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Pedological Development of Soils of Saraswati River Palaeochannels across Haryana through Field Morphology Rating System

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Abstract: To evaluate the pedological development of soils in Saraswati river palaeochannels in Haryana, a study was carried out. Twelve Soil pedons were dug and exposed to study the level of soil development according to field morphology rating system. Six soil profiles were studied in Yamunanagar district, one soil profile in Kurukshetra district, two from Kaithal district, one from Fatehabad district and two from Sirsa district. The slope of the soils from Yamunanagar district was 1-3% (nearly level to gently sloping land). Rest of the soil profiles were in 0-1% sloping lands. The structure of pedons varied from single grain, granular, angular blocky to sub-angular blocky in type, structureless to strong in grade and fine to medium in class. Soils of pedons 1(P-1 Ranipur, Yamunanagar), 2 (P-2 Mugalwali, Yamunanagar), 3 (P-3 Bansewala, Yamunanagar), 5 (Painsal-NB Yamunanagar), 6 (P-6 Mustafabad, Yamunanagar), 7(P-7 Ishargarh, Kurukshetra), 10 (P-10 Birdhana, Fatehabad) and 12 (P-12 Farwai-2, Sirsa) and surface horizon of pedon 11 (P-11 Farwai-1, Sirsa) exhibited sub-angular blocky structure due to low clay and low organic carbon content. The textural analysis of soil particles indicated that pedon 1, 2, 3, 4, 5 and 12 were light, pedon 7 was light to medium and pedon 6, 8, 9, 10 and 11 were heavy in texture. The calcium carbonate concretions were present in pedon 10, 11 and 12 due to calcium containing parent rock which released calcium upon weathering and soil formation. The soils of pedon 2, 3, and 9 have a relative horizon distinctness (total) value of 20 each, pedon 7, 5, 6 and 10 have values 19, 17, 16 and 16 respectively and pedon 12 & 8 14 each. The relative profile development (total) value of the pedons varied from 16 to 33 being minimum in pedon 8 and maximum in pedon 11 and 5. . The horizon boundary, differences in moist colour, texture, structure, consistency, pH, and EC all had a role in the rating variation.

Keywords: Relative horizons distinctness, Relative profile development, Saraswati river, Pedon

Soil morphology offers vital insights into pedogenic processes by revealing variations in horizonation, structure, texture, and colour that reflect the intensity and duration of soil development. In alluvial and paleochannel environments, these morphological patterns not only become more critical but also more complex due to depositional layering and parent-material heterogeneity, which often obscure the signs of true soil development (Lalitha et al., 2023). Field morphology rating system enables quantitative assessments of soil development. These approaches compare profile differentiation and horizon distinctness across geomorphic surfaces, facilitating more rigorous evaluation of paedogenic variations. Despite their ecological significance, the soils within the palaeochannel of the Saraswati River in Haryana remain insufficiently studied. While geophysical investigations have detailed subsurface characteristics of these palaeochannels (Kumar et al., 2024), interpretations of surface morphology and pedogenesis are still lacking. The current study applies a morphological rating system to quantify soil development and assess pedological variation in these paleochannel landscapes of Haryana. The aim of study is to enhance understanding of soil genesis in these unique geomorphic environments and inform regionally tailored land management strategies.

MATERIAL AND METHODS

Twelve representative pedons from palaeochannels of Saraswati River were investigated from Haryana (Table 1).

Table 1. Sites location

Pedon	Location	Latitude	Longitude
P1	Ranipur, Yamunanagar	30°24'47"N	77°19'54"E
P2	Mugalwali, Yamunanagar	30°23'56"N	77°19'33"E
P3	Bansewala, Yamunanagar	30°22'42"N	77°16'58"E
P4	Painsal-1 Yamunanagar	30°18'57"N	77°12'52"E
P5	Painsal-NB Yamunanagar	30°18'57"N	77°12'52"E
P6	Mustafabad, Yamunanagar	30°13'43"N	76°10'6"E
P7	Ishargarh, Kurukshetra	30°0'49"N	76°54'9"E
P8	Mangna, Kurukshetra	29°57'35"N	76°29'43"E
P9	Kaekor, Kaithal	29°57'14"N	76°25'50"E
P10	Birdhana, Fatehabad	29°33'16"N	75°31'45"E
P11	Farwai-1, Sirsa	29°36'40"N	75°6'12"E
P12	Farwai-2, Sirsa	29°36'40"N	75°6'12"E

