryana.

Table 2. Block wise soil fertility status of Kaithal District

Parameters	Range	Mean	Number of samples in the fertility category			NI	Remarks
			Low	Medium	High		
Kalayat (62)							
Organic (g kg^{-1})	0.27-0.92	0.46	24	37	1	1.62	Low
Available N (kg ha^{-1})	90-263	157	61	1	0	1.01	Low
Available P (kg ha^{-1})	6-32	14.7	14	36	12	1.96	Medium
Available K (kg ha^{-1})	58-540	287	6	29	27	2.32	Medium
Available S (mg kg^{-1})	19-430	116	1	1	60	2.95	High
Rajound (48)							
Organic (g kg^{-1})	0.25-0.92	0.47	23	21	4	1.60	Low
Available N (kg ha^{-1})	117-248	164	48	0	0	1.00	Low
Available P (kg ha^{-1})	6-29	13.5	11	32	5	1.87	Medium
Available K (kg ha^{-1})	106-720	270	5	30	13	2.16	Medium
Available S (mg kg^{-1})	48-282	124	0	0	48	3.00	High
Dhand(50)							
Organic (g kg^{-1})	0.24-0.91	0.44	26	23	1	1.46	Low
Available N (kg ha^{-1})	118-249	170	50	0	0	1.00	Low
Available P (kg ha^{-1})	5-30	12.6	15	29	6	1.82	Medium
Available K (kg ha^{-1})	80-454	248	4	33	13	2.18	Medium
Available S (mg kg^{-1})	39-355	121	0	1	49	2.98	High
Pundri (52)							
Organic (g kg^{-1})	0.17-0.95	0.49	16	32	4	1.76	Medium
Available N (kg ha^{-1})	90-268	164	50	2	0	1.03	Low
Available P (kg ha^{-1})	6-32	14.5	10	34	8	1.96	Medium
Available K (kg ha^{-1})	105-606	288	7	36	9	2.03	Medium
Available S (mg kg^{-1})	39-235	113	0	1	51	2.98	High

Table 3. Block wise physico-chemical properties of Kaithal District

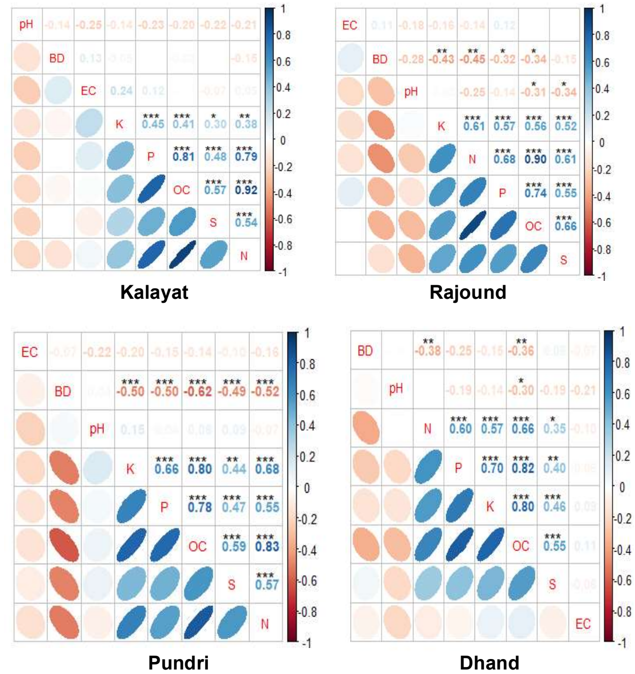
Block	pH		EC (dS m^{-1})		Bulk density (Mg m^{-3})		Texture
	Range	Mean	Range	Mean	Range	Mean	
Kalayat	6.9-9.1	8.09	0.12-1.82	0.45	1.46-1.59	1.50	SL to L
Rajound	6.9-9.0	8.13	0.11-1.39	0.39	1.46-1.59	1.51	SL to L
Dhand	7.1-9.1	8.04	0.13-1.69	0.45	1.32-1.43	1.37	SCL to CL
Pundri	7.4-9.1	8.22	0.11-0.77	0.38	1.35-1.45	1.39	SCL to CL

Available Nitrogen (N): The mean f N of the soils was 157, 164, 170 and 164 kg ha^{-1}, in Kalayat, Rajound, Pundri and Dhand block, respectively (Table 2). In general out of 212 soil samples, 98 % samples fell under low status, 2 % samples were medium and 0 % of the soil samples were high in N category in these blocks. Majority of the samples in Kalayat, Rajound, Pundri and Dhand block was with low N fertility with nutrient index 1.01, 1.00, NI 1.03 and 1.00, respectively (Table 2). The N deficiency in the study could be due to losses of nitrogen by volatilization, runoff, microbial fixation and denitrification. Higher decomposition rate of organic materials due to harsher temperature in the region may also contribute to the less N in the soil (Kumar 2019). Similar result was observed by in the Kaithal district of Haryana, where all the soil samples were deficient in N (Gyawali et al 2016).

Available Phosphorus (P): The mean P of the soils was 14.7, 13.5, 12.6 and 14.5 kg ha^{-1}, in Kalayat, Rajound, Pundri and Dhand block, respectively (Table 2). In general out of 212 soil samples, 23 % samples fell under low status, 61 % samples were medium and 16 % of the soil samples were high in P category in these blocks. Majority of the samples in Kalayat, Rajound, Pundri and Dhand block were found with medium P fertility (NI 1.96, 1.87, 1.96 and 1.82, respectively level) (Table 2). This could be due to the external application of phosphatic fertilizers in the field (Habtamu et al 2014 and Kumar et al. 2012). The findings are consistent with studies carried out in the Kaithal district of Haryana by Singh et al (2011) and Sharma et al (2024).

Available Potassium (K): The value of K of the soils was 287, 270, 248 and 228 kg ha^{-1}, in Kalayat, Rajound, Pundri and Dhand block, respectively (Table 2). In general out of 212 soil samples, 23 % samples fell under low status, 61 % samples were medium and 16 % of the soil samples were high in K category in these blocks. Most of the samples in Kalayat, Rajound, Pundri and Dhand block were with medium K fertility level (NI 2.32, 2.16, 2.03 and 2.18, respectively) (Table 2). It could be probably due to potassium-rich parent material like feldspar and illite may be present in the soil (Sharma et al 2024).

Available Sulphur (S): The mean S of the soils was 116, 124, 121 and 113 kg ha^{-1}, in Kalayat, Rajound, Pundri and Dhand block, respectively (Table 2). In general out of 212 soil samples, 0 % samples fell under low status, 2 % samples were medium and 98% of the soil samples were high in S category in these blocks. Majority of the samples in Kalayat, Rajound, Pundri and Dhand block were with high S fertility level (NI 2.95, 3.00, 2.98 and 2.98, respectively) l (Table 2).The high S status of soil may be due to the continuous application of sulphur containing fertilizer (zinc sulphate) in the cropping system of rice and wheat.

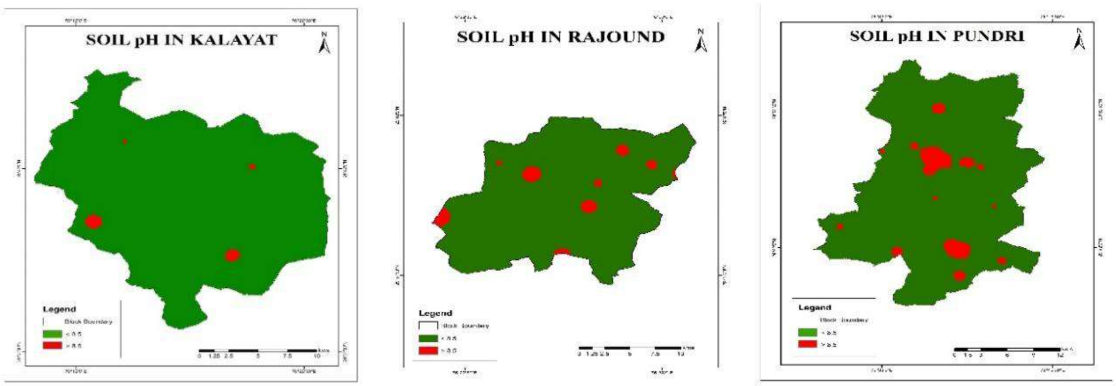


*Significant at the 0.05 level, ** Significant at the 0.01 level and *** Significant at the 0.001 level, NS = Non-Significant

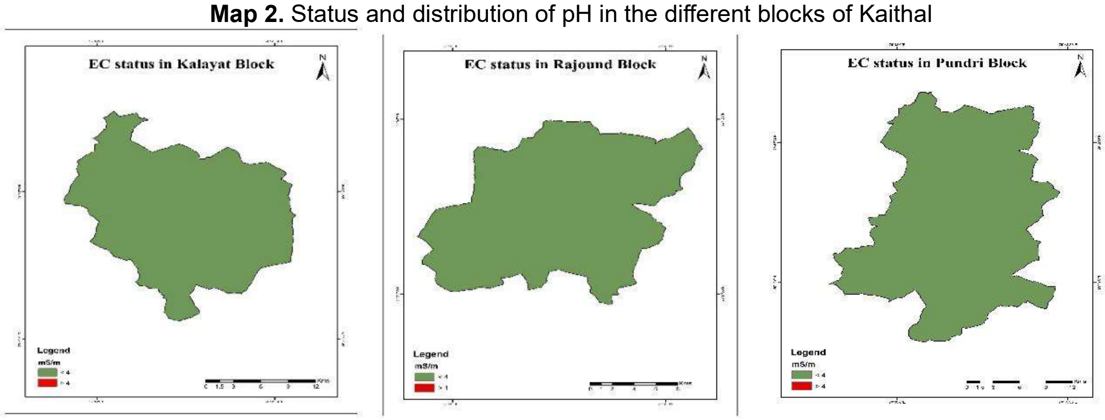
Fig. 1. Correlation of soil properties and nutrients of different blocks of Kaithal

Correlation between Soil Properties and Nutrients

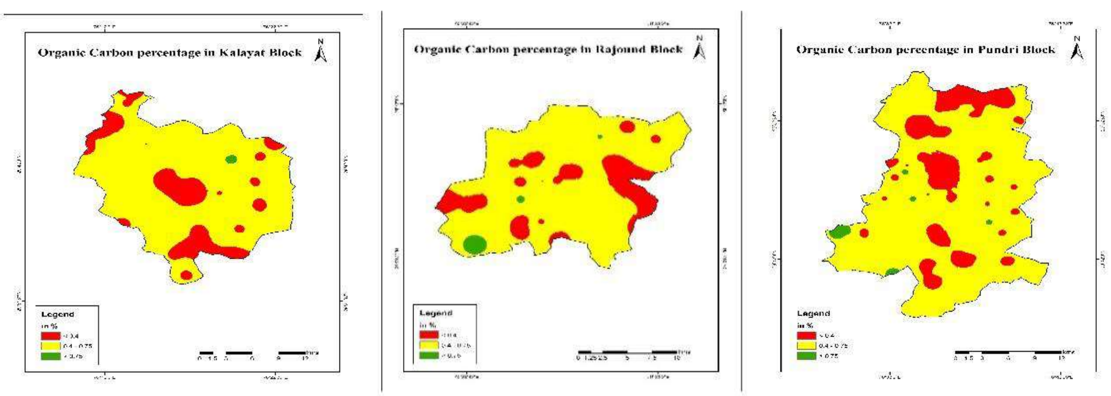
In the correlation matrix of the Kalayat, Dhand, Pundri and Kalayat block, OC exhibited a strong and statistically significant positive correlation with N P K and S content. The distinct correlation exists between OC and N content, as organic matter releases mineralizable N in accordance with its soil concentration. Therefore, the OC status of the soil can predict the availability of N, indicating a positive correlation. Meysner et al (2006) found that about 93 to 97% of the total nitrogen in soil is closely linked to organic matter (OM). Hailu et al (2015) confirmed that the trends observed in total nitrogen closely paralleled those of soil organic matter, underscoring a significant relationship between organic matter and total nitrogen. This was demonstrated by the strong and statistically significant positive correlation with organic matter. It has been reported that S in soils is mostly associated with organic matter (Nor 1981). Elevated levels of available phosphorus are linked to higher organic matter content. Organic matter enhances phosphorus levels by replacing the H_2PO^{4-} ions on adsorption sites through anion replacement, consequently increasing the amount of organic phosphorus that is mineralized into inorganic phosphorus (Havlin et al 2005; Bhat et al 2017). The correlation analysis indicates positive relationship between OC and K (Kumar et al 2023). This association might be explained through the presence of minerals bearing potassium in the silt and clay



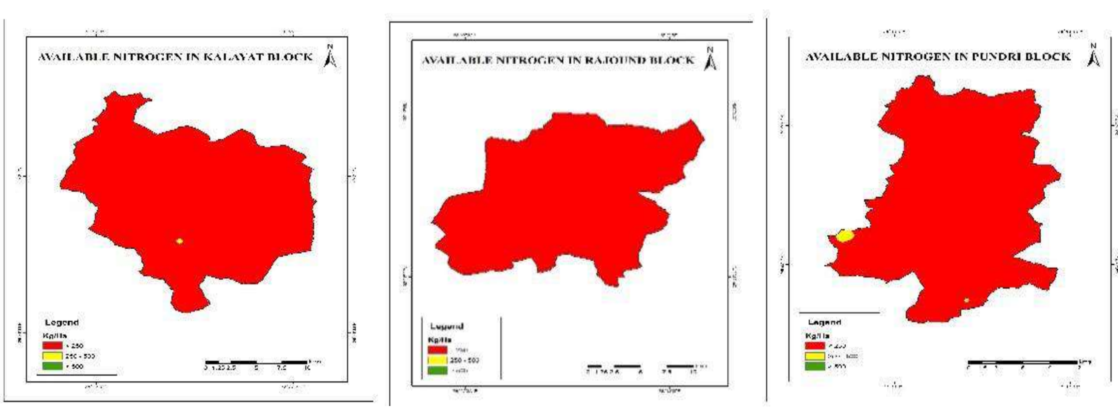
Map 2. Status and distribution of pH in the different blocks of Kaithal



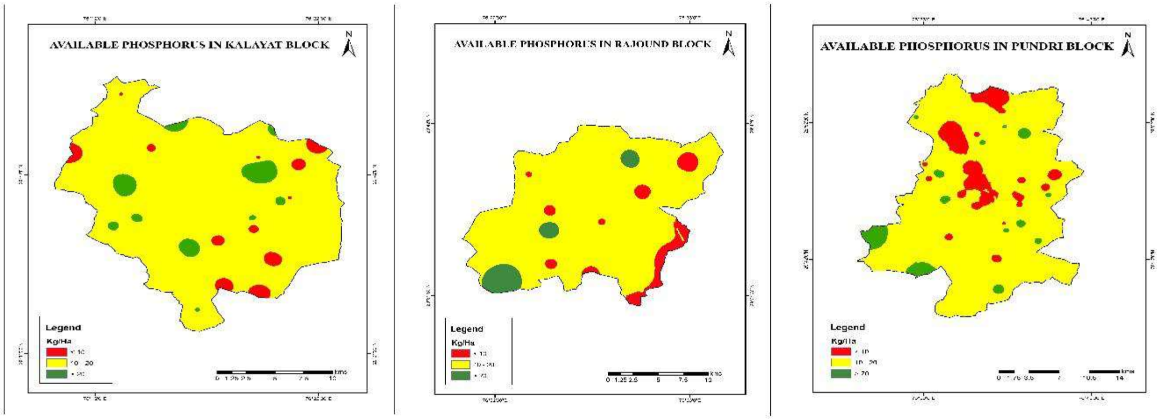
Map 3. Status and distribution of EC in the different blocks of Kaithal



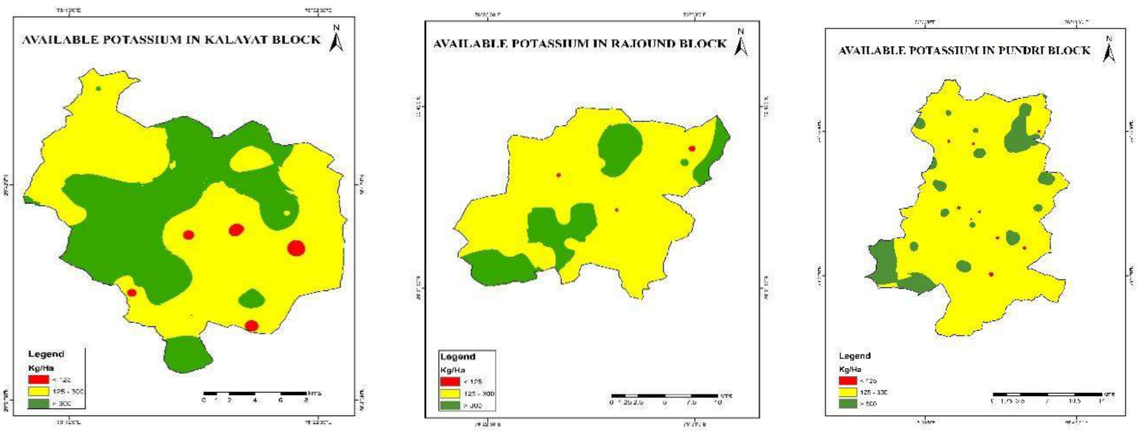
Map 4. Status and distribution of OC in the different blocks of Kaithal



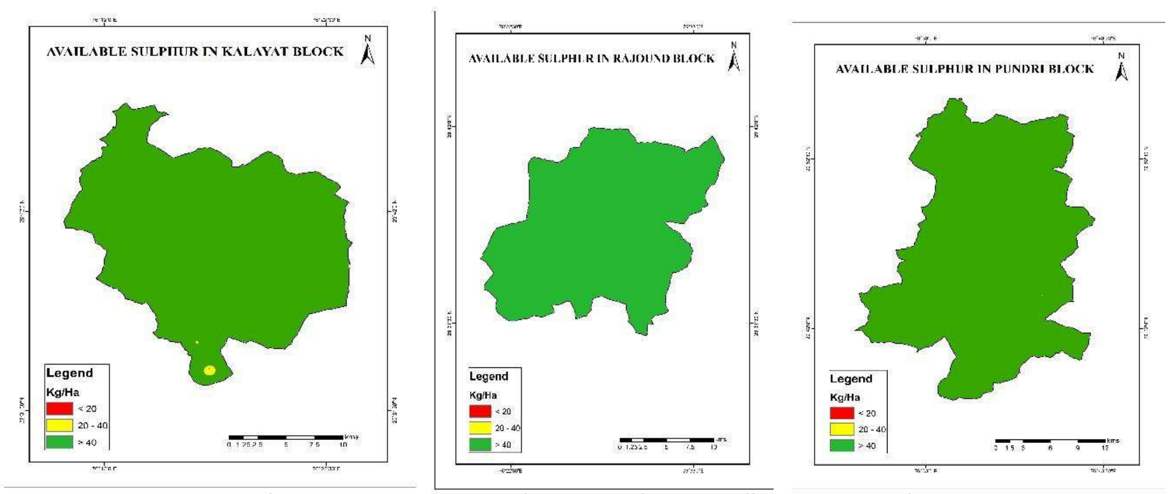
Map 5. Status and distribution of available N in the different blocks of Kaithal



Map 6. Status and distribution of available P in the different blocks of Kaithal



Map 7. Status and distribution of available K in the different blocks of Kaithal



Map 8. Status and distribution of available S in the different blocks of Kaithal

fractions, which include feldspars, illite and mica (Deka et al 1995, Reza et al 2014). Sharma et al (2024) witnessed equivalent findings within the Kaithal region of Haryana.

CONCLUSION

The physical and chemical characteristics of soil were assessed in the Kalayat, Rajound, Pundri and Dhand blocks. Parameters including pH, EC, organic carbon (OC), available nitrogen, available phosphorus, available potassium and available sulphur were examined for the study. In general, the soils in all four blocks exhibited non saline nature with neutral to alkaline pH and low to medium organic carbon content.

They were also non-calcareous. The soil texture varied across the blocks: Kalayat block ranged from sandy loam to loam, Rajound block from sandy loam to loam, Dhand block from sandy clay loam to clay loam and Pundri block from sandy clay loam to clay loam. Soils of all blocks were categorized as low in available N content, medium to high in P and K content and high in S content.

AUTHOR'S CONTRIBUTION

Mohit Sharma: Data curation, Formal analysis, Investigation, Methodology, Software, original draft, Review and editing, R S Garhwal: Investigation, Methodology, original draft, Review and editing, K.K. Bhardwaj: Conceptualization, Review and editing, Anil Kumar: Conceptualization, original draft, Software, Review and editing, Charan Singh: Software, Review and editing, Sunil Kumar: Review and editing, Amit Kumar: Review and editing and Saloni Yadav: Review and editing

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Efficacy of Indole-3-butyric Acid (IBA) on Growth Performance of Terminal Stem Cuttings of Rose-Scented Geranium [*Pelargonium graveolens* (L.) Herit.]

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Abstract: The experiment was conducted to study the effect of different treatments of indole-3-butyric acid (IBA) on the growth performance of terminal stem cuttings of rose-scented geranium [*Pelargonium graveolens* (L.) Herit.] at PAU, Regional Research Station, Gurdaspur (Punjab, India).The basal portion of terminal stem cutting of rose scented geranium were dipped in different treatments of Indole-3-butyric acid (IBA) *viz.* 250,500,750,1000,1250,1500ppm along with control (un-treated) to check the best fit concentration in respect to its growth behaviour after 30 days and 60 days of planting. The terminal stem cuttings of rose-scented geranium treated with IBA 1000ppm significantly reduced the number days to rooting with maximum sprouting percentage, survival percentage, plant height, plant girth, number of leaves, leaf length, leaf breadth, leaf weight, percentage rooting, number of roots, root length, root weight, root girth and number of branches in both 30 and 60 days after planting of cutting.

Keywords: Indole-3-butyric acid (IBA), *Pelargonium graveolens* (L.) Herit, Rose-scented geranium, Rooting, Terminal stem cuttings, Vegetative growth

Rose-scented geranium [*Pelargonium graveolens* (L.) Herit.] is an important high value perennial, aromatic shrub which belongs to the family Geraniaceae (Shawl et al 2006) originated from South Africa and is widely cultivated in Egypt, India, China, and to a lesser extent in Central Africa, Madagascar, Japan, Central America, Belgium, Reunion Islands, Congo and Europe (Shawl et al 2006, Singh et al 2011). There are about 700 different species in the Geraniaceae family (Shawl et al 2006). Out of which rose-scented geranium grows for production of essential oil from its leaves, tender shoots and flowers by using steam or hydro-distillation (Verma et al 2011). The current international demand is more than 600 tons mostly met by countries like China, Morocco, Egypt, Reunion Island and South Africa (Anonymous 1996). Against own requirement of approximately 200 tonnes, India produces less than 20 tonnes of geranium oil annually and meets its requirement by imports (Navale and Mungse 2002). Hence, it is necessary to take up cultivation of this crop on a commercial scale to meet internal demand and make a significant dent in the export trade. Presently two types of geranium called 'Algerian' or 'Tunisian' and 'Bourbon' or 'Re-union' are identified in India. Another cultivar 'Kelkar' has been recently introduced by M/s SH Kelkar and Company Limited-Mumbai, a leading flavour and fragrance company in India (Ram et al 2003). Geranium oil is one of the top 20 essential oils in the world. Mild climate with low humidity is ideal for its growth. The high humidity, heavy rainfall with mist, fog and water logging are detrimental. Quick multiplication of geranium from seeds is difficult. It does not set seeds under Indian conditions and as such vegetative propagation is the only means of perpetuation of this plant. With the recent emphasis on extending geranium cultivation, there has been increased demand for planting material. This warranted an easier and quicker method of propagation. However, no specific information is available in respect to faster multiplication of geranium plants for nursery production. The present investigation was undertaken with the objective to find out the efficacy of indole-3-butyric acid (IBA) on faster rooting/multiplication with improved vegetative growth parameters.

MATERIAL AND METHODS

The present investigation was conducted during the years 2022-23 and 2023-24 at Punjab Agricultural University Regional Research Station, Gurdaspur in sub-mountainous regions of Punjab which is situated between 32°3′ N latitude, 75°22′ E longitude and has an altitude of about 257 m from mean sea level having humid subtropical and dry winter climate. The terminal stem cuttings of rose-scented geranium about 10cm long with 2-4 leaves were taken from matured shoots. The growth substance i.e. indole-3-butyric acid (IBA) at 250,500,750,1000,1250 and 1500 ppm concentrations were prepared in lanolin paste. The basal portions of the prepared geranium terminal stem cuttings were smeared with lanolin having different concentration of IBA. In control, the cuttings were treated with lanolin paste only. The treated stem cuttings were planted during September in poly bags consisting of rooting media and kept in green shade net house. The experiment was laid out in completely randomized block design with three replications. The rooted cuttings (300 cuttings per replication in each treatment) were uprooted carefully without damaging the roots after 30 days and 60 days of planting and washed with water. The data on various growth parameters were recorded both after 30 days and 60 days of planting. Experimental data was statistically analyzed by using SPSS software.

RESULTS AND DISCUSSION

Sprouting and survival percentage: The significantly maximum sprouting and survival were observed in stem cutting treated with IBA 1000ppm as compared to rest of the treatments. The minimum survival and sprouting of stem cuttings were observed in control after 30 and 60days of planting of cutting. This result coincides with the findings of Ali (2018) in guava, Padekar et al (2018) in *Momordica dioica* Roxb, Venugopal et al (2018) in rosemary, Maninderdeep and Singh (2022) in grapes and Ali et al (2022) in dragon fruit. Number of sprouts increases due to better utilization of stored carbohydrates, nitrogen and other factors with the aid of growth regulators (Chandramouli 2001).

Number of days taken for rooting: Stem cutting of geranium treated with IBA 1000ppm took significantly minimum number of days to rooting after 30 days of planting, but control treatment took maximum number of days to rooting (Table 1). Similarly, minimum duration was observed for root initiation with the application of IBA @2000 ppm in stem cuttings of the 'Scented geranium' (*Pelargonium graveolens* L), while the longest duration to rooting was noticed in control (Kumar et al 2023). Similar results were reported in grape (*Vitis vinifera* L.) cuttings treated with IBA @ 3000 ppm (Maninderdeep and Singh 2022).

Growth parameters: The plant height, plant girth, number of leaves, leaf length, leaf breadth, leaf weight and number of branches were significantly higher in the stem cutting treated with IBA 1000ppm after 30 and 60 days of planting. With increased concentration beyond 1000ppm, the growth of plant physiological parameters was decreased. This may due to the inhibitory or phytotoxic effect of IBA at higher concentration (Jamal 2016). Similarly, Rani et al (2018) reported that IBA 3000 ppm treatment on the terminal cuttings of Guava *cv.* 'Taiwan Pink' resulted in highest number of leaves and leaf area of the cuttings. In study, IBA 1000 ppm could be attributed to the rapid hydrolysis of polysaccharides stored in the cuttings into physiologically active sugars by activation of hydrolytic enzymes. These sugars provide energy for the meristematic tissue through respiratory activity leads to early formation of shoots. Ali et al (2022) reported that in dragon fruit cuttings, IBA 7000ppm showed good results with maximum shoot growth, individual shoot length and number of new shoots per cutting.Plant height, number of leaves and collar diameter were also significantly higher in IBA treated patchouli (*Pogostemon cablin* Benth.) cuttings as reported by Kumar et al (2014). The IBA treatment may be complement by activating hydrolyzing enzymes at rooting site which catalyse the starch degradation and thereby enable availability of sugars for rapidly multiplying cells at the site of root initiation (Venugopal et al 2008). The higher number of leaves was due to growth regulators in the soil which increased the activity of lateral meristem and uptake of more nitrogen by plants which were required to intensify vegetative growth (Jadhav et al 2003). Mani et al (2022) also reported that the maximum number of leaves and average leaf area were recorded in cuttings of firethorn shrub treated with 6000 ppm of IBA, while the minimum number of leaves per cutting and average leaf area were observed in control. This may be due to the fact that IBA produced healthier lengthy roots and hence absorbed more nutrients and water contents which has resulted in higher number of leaves produced by the cutting. The increase in number of leaves per cutting might be due to the reason that the plant might diverted maximum assimilate quantities to the leaf buds, since the leaves are one of the production sites of natural auxins in them besides being very important for vital processes like photosynthesis and respiration (Wahab et al 2001). Similarly, Hossain and Gony (2020) reported that the maximum number of leaves per plant, leaf area, leaf length and leaf weight was obtained from IBA treatments to strawberry plants as compared to control. Among all IBA concentrations in bougainvillea cutting, the highest stems number per cutting and longest shoot length were noted under 3000 mg L^{-1} IBA concentration (Sadeeq 2024). Among various concentrations of IBA 7000 ppm to dragon fruit stem cuttings showed better results in terms of maximum shoot growth, individual shoot length and number of new shoots per cutting (Ali et al 2022).

The geranium cuttings treated with IBA 1000ppm recorded significantly highest rooting, number of roots, root length, root weight and root girth as compared to other treatments both in 30 and 60 days after planting (Table 1 and 2). Similarly, the maximum rooting percentage, number of roots , root length and root diameter were obtained in IBA treated cuttings of *Acacia catechu* Willd. & *Toona ciliata* M. Roem (Thakur et al 2018), grapes (Maninderdeep and Singh

2022), dahlia (Singh et al 2023) and bougainvillea (Sadeeq 2024). Increased root length was may be due to increased plant height, number of green leaves which may help in production of photosynthates and further supply to the roots in patchouli (*Pogostemon cablin* Benth.) (Venugopal et al 2008). The effects of auxins are significant on rooting as they facilitate the synthesis of ribonucleic acid and also induce ethylene production which is necessary for cell division and root initiation and hence, more number of roots recorded with auxin treated cuttings. It might be due to the fact that stimulation of cell wall plasticity which accelerates cell division, cambial and metabolic activity and leads to callus development and involved in root initiation by growth regulators as observed in many species (Ullah et al 2005). Auxins promote adventitious root formation by their ability to promote the initiation of lateral roots and also enhanced the transport of carbohydrates to basal portion of the cuttings. This effect may be due to rapid translocation property or fast destruction by auxin, increasing the enzymatic activity resulted in increased root length with IBA treatments of cuttings. But at higher dose of IBA i.e. beyond IBA 1000ppm, performance of all vegetative growth parameters were significantly lower. Similar trend of higher dose beyond IBA 500 ppm treated cuttings of Patchauli (*Pogostemon Cablin* (Blanco) Benth.) was observed *w.r.t* plant height, number of leaves, collar diameter, root length and number of roots (Kumar et al 2014). Similarly, Tien et al (2020) reported that treatments with higher doses of the IBA, remarkable inhibition effects on root number, root length and root weight were obtained among the stem cuttings of *Solanum procumbens*. Meanwhile number of roots at decrease concentration of IBA is attributed to more root length per cuttings and vegetative growth of per cuttings by utilizing applied IBA at concentration in Patchouli. Similarly, IBA treated stem cuttings showed better results in terms of average number of roots per cuttings, individual root length, fresh weight of roots, dry weight of roots and survival percentage of rooted cuttings in dragon fruit (Ali et al 2022),

Table 1. Effect of different treatments of Indole-3-butyric acid (IBA) on the vegetative growth of geranium (*Pelargonium graveolens* (L.) Herit) stem cutting after 30 days of planting

Treatment (ppm)	Survival (%)	Sprouting (%)	Number of days taken for rooting	Plant height (cm)	Plant girth (cm)	Number of leaves	Leaf length (cm)	Leaf breadth (cm)	Leaf weight (gm)	Rooting (%)	Number of roots	Root length (cm)	Root weight (gm)	Root girth (cm)
250	40.18	48.16	24.52	5.56	0.75	6.0	4.25	3.76	0.50	50.0	100.15	8.17	0.50	0.15
500	50.54	55.63	22.25	7.83	0.80	7.55	4.52	4.05	0.70	60.50	145.53	10.25	0.65	0.20
750	65.0	68.10	19.32	9.85	0.95	9.20	4.85	4.31	0.98	68.50	165.5	11.50	0.70	0.25
1000	95.85	92.72	10.0	15.50	2.0	13.0	6.12	5.51	2.06	85.0	250.64	14.55	0.95	0.45
1250	75.50	78.05	15.50	12.45	1.30	11.0	5.56	5.0	1.51	75.20	195.55	13.0	0.80	0.34
1500	70.24	73.35	17.25	11.50	1.0	10.45	5.15	4.65	1.25	72.45	180.77	12.50	0.75	0.28
Control	28.0	40.55	30.0	2.50	0.50	4.0	3.70	3.20	0.30	35.0	40.26	6.0	0.25	0.11
CD (p=0.05)	4.90	5.33	3.10	2.60	0.41	2.25	0.75	0.90	0.25	4.34	8.53	1.84	0.17	0.10

Table 2. Effect of different treatments of IBA on the vegetative growth of geranium (*Pelargonium graveolens* (L.) Herit) stem cutting after 60 days of planting.

Treatment (ppm)	Survival (%)	Plant height (cm)	Plant girth (cm)	Number of leaves	Leaf length (cm)	Leaf breadth (cm)	Leaf weight (gm)	Rooting (%)	Number of roots	Root length (cm)	Root weight (gm)	Root girth (cm)	Number of branches
250	35.30	11.0	1.50	12.50	5.55	4.60	1.45	62.48	145.80	12.45	0.75	0.35	3.12
500	42.40	13.25	2.0	15.0	6.0	4.96	1.70	70.25	180.25	14.15	0.95	0.42	3.52
750	55.0	15.50	2.55	18.55	6.35	5.30	2.16	76.32	215.70	15.55	1.43	0.56	4.0
1000	85.30	26.40	4.92	26.50	9.06	7.85	4.01	95.45	390.25	19.13	3.15	1.13	6.14
1250	70.25	18.0	3.51	22.25	7.65	6.46	3.05	80.42	255.65	17.50	2.10	0.72	5.10
1500	62.23	16.45	2.98	20.0	6.80	5.72	2.53	78.0	236.75	16.75	1.75	0.65	4.52
Control	24.50	5.50	1.12	8.50	5.05	4.0	1.10	42.55	85.60	10.0	0.40	0.20	2.0
CD (p=0.05)	4.61	3.80	0.29	3.19	0.84	0.82	0.34	3.65	7.90	2.15	0.17	0.12	1.62

firethorn (Mani et al 2022) and strawberry(Hossain and Gony 2020) as compared to control. Indole-3-butyric acid initiates the formation of maximum root length, could be due to the hydrolysis of polysaccharides stored in the cuttings into physiologically active sugars, which provides energy through respiratory activity to the root primordia and helps in rapid elongation of meristematic cells and initiate to obtain maximum root length (Singh et al 2014).

CONCLUSION

The terminal stem cuttings of rose-scented geranium [*Pelargonium graveolens* (L.) Herit.] treated with 1000ppm IBA (jndole-3-butyric acid) during September proved to be more effective in terms of various root and physiological growth parameters with minimum days taken for the root initiation, sprouting and maximum survival percentage.

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Residual Effect of Seed Rates and Timing of Knockdown of *Sesbania* and Nitrogen Levels on Growth and Yield of Zero till Maize in Rice-Maize Sequence

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Abstract: Field experiment was conducted during *rabi* seasons of 2020-21 and 2021-22 on a clay loam soil at the Agricultural College Farm, Bapatla to study the residual effect of brown manuring on growth and yield of zerotill maize in rice-maize sequence. The experiment was laid out in split-split plot design and the main plot treatments consisted of brown manure species *Sesbania* (*Sesbania aculeata*) sown at three seed rates (20, 30 and 40 kg ha^{-1}) and sub plot treatments comprising knockdown of *Sesbania* at four stages (20, 25, 30 and 35 DAS) using 2,4-D @ 0.5 kg ha^{-1} applied to *kharif* preceded rice and three sub-sub-plots to receive three nitrogen levels *viz.,* control (no nitrogen), 75 % and 100 % RDN applied to succeeding maize, The higher plant height. cob length, cob girth, 100 kernel weight and grain yield of *rabi* maize was recorded when its preceding rice crop received highest seed rate of *Sesbania* . The brown manuring at 35 DAS followed by BM at 30 DAS registered higher values. The highest plant height. cob length, cob girth, test weight and grain yield was with application of 100% RDN to *rabi* maize., *Sesbania* seed rate @ 40 kg ha^{-1}and knockdown of *Sesbania* at 35 DAS in preceding rice crop and application of 100% RDN to succeeding maize is also essential for accomplishing higher growth and yield of zerotill maize in rice-maize system.

Keywords: Brown manuring, Nitrogen levels, Rice-Maize Sequence and zero till maize

Maize (*Zea mays*. L) is one of the most unique emerging crops under varied agro-climatic conditions ranks third after rice and wheat in India occupying an area of 9.86 M ha, producing 31.51 Mt with a productivity of 3195 Kg ha^{-1} (Directorate of Economics & statistics, Ministry of Agriculture, Government of India, 2021).Having the highest genetic yield potential among the cereals, maize provides food, feed and fodder and also serves as a source of raw material for number of agro-based industries. The changing cultivation scenario of the Krishna Godavari Zone has rendered Rice-Maize as the most commanding cropping system replacing the age old tradition of Rice-Blackgram system because of diversified hurdles decreasing its efficiency and profitability. Nitrogen, an essential primary nutrient for rice, however the sky rocketing prices and the meager availability of inorganic nitrogen has often been beyond the reach of the farmers. Added to this meladay, use of either the organic manures or green manures in the rice-eco system encountered limitations in terms of shift in the season, scarce water availability and delay in time of application. Soil nutrient losses caused by an exhaustive cropping system like rice-maize cannot be fully offset by applying only the recommended doses of NPK fertilizers. To maintain soil productivity under continuous intensive cropping, additional measures are required (Kumari and Kaur 2016). Therefore, by keeping all these in view, present investigation was under taken to study the residual effect of brown manuring on growth and yield of zerotill maize in rice-maize sequence.