Indian Space Research Organization' BHUVAN platform was used for identification of Saraswati River palaeochannels. Additionally, False Colour Composite from Sentinel-2 imagery with 5×5m resolution was used for palaeochannel identification and study of tone and texture of soil of the area. Field traversing was also done before the initiation of the study. Two indices of soil development viz. morphological properties such as soil colour, texture, structure, consistency, pedon reaction and concretions were observed in each pedon in the field (USDA' Soil Survey Manual 2017). Soil pH was determined using pH meter consists the glass electrode in 1:2 soil: water suspension at room temperature (Jackson, 1973). Electrical conductivity was determined using a conductivity meter in 1: 2: soil: water suspension at room temperature 25°C (Jackson, 1973). Relative horizon distinctness (RHD) and relative profile development (RPD) were calculated from the soil morphological data as defined by Bilzi and Ciolkosz (1977). RHD was determined by comparing the morphological features of two adjacent horizons and RPD by comparing of the morphological feature of each horizon with the C horizon within each pedon. Soil pedons were classified in accordance with Key to Soil Taxonomy (Soil Survey Staff 2022). The soils were evaluated and points assigned as described below:

Boundaries: Points are assigned according to the distinctness of the lower or shared horizon as follows: diffuse-0, gradual-1, clear-2, abrupt-3 and very abrupt-4.

Colour (dry and moist): One point is assigned for any class change in hue and for any unit change in value or chroma. For example, a change from 10 YR 4/6 to 5 YR 3/8 would have a value of 5 for the twofold class change, the one – unit change in value, and two – unit change in chroma. Where two colours are observed (other than mottles), each one is compared, and the average difference is used.

Texture: One point is assigned for each class change on the textural triangle. In addition, a change from non-gravelly to gravelly or very gravelly is assigned one or two points, respectively.

Structure: One point is assigned for any change in type of aggregated structure, for each unit change in grade (1, 2, 3), and for each class change in size (vf, f, m, c, vc), irrespective of the aggregate type. For example, a change from weak, very fine subangular blocky (lvfsbk) to moderate, medium angular blocky (2m abk) is assigned a value 4. When the change is from no aggregated-to-aggregated structure (or vice versa), however, only the grade of the aggregate type is evaluated, in addition to the one point assigned for the type change. For example, a change from massive to weak, fine subangular blocky (1f sbk) is assigned a value of 2.

Consistence: One point is assigned for any class change in wet (so, ss, s, vs, po, ps, p, vp) consistence.

Cutans: One point is assigned for each class change in frequency or thickness at any single location.

Coarse fragments/Stoniness (>7.5 cm diameter): Points are assigned according to the volume of coarse fragments (>7.5 cm diameter) present in the matrix of the soil (1 for 80%).

Chemical rating system: This was evaluated and points assigned (Salem et al., 1997).

Soluble salts (dS/m): One point is assigned for each class change in quantity (non, very slightly, moderately, highly, extremely saline).

pH value of soil paste: One point is assigned for each class change in quantity (ultra-acid, extremely acid, very strongly acid, strongly acid, moderately acid, slightly acid, neutral, slightly alkaline, moderately alkaline, strongly alkaline and very strongly alkaline).

RESULTS AND DISCUSSION

The morphological and chemical properties of twelve pedons each covering soils of Saraswati River palaeochannels across Haryana from Yamunanagar to Sirsa district are represented in Table 2. The data were evaluated and prospective points were assigned as described by Bilzi and Ciolkosz (1997), Meixner and singer (1981) and Salem et al. (1997), and the soil rating scales are applied.

Morphological characteristics: The colour of the pedons varied from brown (10YR 5/3) to very pale brown (10YR 7/4) with dominant hue of 10YR. The values ranged from 5 to 7, whereas chromas were 2 to 6. The pale brown soil colour at the surface horizon of the pedons 6, 8, 9 and 11 could be attributed to the relatively higher accumulation and decomposition of OM content of the study site. Teshome et al. (2016) reported that the surface horizons have darker color than the subsurface horizons because of relatively higher soil OM contents. Agbugba (2018) also found similar results. The variation in colour of different pedons is due to different texture, topography, mineralogy and chemical composition of soils (Leelavathi et al., 2009, Sekhar et al., 2019). Horizon boundaries of the pedon 1, 2, 3, 4, 7, 8, 9 & 11 varied from clear and smooth to gradual and smooth, clear and smooth to gradual and wavy to gradual and smooth in pedon 5 & 12, clear and smooth to clear and wavy to gradual and smooth in pedon 6 and abrupt and smooth to gradual and smooth in pedon 10. These differences in surface-subsurface soil layer boundary characteristics may be due to the occurrence of unique morphological features with pedon depth, which would therefore suggest that the study area is still in the early phases of soil development (Ukut et al., 2014).

The textural analysis of soil particles indicated that pedon 1, 2, 3, 4, 5 and 12 were light, pedon 7 was light to medium and pedon 6, 8, 9, 10 and 11 were heavy in texture. Textural variations are due to different parent material and differential degree of weathering. Translocation of finer particles from

Table 2. Morphological and physico-chemical properties of the studied pedons

Soil profile	Horizon	Depth (cm)	Horizon boundary	Colour (Dry)	Texture	Structure	Consistence	Cutans	Roots	Coarse fragment	Reaction	pH	EC (dSm-1)
P1 (Ranipur)	Ap1	0-25	c-s	10YR 5/4	sl	sbk 2 m	SSNP	-		stmf	-	6.53	0.17
	Ap2	25-38	c-s	10YR 5/4	sl	sbk 2 m	SSNP	-	-	-	-	6.41	0.06
	AC1	38-71	g-s	10YR 5/6	sl	sbk 2 m	SSNP	-	-	-	-	6.32	0.16
	AC2	71-86	g-s	10YR 5/6	sl	sbk 2 m	SSNP	-	-	-	-	6.43	0.15
	C1	86-114	g-s	10YR 5/6	sl	sbk 2 m	NSNP		-	-	-	6.52	0.14
	C2	114-135+		10YR 5/4	sl	sbk 2 m	NSNP					6.40	0.15
P2 (Mugalwali)	Ap1	0-32	c-s	10YR 5/4	l	sbk 2 m	SSSP	-	mf	-	-	6.42	0.24
	Ap2	32-57	c-s	10YR 6/6	sl	sbk 2 m	SSNP	-	ff	-	-	6.63	0.17
	AC	57-79	g-s	10YR 6/6	sl	sbk 2 m	SSNP	-	ff	-	-	6.65	0.17
	C1	79-105	g-s	10YR 6/6	sl	sbk 2 m	SSNP	-	ff	-	-	6.35	0.12
	C2	105-150+		10YR 6/4	ls	sbk 1 m	NSNP		-	-	-	6.81	0.10
P3 (Bansewala)	Ap	0-18	c-s	10YR 6/4	l	sbk 2 m	MSSP	-		-	-	6.81	0.24
	B1	18-38	g-s	10YR 7/4	sl	sbk 2 m	SSNP	-	-	-	-	6.84	0.29
	B2	38-56	g-s	10YR 6/5	sl	sbk 1 fn	SSNP	-	-	-	-	6.49	0.12
	B3	56-68	g-s	10YR 6/6	ls	sbk 1 fn	NSNP	-	-	-	-	6.52	0.09
	Cq	68-110+		10YR 7/4	s	sg	NSNP	-	-		-	6.56	0.07
P4 (Painsal)	A	0-11	c-s	10YR 7/3	ls	sg	NSNP	-	-	-	-	8.61	0.29
	AC	11-30	g-s	10YR 6/4	ls	sg	NSNP	-	mp	-	-	6.75	0.15
	C1	30-45	g-s	10YR 7/4	ls	sg	NSNP	-	-	-	-	6.83	0.10
	C2	45-70+		10YR 7/4	ls	sg	NSNP	-	-	-	-	6.40	0.09
P5 (Painsal NB)	Ap	0-18	c-s	10YR 5/3	sl	sbk 2 m	SSSP	-	ff	-	-	6.17	0.12
	B1	18-38	g-w	10YR 6/4	sl	sbk 2 m	SSSP	-	ff	-	-	6.75	0.09
	B2	38-51	g-w	10YR 6/5	sl	sbk 2 m	SSSP	-	ff	-		6.93	0.08
	BC	51-81	g-s	10YR 6/4	sl	sbk 1 m	SSSP	-	ff	-	-	7.27	0.12
	C	81-125+		10YR 7/3	ls	sbk 1 m	NSNP	-	-	-	-	7.66	0.09
P6 (Mustafabad)	A	0-32	c-s	10YR 6/4	sil	sbk 3 m	VSVP	-	mp	-	-	6.60	0.19
	Bt	32-47	c-w	10YR 6/4	scl	sbk 3 m	VSVP	-	mp	-	-	6.90	0.17
	B2	47-70	g-s	10YR 6/4	sil	sbk 3 m	VSVP	-	vff	-	-	7.06	0.22
	B3	70-87	g-s	10YR 7/4	l	sbk 2 m	MSMP	-	vff	-	v sl e	7.45	0.25
	C	87-108+		10YR 7/4	l	sbk 2 m	MSMP	-	-	-	v sl e	7.66	0.35

Cont...