MATERIAL AND METHODS

The experiment was carried out on clay loam soils of Agricultural College Farm, Bapatla during *rabi* seasons of 2020-21 and 2021-22. The soil pH was slightly alkaline in reaction, low in organic carbon, low in available nitrogen, medium in available phosphorus and high in available potassium status. The*kharif* experiment was laid out in a split plot design during both the years of rice crop. The main plot treatments consisted of brown manure species *Sesbania* (*Sesbania aculeata*) sown at three seed rates (20, 30 and 40 kg ha^{-1}) and sub plot treatments comprising knockdown of *Sesbania* at four stages (20, 25, 30 and 35 DAS) using 2,4-D @ 0.5 kg ha^{-1} with three replications. During the succeeding *rabi*, the experiment was laid out on the same site in a split-split plot design to accommodate maize crop wherein, the three sub plot treatments imposed to *kharif* rice were divided into three sub-sub-plots to receive three nitrogen levels *viz.,* control (no nitrogen), 75 % and 100 % RDN to each plot thus, making a total of 12x3=36 treatments during *rabi*. The cultivars used in the investigation were Samba mashuri (rice) and Pioneer P-3396 (maize) respectively.

The average maximum and minimum temperatures during the cropping period were 31.6°C and 20.0°C during 2020-21 and 31.7°C and 18.8°C during 2021-22, respectively. the average relative humidity was 69.8 % and 74.7 % during 2020-21 and 2021-22, respectively. A total rainfall of 23 mm was received in 2 rainy days and 60.3 mm was received in 2 rainy days during *rabi,* 2020-21 and 2021-22, respectively.

Sesbania was grown as co-culture with direct sown rice for brown manuring. Its seeds at three rates (20, 30 and 40 kg ha^{-1}) as per the treatments were broadcasted manually all through the respective plots after sowing of rice in rows and allowed to grow with rice crop. Application of 2,4-D spray @ 0.5 kg ha^{-1} was done uniformly at 20, 25, 30 and 35 DAS by using a knapsack sprayer @ 500 l ha^{-1} of spray fluid to knockdown *Sesbania* as per the respective treatments in the experimental plots which resulted in gradual killing of *Sesbania* plants. As per the treatments, nitrogen (240 kg ha^{-1}) was applied in three equal splits at basal, knee-high and tasseling stage in the form of urea in the respective sub-sub plots. Entire recommended dose of 80 kg P_2O_5 ha^{-1} and 80 kg K_2O ha^{-1} was applied at basal in the form of single super phosphate and muriate of potash, respectively at the time of sowing during the both the years of study.Statistical significance was tested by applying F-test at 0.05 level of probability.

RESULTS AND DISCUSSION

Plant height (cm) at harvest: Plant height was significantly influenced by residual effect of different seed rates and timing of knockdown of *Sesbania* and by the levels of nitrogen applied to maize. The interaction among the seed rates, timing of knockdown of *Sesbania* and levels of nitrogen were non-significant in pooled data (Table 1). The maximum plant height of maize was recorded when the preceding rice was supplied with *Sesbania* seed rate @ 40 kg ha^{-1}which was significantly higher when compared to other treatments. The lower values of plant height of *rabi* maize at all the growth stages were noticed in the *Sesbania* seed rate @ 20 kg ha^{-1} as preceded plots. The taller plants in the higher seed rate plots might be due to enhanced availability of nitrogen from the decomposition of *Sesbania*. Higher seed rate of *Sesbania* might have created a positive effect on availability of nutrients to the succeeding maize crop which resulted in enhanced plant height. The results were in agreement with the research findings of Wolfe and Eckert (2002) and Sujatha et al (2008)

At harvest, significantly the highest plant heightof succeeding maize was observed with *Sesbania* brown manuring at 35 days in preceding rice which was statistically comparable to brown manuring at 30 days. The lowest maize plant height was with the BM at 20 days treatment given to the preceding rice crop. As the decomposition of the aged crop is slow which will help to enhance the period of availability of nutrients that matches the nutrient demand of succeeding crop. That may be the reason for significant difference in plant height of maize due to knockdown of green manures at different ages. These results are in accordance with the findings of Muntasir et al (2010) and Patel and Kumhar (2010).

Plant height increased significantly with increasing levels of nitrogen throughout all the growth stages of *rabi* maize and the tallest plants were recorded when the crop was supplied with 100% RDN. This increase in plant height might be due to better availability and utilization of nutrients resulting in improved assimilation, cell division, cell elongation and plant height at higher levels of nitrogen. Similar result of taller plant at higher nitrogen levels and shorter plants at lower nitrogen was also reported by Kunjir et al (2007), Wasnik et al (2012)

Days to 50 Percent tasseling and silking: Days to 50 percent tasseling and days to 50 percent silking of rabi maize was not affected by the seed rates and timing of knockdown of *Sesbania* in *kharif* rice and nitrogen levels to *rabi* maize during both years of experiment and their interaction was found non-significant (Table 1).

Cob length (cm) and cob girth (cm): The cob length and cob girth in maize was significantly influenced by seed rates and timing of knockdown of *Sesbania* treatments given to preceding rice and by the levels of nitrogen given to maize. The interaction among main plot, sub plot and sub-sub plot treatments was non-significant (Table 2). Among the seed rates of *Sesbania* given to preceding rice, seed rate @ 40 kg ha^{-1}recorded significantly higher cob length and cob girth in maize which was statistically superior over other seed rates of *Sesbania* . The cob length and cob girth observed with the seed rate @ 20 kg $ha^{-1}$was significantly lower and was comparable to seed rate @ 30 kg ha^{-1}.

Significantly higher cob lengthand cob girth recorded with the higher seed rate of *Sesbania* might be due to slow release of nutrients and decomposition of green manure released additional N after mineralization by microbes and increased nitrogen availability in soil which led to better matching between nutrient demand by crops and its supply by soil to result in ultimately higher cob length. These results are in close conformity with the findings of Muntasir et al (2010) and Meena et al (2013).Significantly higher cob length and cob girth of *rabi* maize was recorded when its preceding rice crop received brown manuring at 35 DAS followed by BM at 30 DAS. The lower values were registered when the *kharif* rice received BM at 20 DAS whereas it was statistically differing with BM at 25, 30 and 35 DAS during both the years of study and pooled data. Delayed knockdown of *Sesbania* in preceded rice might have increased the physical and biological properties and availability of nutrients leading to enhanced photosynthesis. Better accumulation of drymatter and photosynthates increased translocation to the sink leading to development of lengthy cobs. The experimental results are in compliance from findings of Arif et al (2011) and Anup Das et al (2016)

There was increase in the cob length and cob girth of *rabi* maize with increase in nitrogen levels during both the years of study. Higher cob length and cob girth of *rabi* maize was recorded with 100% RDN application and remained remarkably superior to all the other levels of nitrogen. Lower cob length and cob girth was registered with the control .The increased yield attributes might be due the increased supply of the major nutrients and the translocation and accumulation of photosynthates in the economic sinks, resulted in increased cob length and cob girth in maize The results are in consonance with the findings of Hari Om et al (2014), Venkata Rao et al (2014) and Pavithra et al (2015).

100 kernel weight (g): No significant differences were observed with the seed rates and timing of knockdown of *Sesbania* (Table 2). The thousand grain weight of maize was changed significantly among the levels of nitrogen. The interaction effect among these three factors was not statistically measurable. Among the levels of nitrogen tested, application of 100% RDN exhibited its better performance in registering significantly higher 100 kernel weight over control plot, while the 100 kernel weight recorded with N_2 was found to be on par with N_1. Though 100 kernel weight is a genetic character, due to its good management, weight of maize grain increased progressively with increased nitrogen levels. This might be due to increased translocation of photosynthates from source to sink. Reduction in nitrogen resulted in the reduced 100 kernel weight of maize. The results confirmed with the findings of Mercy et al (2012) and Owla et al (2015).

Grain yield (kg ha^{-1}): Grain yield was significantly influenced by different seed rates of *Sesbania* imposed to *kharif* rice crop. The highest grain yield of no till maize was registered

Table 1. Growth attributes of zerotill maize as influenced by seed rates and timing of knockdown of *Sesbania* applied to *kharif* rice crop and nitrogen levels to *rabi* maize (Pool data for 2 years)

Treatments	Plant height (cm) at harvest	Days to 50% tasseling	Days to 50% silking
Seed rate of *Sesbania* (M)			
M1- Seed rate of *Sesbania* @20 kg ha^{-1}	224.2	63	69
M2- Seed rate of *Sesbania* @30 kg ha^{-1}	238.9	62	67
M3- Seed rate of *Sesbania* @40 kg ha^{-1}	262.7	62	68
CD (p = 0.05)	16.5	NS	NS
CV (%)	11.5	9.6	8.6
Timing of knockdown of *Sesbania* (S)			
S1- Brown manuring at 20 DAS	222.8	63	69
S2- Brown manuring at 25 DAS	235.4	62	67
S3- Brown manuring at 30 DAS	248.5	61	67
S4- Brown manuring at 35 DAS	259.1	62	68
CD (p = 0.05)	12.5	NS	NS
CV (%)	11.0	9.1	7.1
Nitrogen levels applied to maize (N)			
N0- Control	221.2	63	70
N1- 75% RDN	247.8	62	67
N2- 100% RDN	268.4	61	67
CD (p = 0.05)	11.2	NS	NS
CV (%)	10.9	8.2	7.6
Interaction			
M x S		NS	
M x S		NS	
S x N		NS	
M x S x N		NS	

due to seed rate of *Sesbania* @ 40 kg ha^{-1} imposed to rice crop during *kharif*, which was statistically significant to other seed rates.The significant decrease in grain yield was recorded with the seed rate of *Sesbania* @ 20 kg ha^{-1} but was however comparable to the seed rate of *Sesbania* @ 30 kg ha^{-1}.Brown manure with higher seed rate not only supplements large quantity of organic biomass, but on decomposition has a solubilizing effect of N, P, K, and micronutrients (Zn, Fe, Mn, and Cu) in the soil and alleviating the deficiency of several nutrient elements by way of recycling the nutrients through this practice. Further, it also minimizes the leaching and gaseous losses of N, thus accomplishing the efficiency of applied plant nutrients. The findings are in confirmity with the experimental results of Fabunmi and Agbonlahor (2012), Talebbeigi and Ghadiri (2012) and Usman et al (2013).

The highest grain yield of maize was observed when the brown manuring of *Sesbania* was taken up at 35 DAS in preceding rice crop but was however comparable to BM practice at 30 DAS, which remained significant over BM action at 25 and 20 DAS. The lowest grain yield of maize was tabulated with BM at 20 DAS. Delayed knockdown of *Sesbania* in preceding rice might have supported in justifying the buildup of soil organic matter, which in turn, helped in improving the soil structure, pore size and water-holding capacity, increase in microbial population in rhizosphere of maize which could have rendered better availability of nutrients including micronutrients by reducing the loss of nutrients and improving the fertilizer use efficiency. Increase in the soil microbial population subsequent to the brown manuring at 35 days in rice crop might have led to increased solubilization of all the nutrients for absorption, which also could have resulted in the enhanced yield attributes like number of kernel rows cob^{-1}, kernel weight and test weight and finally kernel yield as compared to early days of brown manuring (Uma Reddy and Sathish 2017).

With increase in nitrogen level supplying to no till maize, the grain yield increased significantly over no N application.

Table 2. Yield attributes and grain yield of zerotill maize as influenced by seed rates and timing of knockdown of *Sesbania* applied to *kharif* rice crop and nitrogen levels to *rabi* maize (Pool data for 2 years)

Treatments	Cob length (cm)	Cob girth (cm)	100 kernel weight (g)	Grain yield (kg ha^{-1})
Seed rate of *Sesbania* (M)				
M1- Seed rate of *Sesbania* @20 kg ha^{-1}	15.6	12.4	22.2	6874
M2- Seed rate of *Sesbania* @30 kg ha^{-1}	16.1	13.0	22.4	7432
M3- Seed rate of *Sesbania* @40 kg ha^{-1}	17.3	14.6	22.6	8387
CD (p = 0.05)	0.8	0.8	NS	610
CV (%)	7.7	8.8	8.5	8.6
Timing of knockdown of *Sesbania* (S)				
S1- Brown manuring at 20 DAS	14.7	12.2	22.2	6519
S2- Brown manuring at 25 DAS	15.8	12.9	22.3	7427
S3- Brown manuring at 30 DAS	17.3	13.9	22.5	7974
S4- Brown manuring at 35 DAS	17.7	14.6	22.8	8229
CD (p = 0.05)	0.8	0.8	NS	352
CV (%)	9.9	8.5	9.3	8.2
Nitrogen levels applied to maize (N)				
N0- Control	14.5	11.9	22.2	6375
N1- 75% RDN	16.1	13.4	22.9	7674
N2- 100% RDN	17.8	16.0	23.1	8435
CD (p = 0.05)	1.0	1.4	0.8	322
CV (%)	8.0	10.9	7.2	8.0
Interaction				
M x S		NS		
M x N		NS		
S x N		NS		
M x S x N		NS		

Significantly the higher and lower grain yield of maize were registered with 100% RDN and control, respectively in both the years of study. The response to increased level of nitrogen may be attributed to faster release of available nutrients from the inorganic sources and maize being an exhaustive feeder could use this nutrient for increasing the physiological processes of plants thereby resulting in higher grain yields. The experimental results corroborate with the findings of Bahar et al (2009) and Baryal et al (2019).

CONCLUSION

On the basis of two years field experiment, residual effect of seed rate of *Sesbania* @ 40 kg ha^{-1} imposed in rice had exhibited significant positive residual effect on increasing the succeeding maize growth and yield. Among the knockdown days, brown manuring at 35 days followed by 30 days displayed superior growth and yield of maize. Further, maize crop requires 100% RDN for realizing superior growth, yield attributes and yield.

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Expression Variability and Comparative Susceptibility of Dual-Toxin Public Sector *Bt* Cotton Hybrids against *Earias vittella* (Fab.) and *Pectinophora gossypiella* (Saunders) in India

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Abstract: In the paradigm of commercially lagging public sector cotton seed corporations, the adoption of novel public sector *Bt* (*Bacillus thuringiensis*) cotton hybrids containing stacked *Bt* genes by Indian growers has not reached the expected scale. To counter the perceived monopolistic dominance of the private sector and address intensified challenges such as plant protection, food and fiber security, and environmental degradation due to indiscriminate application of synthetic pesticides, such research is crucial, particularly for small and marginal rainfed cotton farmers in the country. This study aimed to assess the relative expression of Cry proteins and the biocidal activity of public sector *Bt* cotton hybrids (NHH-44 BG II, PKV Hy-2 BG II, PDKV-JKAL-116 BG II, G. COT-10 Hy. BG II, G. COT-08 Hy. BG II and NHH-44 non-*Bt* as control) against *E. vittella* (Fab.) and *P. gossypiella* (Saunders). Laboratory investigations indicate that the cotton hybrids expressing dual-toxin, Cry1Ac and Cry2Ab genes significantly improved season long expression and contributed to the cessation of bollworm survival. All hybrids demonstrated varying levels of toxins in plant structures at different growth stages of herbivory, resulting in significantly lower survival for early instars compared to later. However, the surviving later instar bollworms flaunted adverse effect on growth and developmental parameters.

Keywords: *Bacillus thuringiensis,* Bollworms, Mortality, Public sector, Transgenic cotton

Cotton (*Gossypium* sp.) is cultivated in more than 80 countries with tropical to temperate agro-climatic conditions (Pathak et al 2023). India hoist its *numero uno* position in cotton cultivation with an area of 125.84 lakh ha under *Bt* cotton and 8.50 lakh ha under non-*Bt*, with production of 360 lakh bales. However, the average productivity of cotton remains low (486 kg per ha) compared to global productivity (CCI 2020). Cotton cultivation alone contributes to the livelihood of 9.9 million farmers (AICCIP 2022) and sustains employment of large labor force in the country, as an industrial commodity. Several factors impede the overall production of cotton, comprised of varied biotic and abiotic stresses (Shuli et al 2018 and Hussain et al 2023). Among biotic factors, globally, this crop shelters over 1326 insect and mite species throughout the growing season (Razaq et al 2013). In India, 162 species have been reported, of which 24 have attained pest status (Arora et al 2011). The lepidopteran pests, spotted bollworm (*Earias vittella* Fabricius) and pink bollworm (*Pectinophora gossypiella* Saunders), are the most vicious constituent of the cotton bollworm complex in India, altering the fitness of cotton produced for textile industries and export (Badiger et al 2011). *E. vittella* is an early to mid-season pest that damages tender growing shoots, bores into stems, and later feeds on squares and bolls (Ahmed et al 2012). Moreover, *P. gossypiella* exhibits most serious threat to cotton production in India, as a borer at boll developing stage of cotton, contributing to considerable reduction of total yield (Likhitha et al 2023). The cotton hybrids exhibit plasticity in getting infested by these insects, where this variation is much pronounced among different *Bt* cotton hybrids (Adamczyk and Gore 2004, Kranthi et al 2006, Dhillon and Sharma 2009, Arshad and Suhail 2010 and Thakre and Bhamare 2023a). Over the years, studies have illustrated the performance of various *Bt* hybrids in different Indian agro-ecological zones, demonstrating broad-spectrum inhibition of bollworm pests on transgenic hybrids (Manjunatha et al 2004, Likhitha and Bhamare 2018 and Thakre and Bhamare 2023a).

In India, these Bollgard cultivars were first approved for commercial cultivation (by the Genetic Engineering Appraisal Committee (GEAC), Ministry of Environment, Forest and Climate Change, Govt. of India) on 26th April, 2002 (Likhitha and Bhamare 2018). Since then, this technology has provided highly effective control against cotton lepidopteran pest complex (Dong and Li 2007 and Knight et al 2016). Over the years, numerous hybrids have been developed simultaneously by private and public sector corporations. Though, the private sector only exhibited monopolistic domination on the cotton seed market, ensuing public seed corporations cast lagging. Keeping this in view, we investigated the effect of season-long expression of Cry toxins in certain public sector *Bt* cotton hybrids on survival of *E. vittella* and *P. gossypiella* during economically critical stages of cotton crop (squares and bolls), grown under rainfed agro-ecological conditions.

MATERIAL AND METHODS

The survival and developmental studies were conducted at the Post Graduate Laboratory, Department of Agricultural Entomology, Latur (Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani), Maharashtra, during *Kharif* 2019-20. The GEAC approved five public sector *Bt* cotton hybrids (NHH-44 BG II, PKV Hy-2 BG II, PDKV-JKAL-116 BG II, G. COT-10 Hy. BG II and G. COT-08 Hy. BG II), as well as a non-*Bt* hybrid (NHH-44 non-*Bt*) as control, were cultivated by following all the recommended operations excluding plant protection practices.

Cry protein expression profiling: The quantification of Cry toxins was performed at Plant Biotechnology Laboratory of Vilasrao Deshmukh College of Agril. Biotechnology, Latur. The expression of Cry toxins was investigated in leaves (60 days old crop), squares (90 days old crop) and bolls (120 and 150 days old crop) of public sector *Bt* cotton hybrids at different crop ages by sandwich enzyme-linked immunosorbent assays (ELISA) using QL 96 quantiplate ELISA kits. The concentration of Cry1Ac proteins was measured using Cry1Ab/Cry1Ac kit (AP 003 QT V50), and Cry2Ab using Cry2A kit (AP 005 QT BC V50) of ENVIROLOGIX 500 Riverside Industrial Parkway Portland, ME, USA, supplied by Amar Immunodiagnostic, Hyderabad, following the manufacturer's instructions (protocol: ENVIROLOGIX 2017).

Bioassay of *E. vittella* and *P. gossypiella:* Larval populations of field-collected *E. vittella* and *P. gossypiella* were reared on a natural diet (non-*Bt* cotton) until pupation to develop initial cultures. After emergence, moths were released into standard oviposition cage (27±1ºC and 70% RH) with cotton swabs dipped in honey solution (10%). The fresh leaves of the host plants were placed as oviposition substrate which was examined for the presence of egg masses and replaced daily with fresh ones. Larvae hatched from eggs were transferred into plastic vials and fed on natural diet to obtain different larval instar (I-V instars for *E. vittella* and I-IV instars for *P. gossypiella*), which were used for further investigations. Plant structures of different public sector *Bt* cotton hybrids were collected from the field in labeled plastic bags, from 90-110 and 120-140 days old crop for *E. vittella* (squares and bolls), and 150-170 days old crop for *P. gossypiella* (bolls). Collected samples were cleaned, placed individually in plastic vials, and then the laboratory-reared larval instars (I-V instars of *E. vittella* and I–IV instars of *P. gossypiella*) were released in each vial. The periodical replacement of *Bt* cotton plant parts with the fresh ones (those on which larvae fed) was ensured till pupation or mortality.

Data analysis: A standard curve was prepared using optical density (OD) values (absorbance at 405 nm) of each calibrator and corresponding concentrations of Cry1Ac and Cry2Ab. The concentration of each sample was determined by its absorbance in an ELISA reader (OD value), and results were multiplied by all dilution factors incurred during extraction, presented as microgram (µg) toxin per gram of fresh tissue. Laboratory bioassays were conducted for each separate instar of bollworms and replicated thrice using ten larvae per replication. The data on per cent mortality, pupation and adult emergence was recorded separately for each instar by feeding them on plant structures of public sector *Bt* hybrids. The weights of surviving instars were registered at 24, 48, and 72 hr of exposure, as well as of pupae soon after pupation. Using the formulae given by Vennila et al (2006), the growth and survival indices were calculated. Concentrations of Cry1Ac and Cry2Ab in different plant structures and the results of bioassays were analyzed in completely randomized design. The data was statistically analyzed by using OPSTAT statistical package by Sheron OP, HAU, Hisar.

RESULTS AND DISCUSSION

Expression of Cry1Ac toxin: The Cry1Ac toxin was estimated using Quantiplate ELISA kit from leaves (60 days old crop), squares (90 days old crop) and bolls (120 and 150 days old crop) of different *Bt* cotton hybrids (Fig. 1). In leaves of 60 days old crop, the highest Cry1Ac expression was observed in PDKV-JKAL-116 BG II (4.71 µg per g fresh tissue), followed by PKV Hy-2 BG II, G. COT-10 Hy. BG II, NHH-44 BG II, and lowest in G. COT-08 Hy. BG II (2.59 µg per g fresh tissue). In squares of 90 days old crop, maximum concentration was recorded in PDKV-JKAL-116 BG II (3.73 µg per g fresh tissue), followed by G. COT-08 Hy. BG II, G. COT-10 Hy. BG II, PKV Hy-2 BG II, and minimum in NHH-44 BG II (2.58 µg per g fresh tissue). Higher Cry1Ac protein expression in bolls of 120 days old crop was found in NHH-44 BG II (1.47 µg per g fresh tissue), followed by G. COT-08 Hy. BG II, G. COT-10 Hy. BG II, PKV Hy-2 BG II and PDKV-JKAL-116 BG II (0.67 µg per g fresh tissue). Whereas, bolls of 150 days old crop registered highest toxin expression with NHH-44 BG II (0.33 µg per g fresh tissue), followed by PKV Hy-2 BG II, PDKV-JKAL-116 BG II, G. COT-10 Hy. BG II, and lowest in G. COT-08 Hy.BG II (0.028 µg per g fresh tissue). The expression profiling indicated a progressive decline in

Cry1Ac concentration from early to later stages of transgenic crop.

Expression of Cry2Ab toxin: The season long expressions of Cry2Ab protein was estimated from leaves (60 days old crop), squares (90 days old crop), and bolls (120 and 150 days old crop) of different public sector *Bt* cotton hybrids (Fig. 2). Among all hybrids, the highest Cry2Ab concentration in leaves was observed in PKV Hy-2 BG II (13.15 µg per g fresh tissue), followed by NHH-44 BG II, PDKV-JKAL-116 BG II, G. COT-10 Hy. BG II and G. COT-08 Hy. BG II (8.18 µg per g fresh tissue). In squares, the maximum expression was found in PKV Hy-2 BG II (15.96 µg per g fresh tissue), followed by G. COT-08 Hy. BG II, G. COT-10 Hy. BG II, PDKV-JKAL-116 BG II, and minimum in NHH-44 BG II (14.32 µg per g fresh tissue). The Cry2Ab protein concentration in bolls of 120 days old crop was highest in NHH-44 BG II (6.40 µg per g fresh tissue), followed by PDKV-JKAL-116 BG II, G. COT-08 Hy. BG II, PKV Hy-2 BG II, and G. COT-10 Hy.BG II (4.25 µg per g fresh tissue). In the bolls of 150 days old crop, higher expression was registered in NHH-44 BG II (4.82 µg per g fresh tissue), followed by G. COT-08 Hy. BG II, G. COT-10 Hy. BG II, PKV Hy-2 BG II, and minimal in PDKV-JKAL-116 BG II (1.77 µg per g fresh tissue). All transgenic cotton hybrids exhibited significant variation in Cry2Ab toxin concentration among plant structures at different stages of crop growth. The highest toxin concentration was detected in early stages of crop growth (leaves and squares), showing progressive decline in later stages (bolls).

Bioassay of *E. vittella* on squares: All treatments showed affirmative results when *E. vittella* fed on different *Bt* cotton hybrids compared to non-*Bt* cotton. Laboratory bioassays indicated significant larval mortality (20.00 to 100.00%) of early instars (I, II and III instar), fed on squares and bolls of *Bt* cotton hybrids at pre-determined intervals than the later instars. However, all larvae from IV and V instars survived till pupation. The first instars fed on squares of PKV Hy-2 BG II and G. COT-08 Hy. BG II, and on bolls of NHH-44 BG II and PDKV-JKAL-116 BG II showed cent per cent mortality. Whereas, the minimum mortality of first instars was observed on squares of NHH-44 BG II (80.00%), and on bolls of G. COT-10 Hy. BG II (76.67%), still higher than the mortality rate of later instars (Table 1). The data followed more or less similar trend for the different growth and developmental parameters of *E. vittella*, when fed on squares and bolls. The larval weights of I-V instars survived beyond 24, 48 and 72 hr after exposure, as well as per cent pupation, pupal weight and per cent adult emergence showed continuing reduction in survival and growth rates (Table 2, 4, 5, 6). The minimum growth index (0.95 and 0.91) and survival index (0.44 and 0.48) values were recorded on squares and bolls of PKV Hy-2 BG II and NHH-44 BG II when fed on transgenic public sector hybrids, respectively (Fig. 3, 4).

Bioassay of *P. gossypiella* on bolls: Larval instars of *P. gossypiella* showed significant survival due to relatively low-toxic reaction against *Bt* cotton hybrids on bolls of 150 days old crop. None of the transgenic hybrids revealed mortality of last larval instar. However, significant mortality rates of 100.00, 86.67 and 60.00 per cent were recorded in I, II and III instars, respectively, when larvae fed on bolls of NHH-44 BG II. Thereafter, mortality rate progressively decreased in G. COT-08 Hy. BG II (100.00, 70.00 and 56.67%), followed by G. COT-10 Hy. BG II, PKV Hy-2 BG II, and minimum in PDKV-JKAL-116 BG II (76.67, 53.33 and 30.00%) (Table 1). Likewise, for other survival and developmental parameters more or less parallel results were evidenced (Table 3, 4, 5, 6). Minimal growth and survival indices of the insect larvae were observed in NHH-44 BG II (0.67 and 0.35), followed by G. COT-08 Hy. BG II, G. COT-10 Hy. BG II, PKV Hy-2 BG II and maximum in PDKV-JKAL-116 BG II (1.33 and 0.55) (Fig. 5).

The dual-toxin expression profiling showed progressive decline in concentrations from early to later stages of crop growth, with higher levels observed at 60 and 90 days (leaves and squares) and lower levels at 120 and 150 days (bolls) of cotton crop. This pattern aligns with previous reports indicating higher expression of Cry proteins in early vegetative and mid-reproductive stages, decreasing in later reproductive stages (Cheema et al 2015 and Zaman et al 2015). Overall, Cry2Ab was found in higher concentration than Cry1Ac, owing to its potential importance in controlling bollworm herbivory at different crop stages (Liu et al 2017 and Manjunatha et al 2017).

The Cry toxins associated with *Bt* hybrids reflected significant mortality and conflicting effect on survival of the bollworms. Among all instars, later instars of bollworms showed higher larval survival than early instars when fed on plant structures of *Bt* cotton. The highest larval mortality was found in newly hatched first instar (neonates) of bollworms, consistent with the findings of Shera and Arora (2016a) and Likhitha and Bhamare (2018). Significant reduction in mortality rate was reported from early crop stages to the later, leading to an incomplete inhibition in bollworm survival, as documented by Kranthi et al (2009), Siebert et al (2009), Hallad et al (2014) and Likhitha et al (2023). *E. vittella* fed on squares showed minimal growth and survival indices when compared with bolls. Therefore, from the illustrated data, these transgenic cotton hybrids may provide control against these pest population in initial crop stages by conferring mortality, especially in early larval instars (Ahmed et al 2012, Hallad et al 2014 and Shera et al 2015). Further, the data showed relatively greater non-toxic response by *P.*

Table 1. Mortality (%) of *E. vittella* on squares and bolls and *P. gossypiella* on bolls of public sector *Bt* cotton hybrids

Treatments	Squares (90-110 days old crop)					Bolls (120-140 days old crop)					Bolls (150-170 days old crop)			
	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar
NHH-44 BG II	80.00 (63.43)*	60.00 (50.77)	20.00 (26.57)	0.00 (0.00)	0.00 (0.00)	100.00 (90.00)	90.00 (71.57)	50.00 (45.00)	0.00 (0.00)	0.00 (0.00)	100.00 (90.00)	86.67 (68.59)	60.00 (50.77)	0.00 (0.00)
PKV Hy-2 BG II	100.00 (90.00)	86.67 (68.59)	40.00 (39.23)	0.00 (0.00)	0.00 (0.00)	80.00 (63.43)	70.00 (56.79)	30.00 (33.21)	0.00 (0.00)	0.00 (0.00)	83.33 (65.90)	60.00 (50.77)	36.67 (37.27)	0.00 (0.00)
PDKV-JKAL-116 BG II	86.67 (68.59)	66.67 (54.74)	26.67 (31.09)	0.00 (0.00)	0.00 (0.00)	100.00 (90.00)	83.33 (65.90)	43.33 (41.17)	0.00 (0.00)	0.00 (0.00)	76.67 (61.12)	53.33 (46.91)	30.00 (33.21)	0.00 (0.00)
G. COT-10 Hy. BG II	90.00 (71.57)	70.00 (56.79)	30.00 (33.21)	0.00 (0.00)	0.00 (0.00)	76.67 (61.12)	56.67 (48.83)	20.00 (26.57)	0.00 (0.00)	0.00 (0.00)	90.00 (71.57)	63.33 (52.73)	40.00 (39.23)	0.00 (0.00)
G. COT-08 Hy. BG-II	100.00 (90.00)	80.00 (63.43)	40.00 (39.23)	0.00 (0.00)	0.00 (0.00)	90.00 (71.57)	76.67 (61.12)	30.00 (33.21)	0.00 (0.00)	0.00 (0.00)	100.00 (90.00)	70.00 (56.79)	56.67 (48.83)	0.00 (0.00)
NHH-44 (Non-*Bt*)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SE (m) ±	0.13	0.19	0.13	-	-	0.13	0.23	0.13	-	-	0.19	0.23	0.19	-
CD (p≤0.05)	0.41	0.58	0.41	-	-	0.41	0.71	0.41	-	-	0.58	0.71	0.58	-
CV %	3.09	5.50	9.02	-	-	3.16	6.50	8.15	-	-	4.44	7.34	8.95	-

* Figures in parenthesis angular transformed values

Table 2. Effect on larval weight of *E. vittella* on squares and bolls fed on public sector *Bt* cotton hybrids

Treatments	Mean larval weight (mg/larva)														
	I instar			II instar			III instar			IV instar			V instar		
	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
Larval weight of *E. vittella* fed on squares (90-110 days old crop)															
NHH-44 BG II	3.7	3.84	4.53	4.89	5.12	9.21	24.92	46.41	73.98	65.64	75.64	96.61	96.22	98.38	111.7
PKV Hy-2 BG II	2.91	3.03	3.26	3.89	4.06	7.41	19.69	33.77	55.74	46.47	62.02	68.26	66.34	74.21	94.66
PDKV-JKAL-116 BG II	3.64	3.78	4.09	4.73	4.98	8.50	23.26	44.47	72.36	60.5	71.77	89.42	83.25	94.66	104.06
G. COT-10 Hy. BG II	3.59	3.75	3.87	4.6	4.93	8.15	21.52	43.38	68.56	58.61	68.61	83.51	79.21	88.59	102.03
G. COT-08 Hy. BG-II	3.55	3.62	3.78	4.56	4.82	7.94	20.43	41.63	66.33	53.92	67.44	81.54	74.08	78.27	98.38
NHH-44 (Non-*Bt*)	4.49	4.67	4.73	5.57	6.40	13.5	31.22	57.53	88.17	78.59	94.81	134.8	124.13	157.56	194.83
SE (m) ±	0.01	0.01	0.03	0.04	0.09	0.23	0.52	1.44	0.42	0.54	1.25	0.71	0.89	1.45	1.46
CD (p=0.05)	0.05	0.04	0.09	0.13	0.27	0.72	1.59	4.37	1.28	1.65	3.79	2.17	2.71	4.40	4.43
CV %	0.79	0.69	1.34	1.59	3.10	4.52	3.86	5.60	1.03	1.55	2.95	1.34	1.78	2.55	2.15
Initial weight	2.76	-	-	3.45	-	-	16.22	-	-	52.53	-	-	58.75	-	-
Larval weight of *E. vittella* fed on bolls (120-140 days old crop)															
NHH-44 BG II	2.94	3.10	3.30	3.91	5.92	8.68	21.42	34.47	56.14	47.82	62.93	68.69	68.09	75.75	95.45
PKV Hy-2 BG II	3.72	3.89	4.21	5.13	6.34	9.34	26.76	47.68	74.67	62.30	73.47	90.57	97.36	95.43	106.76
PDKV-JKAL-116 BG II	3.56	3.78	3.80	4.68	6.17	8.82	22.17	41.53	67.37	55.32	66.92	82.51	77.35	78.54	99.95
G. COT-10 Hy. BG II	3.82	3.93	4.54	5.16	6.82	9.58	26.8	49.75	76.25	67.51	76.06	97.63	99.35	100.79	116.2
G. COT-08 Hy. BG-II	3.63	3.83	4.00	4.87	6.25	9.05	25.39	45.50	69.11	58.98	70.07	84.10	81.86	89.09	103.46
NHH-44 (Non-*Bt*)	4.46	4.56	4.77	5.59	7.32	14.28	34.0	57.75	90.47	80.34	97.45	142.27	126.01	155.54	203.71
SE (m) ±	0.02	0.08	0.08	0.05	0.09	0.35	0.70	0.64	0.70	0.61	0.80	1.56	0.71	0.92	1.94
CD (p=0.05)	0.07	0.26	0.26	0.17	0.28	1.08	2.14	1.95	2.12	1.85	2.42	4.76	2.17	2.80	5.89
CV %	1.11	3.99	3.72	0.34	2.47	6.21	4.69	2.41	1.67	1.71	1.86	2.88	1.35	1.61	2.78
Initial weight	2.83	-	-	3.50	-	-	18.94	-	-	54.55	-	-	71.15	-	-

gossypiella larval instars when fed with bolls of public sector *Bt* cotton hybrids. These results are in conformity with the findings of Soujanya et al (2010) and Naik et al (2014), who also reported progressive decline in the survival and development of the pest on late phenological stages of *Bt* cotton. Meanwhile, surviving later larval instars of both bollworms showed waning effects on growth and developmental parameters, such as reduction in larval weights, per cent pupation with lower pupal weight, and reduced adult emergence. These results of growth inhibition and stunting are supported by the findings of Fabrick et al (2015), Likhitha and Bhamare (2018) and Thakre and Bhamare (2023a, 2023b). The growth and survival indices showed similar trend in results, as the duration and amount of endotoxin consumption confers reduced survival and growth rate (Shera and Arora 2016a).