Table 2. Morphological and physico-chemical properties of the studied pedons

Soil profile	Horizon	Depth (cm)	Horizon boundary	Colour (Dry)	Texture	Structure	Consistence	Cutans	Roots	Coarse fragment	Reaction	pH	EC (dSm-1)
P7 (Ishargarh)	A	0-22	g-s	10YR 6/3	l	sbk 2 m	MSMP	-	fp	-	-	6.78	0.12
	A/B	22-39	g-s	10YR 5/3	l	sbk 2 m	MSMP	-	ff	-	-	6.82	0.08
	B	39-75	g-s	10YR 5/4	ls	sbk 1 fn	NSNP	-	ff	-	-	6.63	0.05
	Bq	75-140	c-s	10YR 7/3	ls	sbk 1 fn	NSNP	-	-	-	-	6.94	0.05
	Cq	140-154+		10YR 6/4	ls	sbk 1 fn	NSNP					7.07	0.05
P8 (Mangna)	A	0-20	c-s	10YR 6/3	sic	abk 3 m	VSVP	-	-	-	-	6.78	0.21
	Bt1	20-39	g-s	10YR 6/3	cl	abk 3 m	VSVP	-	-	-	-	6.69	0.16
	Bt2	39-70	g-s	10YR 7/4	cl	abk 3 m	VSVP	-	-	-	-	6.56	0.12
	B	70-97	g-s	10YR 6/4	cl	sbk 3 m	VSVP	-	-	-	-	7.00	0.21
	C	97-127+		10YR 7/4	cl	sbk 2 m	VSVP	-	-	-	-	6.75	0.21
P9 (Kaekor)	A	0-20	c-s	10YR 6/4	sic	abk 3 m	VSVP	-	-	-	-	6.09	0.10
	B1	20-35	g-s	10YR 5/4	sic	abk 3 m	VSVP	-	-	-	-	6.10	0.10
	B2	35-74	g-s	10YR 5/6	sl	abk 2 m	VSVP	-	-	-	-	6.48	0.06
	B3	74-98	g-s	10YR 7/4	l	sbk 2 m	VSVP	-	-	-	-	6.58	0.08
	C	98-131+		10YR 7/4	l	sbk 2 m	VSVP	-	-	-	-	6.87	0.09
P10 (Birdhana)	Ap	0-15	a-s	10YR 6/2	l	sbk 2 m	VSVP	-	-	-	st e	7.96	0.31
	Bt1	15-38	g-s	10YR 6/3	cl	sbk 3 m	VSVP	-	-	-	sl e	8.35	0.52
	Bt2	38-79	g-s	10YR 6/3	cl	sbk 3 m	VSVP	-	-	-	sl e	8.37	0.55
	C1	79-88	g-s	10YR 6/4	l	sbk 2 m	VSMP	-	-	-	sl e	8.60	0.80
	C2	88+		10YR 6/4	l	sbk 2 m	MSMP	-	-	-	sl e	8.62	0.86
P11 (Farwai)	A	0-25	c-s	10YR 6/3	l	sbk 2 m	VSVP	-	fa	-	st e	6.68	0.24
	AB	25-51	g-s	10YR 6/3	cl	gr 2 m	VSVP	-	fp	-	st e	6.78	0.31
	B1	51-65	g-s	10YR 6/4	sl	gr 2 m	MSMP	-	ff	-	st e	6.83	0.27
	B2	65-109	g-s	10YR 6/4	l	sbk 2 m	SSSP	-	ff	-	st e	6.84	0.26
	B3	109-125	g-s	10YR 5/3	l	sbk 2 m	SSSP	-	-	-	st e	6.95	0.31
	C	125+		10YR 6/2	s	sg	NSNP		-		ve	6.50	0.23
P12 (Farwai NB)	Ap	0-29	c-s	10YR 5/3	sl	sbk 2 m	SSSP	-	-	-	st e	6.06	0.29
	Bu1	29-50	g-s	10YR 6/2	sl	sbk 2 m	SSSP	-	-	-	st e	7.00	0.32
	Bu2	50-80	g-w	10YR 6/2	sl	sbk 2 m	SSSP	-	-	-	st e	7.13	0.31
	C1	80-122	g-s	10YR 6/4	l	sbk 3 m	SSSP	-	-	-	st e	7.26	0.29
	C2	122-140+		10YR 6/4	scl	sbk 3 m	MSMP	-	-	-	st e	7.40	0.29