In addition, previous findings (Olsen and Daly 2000, Cheema et al 2015, Manjunatha et al 2017, Khan et al 2018 and Likhitha et al 2023) suggest that different intrinsic and extrinsic attribute to variation in Cry toxin expression. This would also presuppose that the *Bt* toxin detection by larvae may result in avoidance and apparent feeding preference of the pest, resulting into death by starvation of the bollworms (Shera and Arora 2016b). Therefore, sustainable Cry toxin expression among *Bt* hybrids is essential for their efficacy against bollworms. Therefore, screening and adoption of

Table 3. Effect on larval weight of *P. gossypiella* on bolls fed on public sector *Bt* cotton hybrids

Treatments	Mean larval weight of *P. gossypiella* fed on bolls (150-170 days old crop)											
	I instar			II instar			III instar			IV instar		
	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
NHH-44 BG II	1.46	1.57	2.87	10.44	12.94	13.66	25.39	27.57	28.57	38.49	43.36	48.47
PKV Hy-2 BG II	1.77	3.08	5.41	15.01	16.18	18.08	35.61	36.56	37.82	48.66	53.56	58.36
PDKV-JKAL-116 BG II	1.82	3.23	5.79	16.05	17.75	18.37	36.16	38.00	39.39	50.34	55.32	58.79
G. COT-10 Hy. BG II	1.65	2.96	4.63	14.27	15.72	16.41	32.47	33.46	34.56	47.42	50.57	56.98
G. COT-08 Hy. BG-II	1.57	2.87	4.25	12.18	14.60	14.99	27.19	29.38	31.47	41.39	48.47	53.56
NHH-44 (Non-*Bt*)	2.00	3.29	6.42	19.38	27.38	28.05	38.42	41.3	46.76	59.31	74.63	85.37
SE (m) ±	0.04	0.05	0.06	0.42	0.57	0.40	0.80	0.71	1.33	0.79	1.01	0.55
CD (p=0.05)	0.13	0.16	0.19	1.28	1.73	1.22	2.43	2.16	4.04	2.40	3.07	1.69
CV %	4.51	3.36	2.25	5.04	5.69	3.82	4.26	3.59	6.34	2.88	3.23	1.60
Initial weight	1.11	-	-	9.06	-	-	26.88	-	-	37.75	-	-

Table 4. Pupation (%) of *E. vittella* on squares and bolls and *P. gossypiella* on bolls of public sector *Bt* cotton hybrids

Treatments	Squares (90-110 days old crop)					Bolls (120-140 days old crop)					Bolls (150-170 days old crop)			
	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar
NHH-44 BG II	20.00 (26.57)*	40.00 (39.23)	80.00 (63.43)	100.00 (90.00)	100.00 (90.00)	00.00 (00.00)	10.00 (18.43)	50.00 (45.00)	100.00 (90.00)	100.00 (90.00)	00.00 (00.00)	13.33 (21.41)	40.00 (39.23)	100.00 (90.00)
PKV Hy-2 BG II	00.00 (00.00)	13.33 (21.41)	60.00 (50.77)	100.00 (90.00)	100.00 (90.00)	20.00 (26.57)	30.00 (33.21)	70.00 (56.79)	100.00 (90.00)	100.00 (90.00)	16.67 (24.10)	40.00 (39.23)	63.33 (52.73)	100.00 (90.00)
PDKV-KAL-116 BG II	13.33 (21.41)	33.33 (35.26)	73.33 (58.91)	100.00 (90.00)	100.00 (90.00)	00.00 (00.00)	16.67 (24.10)	56.67 (48.83)	100.00 (90.00)	100.00 (90.00)	23.33 (28.88)	46.67 (43.09)	70.00 (56.79)	100.00 (90.00)
G. COT-10 Hy. BG II	10.00 (18.43)	30.00 (33.21)	70.00 (56.79)	100.00 (90.00)	100.00 (90.00)	23.33 (28.88)	43.33 (41.17)	80.00. (63.43)	100.00 (90.00)	100.00 (90.00)	10.00 (18.43)	36.67 (37.37)	60.00 (50.77)	100.00 (90.00)
G. COT-08 Hy. BG-II	00.00 (00.00)	20.00 (26.57)	60.00 (50.77)	100.00 (90.00)	100.00 (90.00)	10.00 (18.43)	23.33 (28.88)	70.00 (56.79)	100.00 (90.00)	100.00 (90.00)	00.00 (00.00)	30.00 (33.21)	43.33 (41.17)	100.00 (90.00)
NHH-44 (Non-*Bt*)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
SE (m) ±	0.13	0.19	0.13	-	-	0.13	0.23	0.13	-	-	0.19	0.23	0.19	-
CD (p≤0.05)	0.41	0.58	0.41	-	-	0.41	0.71	0.41	-	-	0.58	0.71	0.58	-
CV %	9.86	8.45	3.19	-	-	9.22	10.96	3.31	-	-	13.33	9.18	5.31	-

* Figures in parenthesis angular transformed values

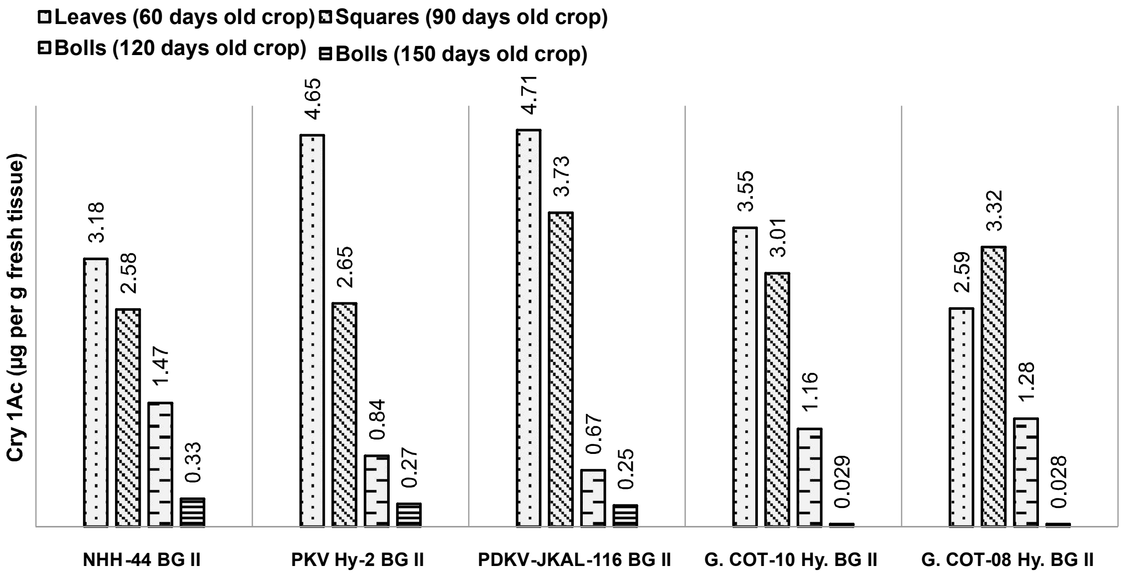

Fig. 1. Expression of Cry 1Ac toxin in different public sector *Bt* cotton hybrids

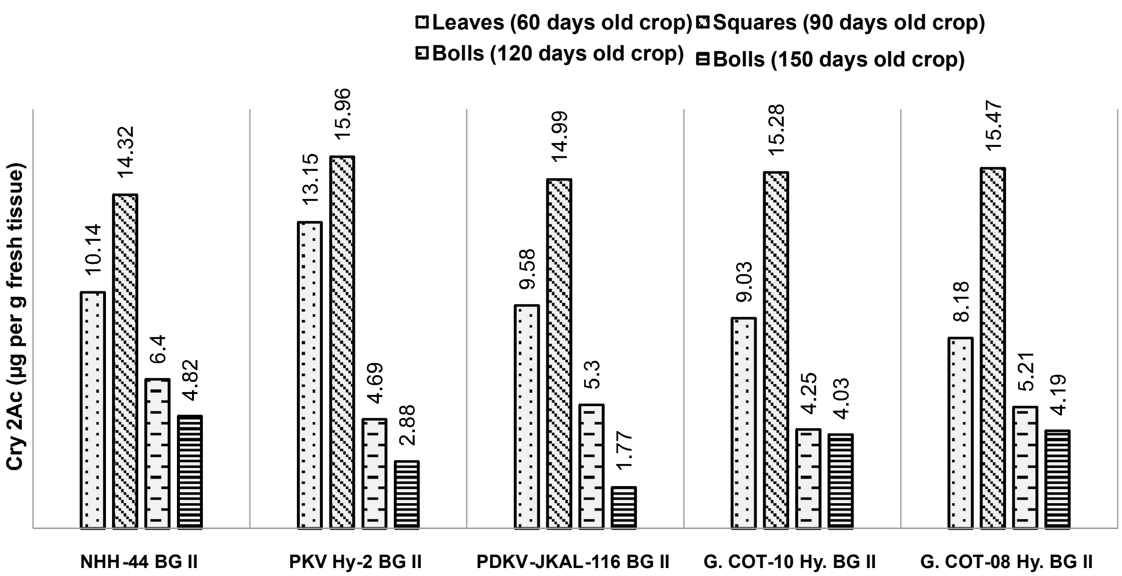

Fig. 2. Expression of Cry 2A toxin in different public sector *Bt* cotton hybrids

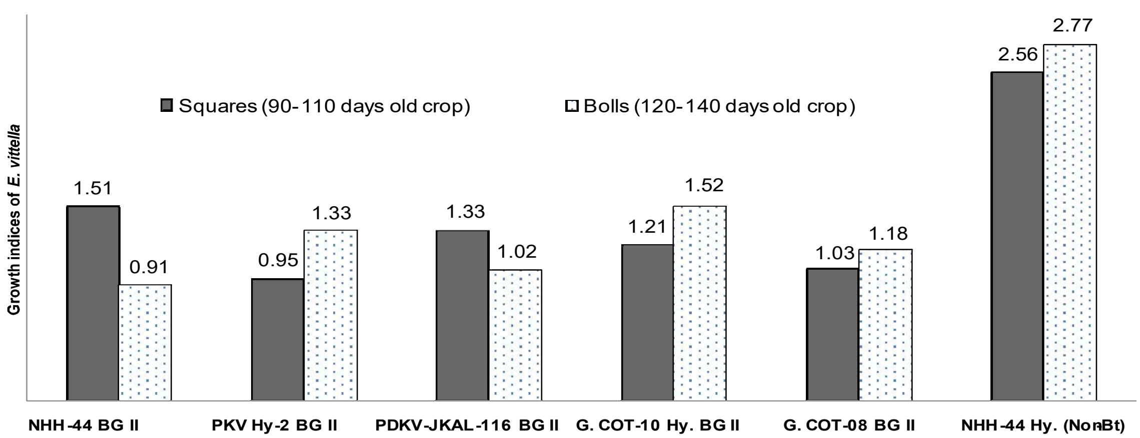

Fig. 3. Growth indices of *E. vittella* reared on different public sector *Bt* cotton hybrids

significant Cry toxin-expressing hybrids play a crucial role in enhancing efficacy against bollworms in specific agro-ecological condition.

This study can be used as baseline notation to detect changes in susceptibility in field populations. Expectedly, in recent past, pink bollworm has developed non-toxic activity against Cry1Ac, first noticed in 2008, and subsequently to Cry2Ab in 2014-15 (Pathak et al 2023). Several factors had

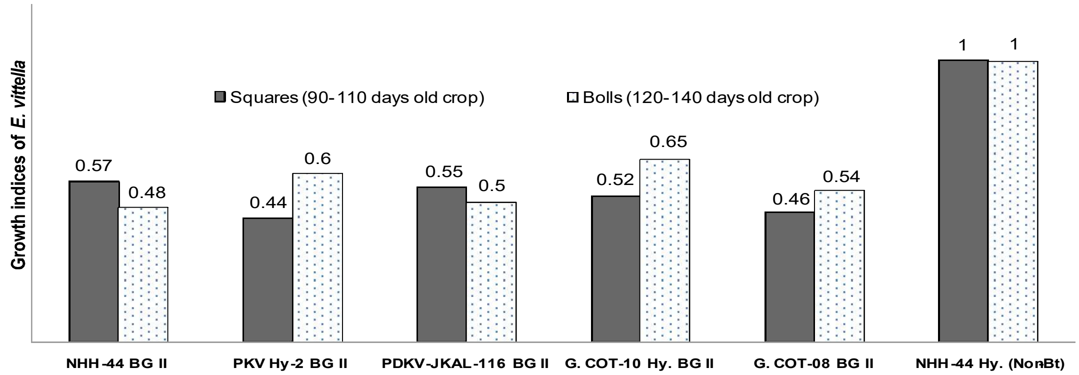

Fig. 4. Survival indices of *E. vittella* reared on different public sector *Bt* cotton hybrids

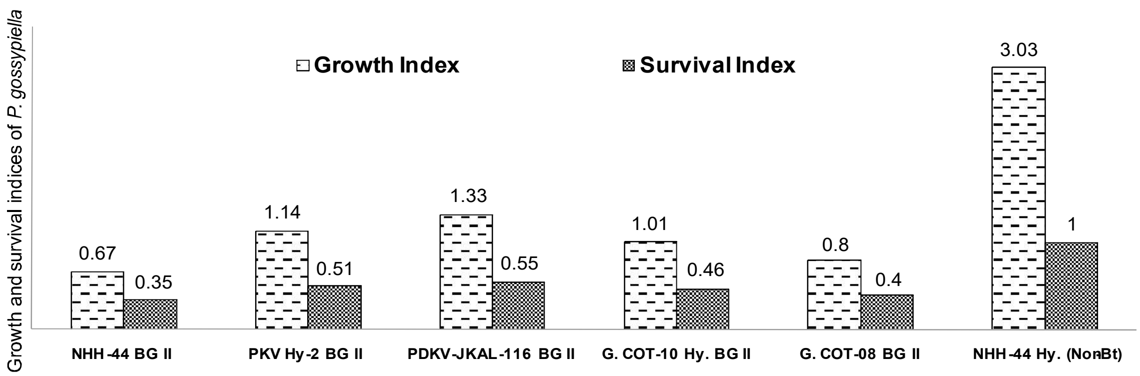

Fig. 5. Growth and survival indices of *P. gossypiella* reared on bolls (150-170 days old crop) sector *Bt* cotton hybrids

Table 6. Adult emergence (%) of *E. vittella* on squares and bolls and *P. gossypiella* on bolls of public sector *Bt* cotton hybrids

Treatments	Squares (90-110 days old crop)					Bolls (120-140 days old crop)					Bolls (150-170 days old crop)			
	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar
NHH-44 BG II	20.00 (26.57)*	30.00 (33.21)	43.33 (41.17)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	10.00 (18.43)	30.00 (33.21)	100.00 (90.00)	100.00 (90.00)	00.00 (00.00)	10.00 (18.43)	33.33 (35.26)	100.00 (90.00)
PKV Hy-2 BG II	0.00 (0.00)	0.00 (0.00)	20.00 (26.57)	100.00 (90.00)	100.00 (90.00)	13.33 (21.41)	30.00 (33.21)	56.67 (48.83)	100.00 (90.00)	100.00 (90.00)	16.67 (24.10)	33.33 (35.26)	56.67 (48.83)	100.00 (90.00)
PDKV-JKAL-116 BG II	13.33 (21.41)	20.00 (26.57)	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	10.00 (18.43)	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	20.00 (26.57)	36.67 (37.27)	63.33 (52.73)	100.00 (90.00)
G. COT-10 Hy. BG II	10.00 (18.43)	20.00 (26.57)	30.00 (33.21)	100.00 (90.00)	100.00 (90.00)	20.00 (26.57)	30.00 (33.21)	70.00 (56.79)	100.00 (90.00)	100.00 (90.00)	10.00 (18.43)	30.00 (33.21)	46.67 (43.09)	100.00 (90.00)
G. COT-08 Hy. BG-II	0.00 (0.00)	6.67 (14.97)	26.67 (31.09)	100.00 (90.00)	100.00 (90.00)	10.00 (18.43)	16.67 (24.10)	43.33 (41.17)	100.00 (90.00)	100.00 (90.00)	00.00 (00.00)	20.00 (26.57)	40.00 (39.23)	100.00 (90.00)
NHH-44 (Non-*Bt*)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
SE (m) ±	0.13	0.13	0.19	-	-	0.13	0.13	0.19	-	-	0.13	0.19	0.36	-
CD (p≤0.05)	0.41	0.41	0.58	-	-	0.41	0.41	0.58	-	-	0.41	0.58	1.09	-
CV %	9.86	8.00	7.69	-	-	9.86	7.19	5.88	-	-	9.64	8.69	11.00	-

* Figures in parenthesis angular transformed values

Table 5. Pupal weight of *E. vittella* on squares and bolls and *P. gossypiella* on bolls of public sector *Bt* cotton hybrids

Treatments	Squares (90-110 days old crop)					Bolls (120-140 days old crop)					Bolls (150-170 days old crop)			
	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar
NHH-44 BG II	210.91	249.63	253.1	263.16	273.16	00.00	219.46	222.96	231.36	242.53	00.00	145.57	176.36	182.8
PKV Hy-2 BG II	00.00	210.83	226.83	227.23	240.9	210.46	232.73	251.96	258.36	268.73	156.27	167.75	192.94	200.74
PDKV-JKAL-116 BG II	206.7	247.26	251	259.85	270.5	00.00	222.4	242	240.06	250.4	157.35	174.93	198.35	204.25
G. COT-10 Hy. BG II	203.2	229.54	248.56	252.56	265.1	219.83	248.67	260.5	265.43	277.83	145.28	162.44	191.87	198.68
G. COT-08 Hy. BG-II	00.00	216.73	233.46	247.96	254.76	208.52	228.46	248.33	249.38	262.73	00.00	159.46	187.01	191.10
NHH-44 (Non-*Bt*)	319.1	329.5	342.3	340.73	347.43	329.13	334.96	341.3	349.13	362.23	257.57	275.36	296.01	300.74
SE (m) ±	0.99	1.32	1.54	1.17	1.37	0.74	0.83	0.55	1.14	1.34	1.79	0.79	1.04	0.93
CD (p≤0.05)	3.02	4.03	4.67	3.55	4.16	2.25	2.54	1.69	3.47	4.08	5.44	2.39	3.15	2.83
CV %	1.10	0.93	1.03	0.76	0.86	0.80	0.58	0.37	0.74	0.84	2.60	0.75	0.87	0.76

flared up the slowly progressing evolution of bollworm resistance in India (Kranthi et al 1999 and Mahesh and Muralimohan 2023). Apart from that, all public sector *Bt* cotton hybrids in the present investigations showed affirmative results against bollworms survival. In general, critical studies in this regard are made with private sector *Bt* hybrids in the past (Gujar et al 2011, Hallad et al 2014, Shera et al 2015, Naik et al 2016, Shera and Arora 2016a, Likhitha and Bhamare 2018 and Likhitha et al 2023), and seldom with public sector hybrids (Dohare and Tank 2014). This might have caused to set a paradigm of commercially lagging public sector cotton seed corporations. The result of the present investigation illustrates the significant efficacy of public sector hybrids against these bollworms. Furthermore, to sustain food and fiber security and avoid environmental degradation through injudicious synthetic pesticides application, authors suggest investigating more potential combination of stacked *Bt*- toxin genes, having altered membrane insertion or pore formation mechanism. As previous reports summarized by Bravo et al (2007), in case of resistant mosquitocidal-Cry proteins, stacking of Cyt proteins results in synergizing or overcoming the resistance. Thus, for the sustainability of this technology, improved alterations in cultivars and adopting refugee planting are recommended.

CONCLUSIONS

All transgenic cotton hybrids exhibited significant variation in the concentration of Cry toxins at predetermined intervals of crop stages. Superior results were registered with PKV Hy-2 BG II when bollworm were fed on squares, and with NHH-44 BG II when fed on bolls. This underscores the potential of these public sector hybrids with dual-Cry toxins as invaluable assets for the efficient management of bollworms, aiming to enhance crop resilience in conventional cotton cultivation.

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Integrative Management of Anthracnose in Mungbean Using Carbendazim Seed Treatment and Botanical Foliar Sprays

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***Abstract*:** This study investigates the synergistic approach for managing anthracnose in mungbean (*Vigna radiata* (L.) R. Wilczek) by integrating carbendazim seed treatment with botanical foliar sprays. The research was conducted to evaluate the efficacy of various botanicals and their combination with carbendazim seed treatment in controlling mungbean anthracnose caused by *Colletotrichum truncatum*. *In vitro* tests were performed using neem (*Azadirachta indica*) oil, neem leaf extract, tulsi (*Ocimum tenuiflorum*) leaf extract, lemon grass (*Cymbopogon citratus*) oil, and moringa (*Moringa oleifera*) leaf extract. Field trials were conducted at Punjab Agricultural University, Ludhiana, and its Regional Research Station, Gurdaspur. Results indicated that among all the botanicals tested, neem oil at 20% concentration exhibited the highest mycelial growth inhibition *in vitro*. In field conditions, neem oil, when combined with carbendazim seed treatment, significantly reduced disease severity and improved yield compared to untreated controls. These findings suggest that integrating carbendazim seed treatment with botanical foliar sprays can effectively manage anthracnose in mungbean, providing an effective and sustainable disease management strategy.

Keywords: Anthracnose, Mungbean, Carbendazim, Botanical extracts, Neem oil, Foliar spray, Disease management

Mungbean (*Vigna radiata* (L.) R. Wilczek) is an important legume crop known for its ecological benefits such as nitrogen fixation, phosphorus mobilization, and improvement of soil health (Tivoli et al 2006), besides nutritional value. In addition to these ecological benefits, it also plays a key role in crop rotation and thus offers a viable option for crop diversification (Pandey et al 2023). However, its cultivation is hindered by diseases like anthracnose, caused by *Colletotrichum truncatum* and/or *Colletotrichum lindemuthianum*, which appears at all the growth stages of the crop, significantly reducing its yield and quality (Lima et al 2023). Traditional management practices primarily rely on chemical fungicides, which pose environmental and health risks. This study explores a more sustainable approach by integrating seed treatment with carbendazim and foliar applications of botanical extracts for the management of anthracnose disease. Previous studies have highlighted the efficacy of botanicals like neem, tulsi, and lemon grass in managing various plant diseases. Not only the crude leaf extract, but also the essential oil derivatives and seed extract have been studied for their disease management potential (Amadioha and Obi 1998, Uddin et al 2013). Besides the foliar application of botanicals, few studies also highlighted the integration of seed treatment and foliar sprays for management of anthracnose of mungbean (Amin et al 2014, Chaudhari and Gohel 2016). Therefore, this research aimed to evaluate the combined effect of carbendazim seed treatment and different botanical foliar sprays on anthracnose in mungbean.

MATERIAL AND METHODS

Study area and experimental design: Field trials were conducted in the year 2022-23 at two different locations, *viz*. Experimental Area, Department of Plant Pathology, Punjab Agricultural University (PAU), Ludhiana (30.898, 75.797 Decimal Degrees) and Regional Research Station (RRS) of Punjab Agricultural University situated at Gurdaspur, Punjab (32.050, 75.423 Decimal Degrees). The experiments were laid out in a randomized complete block design with three replications.

Preparation and evaluation of botanical extracts: Healthy leaves of neem (*Azadirachta indica*), tulsi (*Ocimum tenuiflorum*), curry patta (*Murraya koenigii*), and moringa (*Moringa oleifera*) as depicted in Table 1 were collected, washed, and ground. Extracts were prepared by mixing 100 grams of leaves with 200 ml of distilled water, filtering through muslin cloth, followed by hot water bath treatment and filter sterilization and diluting this stock concentration to desired concentrations (1%, 5%, 10%, 15%, and 20%). Commercial neem and lemon grass (*Cymbopogon citratus*) oil formulations were procured and diluted accordingly, considering the 3000-ppm commercial formulation as 100 per cent stock solution.

Efficacy of botanicals was evaluated in the laboratory using poison food assay (Nene and Thapliyal 1979) on double strength potato dextrose agar (PDA) medium against the pathogen. Required quantity of botanical was mixed with the cooled PDA medium and poured in Petri plates under aseptic conditions. Circular bit (5mm) of actively growing pathogen was inoculated in the centre of Petri plates. Three replications were maintained for each concentration. Medium without any botanical was taken as control and propiconazole 25EC was taken as chemical check. Inoculated Petri plates were incubated at 25±2ºC. The growth of the pathogen in poisoned plates was measured when the control plate exhibited full radial growth (90mm). Per cent growth inhibition was calculated by using the formula given by Vincent (1947):

$$\text{Per cent mycelial growth inhibition} = \frac{C-T}{C} \times 100$$

Where,

C = Radial mycelial growth in un-amended plate (mm)

T = Radial growth in treatment (mm)

Likewise, effect of different botanicals on pathogen's spore production at similar concentrations was worked out by scrapping the mycelial growth from each Petri plate in 1ml of autoclaved distilled water and transferring the mixture into a test tube. This mixture was shaken well so as to dislodge conidia. After mixing, the number of spores was counted with the help of a haemocytometer under the light microscope. The spore count was multiplied with factor 10^4 to calculate the total number of spores/ml.

Table 1. Botanicals used to test *in vitro* efficacy against the pathogen

Botanical (s)	Scientific name
Neem (leaves), Neem oil	*Azadirachta indica*
Tulsi (leaves)	*Ocimum tenuiflorum*
Curry patta (leaves)	*Murraya koenigii*
Lemon grass oil	*Cymbopogon citratus*
Arjuna (leaves)	*Terminalia arjuna*
Sohanjana (leaves)	*Moringa oelifera*

Further, mungbean cultivar ML2056 was used for *in vivo* evaluation of promising botanicals against anthracnose disease in randomized complete block design with three replications in 3×3 m² plots each. Botanicals (neem oil, neem leaves, lemon grass oil, tulsi leaves and sohanjana leaves) that exhibited significant antifungal activity under *in vitro* assays were further evaluated under field conditions at 40 per cent of the stock concentration. The observations were also compared with standard chemical check (0.05% propiconazole 25EC). Two sets of treatments were established *viz*. Set-I: without carbendazim seed treatment and Set-II: with carbendazim-treated seeds (@ 2 g/kg seed). Both the sets were simultaneously given subsequent botanical foliar spray treatments at 40 per cent of stock concentration. The first spray of botanicals was done 14 days before inoculation of the pathogen and the second spray 14 days after pathogen inoculation. Disease severity was recorded 1,2,3,4,5 and 6 weeks after disease appearance using the disease rating scale given by Mayee and Datar (1986) as given in Table 2 and expressed as Per cent Disease Index (PDI).

Per cent Disease Index was calculated using the formula given by McKinney (1923).

$$\text{Per cent Disease Index} = \frac{\text{Sum of numerical ratings}}{\text{Total number of plants observed} \times \text{Maximum rating}} \times 100$$

Further, the Area Under Disease Progress Curve (AUDPC) was calculated for each treatment from PDI using the formula given by Roelfs et al (1992) as given:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \frac{(X_{i+1} + X_i)/2}{t_{i+1} - t_i}$$

Where,

X_{i+1} = PDI at the i+1th observation

X_i = PDI at the ith observation

t_{i+1} = Time after inoculation (day) at the i+1th observation

t_i = Time after inoculation (day) at the ith observation,

n = Total number of observations

Table 2. Disease rating scale for anthracnose of mungbean (Mayee and Datar 1986)

Scale	Description
0	No symptoms on leaves
1	Small size lesions covering 1% or less of the leaf area
3	Small size lesions covering 1-10% of the leaf area
5	Lesions size big but not coalescing, covering 11-25% of the leaf area
7	Lesions on leaves covering 26-50% of leaf area. Cankers on stem and pod infection
9	Lesions on leaves cover 51% or more of leaf area. Defoliation of leaves, deep cankers on stem and pods, blighting of plant occurs

The apparent rate of disease development was computed using the formula given by Van der Plank (1963):

$$r = \frac{2.303}{(t2 - t1)} \log 10 \frac{x2(1 - x1)}{x1(1 - x2)}$$

Where,

r = Apparent infection rate /unit/day,

t1 = Date of first observation,

t2 = Date of second observation,

x1 = Per cent disease incidence at time t1,

x2 = Per cent disease incidence at time t2

Statistical analysis: Data were statistically analysed using RStudio. Factorial ANOVA was worked out and means were compared using the least significant difference (LSD) test at $p \leq 0.05$.

RESULTS AND DISCUSSION

In vitro* evaluation of botanicals against anthracnose pathogen, *Colletotrichum truncatum: The botanicals evaluated against anthracnose pathogen depicted differential response at different concentrations *viz.* 1, 5, 10, 15 and 20 per cent against *Colletotrichum truncatum*. The botanicals significantly restricted the growth of *Colletotrichum truncatum* under *in vitro* conditions (Table 3). The maximum mycelial growth inhibition (50.46%) was in neem oil @20 per cent concentration followed by neem leaf extract @20 per cent . neem oil @15 per cent , tulsi leaf extract @20 per cent, neem leaf extract @15 per cent . Among all the botanicals, neem oil was the most effective antifungal treatment against *C. truncatum* followed by neem leaf, tulsi leaf extract, lemon grass oil and moringa leaf extract. The arjuna leaf extract proved to be least effective with 28.70% growth inhibition at 20 per cent concentration.

The data presented in Table 4 indicate that the botanicals significantly inhibited the conidial count of *C. truncatum* under *in vitro* conditions. Highest mean conidial count (19.2×10^4 spores/ml) was recorded in arjuna leaf extract followed by curry patta leaf extract (18.87×10^4 spores/ml), moringa leaf extract (17.4×10^4 spores /ml) and lemon grass oil (14.34×10^4 spores/ml). Neem oil was found to be the most effective treatment which resulted in the lowest mean conidial count (10.34×10^4 spores/ml), demonstrating significant antifungal activity. Notably, the mean conidial count in the control was (27.00×10^4spores/ml).

Based on *in vitro* evaluation of different botanicals against anthracnose pathogen, the neem oil-based formulation was found to be the most effective botanical in restricting the fungal growth followed by neem leaf extract.

Field evaluation of promising botanicals against anthracnose of mungbean: The field evaluation conducted at PAU, Ludhiana revealed significant variation in the disease severity of anthracnose of mungbean among the botanicals in both sets of treatments (Table 5). In the first set (no seed

Table 3. *In vitro* efficacy of different botanicals against *Colletotrichum truncatum*

Botanical (A)	Radial growth at different concentrations (mm)					Mean	Per cent growth inhibition at different concentrations					Mean
	1%	5%	10%	15%	20%		1%	5%	10%	15%	20%	
T1 -Neem oil (*Azadirachta indica*)	71.25	63.66	57.16	50.41	44.58	57.41[f]	20.83 (27.12)	29.26 (32.73)	36.48 (37.13)	43.98 (41.52)	50.46 (45.25)	36.20 (36.75)[a]
T2-Lemon grass oil (*Cymbopogon citratus)*	76.00	73.08	67.75	64.00	59.83	68.13[c]	15.55 (23.20)	18.79 (25.67))	24.72 (29.80)	28.89 (32.49)	33.52 (35.36)	24.29 (29.30)[d]
T3-Neem leaf extract*(Azadirachta indica)*	74.08	66.91	61.58	56.17	49.16	61.58[e]	17.68 (24.85)	25.65 (30.41)	31.57 (34.16)	37.59 (37.79)	45.37 (42.32)	31.57 (33.90)[b]
T4-Tulsi leaf extract (*Ocimum tenuiflorum*)	76.25	67.33	66.50	59.42	55.82	65.06[d]	15.27 (22.98)	25.18 (30.06)	26.11 (30.71)	33.99 (35.63)	37.96 (38.01)	27.70 (31.48)[c]
T5-Curry patta leaf extract (*Murraya koenigii*)	79.83	76.66	71.00	66.67	62.66	71.36[b]	11.29 (19.62)	14.81 (22.62)	21.11 (27.33)	25.93 (30.59)	30.37 (33.42)	20.70 (26.72)[e]
T6-Moringa leaf (*Moringa oelifera)*	77.17	72.16	68.83	65.17	61.33	68.93[c]	14.25 (22.17)	19.81 (26.41)	23.51 (29.00)	27.59 (31.67)	31.85 (34.34)	23.40 (28.72)[d]
T 7-Arjuna leaf extract (*Terminalia arjuna*)	80.33	76.33	71.83	68.50	64.16	72.23[b]	10.74 (19.12)	15.18 (22.92)	20.18 (26.68)	23.88 (29.24)	28.70 (32.38)	19.74 (26.07)[e]
T 8-Control	90	90	90	90	90	90[a]	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)[f]
Mean	76.41[a]	70.88[b]	66.38[c]	61.47[d]	56.79[e]		15.09 (22.72)[e]	21.24 (27.26)[d]	26.24 (30.69)[c]	31.69 (34.13)[b]	36.89 (37.30)[a]	
	A		B		A×B		A		B		A×B	
CD (p≤0.05)	1.10		0.93		2.46		0.77		0.65		1.73	

Values in the parentheses indicate the arc sine (angular) transformed values,
Different alphabetical letters are significantly different at p<0.05,
A=Botanicals, B=Concentrations

treatment), maximum disease control (55.56%) was from neem oil among botanicals followed by neem leaf extract , tulsi leaf extract , lemon grass oil , and moringa leaf extract . In second set (carbendazim seed treatment), highest disease control (59.73%) among botanicals was in neem oil followed by neem leaf extract, tulsi leaf extract , lemon grass oil and moringa leaf extract when compared to inoculated control. The treatments in second set (carbendazim treated seed) were superior than first set in controlling the disease due to effect of seed treatment with carbendazim which exhibited significantly lowered mean per cent disease index as compared to first set that had no carbendazim seed treatment. However, for both sets, the maximum per cent disease control was for propiconazole 25EC i.e. 74.08 and 75.85 per cent for set I (untreated seed) and set II (carbendazim treated seed), respectively. The observation

Table 4. *In vitro* efficacy of different botanicals on conidial count of *Colletotrichum truncatum*

Botanical (A)	Conidial count at different concentrations (10^4 conidia/ml)					Mean
	1%	5%	10%	15%	20%	
T1 -Neem oil (*Azadirachta indica)*	15.00 (3.93)	11.67 (3.49)	10.34 (3.30)	8.67 (3.03)	6.00 (2.55)	10.34 (3.25)[g]
T2-Lemon grass oil (*Cymbopogon citratus)*	19.00 (4.41)	16.34 (4.10)	14.67 (3.89)	11.34 (3.45)	10.34 (3.30)	14.34 (3.82)[d]
T3-Neem leaf extract(*Azadirachta indica)*	17.00 (4.18)	14.34 (3.86)	11.67 (3.49)	9.00 (3.09)	6.67 (2.68)	11.74 (3.45)[f]
T4-Tulsi leaf extract (*Ocimum tenuiflorum*)	17.67 (4.26)	15.67 (4.02)	13.00 (3.68)	11.34 (3.45)	9.34 (3.14)	13.40 (3.71)[e]
T5-Curry patta leaf extract (*Murraya koenigii*)	21.67 (4.70)	20.67 (4.61)	18.67 (4.37)	17.67 (4.26)	15.67 (4.02)	18.87 (4.39)[b]
T6-Moringa leaf (*Moringa oelifera)*	20.67 (4.60)	19.67 (4.49)	18.00 (4.30)	15.34 (3.97)	13.34 (3.73)	17.40 (4.21)[c]
T 7-Arjuna leaf extract (*Terminalia arjuna*)	22.00 (4.74)	20.67 (4.60)	19.00 (4.41)	18.00 (4.30)	16.34 (4.10)	19.20 (4.43)[b]
T 8-Control	27.00 (5.24)	27.00 (5.24)	27.00 (5.24)	27.00 (5.24)	27.00 (5.24)	27.00 (5.24)[a]
Mean	20 (4.51)[a]	18.25 (4.30)[b]	16.54 (4.08)[c]	14.79 (3.80)[d]	13.09 (3.59)[e]	
	A		B		A×B	
CD (p≤0.05)	0.1		0.059		0.167	

Values in the parentheses indicate the square root (√x+0.5) transformed values
Different alphabetical letters are significantly different at p<0.05
A=Botanicals, B=Concentrations

Table 5. Evaluation of promising botanicals against anthracnose of mungbean during *Kharif* 2022 under field conditions at PAU, Ludhiana

Treatment*	Set-I Seeds not treated with carbendazim		Set-II Seeds treated with carbendazim		Yield (kg/acre)	
	Per cent disease index	Per cent disease control**	Per cent disease index	Per cent disease control**	Seeds not treated with carbendazim	Seeds treated with carbendazim
Lemon grass oil **(***Cymbopogon citratus)*	32.22 (34.54)	35.56	27.78 (31.79)	32.90	364.30	372.00
Neem leaf extract (*Azadirachta indica*)	28.89 (32.49)	42.22	24.08 (29.37)	41.84	395.30	402.34
Tulsi leaf extract (*Ocimum tenuiflorum*)	30.37 (33.42)	39.26	26.66 (31.06)	35.60	374.70	382.67
Moringa leaf extract (Moringa *oelifera*)	35.56 (36.58)	28.88	30.00 (33.17)	27.54	323.30	340.00
Neem oil (*Azadirachta indica*)	22.22 (28.07)	55.56	16.67 (24.06)	59.73	421.70	438.00
Inoculated control ©	50.00 (44.98)	0.00	41.40 (40.07)	0.00	233.30	250.67
Propiconazole 25EC 0.05%	12.96 (21.08)	74.08	10.00 (18.36)	75.85	475.00	480.00
Mean	29.49 (33.02)[a]		24.56 (29.70)[b]		369.67[b]	380.80[a]
	A	B	A×B	A	B	A×B
CD (p≤0.05)	2.616	0.901	4.27	13.033	4.48	21.28

*40 per cent of stock concentration, A=Treatment, B=Carbendazim/no carbendazim seed treatment
Different alphabetical letters are significantly different at p<0.05
Values in the parentheses are arc sine transformed values **Per cent disease control = [Inoculated Control (C) – Treatment (T) /Inoculated Control (C)] × 100
[Note: The values of per cent disease control in set II (seed treated with carbendazim) appear to be lower than set I for treatment (Sr. No. 1 to 4). This apparent reduction is due to decreased per cent disease index in the inoculated control (C) (Sr. No. 6), attributed to the exclusive effect of carbendazim seed treatment]

on yield data also exhibited significant difference amongst the treatments under both sets. In first set, maximum yield (475.00 kg/acre) was in propiconazole 25EC followed by neem oil (421.70 kg/acre), neem leaf extract and tulsi leaf extract. In the second set, maximum yield (480.00 kg/acre) was in propiconazole. Among the botanicals, neem oil reported highest yield (438.00 kg/acre) followed by neem leaf extract, tulsi leaf extract and lemon grass oil.

Similarly, in field trials conducted at RRS, Gurdaspur statistically significant variation was observed in efficacy. There was significant variation in the per cent disease control among the botanicals in both sets. In the first set, highest disease control (70.44%) was in propiconazole 25EC followed by neem oil , neem leaf extract and tulsi leaf extract (Table 6). Similarly, in the second set same trend for disease control was observed with propiconazole 25EC recording maximum disease control (70.26%) followed by neem oil, neem leaf extract and tulsi leaf extract . Among botanicals, neem oil was most effective. The yield in first set was maximum in propiconazole 25EC (443.67 kg/acre) followed by neem oil, neem leaf extract and tulsi leaf extract. Similar trend was seen in second set with propiconazole 25EC recording maximum yield (471.00 kg/acre). The neem oil was the most effective among botanicals (435.00 kg/acre).

Similarly, AUDPC and apparent infection rate (r-value) at PAU, Ludhiana also varied significantly among treatments (Table 7). In the first set, maximum AUDPC (933.67) was observed in inoculated control followed by moringa leaf extract , lemon grass oil and neem leaf extract. However, lowest AUDPC (262.91) was observed in propiconazole 25EC treatment. Similarly in second set, maximum AUDPC (787.16) was reported in inoculated control followed by moringa leaf extract, lemon grass oil, tulsi leaf extract and neem leaf extract whereas lowest AUDPC (244.61) was observed in propiconazole 25EC. In the first set, at PAU, Ludhiana, the highest apparent infection rate (0.056) was reported from moringa leaf extract among botanicals followed by lemon grass oil (0.051). Lowest r-value (0.046) was observed in neem oil among botanicals. Similarly, in second set, maximum r- value (0.049) among botanicals was observed in moringa leaf extract followed by lemon grass oil (0.047) and tulsi leaf extract (0.047) whereas, minimum r-value (0.040) was observed in neem oil treatment among botanicals.

Likewise, AUDPC and apparent infection rate (r-value) at RRS, Gurdaspur, also varied significantly among the treatments. In first set, maximum AUDPC (1064.00) was in inoculated control followed by moringa leaf extract and lemon grass oil. Least AUDPC (293.98) was observed in propiconazole 25EC. In second set, same trend was seen

Table 6. Evaluation of promising botanicals against anthracnose of mungbean during *Kharif*, 2022 under field conditions at RRS, Gurdaspur

Treatment*	Seeds not treated with carbendazim		Seeds treated with carbendazim		Yield (kg/acre)	
	Per cent disease index	Per cent disease control**	Per cent disease index	Per cent disease control**	Seeds not treated with carbendazim	Seeds treated with carbendazim
Lemon grass oil (*Cymbopogon citratus)*	32.23 (34.5)	34.08	30.74 (33.65)	25.24	353.00	369.67
Neem leaf extract (*Azadirachta indica*)	28.89 (32.51)	40.91	27.76 (31.77)	32.49	371.67	404.34
Tulsi leaf extract (*Ocimum tenuiflorum*)	30.37 (33.42)	37.88	29.26 (32.72)	28.84	357.34	378.00
Moringa leaf extract (Moringa *oelifera*)	37.74 (37.88)	22.81	33.34 (35.24)	18.92	318.34	348.67
Neem oil (*Azadirachta indica*)	23.34 (28.86)	52.26	20.00 (26.8)	51.36	415.67	435.00
Inoculated control ©	48.89 (46.05)	0.00	41.12 (41.14)	0.00	225.67	260.33
Propiconazole 25EC 0.05%	14.45 (20.44)	70.44	12.23 (18.4)	70.26	443.67	471.00
Mean	30.90 (33.39)[a]		27.80 (31.39)[b]		355.04[b]	381.04[a]
	A	B	A×B	A	B	A×B
CD (p≤0.05)	1.18	0.63	1.676	10.8	5.791	15.3

*40 per cent of stock concentration, A=Treatment, B=Carbendazim/no carbendazim seed treatment
Different alphabetical letters are significantly different at p<0.05
Values in the parentheses are arc sine transformed values
**Per cent disease control = [Inoculated Control (C) – Treatment (T) /Inoculated Control (C)] × 100
[Note: The values of per cent disease control in set II (seed treated with carbendazim) appear to be lower than set I for treatment (Sr. No. 1 to 5). This apparent reduction is due to decreased per cent disease index in the inoculated control (C) (Sr. No. 6), attributed to the exclusive effect of carbendazim seed treatment]

Table 7. Effect of botanicals on the progression of anthracnose of mungbean (AUDPC and apparent infection rate) at PAU, Ludhiana and RRS, Gurdaspur

Treatment*	PAU, Ludhiana				RRS, Gurdaspur			
	AUDPC		Apparent infection rate (r-value)		AUDPC		Apparent infection rate (r-value)	
	Seeds not treated with carbendazim	Seeds treated with carbendazim	Seeds not treated with carbendazim	Seeds treated with carbendazim	Seeds not treated with carbendazim	Seeds treated with carbendazim	Seeds not treated with carbendazim	Seeds treated with carbendazim
Lemon grass oil (*Cymbopogon citratus)*	672.86	550.27	0.051	0.047	725.12	608.61	0.049	0.049
Neem leaf extract (*Azadirachta indica*)	583.72	522.82	0.048	0.042	618.34	552.13	0.045	0.043
Tulsi leaf extract (*Ocimum tenuiflorum*)	579.84	533.63	0.049	0.047	680.55	602.78	0.046	0.046
Moringa leaf extract (Moringa *oelifera*)	725.13	658.98	0.056	0.049	813.55	711.64	0.051	0.051
Neem oil (*Azadirachta indica*)	439.03	348.02	0.046	0.040	528.89	397.39	0.041	0.040
Inoculated control (C)	933.67	787.16	0.058	0.057	1064.00	894.37	0.056	0.054
Propiconazole 25EC 0.05%	262.91	244.61	0.037	0.029	293.98	260.78	0.038	0.037

*40 per cent of stock concentration

with propiconazole 25EC recording minimum AUDPC (260.78) and inoculated control recording highest AUDPC (894.37). Among botanicals, neem oil recorded lowest AUDPC (397.39). At RRS, Gurdaspur, highest apparent infection rate (0.056) was in inoculated control and lowest apparent infection rate (0.038) was in propiconazole 25EC treatment. Among botanicals, neem oil recorded lowest r-value (0.041). Similar trend was seen in second set with inoculated control recording highest r-value (0.054) followed by moringa leaf extract (0.051). Among botanicals neem oil recorded minimum infection rate (0.040).

The overall results were in conformity with several findings reported previously. Laxman (2006) reported that garlic, neem and eucalyptus oil were effective in managing mungbean anthracnose caused by *C. truncatum*. Uddin et al (2013) reported lowest disease incidence (7.33%) in neem leaf extract treatment at 60 days after sowing, besides giving yield advantage (1.26 t per ha), and higher 1000 seeds weight (27.33g) followed by garlic cloves extract as compared to untreated control. Kulkarni (2019) also reported 10 per cent azadirachtin to be most effective in inhibiting the mycelial growth of *C. truncatum* followed by eucalyptus oil, garlic and neem seed kernel extract.

CONCLUSION

This study highlights the potential of integrating fungicidal seed treatment with botanical foliar sprays, specifically neem oil, neem leaf extract, and tulsi leaf extract, for managing anthracnose in mungbean. The *in vitro* and *in vivo* evaluations revealed that certain botanicals effectively inhibited the growth of *Colletotrichum truncatum* and significantly reduced disease severity in field conditions, particularly in integration with fungicidal seed treatment. Neem oil was the most effective, leading to substantial reductions in Per cent Disease Index (PDI) and Area Under Disease Progress Curve (AUDPC) values, and subsequently, improved yield outcomes. The integrated approach of combining fungicidal (*viz*. carbendazim) seed treatment with botanical foliar applications not only enhances disease control but also promotes sustainable agricultural practices by reducing sole reliance on chemical fungicides. The findings demonstrate potential benefits, as evidenced by higher yields compared to untreated controls, highlighting the practical application and sustainability of this strategy. Future research should explore the synergistic effects of combining botanical extracts with other biocontrol agents and assess their long-term impacts on soil health and crop productivity, more particularly under organic farming setup. Overall, the integration of carbendazim seed treatment with botanical foliar sprays provides an eco-friendly, effective, and sustainable disease management strategy, aligning with the principles of sustainable agriculture and offering a viable alternative to conventional total chemical-based methods.

AUTHOR CONTRIBUTIONS

All authors made significant contributions to the conception and design of the study. SS and YB were involved in conducting the *in vitro* and *in vivo* experiments, analyzing the data, and drafting the manuscript. YB and VKS provided expertise in statistical analysis and contributed to the interpretation of the results. SK assisted with the outstation field trials and the collection of yield data. VKS and AS reviewed and revised the manuscript ensuring its rigor and clarity.

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Transmission and Host Range of Papaya Ring Spot Virus

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***Abstract*:** Papaya belongs to the family Caricaceae. The papaya ring spot virus disease (PRSV) is a well-known aphid and sap transmissible plant pathogenic virus in the genus *Potyvirus* and family *Potyviridae*. Among viral diseases, papaya ring spot virus is a wide spread pathogen that can cause up to 90% yield losses in papaya .The symptoms on mechanical transmission were characterized by vein clearing followed by chlorosis, yellow mosaic, blistering and leaf distortion. Later on necrotic spots developed leading to complete necrosis of leaves, petioles and stem. The result on aphid transmission revealed that, three aphid spp. *viz. Aphis gossypii, Aphis craccivora* and *Myzus persicae* transmit the virus in non - persistent manner from papaya (*Carica papaya*) to papaya. *Myzus persicae* was more efficient (90 %) than *Aphis gossypii* (80%). The, papaya ring spot virus was easily mechanically transmitted in papaya, cucurbits and some other plants. Experimental findings showed that the virus was successfully transmitted by the sap inoculation method in plants belonging to families *viz.* Caricaceae (*Carica papaya*), cucurbitaceae (*C. sativus, Cucurbita moschata*, *C. pepo, Luffa acutangula, L. cylindrica, Lagenaria siceraria, Memordica charantia)* with systemic mosaic mottling symptoms. However, plants of families Chenopodiaceae (*Chenopodium amaranticolor, Chenopodium quinoa*) produced local lesions.

***Keywords*:** Papaya ring spot virus, Transmission, Host range, *Myzus persicae* and *Aphis gossypii*

The importance of papaya in agriculture and in the World's economy is demonstrated by its wide distribution and substantial production in the tropical countries (Anonymous 2015). Many pathogens like viruses, fungi, bacteria and nematodes infect papaya, causing considerable losses in yield and deteriorate the quality of fruits. Besides these, a number of viruses belonging to Cucumo, Gemini, Ilar, Poty, Rhabdo, Tobra, and Tospo virus groups have been recorded on papaya. Important amongst them are papaya leaf curl virus, papaya ring spot virus and papaya mosaic virus. In India, papaya ring spot virus is the major viral disease causing considerable losses in yield and quality of fruits (Jensen 1949). At present papaya ring spot disease has assumed serious proportion and became a major constraint in papaya cultivation, thereby threatening the cultivation of papaya in India, including Maharashtra (Khurana and Bhargava 1970, Rao 1988 and Kale 1999). Symptoms consist of intense yellow mosaic on leaves, small shoestring-like new leaves, dark green and slightly sunken rings on the fruit, numerous oily- looking streaks on the stem and stunting of the plant. Trees infected at a very young age remain stunted and never produce any fruit (Kunkalikar et al 2006 and Reddy et al 2007). The virus was named as PRSV by DeBokx (1965). The virions are filamentous, non-enveloped and flexuous measuring 760-800 x 12 nm. Virus particles contain 94.5% protein and 5.5% nucleic acid. The protein component consists of the virus coat protein (CP), with molecular weight of about 36 kDa. The virus is naturally transmitted by the insect-vector aphids in a non- persistent manner, from papaya to papaya plants infecting all trees in an orchard within a few months. At present, sole cultivation of papaya has become more common after advent of improved varieties and hybrids. The area under this crop is continuously increasing because farmers prefer its cultivation due to its high yield potential, less water requirement and attractive prices in the market. The crop is emerging as an alternative cash crop to banana in Maharashtra. Considering the economic importance of crop and disease present investigation is carried out.

MATERIAL AND METHODS

The present investigations were carried out in the glass house during 2016 at Department of Plant Pathology, College of Agriculture, Latur.

Diseased samples: The papaya ring spot disease samples were collected from the farmers' fields of various villages in Latur district, where papaya fields were found infected with PRSV.

Transmission

Mechanical transmission: For mechanical transmission, sap was extracted by crushing symptomatic leaves of diseased papaya plants with a mortar and pestle in a chilled 0.05M potassium phosphate buffer (P^H 7.4) containing 0.02M 2-mercapto ethanol. Test plants were inoculated by conventional leaf rub method with a cotton swab. Carborandum powder (800 mesh) was used as an abrasive. Immediately after virus inoculation, the leaves of test plants were rinsed with tap water. Test plants used for mechanical inoculation were raised from virus free seeds in earthen pots containing steam sterilized soil, sand and compost (2:1:1) mixture. Test plants were maintained in an insect-free glass house for 4- 6 weeks and observations were recorded with respect of symptom development and incubation period.

Aphid transmission: For aphid transmission, *Aphis craccivora* Koch., *Aphis gossypii* Glov, and *Myzus persicae* Sluz, raised from single aphid colony were used. For raising aphid colony, the healthy leaves of cotton (*Gossypium hirsutum* L.) and groundnut (*Arachis hypogea* L.) were placed in a Petri dishes on slightly wet filter paper and an apterous form of aphids were transferred separately with small camel hair brush to the leaves. Petri dishes were closed for 8 hours and the newly born aphids were used for transmission studies. The apterous forms of aphids were transferred to clean Petri dishes for 2 hours for fasting. This was followed by an acquisition feeding of 40 to 60 seconds on virus infected detached leaves of source plant. Aphids were allowed to make only brief probes of 40 to 60 seconds duration. Aphids still in probing position at 40 seconds were picked up with camel hair brush and transferred in batches of 25 to healthy test plants for inoculation feeding of four hours. The test plants were kept in muslin cages. Later, aphids were killed by spraying with 0.02 per cent imidacloprid (17.8 EC) insecticide and plants were maintained in an insect free glasshouse for three to four weeks. Observations were recorded for the symptoms on test plants.

Host range: For host range studies, plant species belonging to the different families *viz.* Cucurbitaceae, Chenopodiaceae and Solanaceae were raised from healthy seeds in earthen pots containing steam sterilized soil, sand and compost mixture (2:1:1) and maintained in an insect free glass house. Ten plants of each host species were inoculated with the sap extracted from virus infected papaya (Cv. Red lady) plants by conventional leaf rub method and aphid transmission also done simultaneously. All plants were inoculated on the first leaf or fully expanded leaves. The inoculated plants were kept for observation for 4-6 weeks along with the control plants

The following species were used as test plants in host range studies. Family / Host species Amaranthaceae (*Amaranthus caudatus* L), Chenopodiaceae (*Chenopodium album* L.*, Chenopodium amaranticolor*), Compositae (*Helianthus annuus* L.), Cruciferae (*Raphanuns sativus* L.), Cucurbitaceae (*Luffa actungula* L.*, Momordica charantia* L.*, Cucumis sativus* L,*. Lagenaria siceraria, Cucurbita pepo* L.*, Cucurbita moschata*), Leguminoceae (*Phaseolus vulgaris* L*, Pisum sativum* L.*, Vigna mungo, Vigna radiate, Cajanus cajan*), Malvaceae (*Abelmoschus esculenttus,*) and Solanaceae (*Capsicum annum* L, *Nicotina tabacum* L.*, Nicotina glutinosa* L.*, Nicotiana xanthi*)

RESULTS AND DISCUSSION

Collection of PRSV samples: The PRSV infected papaya samples collected exhibited the symptoms *viz.,* severe mosaic, leaf distortion, shoe stringing and fruits with ring spot.

Isolation and maintenance: All the inoculated papaya Cv. Red Lady seedlings showed the PRSV symptoms, which were used as a source of virus inoculum for further studies.

Symptomatology: All the ten inoculated plants showed symptoms within 2 to 3 weeks after inoculation. The initial symptoms observed varied from chlorotic mottling of the leaves to severe rugosity. Infected plant showed chlorosis on the youngest leaves, vein clearing rugosity and mottling of leaf lamina interveinal puckering or bulging of the leaf tissues on the upper surface of young leaves (Plate I) . In the severe cases filiform shoe string and distinct chlorotic streak were found on the leaf tendrils. Most of the field surveyed revealed characteristic symptoms of papaya ring spot virus. Various types of symptoms like mild to severe mosaic, mottling, ring spot on fruits, leaves and stems, distortion of fruits, leaves and stems, filiform leaf, shoestring leaf, vein curling, vein distortion, puckering, leaf curling, leaf rolling, fruit yellowing, vein zigzag and stunting growth of plants were observed during collection of PRSV samples. Several workers have described same type of symptoms for PRSV in mechanical transmission and in field. (Kshirsagar 2014, Surwade 2014, Singh et al 2017).

Transmission studies

Mechanical transmission: The results on sap inoculation indicated that, the virus was readily transmitted by mechanical means under artificial conditions. Cultivar Red lady was mechanically inoculated using 0.1 M potassium phosphate buffer and started developing symptoms 15 days after inoculation. The symptoms always started on newly emerged leaves of papaya seedlings, showing vein clearing, chlorotic spots and chlorotic rings. Later these plants produced varied types of symptoms including leaf reduction to shoestring, leaf distortion, puckering, mosaic pattern and stunted growth. Similar type of results of mechanical transmission i.e. symptoms on leaves, stem and fruits of infected papaya plants WERE reported by several workers. (Roy et al 1999, Reddy et al 2007 and Singh et al 2017)

Aphid transmission:. The three aphid spp. *Aphis gossypii, Aphis craccivora* and *Myzus persicae* transmit the virus in non-persistent manner from papaya (*Carica papaya*) to papaya. (Table 1, Fig. 1) *Myzus persicae* was more efficient (90 percent) than *Aphis gossypii* (80%) and *Aphis craccivora* in transmitting the virus. The appearance of symptoms was fast in case of plants inoculated with *Myzus persicae* as compared with plants inoculated with *A.gossypii* and *A.craccivora.* Similar results regarding *Myzus persicae* as efficient vector were observed by Reddy et al (2007) and Gude et al (2008). The transmission efficiency of aphids i.e. *Myzus persicae* and *Aphis gossypii* was dependent on the number of aphids used / test plant (Table 2, Fig 2 & 3). The percent transmission varied from obtained was 10 to 90%, when inoculated with 1-18 aphids/test plants (*Myzus persicae)* were used. Similarly, when we used same no. of aphids / plant the per cent transmission obtained was 10-

80% by *Aphis gossypii*. Similar results regarding transmission efficiency of *Aphis craccivora* and *Myzus persicae* was earlier reported by Reddy, (2007). Kalleshawaraswamy) 2008) reported that *Myzus persicae* (56%) and *Aphis gossypii* (53%) were significantly more efficient in transmitting PRSV than *A. craccivora* (38%). The systemic symptoms produced on cultivar Red lady of papaya by aphid inoculation were similar to those produced on same cultivar by sap- inoculation.

Fig. a. Leaf distortion

Fig. b. Blistering of leaves

Fig. c. Local lesion on leaves

Fig. d. Mosaic motteling

Plate I. Symptoms of papaya ring spot virus disease produced by mechanical transmission on papaya seedlings

Fig. a. Chlorosis

Fig. b. Vein clearing of leaf

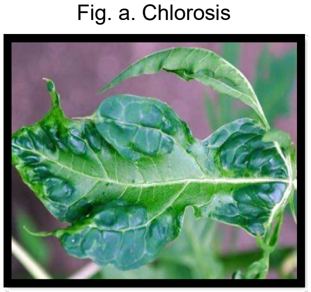

Fig. c. Green islands on leaves

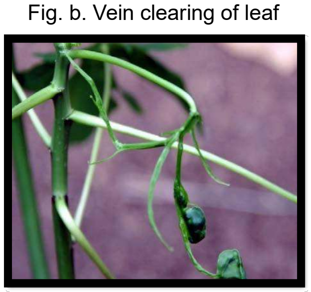

Fig. d. Shoestring of leaves

Plate II. Symptoms of papaya ring spot virus disease produced by mechanical transmission on papaya seedlings

Host range of papaya ring spot virus: The papaya ring spot virus was easily mechanically transmitted in papaya, cucurbits and some other plants. Papaya ring spot virus infected only 9 plant species and failed to infect 12 plant species. Out of 9 plant species infected 2 from Chenopodiaceae, 6 from Cucurbitaceae and one from

Plate III. Symptoms of papaya ring spot virus disease produced by aphid transmission

Table 1. Aphid transmission of the virus causing papaya ring spot virus in papaya Cv. Redlady

Aphid species	Transmission (%)	Reaction on PRSV	
		Local	Systemic
Aphis gossypii	70	------	Vc, MMo, Ld, Ss
Myzus persicae	90	------	Vc, MMo ,Ld, Ss
Aphis craccivora	50	------	Vc, MMo, Ld, Ss

Vc = Vein clearing MMo= Mild mosaic
Ld = Leaf distortion Ss = Shoe string

Table 2. Efficiency of *Myzus persicae* and *Aphis gossypii* vectors in transmitting the papaya ring spot virus in Cv. Redlady

No. of aphids /plant	*Aphis gossypii*	*Myzus persicae*
	Transmission (%)	Transmission (%)
1	10	10
2	10	20
4	20	30
6	40	50
8	50	60
10	60	70
12	70	90
14	70	80
16	70	90
18	80	90

Caricaceae. The virus was successfully transmitted by sap inoculation method in plants belonging to families Caricaceae *viz.* (*Carica papaya*) and Cucurbitaceae (*C. sativus, Cucurbita moschata*, *C. pepo, Luffa acutangula, L. cylindrica, Lagenaria siceraria, Memordica charantia)* with systemic mosaic mottling and leaf distortion symptoms. However, plants of families Chenopodiaceae (*Cheno podium amaranticolor, Chenopodium quinoa*) produced local lesions. Similar results were reported by Kumar et al (2014). The virus under study did not produce any symptoms on *Nicotiana xanthi, Nicotiana glutinosa, Nicotiana tabaccum*,

Table 3. Host range of papaya ring spot virus isolate

Family / Host species	Main symptoms
Amaranthaceae	
Amaranthus caudatus L.	--
Chenopodiaceae	
Chenopodium album L.	LL
Chenopodium amaranticolor	LL
Compositae	
Helianthus annus	--
Cruciferae	
Raphanuns sativus L.	--
Cucurbitaceae	
Luffa actungula L.	VC,M,MO
Memordica charantia L.	MO, M
Cucumis sativus L.	VC,M, MO,LD
Lagenaria siceraria	VC,SM,BL,GVB,C,LD
Cucurbita pepo L.	VC,BL,SM,LD
Cucurbita moschata	MO, M
Leguminoceae	
Phaseolus vulgaris L.	--
Pisum sativum L.	--
Vigna mungo	--
Vigna radiate	--
Cajanus cajan	--
Malvaceae	
Abelmoschus esculenttus	--
Solanaceae	
Capsicum annum L.	--
Nicotina tabacum L.	--
Nicotina glutinosa L.	--
Nicotiana xanthi	--
Caricaceae	
Carica papaya	VC,SM,BL,GVB,C,LD,SS

VC- Vein clearing BL- blistering C - chlorosis
M- Mosaic LD-Leaf distortion Mo- Mottling
LL- Local lesions GVB- Green vein banding SS- Shoe string
SM- Severe mosaic -- Non host

Fig . a. Bitter gourd leaf showing mottling

Fig. b. Mosaic and blisters on Cucurbita pepo

Fig.c. *Chenopodium amaranticolor* showing local lesion

Fig. d. Papaya showing local lesion

Plate IV. Host range of papaya ring spot virus

Fig a. Mild mosaic and blister

Fig b. Leaf distortion

Fig c. Leaf exhibiting vein clearing and green vein banding

Fig d. Mosaic symptom

Plate V. Host range of papaya ring spot virus

Capsicum annum L., *Abelmoschus esculentus*, *Vigna mungo*, *Vigna sinensis* and *Pisum sativum* , which indicated their non-host status. Similar results on host range was reported by Tripathi et al (2008), Kumar et al (2014), Muske et al (2014) and Singh et al (2017). Muske et al (2017) also reported that Zucchini is an indicator plant where as it helps in the early and accurate virus indicator prominently than other host plant. Many cucurbitaceous plants were reported as natural hosts of papaya ring spot virus. (Singh et al 2017).

CONCLUSIONS

Thus, from the results obtained on various aspects during present investigation on papaya ring spot virus disease of papaya, it is concluded that, Based on transmission (insect and mechanical), host range and symptomatology, the virus under present study transmitted by aphids (*M. persicae, A. gossypii, A. craccivora*) and mechanical means. Papaya ring spot virus is restricted to the families such as Caricaceae, Cucurbitaceae and Chenopodiaceae.

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Role of the Riparian Corridor in Conserving Avian Diversity in Intensively Farmed Regions: Evidence from Punjab's Beas River Region

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Abstract: This study examined avian diversity along the Beas River in Punjab, India from April 2019 to March 2021. Three locations were sampled, each divided into agricultural fields, riparian zones, and wetlands. Bird diversity was assessed using point counts and diversity indices. Hierarchical multiple regression revealed habitat type significantly influenced avian ecological indices. Riparian zones consistently showed the highest species richness (51.8-80.1), evenness (0.934-0.946), and Shannon-Wiener diversity (3.677-4.113) compared to agricultural fields and wetlands. Agricultural fields exhibited moderate diversity (3.576-3.889), while wetlands showed lower diversity (2.945-3.609). The Simpson index was highest in riparian zones (0.968-0.980), followed by agricultural fields (0.965-0.974) and wetlands (0.934-0.960). Habitat type explained 45.0%, 40.0%, 45.6%, and 53.9% of the variance in species richness, evenness, Shannon-Wiener diversity, and Simpson index, respectively. Results highlight the importance of preserving riparian zones and wetlands in intensively farmed regions to support diverse bird communities. Despite narrow widths, riparian habitats along the Beas River maintain rich avian diversity, emphasizing their conservation value. Findings underscore the crucial role of habitat type in shaping avian communities and inform strategies for sustainable bird conservation in agricultural landscapes.

Keywords: River Beas, Riparian, Avian, Diversity, Richness, Sustainability

Rivers and their riparian areas play crucial roles in supporting avian diversity (Dallimer et al 2012, Keten et al 2020). They help to regulate the temperature of aquatic habitats, provide woody debris, and create green spaces that enhance landscapes. Beyond these functions, riparian zones act as ecological corridors that connect otherwise isolated habitat patches, including farmland, home gardens, and parks (Bryant 2006). These corridors offer essential food and cover for bird species and serve as colonization and dispersal routes, facilitating the movement of birds from riparian areas to adjacent green spaces (Litteral and Shochat 2017). The width of riparian buffers positively influences bird species richness and abundance, highlighting the significance of maintaining suitable buffer widths for avian habitats. However, extensive agricultural activities in landscapes globally have led to the loss and fragmentation of natural vegetation. This phenomenon is also applicable to riparian ecosystems, where agricultural practices have simplified their structure, resulting in substantial consequences for wildlife diversity (Kontsiotis et al 2019).

Agricultural and urbanization growth has adversely affected the ecology and environment of rivers (Rafie and Kumar 2020, Kumar et al 2023). Agriculture alone has an effect on 87% of the globally threatened bird species. Ecologists have two schools of thought to maintain a balance between landuse and wildlife, i.e., by sparing specific land for wildlife and intensifying production on existing agricultural land or sharing land with wildlife with less intensive agricultural practices (Green et al 2005, Law and Wilson 2015). Sustainable avian diversity can be observed in undisturbed natural areas (Moura et al 2013); however, this is not possible in intensive agricultural farming regions such as Punjab, India. Punjab boasts an impressive gross cropping area (GCA) of 98.5%, (Gulati et al 2021). Such a high level of agricultural development poses significant challenges for biodiversity conservation. However, in the face of this scenario, there is still potential to leverage the natural spaces available within the state, such as areas along rivers (Kumar 2021, Kumar and Kler 2021), canals (Kaur 2018), wetlands (Kumar 2021) and village ponds (Sidhu et al 2022). To harness these spaces effectively for conservation, preliminary studies are essential to gain a deeper understanding of the diversity and ecology of these ecosystems. Punjab, with its prevalent intensive agricultural practices, offers an ideal setting for investigating the role of these natural spaces in avian conservation. The substantial stretch of the Beas River traverses this intensive agricultural region, resulting in the continuous encroachment of the river's boundaries and the narrowing of the riparian zone between the river and agricultural areas (Kumar and Kler (2021). These unique conditions along the Beas River present an exceptional opportunity to study avian diversity within the complex interplay of agricultural, riparian, and wetland habitats, as the ecotone between these three habitats is exceptionally narrow (Kumar 2021). The current study explores avian diversity in the Beas River Conservation Reserve, Punjab, focusing on agricultural, riparian, and wetland regions.

MATERIAL AND METHODS

The study was conducted at three locations along the Beas River, i.e., at the entrance of the river in Punjab state (T1-T) Talwara (31.949 latitude 75.900 long), at the middle of the river in Punjab (T2-B) Beas (31.505 latitude 75.298 long) and before the river merged into Satluj (T3-H) (31.211 latitude, 75.046 long) at Harike (Ramsar Wetland from April 2019–March 2021).Each location was further divided into three sub habitat strata types, i.e., agricultural fields, riparian zones and wetlands along rivers. The stratified random sampling method was used to cover approximately 15 km (5 km× 3 locations) of the total stretch of the river. The point-count method (Verner, 1985) was implemented during the study period. Each sampling consisted of 10 random points with an interval of straight-line distance of at least 500 m between two random sampling points. Each point was counted for 6 minutes within 4 hours after sunrise at each sampling point (Shiu and Lee 2003). The locations under study were visited every fortnightly during the study period. To minimize disturbance to the birds, observations were recorded using binoculars (10×50) and a camera (Nikon D 7200 with a 200-500 mm lens). The study was intended for hierarchical multiple regression analysis; therefore, the experiment was devised to include independent variables, dependent variables, and control variables (Tu et al 2020).

Independent variables: The landscape habitat was divided into three main categories for the purpose of this study:

Agricultural areas located alongside the river: Farmers predominantly engaged in rice-wheat crop rotation along the river, with maize serving as a secondary crop in certain regions. Typically, the chosen fields extend up to 800 meters perpendicularly from the river's edge. Generally, the agricultural fields had very few trees, but if any trees were present, any bird species observed on the trees were included in the overall sampling data.

Riparian zones of the river: These zones were considered the interfaces between the agricultural land and the river. They encompassed a variety of habitats, ranging from sandy banks to lush vegetation consisting of grasses, shrubs, and tall trees. As an agricultural-dominated state, the riparian zone experiences encroachment for agricultural purposes and experiences continuous stress. Therefore, the riparian zone of the river was fragmented and very narrow (<20 mt wide); at some places, such locations were excluded from the study. On average, the width of the riparian habitat under study ranged between 21 and 70 meter.

Wetlands along the river: These wetlands that formed naturally along the riverbanks were supplied with water from the river as well as rainfall. The size of the wetlands varied, ranging from 100 to 500 m^2. A total of 16 wetlands were studied, seven of which were perennial (present throughout the year), while the rest were seasonal (existing only during monsoon periods).

Dependent variables: Species richness, evenness Simpson index and Shannon-Wiener index were selected as dependent variables as they are important components used to measure species diversity in different habitats (Pyron 2010, Harisha and Hosetti 2009, Xu et al 2011, Lembrechts et al 2018) therefore, the combination of all four parameters was used for inclusive information about diversity and habitat health (Tu et al2020). All indices were calculated using the Vegan-2019 package in the statistical software R (version 1.2.5033) (Kumar et al 2023) (Table 1).

Control variables: The precipitation, wind speed, maximum temperature and temperature were downloaded from WorldClim.com Version 4 of the CRU TS monthly high-resolution gridded multivariate climate dataset along the river

Table 1. Diversity, Evenness, Richness, and Simpson indices of sub habitats along the Beas River

Habitat	Location	Diversity	Evenness	Richness	Simpson Index
Agriculture fields	T1	3.889	0.937	64.583	0.974
	T2	3.576	0.933	46.417	0.965
	T3	3.786	0.920	61.667	0.970
Riparian	T1	4.113	0.942	80.083	0.980
	T2	3.677	0.934	51.833	0.968
	T3	4.073	0.946	75.333	0.979
Wetland	T1	3.415	0.910	48.333	0.953
	T2	2.945	0.923	25.167	0.934
	T3	3.609	0.899	58.083	0.960

T1 -Talwara, T2 -Beas and T3-Harike

and subsequently averaged on a monthly basis (Harris et al 2020).

Data analysis: The single river was taken as a single identity during the study period; therefore, climatic variables were taken throughout the length of the river; however, due to human resource limitations, the avian diversity data were taken only from three locations. Similarly, the data recorded from all three locations were also clustered and averaged for the study period. The data were analyzed using the hierarchical multiple regression method to determine the associations between the habitat types and avian ecological indices. The independent variables were assigned as dummy variables, and wetland areas were taken as constant intercepts for comparative analysis with agricultural and riparian zones for all indices. The same process was performed for each hierarchical multiple regression analysis, with the Shannon–Wiener diversity index, species evenness, species richness and Simpson index taken as the dependent variables. Hierarchical multiple regression analysis was conducted to assess the relationship between independent and dependent variables. Results included R^2 and adjusted R^2 for model fit, B values indicating the specific effect of each independent variable, and beta (β) values representing standardized coefficients. The analysis followed procedures outlined by Tu et al (2020) and was analyzed using SPSS version 16.0.

RESULTS AND DISCUSSION

Species richness: Among the three sub habitats, the riparian zones had the highest avian richness (Table 1). Riparian areas support high levels of species diversity, with bird communities shifting toward urban dwelling species due to human modification (Melanie et al 2017). The results of model one for species richness via hierarchical multiple regression indicated a variance of R= 0.450 and R^2=0.230 with an adjusted R^2 = 0.164, which was significantly different from zero. Model 02 was created using independent variables. The R^2 value changed to 0.671, and ΔR^2 = 0.450, indicating a reasonable level of increase in prediction and significant variance can be explained in the dependent variable (richness) on the basis of the independent variables. Simultaneously, a significant change in R^2 of 0.450 inferred that the independent variables had a significant effect on enhancing the model efficiency and helps to compensate for the optimistic bias of R^2 (Miles 2014). The adjusted R^2 for the model was 0.411, indicating the goodness of fit of Model 02. The control variables had no significant contribution at the individual level other than wind speed. The dummy variables used included agricultural and riparian habitats in the intercept, with wetlands taken as constants for comparison. Similarly, compared with those of the constant wetland richness, the B values were 13.771 ($\pm$ 3.782, β=0.312) and 25.299 ($\pm$ 3.782, β=0.573) for agricultural and riparian zone richness, respectively (Table 2). This signifies that the agricultural fields and riparian zones have significantly greater species diversity than the avian diversity available in wetland pockets present along the river. Avian richness varied notably across habitats, with riparian zones harboring the most species, influenced by urban-dwelling species adapting to human-modified environments. Contrary to expectations, agricultural fields also exhibited high species richness, likely due to their proximity to riparian and wetland ecosystems.

Species evenness: This was measured by Pielou's evenness index, reflects the equitability of species distributions in an avian community. Typically, evenness is inversely related to species dominance. In this study distinct patterns in species composition and dominance. In agricultural fields, a total of 110 species were recorded. Among these, the dominant species, listed in descending order of abundance, were rock pigeon, rose-ringed parakeet, bank myna, cattle egret, house crow, plain martin, house sparrow, baya weaver, common myna, plain prinia, ashy prinia, rufous-fronted prinia, red-wattled lapwing, green bee-eater, and paddy field pipit. Moving to riparian zones, where 164 species were documented, a different set of dominant species emerged. These included jungle babbler, yellow-legged green pigeon, house sparrow, red-vented bulbul, common babbler, house crow, plum-headed parakeet, black-headed munia, common myna, scaly-breasted munia, bank myna, laughing dove, alpine swift, black kite, and oriental magpie robin. In wetland areas, with a total of 149 species observed, the dominant species comprised common coot, indian spot-billed duck, purple swamphen, common moorhen, common teal, common pochard, cattle egret, bar-headed goose, little egret, greylag goose, black-winged stilt, northern shoveler, white-breasted waterhen, gadwall, and little cormorant. These highlight the varied ecological dynamics across different habitats, with each exhibiting its unique assemblage of dominant species. Evenness in agricultural fields, riparian zones, and wetlands along the Beas River indicated relatively high evenness in agricultural fields but exhibited some variability, ranging from 0.920 to 0.937. This suggested a relatively balanced distribution of bird species, with no single species dominating the ecosystem. The diverse array of associated resources in agricultural fields likely supports a fairly even distribution of avian species (Hossain and Aditya 2016). The presence of complex edge habitats, such as small trees along field edges, can lead to higher avian abundance within fields and increased avian-mediated pest control services (Kross et al 2016). The riparian zones had higher evenness values (ranging from 0.934 to 0.946), indicating a more equitable distribution of avian species. Lind et al (2019) reported that a 144-meter buffer zone is required to preserve bird diversity in agricultural riparian areas. However, the riparian zone was fragmented and narrower at many locations along the river in the present study. Nevertheless, the overall study of the region has shown that if the riparian zone along the entire length of the river is conserved, this can contribute to the state's efforts to conserve the overall avian diversity of the region. Wetlands exhibited lower evenness values (0.899 to 0.923), suggesting that there might be some species that dominate these ecosystems. Wetlands often have specialized requirements, and water-dependent species may dominate these areas, leading to slightly lower evenness (Mitsch and Gosselink 2000).

Species evenness was taken as another dependent variable along with the control variables in Model one; here, the variance was $R=0.203$, $R^2 = 0.041$, with $F (5,102) = 0.873$. However, after the incorporation of the independent variables in Model 02, the variance increased to $R= 0.633$ and $\Delta R^2=0.400$, with an adjusted $R^2 = 0.359$. Such a high change in R^2 indicates that the independent variable, i.e., type of habitat, strongly influences the even distribution of species and had a significantly strong effect on enhancing model efficiency. The control variables had no significant contribution at the individual level, whereas the habitat variables (independent) had a significantly high variance. The B values were 0.020 ($\pm$ 0.004, β=0.450) and 0.030 ($\pm$ 0.004, β=0.681) for agriculture and riparian zone richness, respectively, in comparison to wetland evenness, which was taken as a constant. In essence, this hierarchical regression model demonstrated that the type of habitat is a key driver of species evenness in avian communities, with agricultural fields and riparian zones significantly enhancing evenness in comparison to wetlands. These findings have implications for habitat management and conservation efforts to promote balanced avian communities in the Beas River region.

Simpson index: Simpson index in agricultural fields (0.965 to 0.974) suggested a relatively balanced avian community, where no single species dominated the ecosystem, which was consistent with the results of the evenness data (Table 1). The riparian zones had higher Simpson indices (0.979 to 0.980), indicating a more even distribution of species and lower dominance of particular species. Due to the high number of some migratory species, wetlands exhibit a lower Simpson index (0.934 to 0.960), suggesting that certain species may be more dominant in these habitats. The variance of $R= 0.450$ and $R^2=0.230$ with an adjusted $R^2 = 0.164$ was recorded for the dependent variable. Model 02 was created using independent variables, and the variance increase $R^2=0.734$, $\Delta R^2 = 0.539$ infers that the variables present in Model 02 can explain the variance with higher accuracy for the Simpson index. An increase in $\Delta R^2 =0.539$ and an adjusted $R^2=507$ infers that the model can explain 50.7% of the variance. The control variables had no significant contribution at the individual level. The independent dummy variables had a significant contribution, wetland area was taken as a constant that infers that agriculture and riparian space provided 2.1 and 2.7 points more of a contribution to variance than wetland area (Table 2).

In agricultural fields, the Simpson index suggests a relatively balanced avian community, while riparian zones exhibit a higher Simpson index, indicating a more even distribution of species and reduced dominance of particular species. In contrast, the wetlands displayed a lower Simpson index, hinting at the dominance of certain species, likely influenced by the presence of numerous migratory birds. The model considering independent variables significantly improved the accuracy of the Simpson index, explaining 50.7% of the variance. The control variables had no individual-level significance agriculture and riparian zones contributed significantly more to the variance than did wetlands (Table 2).

Avian diversity in agricultural fields is moderate to high (3.576 to 3.889). The agricultural fields under study have a wheat–rice cropping pattern throughout their length and avian diversity influenced by various factors, such as water level, flooding period, rice plant structure and size, and pesticide use (Ibáñez et al 2010). Intermediate water levels (10-20 cm) promote the highest bird density and diversity in rice fields during the growing season (Stafford et al 2010). Early flooding and late drying also favor waterbird density

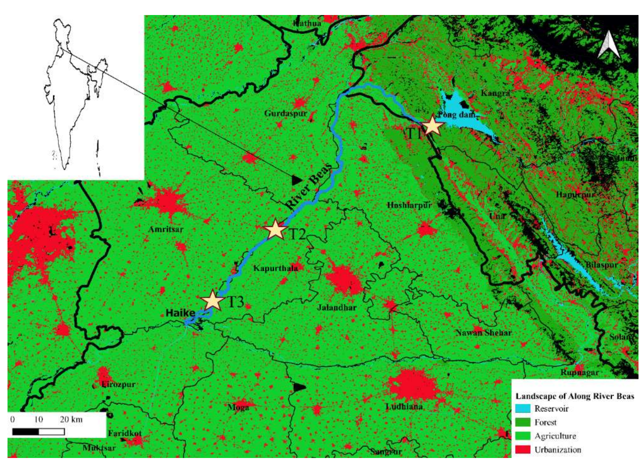

Fig. 1. Landscape along the Beas River

Table 2. Effect of landscape habitat type on the diversity index along the Beas River

Model 02	R (Species Richness)			E (Species Evenness)			D (Shannon–Wiener diversity index)			S (Simpson index)		
	B	Std. Error	B	B	Std. Error	β	B	Std. Error	β	B	Std. Error	β
Constant	51.971**	20.87		0.910**	0.022		122.12**	48.92		0.958**	0.016	
Control variables												
Elevation	0.061	0.021	0.205	0.000*	0.000	0.107	0.001*	0.000	0.177	0.000	0.000	0.134
Temp Max	-1.543	1.237	-0.490	0.000	0.000	-0.082	-0.28	0.023	-0.429	-0.001	0.001	-0.420
Temp Min	0.521	1.220	0.187	0.000	0.000	-0.104	0.0.10	0.023	0.168	0.001	0.001	0.215
Precipitation	-0.057	0.034	-0.259	0.000	0.000	-0.011	-0.001	0.001	-0.236	0.000	0.000	-0.239
Wind	15.230*	7.332	0.179	0.004	0.008	0.053	0.242	0.137	0.139	0.009	0.006	0.120
Independent variables												
Wetland						Constant						
Agriculture	13.771**	3.782	0.312	0.020**	0.004	0.450	13.482**	3.764	0.305	0.021**	0.003	0.558
Riparian	25.299**	3.782	0.573	0.030**	0.004	0.681	25.009**	3.764	0.567	0.027**	0.003	0.19
R^2		0.671**			0.633**			0.675**			0.734**	
ΔR^2		0.450			0.400			0.456			0.539	
Adjusted R^2		0.411			0.359			0.418			0.507	

* P < 0.05; **P < 0.001

and diversity, as well as the stopover of migrating species (Ladha et al 2007). All the factors discussed above, namely, water levels, cropping patterns, the vicinity of the riparian zone and the river, were present in the area of study and might have resulted in the rich avian diversity. The riparian zones exhibited the highest avian diversity among the three sub habitats, with diversity values ranging from 3.677 to 4.113. Riparian areas serve as crucial transition zones between terrestrial and aquatic ecosystems, offering a wide range of resources for avian species. These areas provide water availability, subsidies, and complex habitat structures that support diverse bird populations (Xiang et al 2016). These zones often contain abundant vegetation, water bodies, and a variety of microhabitats that attract diverse avian communities (Naiman et al 2005). The wetlands were studied and exhibited comparatively lower avian diversity (2.945 to 3.609).

The Shannon–Wiener diversity index was taken as the third dependent variable with the same set of control and independent variables. The variance recorded was, R^2 = 0.154 with an adjusted R^2= 0.112, However, the variance increased to R= 0.675 and with an adjusted R^2 = 0.418. A high change in R^2 indicates the significance of independent variables, i.e., type of habitat. Simultaneously, a significant change in the ΔR^2 of 0.456 imply that the independent variables significantly contributed to the diversity index to enhance model efficiency. The elevation variable had some significant effect on diversity, whereas the other control variables did not significantly contribute to diversity at the individual level. The independent variable agriculture was more related to diversity enhancement, with a B value of 13.482 (± 3.764, β=0.305), and to the Riparian zone, with a B value of 25.009 (± 0.3.764, β=0.567), than was wetland (Table 2).

CONCLUSION

The study highlights the critical role of habitat type in shaping avian diversity along the Beas River, with riparian areas and wetlands being particularly important. Habitat type emerged as the primary factor influencing bird communities, while climatic variables showed less significant impact. This aligns with some previous research, though other studies have found climate to be influential. Agricultural, riparian, and wetland habitats each supported distinct levels of bird species richness and evenness. The riparian zones consistently harbored the richest avian communities, even where these zones were narrow. Agricultural areas, while hosting fewer bird species overall, still provided important ecosystem services through occasional bird use. The study emphasizes the importance of preserving riparian zones and wetlands within intensively farmed landscapes. Smaller wetlands, often overlooked and played significant role in supporting bird diversity, especially for migratory species. The legal protection of rivers and riparian zones, as exemplified by the Beas River Conservation Reserve, contributes positively to wildlife conservation efforts. In conclusion, the research underscores the need to prioritize the preservation of riparian areas and wetlands alongside agricultural regions to maintain avian diversity. This approach can establish a sustainable model for bird conservation in intensively farmed landscapes. The findings highlight the importance of understanding the interplay between various ecological factors and bird communities for effective conservation strategies.

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Assessing Avian Diversity and the Impact of Air Pollution on Ecological Communities in Western Haryana, India

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Abstract: Understanding the structure and diversity of avian communities is crucial for elucidating the ecosystem's significance in conservation efforts. Birds, as rapid responders to environmental changes, serve as valuable 'bioindicators' reflecting ecosystem conditions. This study was conducted to explore the avifaunal diversity in the districts of Fatehabad and Hisar in western Haryana. The total of 115 bird species belonging to 18 orders and 46 families were recorded. Passeriformes emerged as the dominant order with 49 species, while families, Muscicapidae & Anatidae were prevalent, each comprising eight species. Examining the impact of fluctuating air quality on avian diversity from 2019 to 2022, including the COVID-19 pandemic period. Negative correlation was observed between the air quality index (AQI) and diversity indices. Elevated AQI values were associated with reduced avifaunal diversity, a trend that was particularly mitigated during the pandemic period due to enforced lockdown measures and decreased vehicular pollution. Of the species recorded, two species- Woolly-necked Stork and Alexandrine Parakeet are as Near Threatened in the IUCN Red List.

Keywords: Avifaunal diversity, Air quality index, Simpson's diversity index, Shannon-Weiner diversity index, Air pollution

Biodiversity is the variety and variability of life in the ecosystem or ecological complex, in which the living organisms are a part. Birds are one of the most diversified creatures living on the earth, having a great diversity of size, form, color and behaviour. The total of 1353 Species of birds are found in India, and 1426 in Indian subcontinent (Praveen and Jayapal 2023). Bird species respond rapidly to any changes in the environment. The avian species diversity, richness, and abundance are determined by various factors such as migration, natality, mortality, and availability of food and niches. Birds are considered the valuable bio-indicators of the environment because they are involved in various essential processes like pollination, scavenging, seed dispersal, pest control and ecosystem engineering (Raj et al 2024).

Air pollution poses a significant threat to biodiversity worldwide, with avian species being particularly vulnerable to its adverse effects. Sanderfoot and Holloway (2017) mentioned birds exhibit heightened sensitivity to air pollutants compared to mammalian species. The ramifications of pollution on biodiversity are profound, contributing to the rapid decline in species populations globally. The interplay between environmental quality and ecological niches shapes the interactions between organisms and their abiotic surroundings. Any alteration in ecosystem structure and function inevitably leads to changes in biodiversity statistics (Bhowmick 2022). Among the myriad impacts of air pollution on wildlife, birds experience direct mortality, physiological stress, and bioaccumulation of toxins. In light of these observations, it becomes imperative to conduct comprehensive surveys of avian faunal diversity to assess the impact of air pollution on bird populations. This paper presents findings from a diversity survey conducted in the districts of Hisar and Fatehabad in the state of Haryana, India.

MATERIAL AND METHODS

Study area: The present study was conducted across various locations in Fatehabad and Hisar districts of Haryana. In Fatehabad district study sites included village Bhodia, Badopal, Dhangar and Chilli Lake. In Hisar district, two locations were studied: Sisai village and Chaudhary Charan Singh Haryana Agricultural University, Hisar.

Data collection: Weekly bird surveys were conducted from June 2019 to May 2022, adopting the line transect method (Gaston 1975; Sales and Berkmuller 1988). The total of 50 transects were studied that covered almost all of the study area. Transect length remained constant i.e. 500m, but the width varied according to survey area and visibility: in forests, 15m; in agricultural fields, 20m; and in other open fields, 50m. The field surveys were conducted in the morning (between 06:00 hours and 10:00 hours) and in the evening (from 16:00 hours to19:00 hours), when birds were found to be most active. Birds were photographed and identified using standard reference books (Grimmet et al 1998). Classification of the recorded bird species residential, abundance and International Union for Conservation of nature (IUCN) status) was done (Praveen and Jayapal 2023). Nikon™ D3300 DSLR camera having 24.2 megapixels sensor along with a Nikkor zoom lens of focal range 70-300 mm and aperture range f/4.5-6.3, was used to photograph the bird species. Nikon™ Aculon A211 binocular was used for bird watching.

Data analysis: Standard biodiversity indices were applied to calculate the species diversity, evenness and richness (Simpson 1949; Shannon Weaver 1963). The diversity indices were calculated using PAST 3.14 software. Each survey was analyzed for relative abundance on the basis of frequency of sightings (Mackinnon and Phillipp 1993): very common- sighted >10times; common- sighted from seven to nine times; uncommon-sighted from three to six times; rare-sighted once or twice. Air Quality Index (AQI) data were collected from the Central Pollution Control Board (CPCB) official website on every single day during the study period ($AQI = max (AQI_{PM2.5}, AQI_{PM10}, AQI_{O3})$). The correlation between AQI and diversity indices was statistically analyzed using Pearson's correlation coefficient in IBM SPSS Statistics 21 and figures were generated using Microsoft Excel.

RESULTS AND DISCUSSION

The total of 115 bird species were identified, representing 18 orders and 46 families (Table 2). Analysis of species distribution by order revealed the dominance of the Passeriformes order, encompassing 49 bird species. Family-wise distribution highlighted the prevalence of the Muscicapidae and Anatidae families, each comprising eight bird species (Fig. 4). Among the recorded avifauna, two species, the Wooly-necked Stork (*Ciconia episcopus*) and Alexandrine Parakeet (*Psittacula eupatria*), are classified as "Near Threatened" (Fig. 2, 3). All other recorded species are categorized as "Least Concern." Among 115 species, 76 species were residents, 28 species were winter visitors, 8 species were summer visitors and four species were

Table 1. Location of selected study sites

Site no.	Name	Co-ordinates
Site 1	Bhodia village pond, Fatehabad	29.491485°N, 75.422804°E
Site 2	Chilli Lake, Fatehabad	29.517824°N, 75.459927°E
Site 3	Dhangar village, Fatehabad	29.470820°N, 75.515744°E
Site 4	Badopal village, Fatehabad	29.427654°N, 75.539440°E
Site 5	Sisai village, Hisar	29.175187°N, 76.009799°E
Site 6	C.C.S. Haryana Agricultural University, Hisar	29.144649°N, 75.707255°E

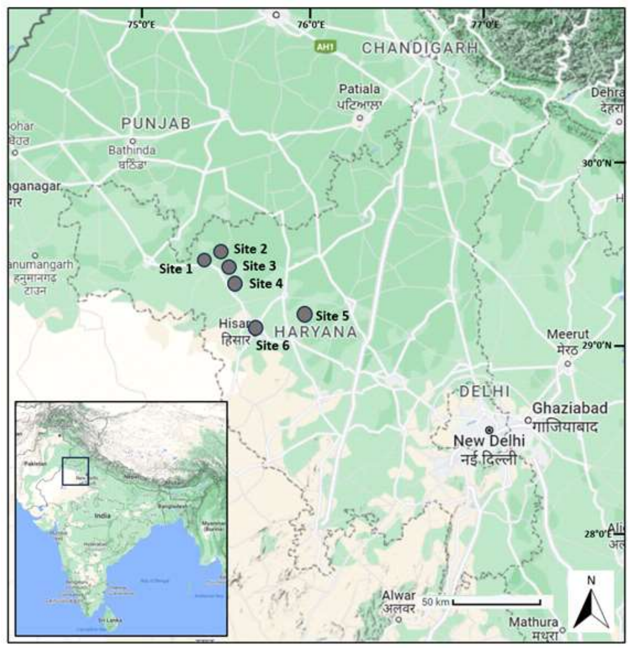

Fig. 1. Location of study sites

2. Alexandrine Parakeet, *Psittacula eupatria*

3. Wooly-Necked Stork, *Ciconia episcopus*

Fig. 2, 3. Recorded bird species with IUCN status of Near-Threatened (NT)

Table 2. List of bird species recorded from selected study sites in the Fatebabad and Hisar districts of Haryana

Order	Family	Common name	Zoological name	IUCN status	Residential status	Abundance status
Colombiformes	Columbidae	Rock Dove	*Columba livia*	LC(DEC)	Resident	Very Common
		Laughing Dove	*Stigmatopelia senegalensis*	LC(STABLE)	Resident	Common
		Eurasian Collared Dove	*Streptopelia decaocto*	LC(INC)	Resident	Very Common
		Yellow-Footed Green Pigeon	*Treron phoenicoptera*	LC(INC)	Resident	Common
		Spotted Dove	*Spilopelia chinensis*	LC(INC)	Resident	Less Common
Charadriformes	Recurvirostridae	Black-Winged Stilt	*Himantopus himantopus*	LC(INC)	Resident	Very Common
	Jacanidae	Pheasant-tailed Jacana	*Hydrophasianus chirurgus*	LC(DEC)	Summer Visitor	Less Common
	Charadriidae	Red-wattled Lapwing	*Vanellus indicus*	LC(UNKNOWN)	Winter Visitor	Very Common
	Scolopacidae	Common Redshank	*Tringa tetanus*	LC(UNKNOWN)	Winter Visitor	Very Common
		Ruff Bird	*Calidris pugnax*	LC(DEC)	Winter Visitor	Very Common
		Common Sandpiper	*Actitis hypoleucos*	LC(DEC)	Winter Visitor	Very Common
		Little Stint	*Calidris minuta*	LC(INC)	Summer Visitor	Very Common
	Burhinidae	Eurasian stone curlew	*Burhinus oedicnemus*	LC(DEC)	Resident	Common
Psittaciformes	Psittacidae	Rose-Ringed Parakeet	*Psittacula krameri*	LC(INC)	Resident	Very Common
		Alexandrine Parakeet	*Psittacula eupatria*	NT(DEC)	Resident	Common
		Plum-headed Parakeet	*Psittacula cyanocepala*	LC(DEC)	Resident	Less Common
Anseriformes	Anatidae	Indian Spot-billed Duck	*Anas poecilorhyncha*	LC(DEC)	Resident	Very Common
		Northern Pintail	*Anas acuta*	LC(DEC)	Winter Visitor	Common
		Northern Shoveler	*Spatula clypeata*	LC(DEC)	Winter Visitor	Common
		Eurasian Green-Winged Teal	*Anas crecca*	LC(UNKNOWN)	Winter Visitor	Less Common
		Gadwall	*Mareca strepera*	LC(INC)	Winter Visitor	Common
		Knob-billed Duck	*Sarkidiornis melanotos*	LC(DEC)	Resident	Less Common
		Lesser Whistling Duck	*Dendrocygnajavanica*	LC(DEC)	Summer Visitor	Common
		Red-crested Pochard	*Netta ruffina*	LC(UNKNOWN)	Winter Visitor	Common
	Podicipedidae	Little Grebe	*Tachybaptus ruficollis*	LC(DEC)	Winter Visitor	Common
Suliformes	Phalacrocorcidae	Little Cormorant	*Microcarboniger*	LC(UNKNOWN)	Resident	Very Common
		Indian Cormorant	*Phalacrocorax fuscicollis*	LC(UNKNOWN)	Winter Visitor	Common
		Great Cormorant	*Phalacrocorax carbo*	LC(INC)	Passage Migrant	Less Common
Galliformes	Phasianidae	Indian Peafowl	*Pavo cristatus*	LC(STABLE)	Resident	Common
		Black Francolin	*Francolinus francolinus*	LC(STABLE)	Resident	Common
		Grey Francolin	*Francolinus pondicerianus*	LC(STABLE)	Resident	Very Common
Ciconiiformes	Ardeidae	Indian Pond-heron	*Ardeolagrayii*	LC(STABLE)	Resident	Common
		Cattle Egret	*Bubulcus ibis*	LC(INC)	Resident	Very Common
		Little Egret	*Egrettagarzetta*	LC(INC)	Resident	Less Common

Cont...

Table 2. List of bird species recorded from selected study sites in the Fatebabad and Hisar districts of Haryana

Order	Family	Common name	Zoological name	IUCN status	Residential status	Abundance status
		Black-crowned Night Heron	*Nycticorax nycticorax*	LC(DEC)	Resident	Less Common
	Ciconiidae	Wooly-Necked Stork	*Ciconia episcopus*	NT(DEC)	Resident	Rare
Strigiformes	Strigidae	Spotted owlet	*Athene brama*	LC(STABLE)	Resident	Common
Pelecaniformes	Threskiornithidae	Red-Naped Ibis	*Pseudibis papillosa*	LC(DEC)	Resident	Common
Gruiformes	Rallidae	Common Coot	*Fulicaatra*	LC(INC)	Resident	Common
		Common Moorhen	*Gallinula chloropus*	LC(STABLE)	Resident	Common
		White-Breasted Waterhen	*Amaurornis phoenicurus*	LC(UNKNOWN)	Resident	Common
Culculiformes	Cuculidae	Jacobin Cuckoo	*Clamator jacobinus*	LC(STABLE)	Passage Migrant	Less Common
		Asian Koel	*Eudynamys scolopaceus*	LC(STABLE)	Resident	Common
		Common Hawk Cuckoo	*Hierococcyx varius*	LC(STABLE)	Resident	Less Common
	Centropodidae	Greater Coucal	*Centropus sinensis*	LC(STABLE)	Resident	Common
Piciformes	Megalaimidae	Brown-headed Barbet	*Psilopogon zeylanicus*	LC(STABLE)	Resident	Less Common
		Copper Smith Barbet	*Psilopogon haemacephalus*	LC(INC)	Resident	Rare
	Picidae	Black-rumpedFlameback	*Dinopium benghalense*	LC(STABLE)	Resident	Common
		Yellow-crowned Woodpecker	*Leiopicus mahrattensis*	LC(STABLE)	Resident	Less Common
		Eurasian Wryneck	*Jynx torquilla*	LC(DEC)	Winter Visitor	Rare
Upupiformes	Upupidae	Common Hoopoe	*Upupa epops*	LC(DEC)	Resident	Common
Burcerotiformes	Burcerotidae	Indian Grey Hornbill	*Ocyceros birostris*	LC(STABLE)	Resident	Common
		Oriental Pied Hornbill	*Anthracoceros albirostris*	LC(STABLE)	Summer Visitor	Rare
Coraciformes	Coraciidae	Indian Roller	*Coracias benghalensis*	LC(INC)	Resident	Less Common
	Halcyonidae	White-Breasted Kingfisher	*Halcyon smyrnensis*	LC(INC)	Resident	Common
	Meropidae	Green Bee-eater	*Merops orientalis*	LC(INC)	Summer Visitor	Less Common
		Blue-tailed Bee-eater	*Merops philippinus*	LC(STABLE)	Passage migrant	Less Common
Accipitriformes	Accipitiridae	Shikra	*Accipiter badius*	LC(STABLE)	Winter Visitor	Less Common
		Besra Sparrowhawk	*Accipiter virgatus*	LC(DEC)	Resident	Common
		Crested Honey Buzzard	*Pernis ptilorhynchus*	LC(DEC)	Resident	Common
		Black-Shouldered Kite	*Elanus axillaris*	LC(INC)	Winter Visitor	Less Common
		Brahminy Kite	*Haliasturindus*	LC(DEC)	Resident	Common
		Changeable Hawk Eagle	*Nisaetus cirrhatus*	LC(DEC)	Winter Visitor	Less Common
		Booted Eagle	*Hieraaetus pennatus*	LC(UNKNOWN)	Winter Visitor	Less Common
Passeriformes	Muscicapidae	Brown Rockchat	*Oenanthe fusca*	LC(STABLE)	Summer Visitor	Less Common
		Oriental Magpie Robin	*Copsychus saularis*	LC(STABLE)	Winter Visitor	Less Common
		Black Redstart	*Phoenicurus ochruros*	LC(INC)	Resident	Common
		Pied Bushchat	*Saxicola caprata*	LC(STABLE)	Winter Visitor	Less Common

Cont...

Table 2. List of bird species recorded from selected study sites in the Fatebabad and Hisar districts of Haryana

Order	Family	Common name	Zoological name	IUCN status	Residential status	Abundance status
		Red-breasted Flycatcher	*Ficedula parva*	LC(INC)	Resident	Very Common
		European Stonechat	*Saxicola rubicola*	LC(DEC)	Summer Visitor	Common
		Verditer Flycatcher	*Eumyias thalassinus*	LC(STABLE)	Resident	Common
		Blue-Throat	*Luscinia svecica*	LC(STABLE)	Resident	Very Common
	Dicruridae	Black Drongo	*Dicrurus macrocercus*	LC(UNKNOWN)	Resident	Common
		Ashy Drongo	*Dicrurus leucophaeus*	LC(UNKNOWN)	Passage Migrant	Common
	Pycnonotidae	Red-Vented Bulbul	*Pycnonotus cafer*	LC(INC)	Resident	Common
	Hirundinidae	Wire-tailed Swallow	*Hirundo smithii*	LC(INC)	Resident	Common
		Streak-throated Swallow	*Petrochelidon fluvicola*	LC(INC)	Resident	Common
	Sturnidae	Common Myna	*Acridotheres tristis*	LC(INC)	Resident	Common
		Brahminy Starling	*Sturnia pagodarum*	LC(UNKNOWN)	Summer Visitor	Rare
		Rosy Starling	*Pastor roseus*	LC(UNKNOWN)	Winter Visitor	Common
		Asian Pied Starling	*Gracupica contra*	LC(INC)	Resident	Common
	Corvidae	House Crow	*Corvus splendens*	LC(STABLE)	Resident	Common
		Large-Billed Crow	*Corvus macrorhynchos*	LC(STABLE)	Resident	Less Common
		Rufous Treepie	*Dendrocitta vagabunda*	LC(DEC)	Resident	Less Common
	Oriolidae	Indian Golden Oriole	*Oriolus kundoo*	LC(UNKNOWN)	Winter Visitor	Common
	Acrocephalidae	Clamorous Reed-warbler	*Acrocephalus stentoreus*	LC(STABLE)	Winter Visitor	Less Common
	Estrildidae	Scaly breasted Munia	*Lonchura punctulata*	LC(STABLE)	Resident	Common
		Indian Silverbill	*Euodice malabarica*	LC(STABLE)	Winter Visitor	Less Common
	Laniidae	Long-Tailed Shrike	*Lanius schach*	LC(UNKNOWN)	Winter Visitor	Less Common
		Bay-backed Shrike	*Lanius vittatus*	LC(STABLE)	Winter Visitor	Common
	Motacillidae	White Wagtail	*Motacilla alba*	LC(STABLE)	Resident	Common
		GreyWagtail	*Motacilla cinerea*	LC(STABLE)	Resident	Common
		White-browed Wagtail	*Motacilla maderaspatensis*	LC(STABLE)	Resident	Common
		Yellow Wagtail	*Motacilla flava*	LC(DEC)	Resident	Common
		Citrine Wagtail	*Motacilla citreola*	LC(INC)	Resident	Common
		Tree Pipit	*Anthus trivialis*	LC(DEC)	Resident	Common
		Paddyfield Pipit	*Anthus rufulus*	LC(STABLE)	Resident	Common
	Nectariniidae	Purple Sunbird	*Cinnyris asiaticus*	LC(STABLE)	Resident	Common
	Cisticolidae	Ashy Prinia	*Prinia socialis*	LC(STABLE)	Resident	Very Common
		Rufous-Fronted Prinia	*Prinia buchanani*	LC(STABLE)	Resident	Less Common
		Graceful Prinia	*Prinia gracilis*	LC(STABLE)	Resident	Very Common
		Striated Prinia	*Prinia crinigera*	LC(STABLE)	Resident	Very Common
		Plain Prinia	*Prinia inornata*	LC(STABLE)	Resident	Common
		Common Tailor bird	*Orthotomus sutorius*	LC(STABLE)	Resident	Less Common
	Passeridae	House Sparrow	*Passer domesticus*	LC(DEC)	Winter Visitor	Common

Cont...

Passage migrants. Regarding abundance status, 19 species were classified as very common, 58 species as common, 33 species as less common and 5 species as rare.

The impact of fluctuating air quality on avian faunal diversity was investigated throughout the study period from 2019 to 2022, including the Covid-19 pandemic. Month-wise AQI data for the study areas were analyzed (Table 3) revealing a negative correlation between AQI and avian diversity indices., Specifically, the correlation coefficients between AQI and Simpson's Diversity, as well as Shannon-Weiner diversity index, were -0.677 and -0.796, respectively (Table 4). AQI peaked in November, and was lowest in August. Notably, AQI decreased significantly during the pandemic due to lockdown measures and reduced vehicular pollution (Fig. 5, 6). The negative correlation coefficients indicate that species diversity, richness, and evenness

Table 2. List of bird species recorded from selected study sites in the Fatebabad and Hisar districts of Haryana

Order	Family	Common name	Zoological name	IUCN status	Residential status	Abundance status
		Sind Sparrow	*Passer pyrrhonotus*	LC(STABLE)	Winter Visitor	Less Common
	Ploceidae	Baya Weaver	*Ploceus philippinus*	LC(STABLE)	Resident	Common
	Leiothrichidae	Jungle Babbler	*Turdoides striata*	LC(STABLE)	Resident	Very Common
		Large Grey Babbler	*Turdoide smalcomi*	LC(STABLE)	Resident	Common
		Striated Babbler	*Turdoide searlei*	LC(DEC)	Winter Visitor	Less Common
	Sylviidae	Lesser Whitethroat	*Sylvia curruca*	LC(STABLE)	Resident	Common
	Phylloscopidae	Hume's Leaf Warbler	*Phylloscopus humei*	LC(STABLE)	Winter Visitor	Less Common
	Zosteropidae	Oriental white-eye	*Zosterops palpebrosus*	LC(DEC)	Resident	Less Common
Apodiformes	Apodidae	Little Swift	*Apus affinis*	LC(STABLE)	Resident	Common
		Common Swift	*Apus apus*	LC(STABLE)	Resident	Common

IUCN-International Union for Conservation of Nature; LC-Least Concern; NT-Near Threatened; DEC-Declining; INC-Increasing

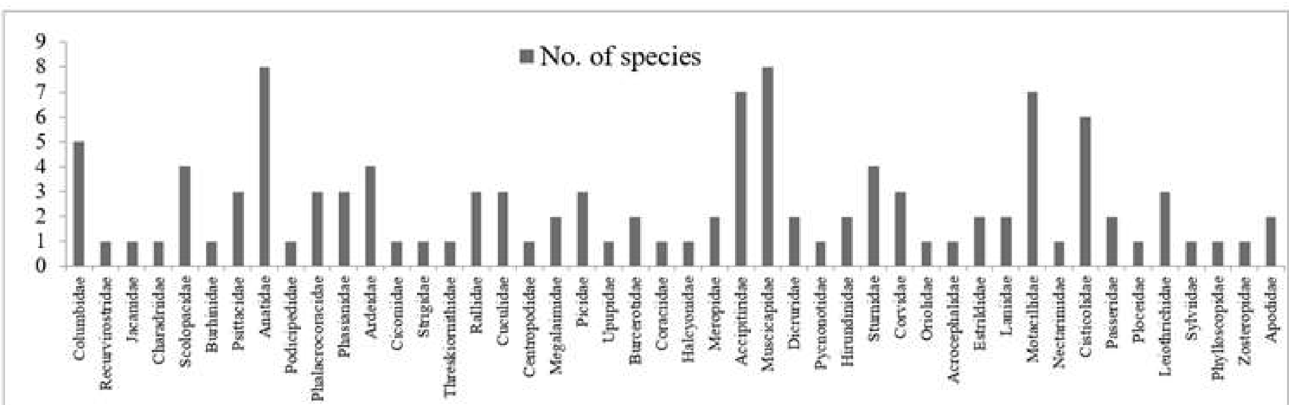

Fig. 4. Family-wise distribution of bird species in the study area

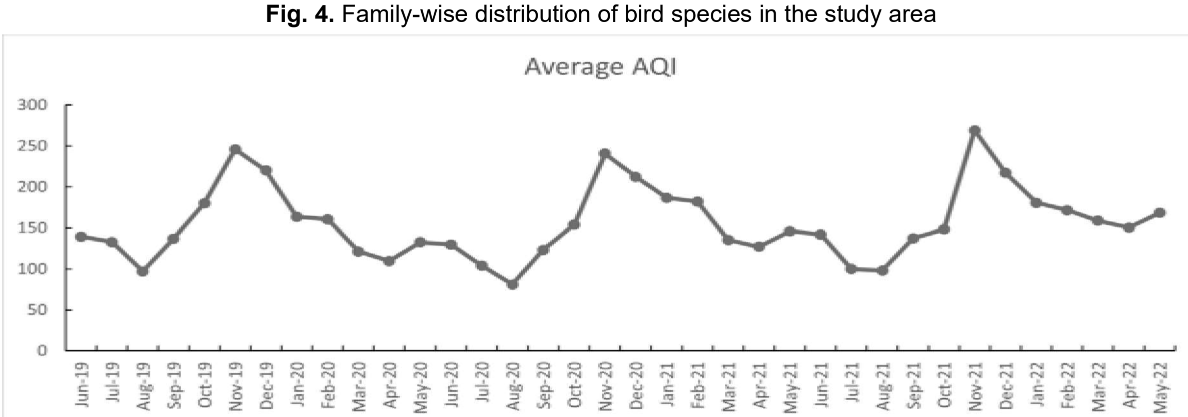

Fig. 5. Month-wise average AQI data during the study period

Table 3. Month-wise air quality index (AQI), bird diversity and species evenness of the study area

	Average AQI	Simpson Index of diversity	Species evenness index
2019			
June	139.6	0.711	2.198
July	133.1	0.814	2.291
August	97.3	0.821	2.313
September	136.9	0.734	2.211
October	180.2	0.648	2.124
November	246.1	0.883	2.440
December	220.1	0.951	2.501
2020			
January	163.7	0.629	2.103
February	160.5	0.640	2.119
March	121.4	0.751	2.224
April	109.7	0.799	2.256
May	132.7	0.699	2.136
June	129.9	0.819	2.212
July	104.4	0.856	2.411
August	81.3	0.887	2.450
September	123.4	0.821	2.242
October	154.0	0.781	2.140
November	240.8	0.950	2.512
December	212.2	1.110	2.663
2021			
January	186.6	0.723	2.089
February	182.1	0.746	2.136
March	135.5	0.833	2.356
April	127.1	0.842	2.399
May	146.1	0.802	2.198
June	141.9	0.804	2.223
July	100.1	0.842	2.312
August	98.2	0.864	2.397
September	137.5	0.811	2.236
October	148.2	0.759	2.189
November	269.2	0.822	2.429
December	217.5	0.919	2.333
2022			
January	180.8	0.710	2.091
February	171.8	0.721	2.113
March	158.9	0.821	2.291
April	150.5	0.836	2.302
May	168.3	0.786	2.219

decline with increasing AQI levels, suggesting birds' efficacy as bioindicators of air pollution. Furthermore site 2, Chilli Lake, 30 species of birds were recorded. This lake is shrinking at a very fast rate because of garbage dumping in it by the local people and use of land for agriculture practices nearby the lake.

Ther documentation of 115 bird species across 18 orders and 46 families aligns with similar studies conducted in various wetland habitats across India, and underscores the importance of these ecosystems for avian conservation. Muralikrishnan et al (2023) recorded 90 bird species belonging to 21 orders, 42 families in the Koonthankulam village pond in Tirunelveli district, southern Tamil Nadu. Similarly, Raj et al (2023) recorded 262 bird species from the Bharathapuzha River Basin, the second largest, west-flowing river in Kerala, Western Ghats. Kumar et al (2016) documented 69 wetland birds belonging to 20 families in the six rural ponds of District Kurukshetra, Haryana. Koli (2014) identified 150 bird species in the Todgarh-Raoli Wildlife Sanctuary, Rajasthan, India., Gupta et al (2009) reported 92 bird species at Kurukshetra University, Haryana. Yadav & Chauhan (2018) reported 181 bird species belongs to 22 orders in Jhalawar forest division, Rajasthan. Yadav et al (2023) reported 59 birds along Yamuna River, Haryana. Brraich et al (2023) observed 204 bird species in Patiala district, Punjab. Kumar (2021) reported 114 species of birds in Central University campus, Himachal Pradesh. Sharma & Tripathi (2023) found 102 species of avifauna in Bhilwara, Rajasthan. The variations in bird species composition across different regions can be attributed to factors such as habitat type, climate, and geographical location. The presence of 152 bird species in the Indian Institute of Technology - Guwahati campus highlights the importance of secondary growth and eco-forests in supporting avian diversity. Order Passeriformes emerged as the most dominant avian taxa with 49 species, indicating its ecological significance in the region. This finding is in line with, identifying Passeriformes as particularly species-rich across different habitats in India (Kohli 2014; Rai et al 2017; Singh et al 2021). The total of 40 points were laid and 140 species were recorded, with Passeriformes as the most dominating order (Rana and Khan 2024). Overall, 59 species of birds including 45 resident, 11 resident migratory and 3 migratory species were recorded (Sekhon et al 2023). A total of 506 nests were

Table 4. Correlation between AQI with diversity indices during the study period

Correlation coefficient value of Mean AQI with:	r
1. Simpson's Index of Diversity	-0.677
2. Shannon-Weiner Diversity Index	-0.796

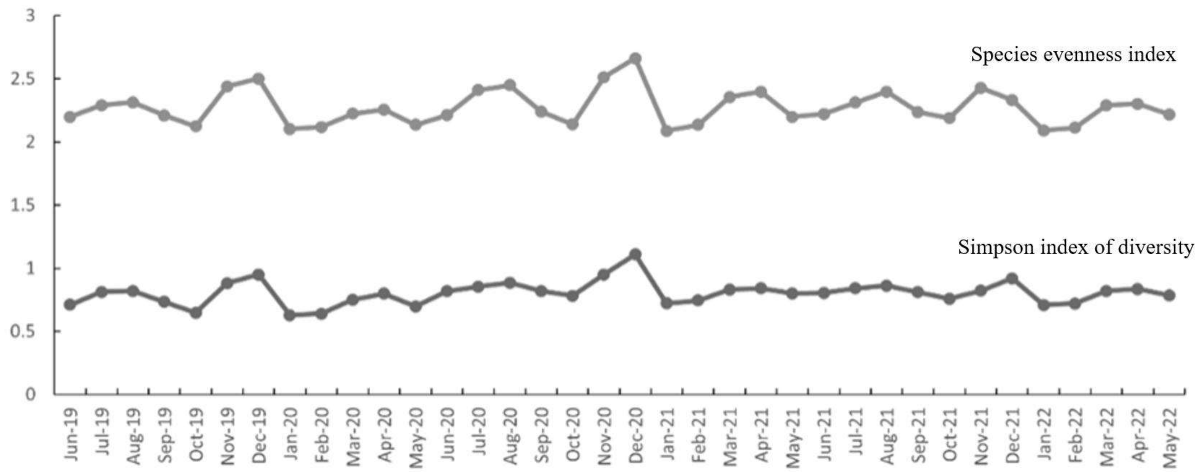

Fig. 6. Month-wise diversity indices during the study period

counted from the study areas which shows proximity to water bodies and among these 33% were observed from Krishnakumarsinhji Town Hall followed by 30% from Manila bag (Gohel et al 2021). A total of 201 species belonging to 44 families were recorded in the area. The family Muscicapidae was dominant followed by Corvidae (Kukreti 2021). The observed negative correlation between air quality index (AQI) and bird species diversity is an important finding, reflecting the interconnectedness of environmental factors and biodiversity. The improvement in air quality during the COVID-19 lockdown period coinciding with increased bird species diversity underscores the vulnerability of avian populations to anthropogenic activities, particularly those impacting air quality. Furthermore, studies such as Bhowmick (2021) emphasize the complex relationship between air pollution and biodiversity, suggesting that efforts to mitigate air pollution could have positive implications for biodiversity conservation. By elucidating the relationships between environmental factors, such as air quality, and bird species diversity, researchers can inform conservation efforts aimed at preserving ecosystems and safeguarding avian populations for future generations.

CONCLUSIONS

115 bird species were found comprising 18 orders and 46 families which shows that the study areas have a great diversity of birds. Rural ponds in the villages are preferred habitat of aquatic birds so, they should be protected. Order Passeriformes was dominant with 49 species. Family Muscicapidae & Anatidae were dominant, each comprising eight species. Two species were found Near Threatened - Woolly-necked Stork and Alexandrine Parakeet. Elevated AQI values were associated with reduced avifaunal diversity. It shows that birds are valuable bioindicators of the air quality.

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Flood and Hydrological Water Discharge in Rishikesh City Assessment of Flood and Hydrological Water Discharge in Rishikesh City

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Abstract: The rivers are life line for human being and these are also a disaster for human as well in monsoon season rivers become disaster in India. The study is focused on occurrence of flash flood in Rishikesh city with rainfall data and water discharge level data of Ganga River. The climate data was processed with the help of Mann Kendall test to observe the trend of surge in water level which is potential threat of flash flood in the city. There was increasing trend in the rainfall of the months of July (0.21), August (1.11), September (0.98) and October (2.21) for last 44 years which was more than significant value. The flood frequency analysis method was used to observe the frequency of occurrence of flash flood in the city. The study shows that 448 occurrence of surge is observed in last 44 years. There was 13 occurrence of flash flood events observed in last 448 surges. The river's normal flash floods is occur at 28177 m3/s, with an arrival time of around every three years, as shown by the flood peak data. The data analysis shows that the most extreme flood was recorded in 2013.

Keywords: Himalaya, Rishikesh, Flood occurrence, Flood frequency, Hydrological-system

The risks of climate change and extreme climatic events such as droughts and floods impact on economy and natural systems (Saha and Malkar 2024). The mountains are more vulnerable than the other places in the world (Dimri et al 2021). The rapid urban growth of the past three decades and resulting increased flooding problem are common in many cities, especially in humid areas (Rawat et al 2017). Hydroelectric power plants are being studied as a possible contributor to the Himalayan flood threats and consequences intensifying (Vishwanath and Tomaszewski 2018). Mountain environments are susceptible to climate change because small changes in winter precipitation, summer solar radiation receipts, and summer air temperatures can have major effects on glacier mass balance, dynamics and geometry (Elalem and Pal 2015). The implications of global change on the tropical montane ecosystem, in particular the composition of the angiosperm and vertebrate communities is widespread (Gupta et al 2022). The type of soil, large river networks, and high elevations have all contributed to the increased intensity of flash floods (Payra et al 2022). Global climate change has an impact on water resources through changes in rainfall, temperature and energy balance (Kundzewicz et al 2014). Increasing trend of rainfall can be resulted as increase in floods and could thereby affect water quality (Tabari et al 2011). Himalayan Mountain are facing the most disastrous events worldwide and the concern for sustainability has emerged. The cities which are built in the mountains have weak hydrology and improper management of water resources (Chauhan et al 2021). Climate change has great impact on the Indian sub-continent because its economic performance and social progress are dependent on rainfall and climate change is likely to affect rainfall. India possesses a great variety and diversity of climate, varying from extremely hot to extremely cold, from extremely arid regions to extremely humid regions and drought-prone areas to flood-prone areas (Roy et al 2021). Climatic conditions govern to a great extent the operation of water resources in the country. A one percent rise in floods can cause a 2.7% decrease in economic growth (Ayog et al 2021). The study assess impact of climate change on hydrological system and flood occurrence with changes in temperature, rainfall and evaporation.

MATERIAL AND METHODS

Study area: Rishikesh city is situated at 30° 10' 33" N latitude and 78° 29' 47" E longitude with average height of 442 meters (1,745 feet) (Statistical Abstract of Dehradun, 2016). Rishikesh city is one of the mountain city which lies along the bank of river Ganga (Fig. 1).

Methodology

Data source: The climate data such as rainfall, temperature, humidity, evapotranspiration. was collected from India Meteorological Department (IMD) for last 44 years has been collected from 1980 to 2024 .

Data Analysis

Mann Kendall method: The Mann-Kendall Test used to detect monotonic trends in series of environmental data, climate data or hydrological data. Each data value is compared to all subsequent data values. Let *x*1 *x*2, *x*3 ……xn represent n data points where *xj* represents the data point at time j. Then the Mann-Kendall statistic (S) is given by (Kendall 1975):

$$S = \sum_{k=1}^{n-1} \sum_{j=k+1}^{n} \operatorname{sgn}(x_i - x_j)$$

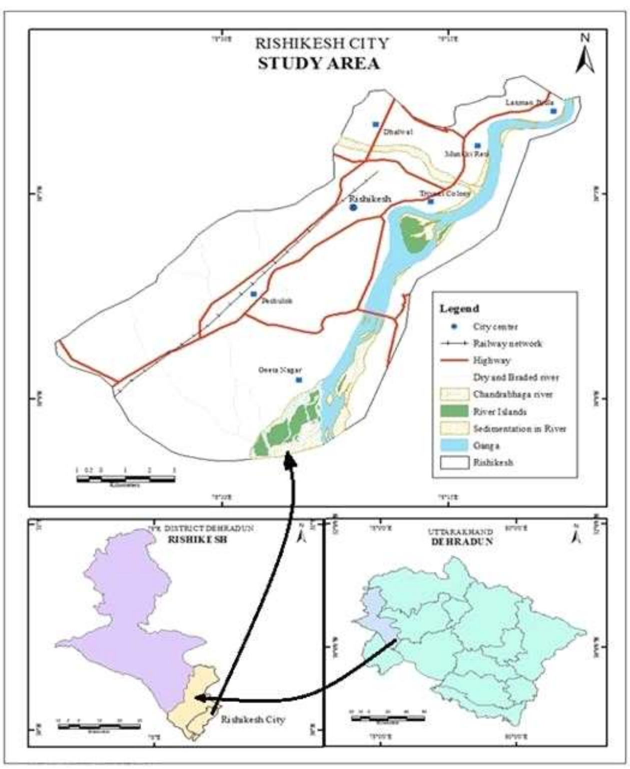
Where,

$$\operatorname{sgn}(xi - xk) = \begin{matrix} +1\ i(xi - xk) > 0 \\ \text{if}\,(xi - xk) = 0 \\ -1\ \text{if}\,(xi - xk) < 0 \end{matrix}$$

A very high positive value of S is an indicator of an increasing trend, and a very low negative value indicates a decreasing trend. It is necessary to compute the probability associated with S and the sample size, n, to statistically quantify the significance of the trend. If n is at least 10, the normal approximation test is used. However, if there are several tied values (i.e. equal values) in the time series, it may reduce the validity of the normal approximation when the number of data values is close to 10. First the variance of S is computed by the following equation which takes into account that ties may be present:

$$VA(S) = \frac{1}{18}\left[n(n-1)(2n+5) - \sum_{p=1}^{q} tp(t_p - 1)(2t_p + 5) \right]$$

Where *q* is the number of tied groups and *tp* is the number



Source: Census of India, 2011

Fig. 1. Location of study area

of data values in the *pth* group. The values of *S* and *VAR(S)* are used to compute the test statistic Z as follows:

$$Z_S = \frac{S-1}{[VAR(S)]^{1/2}} \text{ If } S > 0$$

$$Z_S = 0 \text{ If } S = 0$$

$$Z_S = \frac{S+1}{[VAR(S)]^{1/2}} \text{ If } S < 0$$

Probability density function for a normal distribution with a mean of 0 and a standard deviation of 1 is given by the following equation:

$$f(z) = \frac{1}{\sqrt{2\pi}} e^{-\frac{z^2}{2}}$$

The trend is said to be decreasing if Z is negative and the computed probability is greater than the level of significance. The trend is said to be increasing if Z is positive and the computed probability is less than the level of significance. If the computed probability is less than the level of significance, there is no trend.

The Mann-Kendall test has two parameters that are of importance to trend detection. These parameters are the significance level that indicates the trend's strength and the slope magnitude estimate that indicates the direction as well as the magnitude of the trend. In MAKESENS the tested significant levels are 0.001, 0.01, 0.05 and 0.1. For the four tested significant levels, the symbols used in the trend statistics worksheet are:

*** if trend at $\alpha = 0.001$ level of significance,** if trend at $\alpha = 0.010$ level of significance,

* if trend at $\alpha = 0.050$ level of significance, and + if trend at $\alpha = 0.100$ level of significance.

If the cell is blank, the significance level is greater than 0.1. The presence of a statistically significant trend is evaluated using the Z value. A positive value of Z indicates an upward trend and a negative value of Z indicates a downward trend.

Probability -Flood frequency analysis: The main purpose of probability frequency analysis is to obtain a relation between the magnitude of flood or storm and its probability of occurrence.

This analysis is done through empirical Gumble's method (1958):

and

Whereas, p = Probability exceedance

p = i/T

T = N+1/m

T = Recurrence interval or Frequency or return period

m = Ranking of flood

The Maya Kund station (30°10'53” and 78°30'02”) annual peak flood data from 1980 to 2024 is presented in descending order, and each flood event is given a ranking number (m). Thus, the flash flood that was most catastrophic ranked first and placed at the top and second flash flood was ranked second and smallest level of peak flow was positioned last. Equation T = N+1/m is then used to determine the frequency (T), or return N + 1 period, and a graph is shown between m frequencies and flood discharge. If needed, the graph can be expanded to extrapolate the flood magnitude value corresponding to any high frequency value.

RESULTS AND DISCUSSION

Mann-Kendall trend analysis of rainfall: The overall annual and non-monsoon trends was not significant as per Mann Kendall trend analysis of 44 years of climate data of Rishikesh city. The rainfall data of annual, monsoon season and non-monsoon season were reflecting the increasing trend in rainfall with Z of 0.62, 0.06 and 1.44 respectively which was showing that the rainfall in monsoon season was significant with 0.06 value. An increasing trend in the rainfall of the months of July, August, September and October was significant (Table 1). An increasing trend for the annual rainfall was seen where as a decreasing trend for the rainfall of February (-0.95) and December (-88) was also observed, both at a significance level of 0.10 and in June (-.71) at 0.50 significance level.

There was an increase of 90% in rainfall in Rishikesh city during the pre-monsoon season, which follows an increasing pattern. The annual rainfall is trending upward and is increasing partially. The monsoon season has shown a rising trend in rainfall, which is significant since it provides the basis for the yearly increase in total rainfall. The decreasing trend is shown in the post-monsoon season. Although there is not much rain throughout the winter, but some rainfall took place in this area because to western disturbances. Since winter rainfall makes up a very small portion of the overall growing trend in annual rainfall, it does not indicate a significant trend; the annual trend of rainfall describes the increasing trend at level 0.05 and it is annual rainfall increasing trend with significant seasonal declining trend of rainfall (Table 2).

Mann-Kendall trend analysis of maximum and minimum temperature: Increasing trends was observed for maximum temperature at Rishikesh city and decreasing trends for minimum temperature. The computed Mann-Kendall for Tmax, during winter, was significant, while for annual, North-eastern Monsoon, and summer, and was insignificant. These statistics indicated that there was an increasing trend in Tmax for winter, while the decreasing trend in Tmax during the South-west Monsoon season was observed. The Tmin, during summer season, was insignificant and during annual and winter, was significant, whereas during South-west Monsoon and North-eastern Monsoon was insignificance (Table 3).

The Tmean during winter, summer, and annual were significant whereas the NEM was not significance.

Flood frequency and return period: There is an increase in water levels in Rishikesh city of more than 50% (R^2=0.52). On the basis of returning period data, the tendency of flash flood very feasible. The occurrence data of 448 surges in river Ganga observed in last 44 years is extremely valuable for building water management purposes to prevent the loss caused by flash flood (Izinyon and Igbinoba 2011). The present study comprises various aspect of measurement of surges in the river basins such as length, shape, and cross profile, and rainfall in the catchment area. The catchment area explain over 50 percent of the variation in surge extent. There was 13 occurrence of flash flood events observed in last 448 surges. The Gumbel method is used in this study

Table 1. Mann-Kendall trend statistics for rainfall

Time series	No. of years	Test Z	Significance levels
January	44	0.18	
February	44	-.95	+
March	44	0.16	
April	44	0.13	
May	44	0.48	*
June	44	-.71	*
July	44	0.21	
August	44	1.11	*
September	44	0.98	*
October	44	2.21	*
November	44	0.17	
December	44	-.88	+
Annual	44	1.74	+
Monsoon	44	1.23	
Non-monsoon	44	1.38	

+: significance level: 0.1; *: significance level: 0.05

Table 2. Mann Kendall Trend Statistics (Z) Rainfall in Rishikesh city

Seasons	Trends
Annual	↑*
Pre-Monsoon (Mar-May)	↑+
Monsoon (June-Sept)	↑*
Post-Monsoon (Oct-Nov)	↓
Winter (Dec-Feb)	↑

Where, (↑) shows increasing trend; (↓) shows decreasing trend;
* 0.05 level of significance; + 0.1 level of significance
Source: Calculated by Researcher

because the distribution of the extremes events, each selected from a set of occurrences, exponentially approaches the Gumbel distribution as r approaches infinity.

The result of analyses of monthly time series of discharge at Maya Kund gauge station in annual, winter and summer time period with the hydrological years 1980-2024 have been presented in Figure 2. The results describe various aspects such as in between 1990 to 1996 there were trend of extreme water discharge and occurrence of floods this water discharge is nearby 1.5 lakh cusec which was highest ever discharge in this trend of discharge data. There is probability of occurrence of highest discharge once in last thirty years except year 2013 which is part of another thirty years of trend at that time discharge level was 2.5 lakh cusec. The probability of highest discharge reflects the increasing trend of highest discharge with increasing total discharge in the year. This is the result of highest probability analysis in which the total number of event has been divided by number of highest discharge level. The probability of occurrence of flood is 0.03 throughout the months in the duration of 44 years. The plotting position method has been used to describe the probability of occurrence of flood.

The maximum river flow of 233 m3/s was at Mayakund in 1980, 1992 and 2013 while minimum of 8.83 m3/s was in 1998. The 44 year mean average peak river was 75.05 m3/s the most extreme flood of 246893 m3/s was recorded in 2013 while the least river flow of 5182 m3/s was in 1983. The 44 year mean rapid river flow is 28177 m3/s with a less fluctuation in water flow. The Gumbel dispersion capacity's remarkable ability to predict the amount the flood flows (Fig. 3). Estimated R^2 of 0.9543 indicates that Gumbel's dispersion technique is predicting the expected flood in the river and that

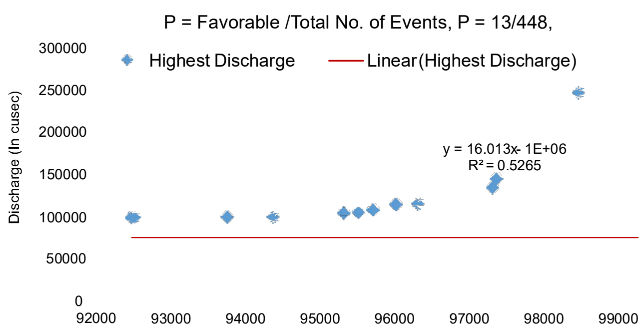


Fig. 2. Probability of flood in Rishikesh City

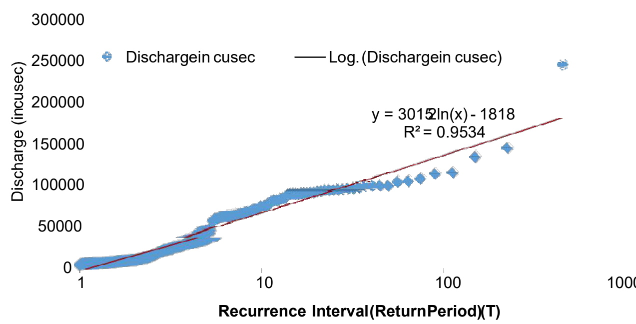


Fig. 3. Frequency curve and return period of flood in Rishikesh city

Table 3. Mann-Kendall trend statistics for maximum and minimum temperature

Time series		Maximum temperature		Minimum temperature	
	N	Test Z	Significance	Test Z	Significance
January	44	2.83	+	-.84	+
February	44	2.22	*	-2.09	*
March	44	3.14		-1.48	*
April	44	0.29	*	-2.43	*
May	44	2.04	*	-3.06	
June	44	2.12	*	-2.19	
July	44	1.21		-2.25	*
August	44	2.16	*	-1.41	+
September	44	1.75	+	-3.42	*
October	44	2.14	*	-1.87	*
November	44	0.31	*	-2.87	
December	44	0.47	+	-2.16	*
Annual	44	1.40		-2.17	*
Monsoon	44	1.01	*	-2.57	+
Non-monsoon	44	2.07	+	-2.21	*

+: significance level: 0.10; *: significance level: 0.05

the example of the dispersion is limited. Furthermore, the river's normal flash floods is occur at 28177 m3/s, with an arrival time of around every three years, as shown by the flood peak data. This indicates that the basin's flood forecast is somewhat precise. The planning of important pressure-driven structures and additions in the river reach can be done using this flood forecast. The flash flood prediction is accurate in Rishikesh city. This forecast of flood can be used in the planning of critical pressure driven structures and extensions in the river reach.

CONCLUSION

The Mann Kendall shows an increasing trend in the rainfall was significant of the months of July, August, September and October. An increasing trend for the annual rainfall was seen where as a decreasing trend for the rainfall of February and December was observed. The catchment area explain over 50 percent of the variation in surge number. There was thirteen occurrence of flash flood events observed whole period of time. From 1990 to 1996, trend of extreme water discharge and occurrence of floods was very high with exceptional value of water discharge level. There was probability of occurrence of extreme flash flood once in thirty years. The river's normal flash floods occur at water level of 28177 m3/s, with an arrival time of around every three years, as shown by the flood peak data.

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Spatiotemporal Fluctuation in Water Quality Parameters and Correlation with Phytoplankton Community at Sambhar Salt Lake, India

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Abstract: This research brings input on information regarding the effects of seasonal fluctuation in the physicochemical properties of water and phytoplankton communities. Water quality parameters and nutrients, including water temperature, pH, total alkalinity, DO, conductivity and heavy metals were quantitatively monitored from 2020 to 2021 and exhibited significant differences between locations and seasons. Based on the observed data of the temporal and spatial variations of physicochemical properties and phytoplankton abundance chlorophyceae, cyanophyceae and bacillariophyceae were the predominant groups, respectively. Heavy metals revealed the presence of cadmium, zinc, iron, lead, manganese and chromium and were found above the upper permissible limit (as per Indian standard IS 10500: 2012). The comparative analysis using the Pearson's correlation, the results showed that most phytoplankton group's density significantly correlates with water parameters. These results suggested that seasonal differences are major factors influencing water quality causing algal bloom and increased amount of heavy metals which should be taken as important criteria under consideration for effective water management. Therefore, strengthening the supervision for controlling damage to India's largest inland saline lake and the Ramsar site is urgently needed.

Keywords: Ramsar site, Seasonal fluctuations, Physicochemical parameters, Phytoplanktons, Correlation

Saline lakes are widespread globally in diverse sizes and found in arid and semiarid climates. These are mainly fed by small streams or rainfall with water lost by seepage or evaporation. The water chemistry of saline lakes fluctuates with seasons, time, temperature and other climate factors. Ensuring water security and accessibility is vital to the well-being of all living organisms and essential for the preservation of natural ecosystems worldwide (Devi and Tiwari 2024). Saline lakes, despite being widely distributed, have received less attention in terms of characterization compared to freshwater lakes (Baatar et al 2017). Due to the increasing discharge of industrial, municipal, and agricultural wastewater, the water quality of the lake has degraded (Elshemy 2016). Wetlands are widely recognized as dynamic ecosystems with diverse features, including unique biodiversity that provide abundant shelter and food for bird populations year-round (Harshavardhan and Girish, 2024). The health of aquatic ecosystems is entirely dependent on the correct proportions of nutrients in water and sediment. It is absolutely crucial to maintain this balance to preserve water quality and sustain life (Tibebe et al 2022). The study was conducted in India's largest inland saline lake which is most valuable for commercial salt production sources using several multi-pond solar salt pans. At the moment the lake is undergoing rapid industrialization and urbanization suffering from exposure to high inputs of domestic, industrial and agricultural pollutants. The brines are enriched with phytoplanktons and other microbes that facilitate a unique opportunity to study microbial successions along salinity gradients similar to the transition in water quality of the hypersaline lake. At present very limited Microbial exploration study with isolation and culture of halophilic bacteria from different bioprospecting outlooks of Sambhar lake is performed (Cherekar and Pathak 2016).

The degradation of water quality poses a threat to both aquatic life and human health by affecting underground water quality. It is important to consistently monitor spatiotemporal variations in water quality parameters and biological characteristics in order to gain a comprehensive understanding of a lake's environmental conditions (Maansi and Wats 2022). The quality of groundwater in a specific area varies depending on physical and chemical parameters, which are significantly affected by geological formations and human activities (Ganiyu et al 2018). Water pollution is a global environmental issue that causes a decline in water quality (Xu et al 2019). Effectively preventing and controlling eutrophication is of utmost importance to uphold the overall water quality and ensure the safety of the aquatic biota (Wei et al 2022).

Phytoplankton contributes 95% of primary production in aquatic ecosystems and is a natural bioindicator for water quality assessment. It responds rapidly to environmental changes, making it useful for assessing temperature, pH, salinity, nutrients, and turbidity concerning contamination (Clark et al 2017). Certain types of algae are crucial for purifying water bodies contaminated with organic waste.

Industrial waste can be recycled by producing microalgae using industrial wastewater, creating new sources of raw materials for various purposes. Pollution, ecological conditions, and human impact can affect the characteristics and growth of phytoplankton (Neelam et al 2019). Thus, phytoplankton based biomonitoring can be employed as an efficient, quick and affordable method for estimating water pollution (Konanc 2023, Subbaiah and Kaledhonkar 2024). To the best of our knowledge no seasonal and spatial fluctuation in microbial community and water quality parameters has been studied to date to understand the correlation of this very distinctive lake. Therefore, the present study, analyzes the temporal and spatial changes in water quality and vegetation coverage with the impact and apparent relevance of physicochemical properties on phytoplankton communities is discussed to develop and conserve this wetland.

MATERIAL AND METHODS

Site location: Sambhar lake, the largest inland hypersaline lake (Latitude 26.5760 N and Longitude 75.0500 E) in Rajasthan, India, with a catchment area of 230 km^2 is located in the eastern part of Rajasthan state in a closed depression of Aravalli schists (Bhatt et al 2016, Sinha 2004). The only Hypersaline Lake in India was declared a Ramsar site (wetland of international importance) in 1990 for hosting thousands of migratory birds including famous pink flamingos from northern Asia and Siberia every year. Sambhar salt lake falls in the rain shadow of the southwest monsoon, receiving an average annual rainfall ranging from 100 to 500 mm. The lake basin is primarily fed by atmospheric precipitation and seasonal streams, namely Mendha and Roopangarh rivers, during the monsoon season from July to September. Samples were collected from various locations at Devyani kyars and near pump house. Precipitations leaves kyars concentrated in salinity resulting pink color and other kyar rich with algal bloom in green color.

Sample collection and analysis: Brine samples were collected in all seasons of year 2020 and 2021 from different collection sites enriched with microflora. The microalgal diversity and color of brines change with seasons throughout the year with salinity ranging from 10.0-30.0 (% w/v, NaCl) and high pH (8-11). The lake water was sampled for physicochemical and biological analysis in prewashed plastic bottles from areas with different degrees of brine concentration. The temperature, pH and color of water samples were measured using a laboratory glass thermometer and pH meter respectively at the collection point. Ammonical nitrogen, COD (Chemical oxygen demand), BOD (biological oxygen demand), electrical conductivity, total hardness, total alkalinity, and dissolved oxygen were measured at laboratory according to APHA (Anonymous 1996). The presence of heavy metals was estimated by AAS (Atomic absorption spectrophotometry) (Bhateria and Jain, 2016). Permissible limit studies on physico-chemical characteristics were determined as per Indian standards (IS 10500: 2012) (Sankaranarayanan et al 2021).

The microscopic analysis was carried out to find out the presence of microalgae. Different algae cells were identified using a fluorescent microscope with inverted digital microphotography (Leica-1000) at 40X and 100X magnification as per the morphological description given by Iyenger and Desikachary (Raji and Abraham, 2018).

Statistical analysis: A two-way ANOVA test was used to analyze the collected data. A two-tailed Pearson product-moment correlation was performed using SPSS version 22 with Duncan's multiple range test.

RESULTS AND DISCUSSION

Physicochemical analysis of water: The water temperature varied between 5°C and 29°C at different sites. Minimum value was in December on site II while maximum in June at site I. The phytoplankton diversity and succession are affected by these variations of temperature. The positive correlation was studied between cyanophyceae and temperature (Table 2). The positive correlation between chlorophyceae and cyanophyceae group and temperature was reported by Deyab et al (2019). In most sites, salinity variations were due to the brine used for salt production. Salinity was highest at in summer and may be due to the high evaporation rate and lowest in September because of dilution with rain water. The maximum was at site II while minimum at site II. High salinity indicates increased pollutants in discharged water. The salinity was correlated negatively with phytoplankton groups as less number of algae were reported when salinity was highest in summer. There weak correlation with BOD, TN, Cr and was moderately correlated with Zn.

The mean Ammonical nitrogen (AN) ranged between 3 and 29.35 mg/L throughout the year. Minimum value of Ammonical nitrogen reported in summer at site I while maximum in winter at site III. Same results were observed by Gammal et al (2017). Bacillariophyceae and DO were correlated positively with ammonia while negatively correlated with pH COD and BOD. It is a decomposed product of organic nitrogen by bacteria showed maximum degradation in winter season. High pH was in winter season at 9.9 and lowest in rainy season 7.3. The variation in pH values shows high productive nature of the lake water (Gyanendra and Alam, 2023). All sites were reported alkaline may be due to domestic and agricultural runoff with lowest value at site I and highest at site IV. The pH value showed a positive correlation with Bacillariophyceae in winter while weak correlation with Mn. It was negatively correlated with Hardness, Mg and TN.

The COD varied between 154 to 706 mg/L. Maximum value was reported at site II while minimum at site I. The positive correlation was between COD and BOD and Cl. COD values indicated negative correlation with phytoplankton groups and total hardness, DO, TN. BOD (biological oxygen demand) is influenced by time and temperature. The level of pollution in a body of water is directly proportional to the BOD and was highest at site II with mean of 56.33 in rain and lowest with 23 mg/L at site I. It was positively correlated with cyanophyceae while negatively correlated with temperature and AN. BOD was significantly positive correlated with salinity and pH.

Chloride was lowest at site II with a mean of 5139 and highest at site IV with 127300 mg/L. The presence of chloride was decreased during the rainy season due to dilution and indicated a positive correlation with conductivity and salinity. Chloride showed moderate correlation with phosphate and negative correlation with phytoplankton groups in summer. The annual mean ranged between 1327 to 29788 mg/L. It was reported maximum in the winter season at site III and a minimum in the rainy season at site I.

Sulphate not only impairs the quality of drinking water, but also impacts the cycling of carbon, nitrogen, and phosphorus. This can lead to increased nutrient levels in water bodies, promoting the growth of plants and algae, and providing more food for aquatic organisms (Melese and Debella 2023). The average annual value of sulphate was 11453.31 mg/L with a mean minimum of 1327.5 and a mean maximum of 29788 mg/L. It was detected minimum in rain and maximum in winter at site III. Sulphate indicated negative correlation with Mg and TN and weak correlation with AN. Chlorophyceae and bacillariophyceae were positively correlated with Sulphate.

The annual mean conductivity was 14440 µs/cm low at site I in September month and high at site III. It was increased in summer with 333000 in May. Conductivity was attributed to the biogeochemical cycle, biodegradation and human activities and high dissolved solids resulted decrease in phytoplankton growth (Rus et al 2020). The positive correlation was reported between conductivity with salinity, chloride and sulphate and negative correlation with chlorophyceae, cyanophyceae, temperature and Mg. The total alkalinity mean ranged 448 to 17140 mg/L. The lowest value was during rainy season while the highest was in winter. It was positively correlated with all phytoplankton

Table 1. Seasonal fluctuation in physicochemical parameters

Para/ month	Temp	Sal	AN	pH	COD	BOD	Cl	SO_4	Hard	Mg	Con	ALK	DO	TN	Phos	Cd	Fe	Pb	Mn	Cr	Zn
January	7.66	143.88	7.26	9.11	609	46.66	79700	29788	1256	220.33	331000	9333.33	3.8	509.07	886.05	0.10	14.51	0.81	0.86	0.34	1.68
February	17.5	141.55	14.85	9.44	501.5	23	78400	13227.5	1350	245	274500	9055	5.0	694.87	843.14	0.02	10.63	2.54	0.81	0.28	2.01
March	8.5	229.8	21.6	8.75	627.5	53.5	127300	7336.5	365	65	374000	9590	3.4	501.74	494.37	-	4.63	1.03	0.52	0.45	1.46
April	12	204.4	24.65	8.97	487.5	40	113250	10250	318	47.5	323500	10820	4.2	523.54	432.94	0.08	2.61	0.94	0.58	0.35	0.34
May	14.5	204.8	29.35	8.68	568.5	42	116740	10885.5	91.5	37	333000	13570	4.1	403.52	387.06	0.56	3.15	0.98	0.60	0.32	0.43
June	28.5	122.58	7.15	9.75	594.5	43	67900	7204.5	45	7.5	204000	5635	3.9	432.75	356.28	0.16	7.69	0.24	0.34	0.23	0.48
July	23.5	96.04	3	9.27	595	51	108600	12852	90	15	292500	17140	2.9	785.98	313.65	0.28	4.39	0.39	0.36	0.33	0.60
August	21	87.74	7.06	9.04	593	56.33	46935.67	5094	58.66	34.66	131433.3	2125.33	2.6	867.49	224.65	0.21	1.25	0.72	0.23	0.05	0.81
September	21.5	9.3	9.8	8	193	27	5139	1327.5	54	30	14440	448	3.6	883.01	232.44	-	8.39	-	0.14	0.05	0.14
October	18.33	66.38	14.8	8.79	421.33	41	36766.67	6444	1134.66	769.33	116745	3500	3.8	763.02	432.67	0.19	13.64	0.14	0.83	0.58	0.90
November	12	66.69	20.93	8.57	392	37.33	36933.33	6211.66	1413.33	215	128333.3	5933.33	4.6	643.76	675.87	0.19	14.8	0.05	0.83	0.14	1.44
December	5.5	108.14	15.15	9.28	504	39	59900	26818.5	115	20	209000	9470	4.9	612.03	857.55	0.18	10.1	0.14	0.72	0.27	1.94

Table 2. Pearson's correlation between phytoplankton groups and physicochemical parameters

	CH	CY	BA	Temp	Sal	AN	pH	COD	BOD	Cl	SO_4	Hard	Mg	Con	Alk	DO	TN	Ph	Cd	Fe	Pb	Mn	Cr	Zn
CH	1																							
CY	0.483	1																						
BA	0.580	0.22	1																					
Temp	0.387	0.295	0.601	1																				
Sal	0.390	0.564	0.149	0.425	1																			
AN	0.300	0.580	0.098**	0.519	0.575	1																		
pH	0.103	0.344	0.194	0.186	0.279	0.348	1																	
COD	0.257	0.269	0.009	0.106	0.668	0.067	0.690	1																
BOD	0.208	0.172	0.220**	0.038	0.302**	0.178	0.190	0.701	1															
Cl	0.408	0.434	0.242	0.266	0.908	0.376	0.347	0.730	0.407	1														
SO_4	0.519	0.204	0.787	0.595	0.246	0.126	0.431	0.401	0.063	0.258	1													
Hard	0.070	0.368	0.590	0.317	0.085	0.099**	0.008	0.123	0.340	0.183	0.205	1												
Mg	0.251**	0.306	0.378	0.043	0.249	0.038	0.105	0.219	0.172	0.317	0.052	0.684	1											
Con	0.282	0.490	0.005	0.412	0.912	0.330	0.408	0.757	0.339	0.956	0.470	0.007	0.240	1										
Alk	0.162	0.354	0.006	0.244	0.602	0.212	0.358	0.562	0.227	0.838	0.455**	0.103	0.288	0.814	1									
DO	0.193	0.641	0.548	0.431	0.121	0.476	0.134	0.249	0.721	0.056	0.345	0.452	0.150**	0.072	0.089	1								
TN	0.356	0.771	0.070	0.357	0.771**	0.516**	0.317	0.510	0.132	0.630	0.343	0.003	0.204	0.692	0.452	0.370	1							
Phos	0.423*	0.445	0.877**	0.683	0.188	0.126	0.303	0.141	0.311*	0.072*	0.766	0.650	0.196	0.319	0.217	0.722	0.314**	1						
Cd	0.202	0.022	0.392*	0.099	0.201	0.353	0.426	0.219	0.354*	0.323	0.196	0.473	0.203	0.129	0.351	0.263	0.223	0.467	1					
Fe	0.240	0.283	0.772	0.254	0.420	0.134	0.040	0.343	0.435*	0.501	0.332	0.786	0.613	0.292	0.268	0.514	0.033	0.652	0.406	1				
Pb	0.041**	0.094	0.078	0.052	0.483	0.182	0.170	0.183	0.453	0.388	0.037	0.245	0.072	0.483	0.189	0.241	0.043	0.270	0.254	0.164	1			
Mn	0.006	0.676	0.748	0.623	0.238	0.381**	0.158**	0.107	0.235	0.124	0.534	0.810	0.591	0.312	0.236	0.669	0.354	0.819	0.272	0.674	0.143	1		
Cr	0.435	0.637	0.184	0.293	0.475**	0.282	0.181	0.336	0.185	0.504	0.241	0.276	0.574	0.527	0.424	0.097	0.370	0.217	0.017	0.158	0.010	0.544	1	
Zn	0.444	0.275	0.778	0.612**	0.135*	0.012	0.318	0.249	0.112	0.038	0.607	0.601**	0.216	0.247	0.108	0.503	0.104	0.891	0.484	0.542	0.302	0.686	0.183**	1

Ch. Chlorophyceae, Cy. Cyanophyceae, Ba. Bacillariophyceae.
*Significant correlation at p<0.01, **significant correlation at p<0.05, bold text indicates negative correlation

groups. Total alkalinity was moderately correlated with temperature, hardness, Mg, TN and Fe.

Nitrate is considered as predominant and most stable inorganic nitrogen form in salt water bodies and known as one of the main nitrogen sources for phytoplankton while the intermediate oxidation state between nitrate and ammonia makes nitrite concentrations useful in the aquatic system. High annual mean value of total nitrogen (TN) was detected at site III, while low values were detected at site II. It showed positive correlation with Chlorophyceae and Bacillariophyceae while negatively with BOD, Cl, conductivity, alkalinity and DO. TN at all sites increased from June to September as nitrifying bacteria increased with water temperature. The oxidation–reduction reactions of bacterial activity affected the concentration of total nitrogen in Lake area. The annual TN varied between 432.75 to 883.01 mg/L. Total nitrogen showed a correlation with cyanophyceae may be related to dependency on nitrogen. Deyab et al (2019) observed the highest values of TN in rainy season.

Phosphorus is considered an essential element for the primary production and growth of phytoplanktons. The annual mean value of phosphate ranged from 224.65 to 886.05 mg/L in the rainy season (minimum) and 0.8 to 6.05 mg/L in winter (maximum). It was reported positively correlated with chlorophyceae and BOD while weakly correlated with bacillariophyceae and TN. The cyanobacterial blooms occurrence is closely linked to the contents of phosphorus and nitrogen in water (Tang et al 2021). Excessive use of fertilizers in agriculture contributes greatly to water pollution due to nitrogen and phosphorus concentrations (Sarkar et al 2020). According to Margalef the phosphorus concentration ranged 0.2 to 2.8 mg/l is suitable for phytoplanktons especially bacillariophyceae and cyanophyceae (Rahman et al 2015). It was maximum at site III and minimum at site I.

Dissolved oxygen is essential for a well-balanced aquatic life. The concentration above 5 mg/L of dissolved oxygen is considered suitable for aquatic animals (Baleta and Bolaños 2016). During summer and autumn, there is almost no vertical water circulation due to thermal stratification and mineral stratification occurring at significant depths. This results in a reduction of the amount of dissolved oxygen (DO) towards the lake's bottom. This action has the potential to upset the delicate balance of aquatic ecosystems and harm the quality of water (Avram et al 2022, Xu et al 2022). Dissolved oxygen was reported less than 5 gm/l at most of the sites. The mean value of DO ranged between 2.6 to 5.0 mg/L at all sites. The maximum was observed in winter while minimum in August. DO has positively correlation with chlorophyceae and bacillariophyceae while negative correlation with temperature, BOD, COD, TN, Cd and weak correlation with Mg. A rise in urbanization and population growth results in stochastic anthropogenic nutrient supplies to the water, causing depletion of the aquatic oxygen supply (BR and Sivakumar 2024).

Apart from heavy metals, pollutants such as fluorides (F^-) and nitrates (NO_3^-) can harm human health and aquatic ecosystems (Githaiga et al 2021). Trace metal concentration was recorded by collecting water samples once a month seasonally. The results revealed the presence of cadmium, zinc, iron, lead, manganese and chromium. Cadmium was in upper permissible limit from 0.027 to 0.56 mg/L and was completely absent in March and September. Positive correlation was observed between cadmium and cyanophyceae but negative with chlorophyceae and bacillariophyceae. It was significantly correlated with BOD, Cl and Cr. Iron (Fe) ranged between 1.2 to 14.5 mg/L. It was reported positively correlated with chlorophyceae, bacillariophyceae, SO_4 and hardness. lead (Pb) and was between 0.24 to 2.5 mg/L maximum at site III in winter and lowest at site I in summer season. The positive correlation was observed between salinity, pH and COD but negative correlation with all phytoplankton groups with BOD and Cd. Manganese (Mn) was minimum at site II with mean value 0.14 mg/L and maximum 0.86 mg/L in winter season. Mn was positively correlated with bacillariophyceae, moderately correlated with AN, pH but negatively correlated with BOD and TN. Chromium (Cr) ranged between 0.51 to 0.58 mg/L. Waste from chromate-processing facilities can contaminate water bodies if improperly disposed of in landfills where chromium may be deposited for several years. Cr was positively correlated with bacillariophyceae and all presented heavy metals but negatively correlated with temperature and TN. Zinc (Zn) is an essential element for animals and plants but its presence in excess amounts may be harmful (Baricz et al 2021). The values of Zinc varied from 0.1 to 2.01 mg/L. The positive correlation was studied between chlorophyceae and bacillariophyceae while a negative correlation with BOD and TN. The significant correlation was with temperature, salinity and hardness. The presence of cadmium, chromium, manganese and lead was in upper permissible limit throughout the year. These results of fluctuation in physicochemical parameters with seasons attributed to the impact of various contamination sources.

Biological analysis: Chlorophyceae also known as blue green algae plays a crucial role in the global nitrogen, carbon and phosphorus cycle due to its high tolerance for weather conditions. Cyanophyceae, found in this study is a group of major photosynthetic organisms that is found in various aquatic environments. The cyanophyceae has photosynthetic pigments that result in a distinct turquoise color. Certain groups of cyanophyceae have the ability to fix nitrogen, which makes them significant for aquatic environments (Arsad et al 2021). Cyanophyceae are highly sensitive to copper, cadmium, and zinc metals (Agawany and Kaamoush 2023). To study the presence of halophilic microalgae the water samples were collected from brines of the Sambhar Lake area in all seasons of the year 2020 and 2021. The variety of colors indicated the dominance of algal diversity during the winter season (green and orange color) and bacterial diversity was dominant (pink color) in summer depending on pH and salt concentrations. Depending on pH and salt concentrations, a variety of colors showed that microalgal diversity was predominated in the winter (green and orange color).

Three phytoplankton groups were identified in all seasons. Chlorophyceae, cyanophyceae and bacillariophyceae were the predominant groups. Cyanophyceae was maximum in number followed by Chlorophyceae. The maximum number of total phytoplankton was in winter at site II and III while the minimum in rainy season at site I. The growth of chlorophyceae and cyanophyceae algae was heightened by the availability of Nitrogen and Phosphorus content in the winter and rainy seasons at all collection sites. These elements limited the primary productivity of algal biomass in the lake area (Carstensen et al 2018). Phosphorus is considered an essential element for the primary production and growth of phytoplankton in aquatic ecosystems (Turner et al 2023). The positive correlations were observed between the physicochemical parameters and biomass of most phytoplankton groups (Table 2). Members of chlorophyceae were positively correlated with cyanophyceae and bacillariophyceae. Chlorophyceae was moderately correlated with phosphate, Mg, DO, TN, alkalinity. Cyanophyceae phytoplanktons showed a significant correlation with temperature, BOD, alkalinity and TN. Bacillariophyceae indicated a positive correlation with pH, sulphate, conductivity and DO and a moderate correlation with AN, BOD, phosphate and Cd. According to this research all phytoplankton groups reported positive correlation with each other.

CONCLUSION

The physicochemical parameters investigated in the present study showed variability depending on season and location and correlation with phytoplankton groups. The high concentrations of physicochemical parameters during the dry seasons in the lake area were due to the low incidence of rainfall that may be caused by overexploitation of catchment areas for salt production and climate change. Three Phytoplankton groups were identified on most of the studied sites during all seasons and considered as natural bioindicators of pollution and water quality status due to their high proliferation rate, ease of handling and low cost.

The variability of different physicochemical parameters affected phytoplankton distribution and had a significant positive relationship with water pH, temperature, salinity, nitrogen, total alkalinity, ammonia and phosphorus. However, the information gathered from this study can serve as a baseline for additional research in the future, which can be utilized to monitor the situation, make management plans, and create mitigating measures for the conservation of water and biodiversity.

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Corncob Biochar Production Using Super Sun Retort Combined with Kon-Tiki Kiln

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Abstract: In Thailand, crop residues are abundant but underutilized, creating a need for better valorization methods like biochar production. Firewood is commonly used to initiate pyrolysis in biochar retorts, but this process is inefficient and produces high emissions. This study evaluated biochar production from corncobs using the Super Sun retort with heat from the Kon-Tiki kiln, aiming to reduce pollution from firewood burning. Pyrolysis temperature and biochar properties, including electrical conductivity, calorific value, and iodine value, were measured. Each experiment used 20 kg of firewood and 25 kg of corncobs. Results showed complete conversion of corncobs into biochar, with a yield of up to 26%.The Super Sun retort produced biochar at high temperatures (>800 °C) more efficiently than conventional methods. The corncob biochar demonstrated good electrical conductivity and an iodine value of 230 mg/g. Its calorific value was 7300 cal/g. The findings indicate that the corncob biochar obtained in this study has potential applications in soil amendment and in the production of deodorizing charcoal and charcoal briquettes. The results also suggest that the combination of the Super Sun retort and Kon-Tiki kiln could offer a more sustainable and cost-effective solution for biochar production in developing countries.

Keywords: Biochar, Corncob, Crop residues, Kon-Tiki kiln, Super Sun retort

Maize (*Zea mays* L.) residues, especially corncobs are abundant, but they remain underutilized in Thailand (Schweikle et al 2015). Many farmers still practice open field burning of crop residues, which causes both air pollution and health problems (Junpen et al 2018). Therefore, the need exists for a better valorization of biomass residues such as corncobs. Production of biochar from crop residues has been promoted by the government, research institutions, and non-governmental organizations, which intended to eliminate the open field burning of crop residues and create value-added products from biomass waste (Bhattet al 2022, Mbah et al 2022). Biochar retort made of 200 L steel drum is widely used in Thailand. Firewood was widely used as an energy source to initiate the pyrolysis process in the biochar retort. The current use of firewood in an open fire is associated with low efficiency and high emission. A novel technology called the Kon-Tiki kiln combines the simplicity of traditional kilns with the combustion of pyrolysis gases in a flame curtain similar to retort kilns, which could be used to generate heat for starting-up the pyrolysis process in the biochar retort. This study aimed to evaluate the production of biochar from corncobs with the Super Sun retort by using heat generated from the Kon-Tiki kiln. This approach would help reduce air pollution caused by biomass burning during biochar production. The data on pyrolysis temperature was collected. Biochar properties such as electrical conductivity, calorific value, and iodine value were determined.

MATERIAL AND METHODS

Biomass feedstock: The corncobs were collected from the National Corn and Sorghum Research Center, Faculty of Agriculture, Kasetsart University (Fig. 1a). They were the biomass residues from seed production. The corncobs had already been dried during the seed production process and thus did not require further drying before biochar production. Their moisture contents (wet basis, wb) were less than 9%. The whole corncobs without size reduction were used as the feedstock for biochar production. The corncobs had an average diameter of 25 mm and length of 133 mm. The firewood used in this study was obtained from a wood furniture manufacturer located near Kasetsart University (Fig.1b). The wood furniture manufacturer provided the biomass waste with no charge. The firewood consisted of different kinds of wood and had inhomogeneous size distribution. The moisture contents of the firewood were less than 4%.

Biochar production equipment: The Super Sun retort and the Kon-Tiki kiln were the biochar production technology used in this study (Fig. 2). The Super Sun retort was made of a standard 200 L steel drum. The dimension of the steel drum was 89.5 x 59.5 cm (height x diameter). A ceramic fiber was used as insulation material for the biochar retort. The thickness of the insulation material was 2 cm. A metal grate made of steel bars was place at 10 cm above the bottom of the biochar retort to prevent the blocking of the gas outlets and carry the weight of the biomass feedstocks. In addition, a perforated metal sheet with round holes of 6.5 mm (diameter) and thickness of 0.5 mm was placed on top of the metal grate, which prevented the corncobs from falling under the metal grate. A chimney with a dimension of 10 x 10 x 93 cm (length x width x height) was installed at the center of the steel drum. There were 12 holes with a diameter of 9 mm at the bottom of the chimney, which were the outlets of the pyrolysis gas. The pyrolysis gas was burnt at the bottom of the Super Sun retort, which heated up the retort and sustained the pyrolysis process.

The Kon-Tiki kiln was made of stainless steel 304 with a thickness of 1.5 mm. The kiln had a cone shape with a wall inclination of 60°. The upper diameter was 72 cm and the height was 35 cm. The diameter at the bottom of the Kon-Tiki kiln was 32.5 cm. A metal frame was constructed with rectangle metal bars. The dimension was 77 x 77 x 41 cm (length x width x height). The metal frame was placed above the Kon-Tiki kiln and the Super Sun retort was put on the frame.

Biochar production process: In each experimental run, 25 kg of corncobs were loaded in the Super Sun retort around the chimney. The lid was tightly closed with a galvanized locking ring. A thermocouple was installed in a hole on the lid of the biochar retort. A layer of sand was put on the lid as an insulation material. In the Kon-Tiki kiln, 20 kg of firewood was used. At first, the firewood was loaded until the top of the Kon-Tiki kiln. The fire was ignited on the top of the firewood with some tinder. A thermocouple was attached to the metal frame and used to measure the temperature above the Kon-Tiki kiln. A data logger was used to record the temperature in the Super Sun retort and Kon-Tiki kiln every 10 min. During the experiment, when the temperature in the Super Sun retort decreased, more firewood was loaded in the Kon-Tiki kiln. The pyrolysis process in the Super Sun retort was considered completed, when there was no combustible gas released from the chimney. After the temperature in the Super Sun retort was below 100 °C, the corncob biochar was carefully transferred to an empty 200 L steel drum for cooling. The lid of the steel drum was closed with a galvanized locking ring. The fire in the Kon-Tiki kiln was extinguished with clean water. The weight of the corncob biochar was recorded after 12 h of cooling at room temperature. The biochar was stored in an airtight plastic bag until use.

Characterization of biochar: The biochar yield $Y_{biochar}$ was calculated on air-dried basis (ad), as follows:

$$Y_{biochar,\ ad}\ (\text{wt. \% ad}) = 100 \times \frac{M_{biochar}}{M_{biomass}}$$

where $M_{biochar}$ is the mass of biochar (kg), $M_{biomass}$ is the total mass of biomass (kg), and $Y_{biochar,\ ad}$ represents the air-dried basis yield of biochar (%).

The electrical conductivity of the corncob biochar was tested using a conductivity tester with a light bulb. The oven drying method for measuring moisture content (MC) was carried out based on the standard method of German Institute for Standardization (DIN 51718 2002). The determination of iodine number was performed according to the standard method ASTM D4607-14 (2021). The gross calorific value was measured based on the standard method ASTM D5865 (2021).

RESULTS AND DISCUSSION

Temperature profiles in the Super Sun Retort and Kon-Tiki kiln: In the first experimental run, after 30 min the temperature in the Super Sun retort reached 249 °C (Fig. 3). At this temperature, the volatile gases were released from the corncobs. It was observed that at 40 min there was thermal overshoot, where the temperature in the Super Sun retort

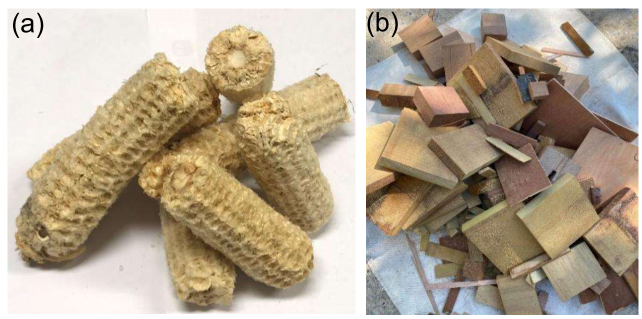

Fig. 1. (a) Corncobs used as feedstock in the Super Sun retort (b) firewood used in the Kon-Tiki kiln

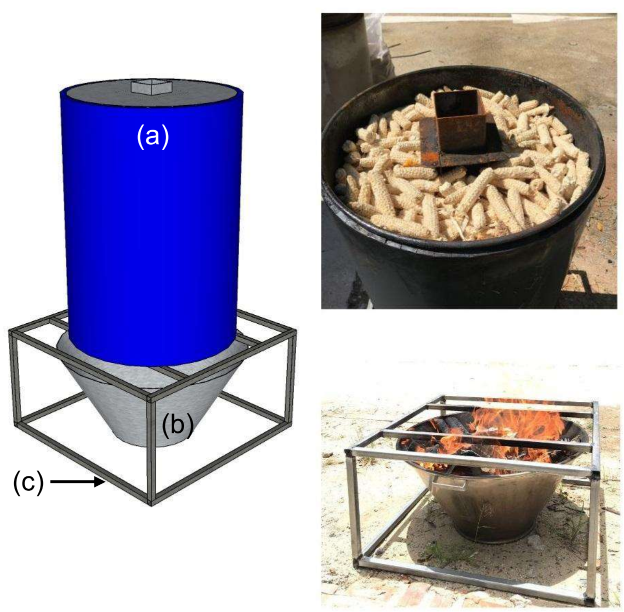

Fig. 2. Biochar production equipment including (a) Super Sun retort, (b) Kon-Tiki kiln and (c) metal frame

was significantly higher than that of the Kon-Tiki kiln. This phenomenon was resulted from the exothermic reactions of the pyrolysis process. This result was also reported in the previous study (Intani et al 2016). However, the thermal overshoot in the first experimental run was quite extreme. Therefore, more experiments need to be carried out in the future to verify this phenomenon. In another study, a thermal runaway or uncontrolled ignition/combustion was observed in the pyrolysis of some lignocellulosic biomasses. The thermal runaway happened when there was a sudden release of large quantities of volatile products from the biomass in pyrolysis process (Di Blasi et al 2014). It was also reported that the size of the biomass feedstock had an effect on the thermal runaway (Di Blasi et al 2015). The whole corncobs used in this study might promote high reaction exothermicity and lead to the thermal runaway. The thermal runaway resulted in the highest pyrolysis temperature of 868 °C in the Super Sun retort, while the highest heating temperature in the Kon-Tiki kiln was 469 °C. After 120 min, the pyrolysis temperature reached 868 °C, when all volatile gases in the corncobs were released. Subsequently, the pyrolysis temperature significantly decreased, while no additional firewood was loaded to the Kon-Tiki kiln.

The temperature profiles in the Super Sun retort and Kon-Tiki kiln were different in the second experimental run compared to those of the first experimental run (Fig. 4). This result indicated that the precise control of the temperature in the Super Sun retort and Kon-Tiki kiln is very difficult. In particular, the temperature in the Kon-Tiki kiln showed high fluctuation. Therefore, the position and the method of measuring temperature above the Kon-Tiki kiln should be reconsidered and improved. In additional, the manual control of the combustion process in the Kon-Tiki kiln was also very challenging. It needs experience, good attention and careful observation. The highest heating temperature in the Kon-Tiki kiln was 561°C, while the highest pyrolysis temperature in the Super Sun retort was 563°C. Therefore, the thermal overshoot and thermal runaway were not observed in the second experimental run. However, the pyrolysis temperature reached the highest value (563°C) after 140 min, which was only 20 min different from the first experimental run. This indicated that the corncobs released all volatile gases within 140 min after starting the pyrolysis process. This information would help for the design of future experiments.

Biochar yield: The corncob biomass was successfully converted into biochar in the Super Sun retort using the heat generated from the Kon-Tiki kiln (Fig. 5). Super Sun retort and Kon-Tiki kiln generated lower emission compared to the conventional methods of biochar production in Thailand. In a previous study, it was evident that the Kon-Tiki kiln showed the lowest gas emissions including mainly methane and carbon monoxide (Cornelissen et al 2016). The corncob biochar yield was 26.4 and 26.8% in the first and second experimental run, respectively (Table 1). The corncob biochar yield was similar to the value reported in the previous study (Intani et al 2016). Interestingly, the difference between the biochar yields from the two experiments was not significant, despite of the thermal overshoot and thermal runaway in the first experimental run. This could be due to the size of the biomass feedstock (Di Blasi et al 2015). In this study, the size of the corncobs was not reduced. Therefore, the thermal overshoot and thermal runaway did not significantly affect the biochar yield. Except from the corncob

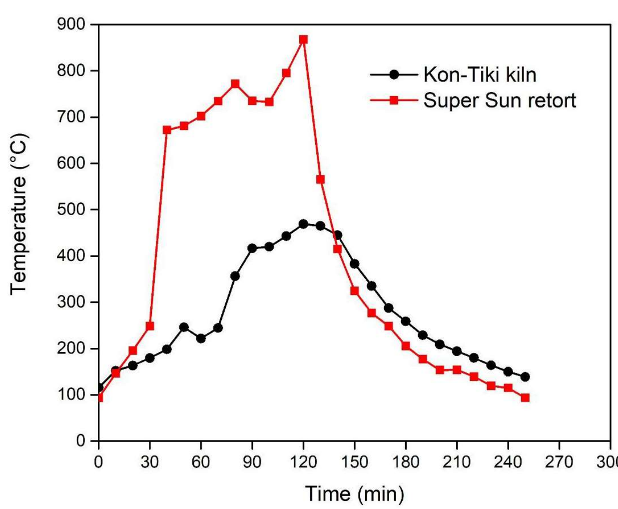

Fig. 3. Temperature profiles in the Kon-Tiki kiln and Super Sun retort in the first experimental run

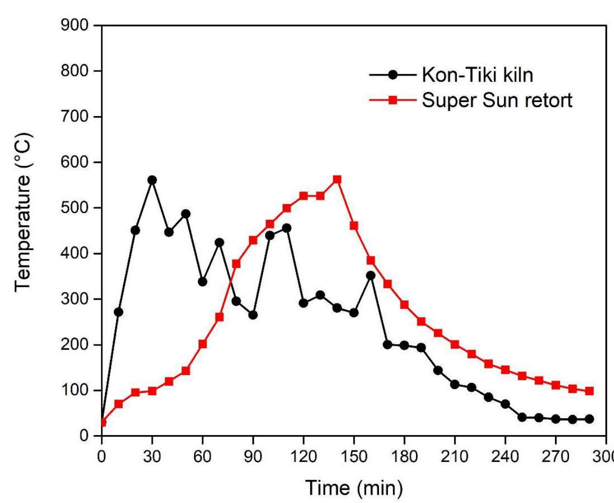

Fig. 4. Temperature profiles in the Kon-Tiki kiln and Super Sun retort in the second experimental run

biochar obtained from the Super Sun retort, the biochar from firewood was also produced in the Kon-Tiki kiln. The biochar from the Kon-Tiki kiln was found to be suitable for soil amendment (Pandit et al 2017).

Biochar properties: The electrical conductivity of the corncob biochar was moderate. In total 10 samples were tested using a conductivity tester. The result showed that 5 samples had a good electrical conductivity (Table 2). It was reported that the biochar produced at high temperature (>700 °C) had high electrical conductivity (Bartoli et al 2022). The moisture content of the corncob biochar was 7%, which was lower than that of the corncob biomass (8.4%). The iodine number was 230 mg/g, indicating a good adsorption capacity. This iodine value of corncob biochar was higher than that of the biochar of nut shell (Gorshkov et al 2021). This result implied that the corncob biochar had high porosity. The gross calorific value of the corncob biochar was 7300 cal/g, which was high and indicated the potential to be used as a biofuel. The calorific value of the corncob biochar in this study was comparable to the value (6872 cal/g) reported in the previous study (Intani et al 2016).

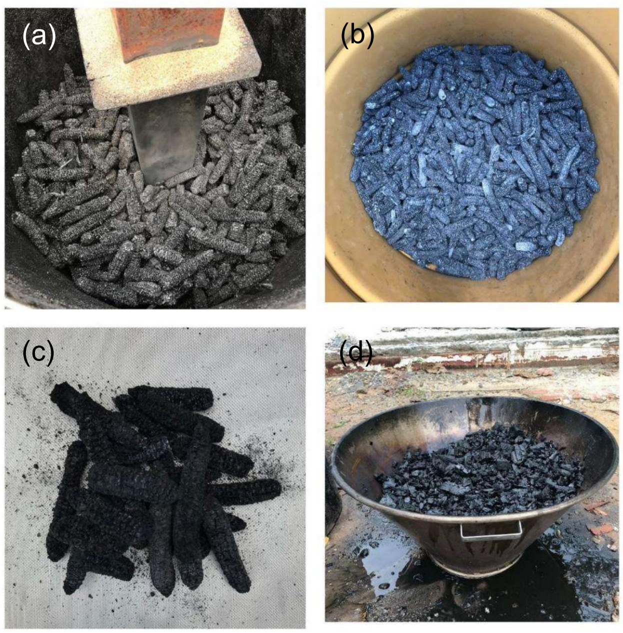

Fig. *5* . (a) Corncob biochar produced in the Super Sun retort, (b) corncob biochar kept in a sealed steel drum for cooling, (c) close-up image of the corncob biochar, (d) biochar produced in the Kon-Tiki kiln

Table 1. Biochar yield

Experimental run	Corncobs (kg)	Biochar (kg)	Biochar yield (%)
1	25.0	6.6	26.4
2	25.0	6.7	26.8
Mean	25.0	6.7	26.6

Table 2. Biochar properties

Parameter	Value
Electrical conductivity (%)*	50
Moisture content (wt.% wb)	7
Iodine number (mg/g)	230
Gross calorific value (cal/g)	7300

* 5 out of 10 samples showed good electrical conductivity

CONCLUSION

The heat provided by the Kon-Tiki kiln was sufficient to initiate and sustain the pyrolysis process in the Super Sun retort. The amount of corncob biomass was 25 kg, while 20 kg of firewood was consumed. The highest temperature measured in the Super Sun retort was 868 °C. The highest biochar yield of 26.8% was obtained. The corncob biochar showed high adsorption capacity with iodine value of 230 mg/g. The calorific value of the biochar was also high (7300 cal/g). The results indicated that the corncob biochar produced with the sustainable and low-cost techniques in this study had potential to be used for soil amendment and the production of deodorizing charcoal and charcoal briquette.

AUTHOR'S CONTRIBUTION

The experiment was conceptualized by K.T., K.I., D.J. and S.S., and the methodology was applied by K.T., K.I. and D.J.; software was employed by K.T., K.I., P.S. and R.T.; the manuscript was validated by K.T., K.I., D.J., P.S., R.T. and S.S.; formal analysis and investigation were mainly conducted by K.T. and K.I.; resources were acquired and provided by K.I., D.J., R.T., P.S. and S.S.; data curation was conducted by K.T. and K.I.; writing-original draft preparation, K.T. and K.I.; writing-review and editing, K.I., R.T. and S.S.; data visualization was realized by K.T. and K.I.; supervision of the project was conducted by K.I., D.J., P.S., R.T. and S.S.; project administration, K.I. and S.S.; funding acquisition, K.I. and S.S. All authors have read and agreed to the published version of the manuscript.

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Artificial Intelligence Robotics Technologies for Harvesting Horticultural Crops: An Alternative Management Approach

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***Abstract*:** The horticulture sector is very important in the agricultural industry of the Indian economy. The sector has huge potential to make a significant contribution to agricultural exports, but product quality is often a debatable issue in global markets. Global production of fruit has been growing rapidly, leading to increased competition in export markets. The global fruit industry can increase its competitiveness by adopting more effective fruit production and competent harvesting systems. Fruit production for the fresh market accounts for 60 % of the total labour requirement. Fruit harvesting is a seasonal activity and skilled labour in harvesting on time is quite problematic. Many farmers are concerned about the fact that there is uncertainty and a shortage enough labour during the peak season of harvesting. Most processes are done manually using traditional tools, increasing the total cost of production, and a decrease in net income for farmers. Therefore, there is a need to adopt the proper technologies to ensure that apple growers get benefits.One solution to these problems is AI robotic technologies capable of meeting complex tasks and having the potential to modernize the horticulture sector in the world. The goal of this paper is to provide an overview of the global growth and current status of development and scope of robotics in horticulture crops based mainly on literature established in several countries in recent years.

***Keywords*:** Robotics fruit harvesting, Robotic picking, Atomization, Apple harvest, Fruit sensors, Horticulture

The Indian agriculture sector is developing enthusiastically day by day. Horticulture has been an emerging sector in agriculture and is increasingly recognized as a sunrise sector accounting for 90% o of the total horticulture production in the country (Herrick 2017). Horticulture is increasingly recognized as a sunrise sector, owing to its potential to raise farm income, provide livelihood security and earn foreign exchange through export as shown in Figure 1. The diverse agro-climatic conditions and rich diversity in crops and genetic resources enable India to produce a wide range of horticultural crops, which consists of crops like vegetables, fruits, flowers, mushrooms, tuber crops, spices, plantation, aromatic, and medicinal plants. The horticulture sector encompasses a wide range of crops like fruits, vegetables, flowers, spices, and plantation crops like coconut, beverages like tea and coffee, and some medicinal and aromatic plants. Statistics provided by the National Horticulture Development Board indicate that, by accounting for 13% of the global production of fruits and 21% of vegetables, India is the second largest producer, after China, in both the commodity groups (Horticultural Statistics at a Glance 2021, Anonymous 2021). State-wise production of fruit crops is shown in Table 1. In recent years, horticulture has made significant progress in terms of increased area and production under various crops, increased productivity, crop diversification, technological interventions for production, and post-harvest and forward association through value addition and marketing. As per the report, the total horticulture production in the country is estimated to be 313.85 million tonnes which is 0.69% higher than the horticulture production of 311.71 million tonnes in 2017-18. The area under horticulture crops has increased to 25.49 million hectares in 2018-19 from 25.43 million hectares in 2017-18. The increasing population and the mindset of adopting a healthy lifestyle have increased the demand for nutritional requirements in people, which provides vast chances for sustaining a large number of agro-based industries which creates substantial employment chances.

The horticulture sector has the potential to generate multiple sources of income, thereby boosting the economic growth of a country. Horticulture can be undertaken as market-driven cultivation of vegetables, fruits, and flowers, as eco-tourism, as therapeutic medicinal plant harvesting, and as a part of multiple farming to complement the main source of income. In tune with the emerging demands, India brought forth several technology and policy initiatives for promoting horticulture. The most important among them is the newer technology packages spanning from production to post-harvest (Anonymous 2019a). Protected cultivation, precision technologies including automation, and usage of biotechnology are some of the examples in this direction. Also, newer initiatives were made in the sphere of infrastructure development including cold storage, quality assurance, and streamlining and handholding to participate in the export markets. Further, the Government has facilitated the emergence of newer institutional mechanisms to strengthen vertical and horizontal linkages through contract farming. Another significant dimension is to capitalize on the power of collectives. The formation of Farmer Producer Companies that could bring about the sea change in the input and service delivery systems is promoted. Evidence suggests that the net return in horticultural crops is higher than in other crops. The government of India has proposed to double farmers' income by the year 2025. It is increasingly being recognized that horticulture will remain an integral component of the strategy to achieve this goal.

The mechanization of fruit harvesting, especially those assigned to fresh or new markets, is extremely needed in almost all horticultural countries because of the low seasonal labour service in many countries. Some fruit-harvesting technologies are designed particularly for processing purposes only with limitations in their use for soft and fresh fruits because the fruit is susceptible to mechanical damage during mechanical harvesting (Anonymous 2019b). The alternative to modern mechanical harvesting systems, very superior but much more ambitious in terms of fruit quality, is an automated robotic system for fruit harvesting or picking. Robots can work well in a well-designed or controlled environment in which the direction and location of the target are very well known, or the object can be placed in the right place and desired direction. The detailed representation of the conventional arrangement and attributes associated with a harvesting robot that enable these machines to efficiently perform their tasks in the field is shown in Figure 2.

But nowadays, in the scientifically and technologically advanced environment, robotic systems or automated machines are used in unusual places or non-traditional zones, where the environment is used in optically guided warfare, medical robots, and agricultural robots (Kumar and Bector 2022). Currently, the Center of attraction and attention of much research on robotic fruit harvesting is the design of a harvesting system that copies the accuracy of a human harvester while reducing the labour requirements and increasing the efficiency of operations of a purely mechanical harvester. The comparison between traditional and modern automated harvesting methods is shown in Figure 3, which helps to gain insights into the various aspects of both traditional and modern automated harvesting, including efficiency, cost-effectiveness, and environmental impact. The classic design of a robotic fruit collector consists of the visual system of fruit detection, the stimulus to move towards fruit, and the end effecter for fruit plucking and harvesting. Connecting the fruit detection algorithm to an automatic harvester is an important part of the vision-based robotic fruit harvester. The idea is to extract information from the vision-based system about the spotted fruit and turn this information into instructions to direct the automated system to the right situation and to make harvesting a reality (Patel and George 2012).

The challenge of developing a cost-effective robotic system for fruit picking has been taken up by researchers at several places in the world. The major problems that have to be solved with a robotic picking system are recognizing and locating the fruit and detaching it according to prescribed criteria, without damaging either the fruit or the tree. In addition, the robotic system needs to be economically sound to warrant its use as an alternative method over manual methods. Researchers in several places around the world face the challenge of developing a more efficient robotic fruit collection system. The main issues to be solved using an automated collection system are the recognition, detection, and distribution of fruit according to established criteria without harming fruit or tree (Fu et al 2020). In the nineties of the 20th century, the new improved mechanical technologies in harvesting, achievements in computer interference, image interpreting technologies, and developments in tree design and fruit processing systems led to the development of a new generation of robotics in harvesting. Such technologies were more suitable and effective for fresh fruits, with higher yields and minimum damage. The major contributors to the development of such technologies were Europe, the United States, and Japan.

METHODS OF ROBOTIC HARVESTING

Two types of harvesting are used by horticultural practitioners to decrease the overhead output of horticulture from labour costs:

Selective harvesting: This is a selective method of harvesting by the robotic systems that use robotic manipulators equipped with an end-effector for grasping the fruits. They are usually installed on a mobile platform with machine vision technology for vision and the end-effector selectively separates mature fruit (Bac et al 2014) as shown in Figure 4 and 5. Since robotic systems can combine machine efficiency with long-term goal line (Shevfelt et al 2014), an automated harvesting technique is thought to have the potential to completely substitute the human pickers (Sanders 2005). Therefore, this method of selective harvesting has received widespread attention from both academia and industrial sectors and emerged as the ideal method of harvesting horticultural crops among fruit growers. The rapid development in artificial intelligence (AI) and robotics technologies has paved the way for commercial automated techniques for selective harvesting.

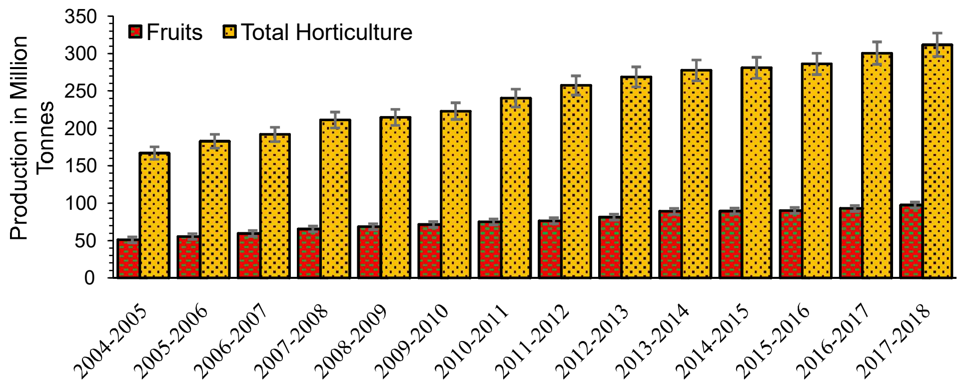

Fig. 1. Increasing trend inthe horticulture sector of India (Anon 2020a)

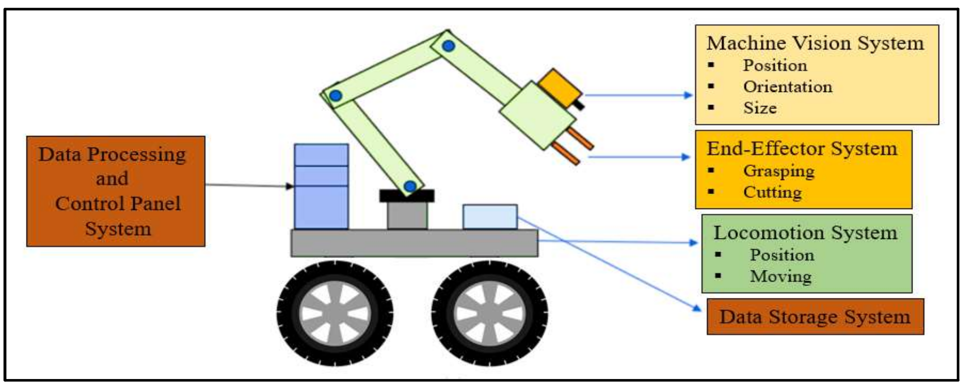

Fig. 2. Standard design and elements of a harvesting robot

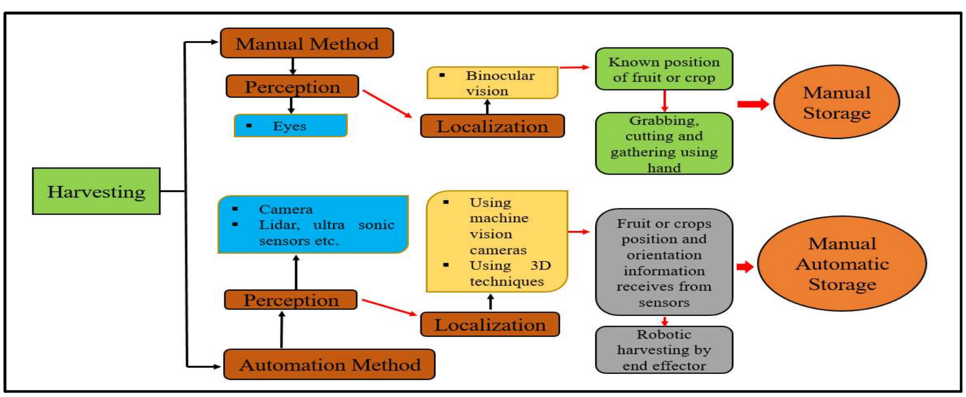

Fig. 3. Manual Vs Automated Harvesting

Fig. 4. Selective harvesting of apples with vision technology and end-effector manipulator

Bulk harvesting: The bulk harvesting method is based on the principle of using oscillation or vibration force on fruit trees to force the fruits from the trees (Mehta 2016) as shown in Figure 6. This type of harvesting method is implemented by many apples and cherries fruit growers (De Kleine and Karkee 2015, Zhou et al 2016). Although large-scale bulk harvesting systems are highly efficient (Sola-Guirado et al 2020), there are significant drawbacks. Farmers have expressed concern about extreme damage to canopies and fruit caused by mechanisms (Moseley et al 2012). The research studies to reduce bulk harvest damage remains active as fruit spoilage affects its market acceptance (Pu et al 2018, Wang et al 2019). Another big disadvantage of the bulk harvesting method is that the quality of the harvested fruits can change dramatically since less mature fruits are also harvested with mature fruits. The coordination of fruit and vegetable maturity rates throughout the entire field is not a trivial job, and under a huge harvest scheme, harvest time can be based on minimizing the losses because of collecting immature and over-mature fruits during harvesting.

Table 1. State-wise production of fruit crops in India

States/UTs	Area ('000 ha)	Production ('000 ha)
Andra Pradesh	718.91	1761.67
Arunachal Pradesh	48.14	125.84
Assam	167.2	2518.89
Bihar	313.95	4384.46
Chhattisgarh	225.24	2580.31
Gujarat	433.79	9927.26
Haryana	67.28	712.02
Himachal Pradesh	230.852	571.739
Jammu & Kashmir	345.39	2564.27
Jharkhand	105.39	1111.96
Karnataka	395.55	6567.293
Kerala	321.36	1885.97
Madhya Pradesh	357.01	7464.97
Maharashtra	756.97	10822.77
Manipur	46.74	451.23
Meghalaya	35.75	331.67
Mizoram	62.91	339.18
Nagaland	33.94	315.34
Odisha	337.29	2361.13
Punjab	94.8	2001.69
Rajasthan	62.35	919.9
Sikkim	19.54	55.45
Tamil Nadu	293.97	5767.95
Telangana	175.9	2034.29
Tripura	53.702	555.473
Uttar Pradesh	480.53	10651.26
Uttarakhand	178.8	670.63
West Bengal	266.33	3829.85

Robotic Harvesting Technologies for Horticulture Crops

Robots in the horticultural sector are widely utilized in harvesting, drone spraying, and field monitoring, sorting, grading, and packing of final horticultural produce, nurseries, and greenhouses to some extent. Several robots are already being designed for fruit cultivation as well. These AI robots can perform many heavy tasks and repetitive work with good efficiency in no time without any tiredness.

Fruit recognition, end-effector, and detachment: The first step in automated robotic harvesting is to spot the fruits and estimate their 3D location in the canopy of the tree so that the end effector can grasp the target fruit and separate it from the tree. Extensive research studies on the detection of fruits and obstacles using precise features such as shape, colour, edge, size, and texture, including different thresholds and classification techniques such as neural networks and Bayesian classifiers (Silwalet al 2014, Tabb et al 2006). However, these technologies based on the precise feature techniques, have limited success due to issues such as clogging, fruit gathering, unstructured, variable lighting conditions, various uncertainty conditions, and crop and canopy variability. To meet the challenge of fruit gathering, the convex hull technique is being used for the identification of individual citrus fruits and their center in the images with overlapping bunches of fruits with an assumption that the shape of the fruit is round in images (Changhui et al 2017). This is particularly useful when dealing with images where multiple fruits are closely packed together, making it difficult to distinguish between them. The convex hull technique helps in accurately identifying and locating each fruit's center, which is essential for various applications such as fruit grading, yield estimation, and quality assessment. Similarly, Wang et al (2017) developed an image enhancement technology that involved the Retinex principle and wave conversion to reduce problems related to fruit identification under changing lighting conditions.

Approaching the fruit is a key step in the robot's harvest, which mainly involves determining the optimal path and shifting the provincial final end-effector to the target fruit to complete the separation of the fruit from the tree. Approaching the fruit with visual surveying involves frequently identifying the fruit and changing its position using a recent end-effector based on the imaging system, as well as changing the position of the manipulator's joint (Mu et al 2020). An alternative to visual surveying is the use of a universal camera system, installed in a fixed position for capturing the images at the beginning of several harvest cycles. Next, the fruit condition of all fruits in the field of view or the given working space at the beginning of the harvest cycle is assessed. Once the live position of fruits and end-effector is assessed, the reverse kinematics is used for estimation of the new position of all robotic manipulator joints to finally move the end-effector to the chosen final position and direction (Fig. 7). Among the challenges facing this technology are accurate detection and detection of fruit from the beginning, and correct calibration between cameras coordinates and processor coordinates so that the end-effector can accurately reach the fruit.

A variety of end-effector techniques are used to harvest the fruit. One of the techniques is to isolate fruits using a mechanical end-effector with hands and fingers like humans. A soft Palm is used to prevent the fruits from being damaged. The fingers of the end-effectors of the robotic harvesters are designed with a hollow space finger from the inside and have a wrinkled surface on the outside. When the hollow space of the finger area is filled with air, the fingers approach the target fruit as the compressed air extends beyond the curved surface. This type of hollow finger can work faster than an electric or motor-operated manipulator (Figure 8). In addition, the soft hand provides a degree of cushion for the separated fruit. In general, however, these fingers are thicker than traditional fingers, making it difficult to harvest tightly packed fruits or fruits in groups (Shintkek et al 2018).

Another variety of end-effectors uses scissors types that cut the stem off to separate the fruit.However, it is acknowledged that the detection and location determination of stems for cutting purposes with scissors-type end-effectors is an extremely challenging task. To solve this problem, the cup-shaped scissors closed around the fruit can be used to cut the stem regardless of its position (Li et al 2011). This type of technique is more appropriate for fruit types with long stems. Different designs are used in mechanical hands, including a different number of fingers and actuators to control the fingers. One way to fit the finger is electric motors that carry each generation within reach, which requires multiple actuators in one hand, making them relatively slower, more complex, and more expensive. Another way is to use a tandem design, in which a single central motor pushes the fingers with a cable so that the hand can remain close to the target fruit to confirm the shape and size of the fruit when the desired quantity of force is applied in the cable (Davidson et al 2016).

Most of the technologies use one or two actuators which are either pneumatic or electric, whereas the number of actuators varies from a maximum of up to four in the current robotic harvesting technologies for the detachment of fruits. The choice of manipulative degrees of freedom plays an important role in harvesting processes. The ergonomics of different fruits are different. Seven out of 39 robotic processor technologies use electric actuators. Electric servo motors and stepper motors are used to process places with low weight and load**.** Hydraulic and pneumatic actuators are used for heavy payloads because of their high power-to-weight ratio. The breakdown of actuators used in the robotic harvesting technologies is shown in Figure 9.

Deep learning methods based on the artificial neural network: Deep learning has been successfully used to address various fruit recognition challenges in recent years. These methods based on artificial neural networks have been extensively studied and explored. With a multi-layered future, deep learning methods can form more high-level traits. Both low-level and high-level functions can be analysed and used to reveal the end goal. The deep learning method was used to detect and localize mangoes (Stein et al 2016). Similarly, Chen et al (2017) detected and counted oranges and apples with a fully convolutional neural network (F-CNN). Among many types of deep learning techniques, convolution neural network (CNN) is a more sophisticated method involving convolution and back dissemination to extract and capture the target goal (Fig. 10), thus significantly improving the accuracy and generalization of the recognition algorithm (Koirala 2019).

Depth images taken by the RGB-D camera can be used to detect the fruit. Fruit spotting in RGB images can be affected by changes in ambient lighting, maturity status, and uncertain background structures. RGB and depth images were integrated to detect kiwi fruit, and a 3% higher recognition rate was documented (Sa et al 2016). Researchers are working to use multimedia sensors and multiple images to detect fruit in complex orchard environments. Even though, these deep learning techniques and methods can be employed in various types of raw data, such as infrared images, depth images, RGB images, or various even combinations of them, to achieve high accuracy in fruit detection, the algorithm training requires a long amount of quality time as well as a huge number of raw images with labelling.

Robotic Technologies for Management of Orchards

In horticulture, robots are widely used in harvesting, field surveillance, drone spraying, grading, sorting, and packaging of horticultural products, greenhouses, and nurseries. Robotics Plus is working on versatile Orchard AI-Robotics projects. The objective of the project is to automate the fruit harvesting and pollination of apples and kiwi fruits by developing a centralized system in which other modules can be supplemented for various purposes such as spraying,

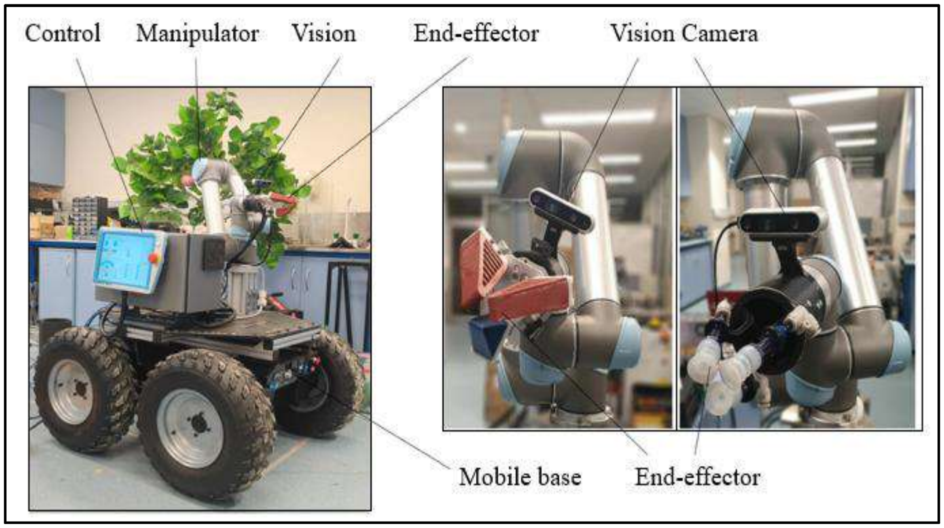

Fig. 5. Robotic harvesting system with vision sensors and different designs of end-effector

Fig. 6. Bulk harvesting method using mechanical tree shaker

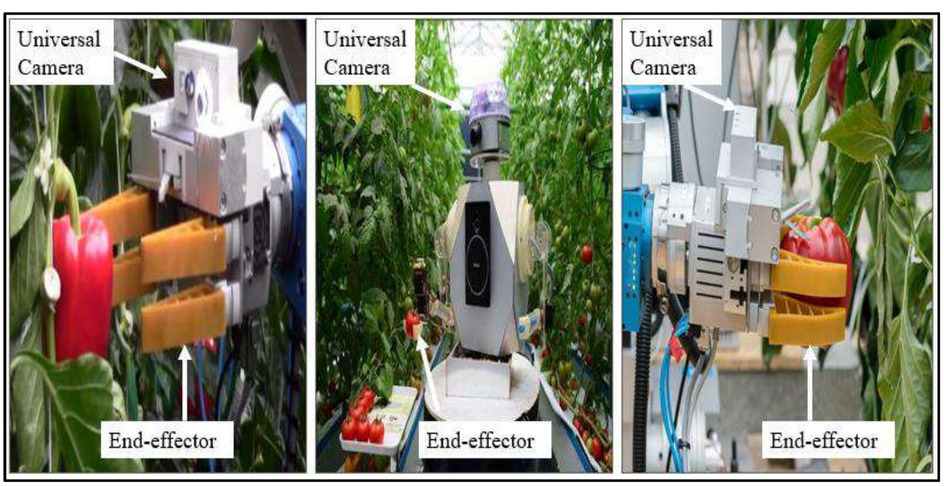

Fig. 7. Robotic harvesting with end-effector installed with universal camera

pollination process, and fruit harvesting. A lot of research is also going on greenhouse-grown horticultural as well. Many robots have been developed for the harvesting of tomatoes and several crop functions such as leaf cutting and pollination (Charles 2018). Methods are also being developed that can predict the harvest and can measure the yield. All these data and measurement generators need to be able to manage their crops as effectively as possible in the greenhouse. A large number of companies around the world are investing in this aspect. Researchers have also developed mobile robots that use artificial intelligence to assess crop conditions such as almonds and apple orchards. Robots use artificial intelligence to evaluate the size of the canopy, which is directly related to crops which can be compared with the historical data to estimate flower and fruit concentrations. Therefore, it can help in quick counting of the pre-harvested fruits and anticipating the yield to be harvested. The various types of harvesting robotics technologies all over the world for harvesting several types of crops are shown in Figure 11.

It is recommended to use robotic conveyors to carry out automated harvesting with a minimum of seasonal staff, to transport empty or fruit containers to and from the workplace. An efficient automated bus requires i) autonomous navigation to have a basic function; ii) intelligent management within the orchard; and iii) container handling.Various types of harvesters currently being used for collection and transportation are shown in Figure 12. Fruit containers used in the Pacific Northwest region of the United States are typically designed to hold about 400 kilograms of fruit during harvesting for transportation, and storage. Therefore, the two basic requirements of robotic container carriers are the ability to handle a load of up to 500 kilograms and the effective movement within the orchard environment (Ye et al 2017). Current robotic technology has been able to meet this requirement by providing a self-propelled automated conveyor system for autonomous transport boxes (Hamner et al 2010).

The robot bin is involved in the navigation functions of carriers must guide the container holder with an empty pot to the tree trunk where the harvest is harvested, proceed to distribute and install the empty container in a passageway to a suitable place in the harvest area and carry a full container from the passageway and distribute it to a storehouse station.

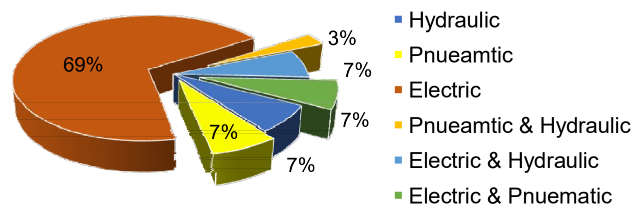

Fig. 9. Breakdown of manipulator actuators used in the robotic harvesting technologies

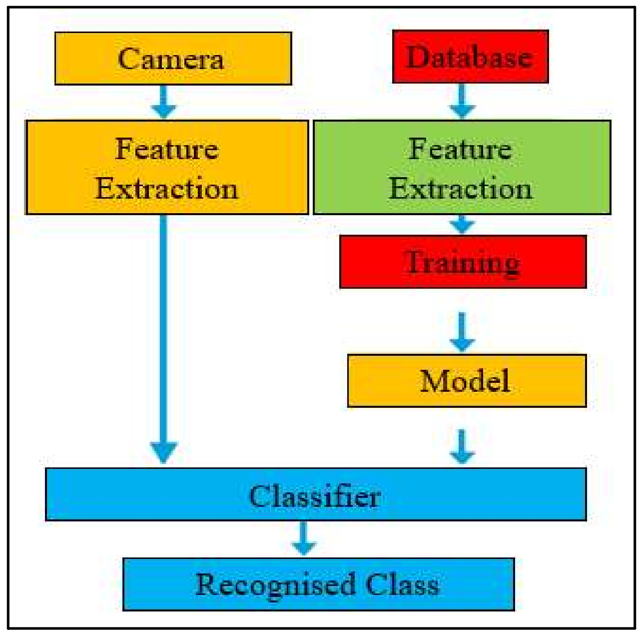

Fig. 10. Fruit classification and quality detection using deep convolutional neural network

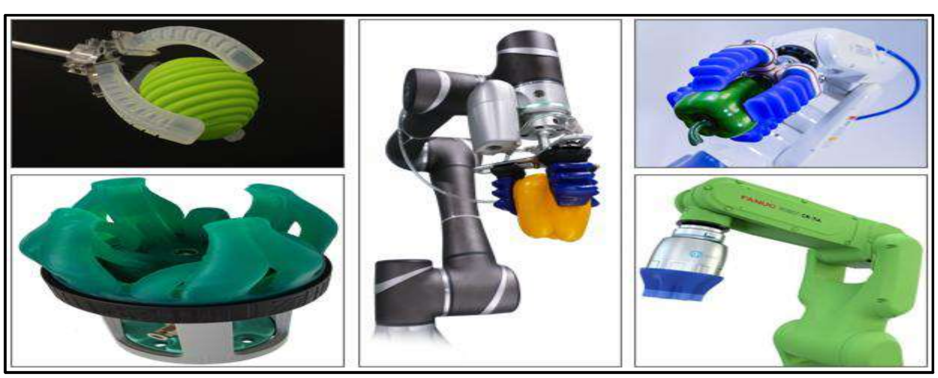

Fig. 8. Different types of soft and hollow fingers used in end-effector

Fig. 11. Robotic harvesting applications. "**sb**", "**tm**", "**ap**", "**sp**", "**cc**", "**kw**", "**ct**", "**rb**", "**lc**", "**mg**", "**pl**" represent strawberry, tomato, apple, sweet pepper, cucumber, kiwifruit, citrus, raspberry, litchi, mango, and plum, respectively., **sb-1** Hayashi, 2010, **sb-2** Feng, 2012, **sb-3** Shibuya Seiki, 2014, **sb-4** Yamamoto, 2014, **sb-5** DogTooth 2018, **sb-6** Agrobot-2018, **sb-7** Xiong 2019, **sb-8** Traptic-2019, **sb-9** Harvest CROO-2019, **Sb-10** Octinion-2019, **sb-11** Advanced Farm-2019, **sb-12** Tortuga-2020, **tm-1** Kondo, 2010, **tm-2** Yaguchi, 2016, **tm-3** Zhao, 2016, **tm-4** Wang, 2018, **tm-5** Feng, 2018, **tm-6** Panasonic-, 2018, **tm-7** MetoMotion-2019, **tm-8** Botian-2019, **tm-9** ROOT AI-2019, **ap-1** Baeten, 2008, **ap-2** Zhao, 2011, **ap-3** Nguyen, 2013, **ap-4** Siwal, 2017, **ap-5** Abundant Robotics-2019, **ap-6** FFRobotics-2020, **ap-7** Ripe Robotics-2020, **ap-8** Kang, 2020, **sp-1** Bac, 2017, **sp-2** Lehnert, 2016, **sp-3** SWEEPER-2018, **kw-1** Scarfe, 2012, **kw-2** WilliamsSavoie, 2019, **kw-3** Mu, 2020 **cc**, **-1** Ven Henten 2002 **cc**, **-2** Ji, 2011 **cc**, **-3** IPK, 2018, **ct-1** Muscato, 2005, **ct-2** ENERGID, 2012, **rb** Fieldwork Robotics-2020, **lc** Xiong, 2018, **pl** Brown, 2020, **mg** Walsh, 2019.(Source: Zhou et al 2022)

An efficient navigation system requires a GPS to shift the carrier between the storehouse and the target passageway, and an ultrasonic or lidar-based distance scanning sensor system to identify boundaries and barriers within the passageway where GPS signals are frequently disturbed by the fruit trees (Ye et al 2017). An intelligent management system was used (Zhang et al 2015) to support the efficient harvesting by autonomous containers to independently manage the various operations in the orchard. To plan for effective coordination in a multi-robotics management system, the automated prototype was developed and authenticated an algorithm based on a market-based framework, which had the industrial market value. The developed prototype was capable of making a decision, it will proceed to complete its specific task and make another decision only after completing its current work. It can be very difficult to carry out all basic work tasks effectively and reliably in a commercial environment, mainly due to enclosed premises with fruit tree passageways, and randomly growing and deformed tree canopy interventions.Kanget al(2020) developed a real-time robotic apple harvesting that included four steps: sensing, verification, grasping, collection, and transportation, as shown in Figure 13.

Future Scope of Artificial Intelligence Robotics Technologies

Market statistics on automation systems in robotics for various agricultural applications are expected to increase from US$ 7.4 billion in 2020 to US$ 20.6 billion worldwide by the end of 2025 (Anonymous 2022). Factors such as a reduction in labour, a growing population, and high

Fig. 12. Different types of robotic harvesters with autonomous collection and transportation mechanism

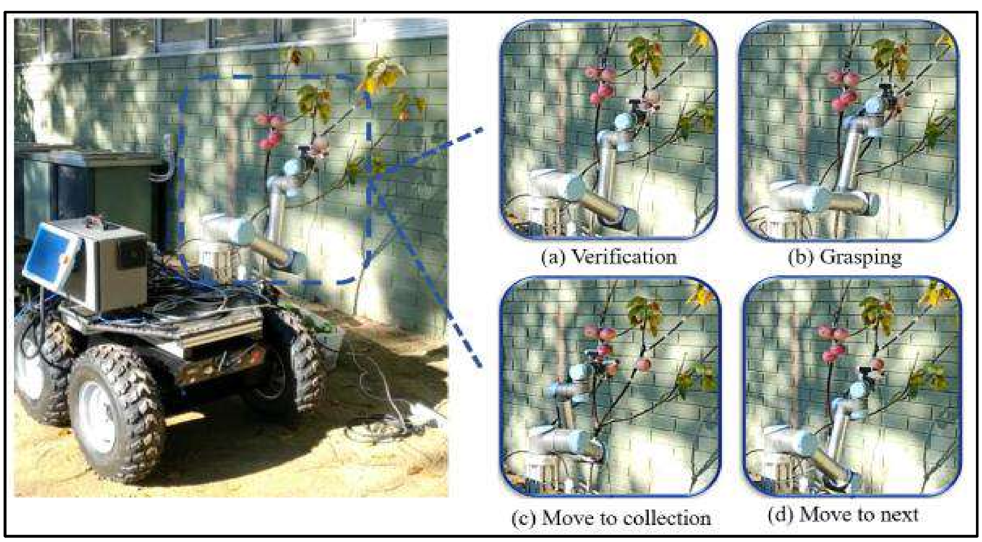

Fig. 13. The process for robotic harvesting experiment in the outdoor environment

productivity needs have enabled the development of agricultural automation and robotics. The difficulty of developing a cost-effective robotic system for fruit harvesting has been discovered by researchers in several parts of the world. The core problems that need to be solved by the robot selection system are to identify, recognise, locate, and detach the fruit without harming the fruit or tree, according to the specified criteria (Anonymous 2020). The development opportunities require tremendous technology maturity to ensure that automated robotics products designed to be implemented in various agricultural activities are reliable and robust. Therefore, a lot of research is still actively being done to overcome many challenges in managing different agricultural activities in different work environments and situations. In addition, the robotic system must be economically sound so that it can be used as an alternative method to manual or conventional methods of fruit harvesting. Despite many challenges in various agricultural activities, the farmers are also more concerned about the total costs required to invest in agricultural robotic systems. Some farmers are afraid to spend their money on technology that won't benefit them in the future. Agricultural researchers must therefore come up with more innovative ideas for designing a multifunctional robotic system at an affordable cost.

CONCLUSION

Robotics and automation play a key role in the horticulture sector for sustaining and boosting food security in the future. The application of robotics equipment lets farmers conduct agricultural operations promptly using a wide range of technologies provided by the advanced system. The artificial intelligence system makes the transition to precise cultivation and harvesting of horticultural crops while helping farmers automate their farms while achieving higher yields and better-quality crops while using fewer resources. Labour is very expensive for gardening. Intensive horticultural products require a more professional workforce than a large-scale agriculture farming approach. Approximately, 50 percent of retail spending is covered by wages on the hired labour leased for various operations. The artificial intelligence-enabled robotic system can reduce farming costs by regulating labour use, efficient use of pesticides and fertilizers, and minimising crop losses by harvesting horticultural crops on time. The application of artificial intelligence robotic harvesting systems in all areas of application will now bring a perfect change in the way it explores and evolves in horticulture. The manufacturing of AI-based products and services such as automated robots, acquiring data through deep learning, flying drone harvesters, etc., will make technical advances in the future and provide the sector with more useful applications to improve efficiency with the primary objective of producing high amounts of agricultural production in the future in the protection of food security for the world.

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Ladybird Beetles (Coccinellidae: Coleoptera) in Southern Telangana: Diversity and Habitat Distribution

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Abstract: This research paper investigates the diversity and distribution patterns of ladybird beetles (Coccinellidae) in agricultural ecosystems of Southern Telangana, with a specific focus on the okra crop. A total of 735 specimens representing 12 species were collected and examined, revealing a diverse assemblage of coccinellids. Among the recorded species, eight belonged to the subfamily Coccinellinae, while the remaining species were distributed across other subfamilies. Notably, the most abundant species in the okra ecosystem was *Illies cincta,* constituting 35.25 % of the total specimens, followed by *Cheilomenes sexmaculata* (15.10 %) and *Propylea dissecta* (12.23 %). The study further elucidates the species richness and abundance of coccinellids across various crops, with ragi and okra hosting the highest number of species. Additionally, diversity indices such as Shannon index (H'), Simpson's index of diversity (1-D), Simpson dominance index (D) and Pielou's evenness index (E) were calculated to assess the diversity of ladybird beetles in the okra ecosystem. The results revealed a high diversity index, indicating a stable ecosystem with complex food webs. These findings underscore the importance of studying coccinellid diversity and distribution in agricultural ecosystems, as they play a crucial role in natural pest control and ecosystem stability. Understanding the dominance patterns of species, species richness and diversity indices can inform effective pest management strategies, ultimately promoting sustainable agriculture.

Keywords: Ladybird beetles, Biodiversity, Habitat distribution, Southern Telangana, Sustainable agriculture

Ladybird beetles, members of the family Coccinellidae within the order Coleoptera, represent a vital component of natural pest control mechanisms in horticultural and agricultural ecosystems (Dixon 2000, Omkar and Pervez 2000). Exhibiting remarkable habitat diversity, these beetles thrive in various environments, including forests, fields, grasslands and gardens. With a global presence, the Coccinellidae family encompasses 490 genera and approximately 6000 described species, classified into six subfamilies: Sticholotidinae, Chilocorinae, Scymninae, Coccidulinae, Coccinellinae and Epilachninae, with recent phylogenetic studies suggesting the addition of a seventh subfamily, Ortaliinae (Slipinski 2007). Within the Indian subcontinent, excluding the Epilachninae subfamily, there exists a rich diversity comprising 400 species (including six subspecies), distributed among 79 genera, 22 tribes and 5 subfamilies (Poorani 2002). Owing to their predatory habits, ladybird beetles play a significant role in regulating populations of various pests such as aphids, leafhoppers, whiteflies, mealybugs and scales, thus contributing to biological pest control strategies. However, certain members of the Epilachninae subfamily exhibit phytophagous behavior, posing a threat to vegetable crops. *Henosepilachna vigintioctopunctata* (Fabricius) and *Epilachna implicata* (Fabricius) are particularly damaging to solanaceous and cucurbitaceous plants, respectively (Megha et al 2015).

The composition of predatory coccinellids varies widely across different agroecosystems. The dependence of any species in a given habitat is mainly determined by the occurrence of prey and abiotic factors. The relationship of many species to a habitat varies in different regions of their distribution and also in different ecosystems. As information on the species composition of coccinellids in agricultural fields of Southern Telangana is not available and considering the importance of these beneficial predators, the present study was conducted to list the species of ladybird beetles with the objective of exploring the beetle fauna, their species composition in okra in particular, along with other existing crop ecosystems in Southern Telangana.

MATERIAL AND METHODS

Ladybird beetle samples were collected from various fields in Southern Telangana, located at Latitude: 17.1231° N and Longitude: 79.2087° E, using net sweeping and handpicking techniques at fortnightly intervals during the *Kharif* season of 2023. Intensive collections were conducted in fields cultivating millets, vegetables, fiber crops, oilseeds and plantation crops. After collection, the beetles were killed using ethyl acetate. The specimens were then thoroughly dried in a hot air oven at 45-50 °C for 4 to 6 hours. The collected specimens were maintained in the Department of Entomology, College of Agriculture, Rajendranagar, for further studies.

During the *Kharif* season of 2023, okra crop was cultivated over an area of 300 square meters using standard agronomic practices (Bhatt et al 2018). Ladybird beetle samples were collected at 15-day intervals over a period of three months, covering an area of one square meter with five replications. The samples were collected into polythene bags and stored in a standard freezer. At the end of the sampling period, the samples were pooled together. Ladybird beetles were subsequently extracted from the samples and identified to the species level, with the recorded species composition documented. Identification of beetle species was conducted based on their morphological characteristics and genitalia.

Species Diversity/Index

Species richness: This term refers to the number of species in a community, directly reflecting the diversity of species in a given area.

Species richness (S) = number of species/genera collected

Species diversity (H'): This computed using the Shannon-Weiner index of diversity (Shannon, 1948):

$$\text{Species diversity (H')} = -\sum^{k} p_i \ln p_i$$

Where p_i represents the proportion of individuals found in species i. For a well-sampled community, p_i can be estimated as n_i/N, where n_i is the number of individuals in species i and N is the total number of individuals in the community. The natural log makes all terms of the summation negative, for which we take the inverse of the sum.

Simpson's dominance index (D): This index measures the probability of two individuals randomly selected from a sample belonging to the same species.

$$D = \sum n_i (n_i - 1) / N (N - 1)$$

Where n_i represents the number of individuals of each species in a sample and N is the total number of individuals of all species in the sample.

Simpson's index of diversity (1 – D): This index represents the probability that two individuals randomly selected from a sample belong to different species was calculated by:

$$1 - D = 1- [\sum n_i (n_i - 1) / N (N - 1)]$$

Where n_i represents the number of individuals of each species in a sample and N is the total number of individuals of all species in the sample.

Simpson's reciprocal index (1/D): This index provides the number of equally common species that would produce the observed Simpson's index, calculated by:

$$1/D = 1/ \sum n_i (n_i - 1) / N (N - 1)$$

Where n_i represents the number of individuals of each species in a sample and N is the total number of individuals of all species in the sample.

Pielou's evenness index or equitability (E): This index measures diversity along with species richness and is calculated by:

$$E = H'/\ln (S)$$

Where ln(S) represents the natural logarithm of the number of species present. The value of E falls between 0 and 1, with the maximum value achievable in a community where all species are equally abundant.

RESULTS AND DISCUSSION

A total of 735 specimens were examined and 12 species were recorded. Among these, eight species, belonged to the subfamily Coccinellinae and tribe Coccinellini. Two species, belonged to the subfamily Chilocorinae and tribe Chilocorini. One species, to the subfamily Scymninae and tribe Scymnini and another species, *Henosepilachna vigintioctopunctata* (Fabricius) to the subfamily Epilachninae and tribe Epilachnini. Among the millets, seven species were found in brown top millet, five species in maize and six species in ragi. Among the vegetable crops, two species were found in bitter gourd, eight species in okra three species in brinjal and three species in cabbage. Among fiber crops, five species were found in cotton. Among oilseed crops, four species were found in safflower. Among plantation crops, one species (*Illies cincta*) was found in mulberry and one species (*Chilocorus nigrita*) was found in coconut (Table 1).

The diversity of predaceous coccinellids has been extensively documented in various regions across the globe. For instance, Usman and Puttarudriah (1955) reported an impressive total of 48 species of predaceous coccinellids from Mysore State, highlighting the rich diversity of these beneficial insects in the region. Similarly, Sathe and Bhosale (2001) documented 21 predatory coccinellid beetles feeding on aphids and other soft-bodied insects in Maharashtra, emphasizing the ecological importance of coccinellids as natural enemies of agricultural pests. In the northern region of India, Joshi and Sharma (2008) recorded 31 species of coccinellid beetles from Haridwar, indicating a diverse assemblage of these predators in the area. Furthermore, Sharma and Joshi (2010) reported approximately 25 species, while Joshi et al. (2012) documented 23 species of coccinellids from the Dehradun District of Uttarakhand, underscoring the regional variation in species composition. Outside of India, Rahatullah et al (2011) conducted a study in Pakistan and recorded 14 species of ladybird beetles, contributing to the knowledge of coccinellid diversity in the country. Similarly, Biranvand et al (2014) documented 22 species of coccinellids from Iran, providing valuable insights into the distribution of these insects in the region. Present study contributed significantly to our understanding of the species richness and distribution patterns of coccinellid beetles in different ecosystems. There was the significant variation in coccinellid species richness and composition across different geographical regions.

Species composition and abundance of ladybird beetles in okra crop ecosystem: The species composition and abundance of ladybird beetles were recorded by collecting beetle specimens in the bhendi ecosystem over an area of 300 square meters during the *Kharif* season of 2023. A total of 139 specimens representing eight species, as mentioned above, were captured. These coccinellids were found in the okra crop, feeding on leafhoppers (*Amrasca biguttula*),

Table 1. Distribution of coccinellid beetles across various crop ecosystems

Crop	Coccinellid species	Prey	No. of species
A. Millets			
Brown top millet	*Harmonia octamaculata, Micraspis discolor, Cheilomenes sexmaculata, Coccinella transversalis, Scymnus nubilus, Propylea dissecta, Hippodamia variegata*	*Rhopalosiphum maidis*	7
Maize	*Cheilomenes sexmaculata, Harmonia octamaculata, Micraspis discolor, Scymnus nubilus, Illies cincta*	*Rhopalosiphum maidis,* Mildew (*Illies*)	5
Ragi	*Harmonia octamaculata, Cheilomenes sexmaculata, Hippodamia variegata, Propylea dissecta, Coccinella transversalis, Brumoides suturalis*	*Rhopalosiphum maidis*	6
B. Vegetables crops			
Bitter gourd	*Henosepilachna vigintioctopunctata, Aneglies cardoni*	Phytophagous (*Henosepilachna*), *Bemisia tabaci*	2
Okra	*Brumoides suturalis, Hippodamia variegata, Harmonia octamaculata, Illies cincta, Cheilomenes sexmaculata, Micraspis discolour, Coccinella transversalis, Propylea dissecta*	*Amrasca biguttula, Bemisia tabaci, Myzus persicae*	8
Brinjal	*Cheilomenes sexmaculata, Coccinella trasnversalis, Propylea dissecta*	*Cestius phycitis*	3
Cabbage	*Cheilomenes sexmaculata, Harmonia octamaculata, Scymnus nubilus*	*Brevicoryne brassicae*	3
C. Fibre crop			
Cotton	*Harmonia octamaculata, Cheilomenes sexmaculata, Micraspis discolor, Coccinella transversalis, Hippodamia variegata, Illies cincta, Scymnus nubilus*	*Aphis gossypii, Amrasca biguttula, Bemisia tabaci,* Powdery mildew *(Illies)*	5
D. Oilseed crop			
Safflower	*Cheilomenes sexmaculata, Harmonia octamaculata, Micraspis discolor, Coccinella tranversalis*	*Uroleucon compositae*	4
E. Plantation crops			
Mulberry	*Illies cincta*	Powdery mildew	1
Coconut	*Chilocorus nigrita*	*Aspidiotus destructor*	1

whiteflies (*Bemisia tabaci*) and aphids (*Aphis gossypii*). Additionally, a fungal feeder, *Illies cincta*, was observed feeding on spores of *Erysiphe cichoracearum*, which caused powdery mildew in bhendi. Among all the ladybird beetles, *Illies cincta* (35.25 %) was the most abundant coccinellid, followed by *Cheilomenes sexmaculata* (15.10 %) (Table 2). The high percentage (35.25 %) of occurrence of *I. cincta* might be due to the incidence of powdery mildew during the October to November, as the temperature ranged from 29 °C to 30.6 °C, which was conducive to fungal infection (Rajalakshmi et al., 2016). Similar to the present result, a higher population of *I. cincta* was recorded by Thite et al (2013) during September and October, coinciding with high incidences of powdery mildew on *Dalbergia sissoo* and *Xanthium strumarium.* Among the eight species, *C. sexmaculata* was the most abundant predatory coccinellid beetle in okra ecosystem. Robert et al (2012) in cowpea and those of Rani et al (2013), Shailaja et al (2014), Megha et al (2015), and Rani (2016) reported *C. sexmaculata* as the predominant species.

Chanmamla (2009), recorded 12 species of coccinellids, among which *Coccinella transversalis* (38 %) and *Cheilomenes sexmaculata* (34 %) were the most abundant species, while *Brumoides suturalis* population was very low (1 %). Similarly, Rajan et al (2019) reported that Bhendi harbored the maximum number of coccinellids, including *Coccinella transversalis, Cheilomenes sexmaculata, Hippodamia variegata, Micraspis discolor, Harmonia octomaculata, Illeis cincta, Brumoides suturalis, Stethorus* sp., and *Scymnus coccivora*, with *Coccinella transversalis* and *Cheilomenes sexmaculata* being the two most abundant species. Sharma et al (2017) registered a total of 65 predatory coccinellids associated with different sucking pests and found *Coccinella septumpuntata*, *Hippodamia variegata* and *Cheilomenes sexmaculata* as the most widely distributed coccinellids in all agro-climatic zones of the state. In contrast, Gurung et al (2018) recorded only four coccinellids in okra, namely *Brumoides suturalis, Cheilomenes sexmaculata, Coccinella transversalis* and *Micraspis discolor*, among which *Micraspis discolor* was the most abundant species.

Shah and Ali (2014) conducted a survey on coccinellid biodiversity under pesticide pressure crop ecosystems and reported fewer lady beetle species in pesticide-treated vegetable ecosystems. Similarly, Chakrabothy et al (2014) recorded a higher population of coccinellids in untreated plots (0.47 and 0.50/plant in *Kharif* and *Rabi*, respectively) compared to treated plots (0.18 and 0.28/plant in *Kharif* and *Rabi*, respectively). They also reported a reduction in the population of *C. sexmaculata, C. transversalis, H. octamaculata, M. discolor* and *B. suturalis* from 21.87 % to 60.94 % due to the application of herbicides, insecticides and fertilizers in okra.

Diversity of ladybird beetles in okra crop ecosystem: The present study confirmed the occurrence of 139 specimens of ladybird beetles in bhendi, which belonged to two different subfamilies, Coccinellinae and Chilocorinae (Table 3). Among the two subfamilies, Coccinellinae was more dominant with high species richness comprising seven species belonging to seven genera, followed by the subfamily Chilocorinae with one species belonging to one genus. The diversity indicated a diverse community of coccinellids in the bhendi ecosystem. The high diversity of coccinellids was attributed to a greater number of successful species, a more stable ecosystem, complex food webs and environmental changes less likely to be damaging to the ecosystem as a whole. The structural complexity of habitats has a significant impact on the abundance and diversity of coccinellids (Langellotto and Denno, 2004). Similar results were reported by Ankalgi and Jadesh (2016) with dominance (D) = 0.151, Simpson index of diversity (1-D) = 0.848, Shannon (H) = 2.105, Simpson's reciprocal (1/D) = 6.591 and evenness of 0.745 indicating greater diversity. Rekha et al. (2009) showed more species heterogeneity with richness (3.27), species evenness (1.23) and diversity (0.96) in

Table 2. Species composition and abundance of the coccinellid beetles in okra ecosystem

Subfamily	Species	Abundance	Frequency (%)
Chilocorinae	*Brumoides suturalis*	9	6.47
Coccinellinae	*Hippodamia variegata*	5	3.59
	Harmonia octamaculata	14	10.07
	Illies cincta	49	35.25
	Cheilomenes sexmaculata	21	15.10
	Micraspis discolor	10	7.19
	Coccinella transversalis	14	10.07
	Propylea dissecta	17	12.23

Table 3. Diversity indices of ladybird beetles (Coleoptera: Coccinellidae)

Diversity indices	Values
No. of species (n)	8
No. of specimens (N)	139
Shannon (H')	1.85
Simpson index (D)	0.18
Simpson index of diversity (1-D)	0.82
Pielou's evenness index (E)	0.89

tomato. This highlights the pivotal role of biodiversity conservation efforts in maintaining healthy ecosystems and underscores the importance of continued research to understand and preserve the intricate dynamics of natural habitats.

CONCLUSION

The investigation revealed a diverse assemblage of ladybird beetles across different crop ecosystems, highlighting their adaptability and ecological significance. A total of 12 species of ladybird beetles were reported with *Cheilomenes sexmaculata* (Fabricius) is the most abundant species with 196 specimens followed by *Harmonia octamaculata* (Fabricius) with 131 specimens. In the okra ecosystem alone, a total of 139 specimens representing eight species were recorded, showcasing the prevalence and importance of these predators in controlling common pests such as leafhoppers, whiteflies and aphids. Among the recorded species, *Illies cincta* emerged as the most abundant, followed by *Cheilomenes sexmaculata, Propylea dissecta* and others, with their respective contributions delineated. The dominance of Coccinellinae over Chilocorinae in terms of species richness underscores the varied composition within the family, with each species potentially playing a vital role in maintaining ecological balance. The diversity indices reflected a high diversity of coccinellids, indicative of a stable ecosystem with multiple successful species coexisting synergistically in okra ecosystem. Overall, the study underscores the crucial role of ladybird beetles in integrated pest management and highlights the need for continued research and conservation efforts to safeguard these invaluable allies of agriculture.

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