Horizon Boundary {[Distinctness (a: abrupt, c: clear, g: gradual and d: diffuse)] and [Topography (s: smooth and w: wavy)]}, {[Colour (YR-yellow red)]}, {[Texture (s: sand, ls: loamy sand, sl: sandy loam, l: loam, sl: silt loam, sic: silty clay, scl: sandy clay loam and cl: clay loam)]}, {[Structure (S: Size (m: medium, f: fine and c: coarse)]}, [(G: Grade (1: week, 2: moderate and 3: strong))], [(T: Type (abk: angular blocky, sbk: sub angular blocky and sg: single grain)]}, {Consistency [Stickiness (ns: non-sticky, ss: slightly sticky, ms: moderately sticky and vs: very sticky,)] and [Plasticity (np: non plastic, sp: slightly plastic, mp: moderately plastic and vp: very plastic)]}, {Roots [S: size (vf: very fine, f: fine, m: medium and c: coarse)] and [Q: Quantity (f: few, c: common and m: many)]}, {[Coarse fragment (Nature (st: stone and gr: gravel), [Size (vf: very fine, f: fine, m: medium and c: coarse)] and [Abundance (f: few, c: common and m:many)]} and {[Effervescence (e: slight, es: strong and ev: violent)]} upper horizons to lower horizons by percolating (Sawhney et al., 2000). The clay content increased with depth due to downward translocation of finer particles from the surface layers as reported by Nasre et al., (2013).

The structure of the studied pedons varied from single grain, granular, angular blocky to sub-angular blocky in type, structureless to strong in grade and fine to medium in class. Soils of pedons 1, 2, 3, 5, 6, 7, 10 and 12 and surface horizon of pedon 11 exhibited sub-angular blocky structure due to low clay and low organic carbon content. Similar results were also observed by Sitanggang et al. (2006). Pedon 8 and 9 showed angular blocky to sub-angular blocky structure and B horizon of pedon 11 showed granular structure due to more clay content and increased amount of organic matter. Pedon 4 showed single grain structure with structureless grade due to very coarse texture and lack of organic matter. Kumar and Philip (2021) also obtained similar findings. The structure grade in all other pedons was varying from to moderate to strong. The existing minor variability in structure could be related to the topographic position of the profile in the landscape and horizons in the profile, and the contents of OM. (Singh and Aggarwal 2005, Rao et al., 2008).

The soils of pedon 1, 2 and 5 showed slightly sticky non-plastic consistence in upper horizon, non-sticky non-plastic in middle & lowermost horizon and in pedon 12, the consistence was slightly sticky slightly plastic in all horizons due to low amount of clay content except in C2 horizon where moderately sticky moderately plastic consistency was because of more clay content in these horizons as a result of eluviation process. The soils of pedon 4 showed non-sticky non-plastic in all the layers because of very coarse texture of soil. The soils of pedon 3 showed moderately-sticky slightly plastic in the first layer due to higher amount of clay content as compare to the pedon 1,2,5 and 12. In pedon 7, the consistency was moderately-sticky moderately plastic in the first two layer and non-sticky non-plastic in rest of the layers. The consistence soils of pedon 6, 8, 9, 10 & 11 was very sticky very plastic to moderately sticky moderately plastic throughout the solum because of high amount of clay in the soil. This physical behaviour of soils influenced by dry, moist and wet conditions was not only due to the textural make up but also due to type of clay minerals present in these soils (Dasog and Patil 2011). Coarse fragments were absent in all the horizons of all the pedons except surface horizon of pedon 1.

The calcium carbonate concretions were present in pedon 10, 11 and 12 due to calcium containing parent rock which released calcium upon weathering and soil formation. Relatively higher calcium carbonate content was found in the subsurface layers as compared to the surface soil; this might be due to the parent material. Similar findings were recorded by Sebnie et al. (2021).

Relative horizon distinctness: The RHD value of soils of P1 to P3 ranged from 1-9 whereas for P4 the values ranged from 1-4. The slope of the land ranged from 3-5% (Gently sloping lands) in P1-P4 profile sites (Table 3, Fig. 2). The

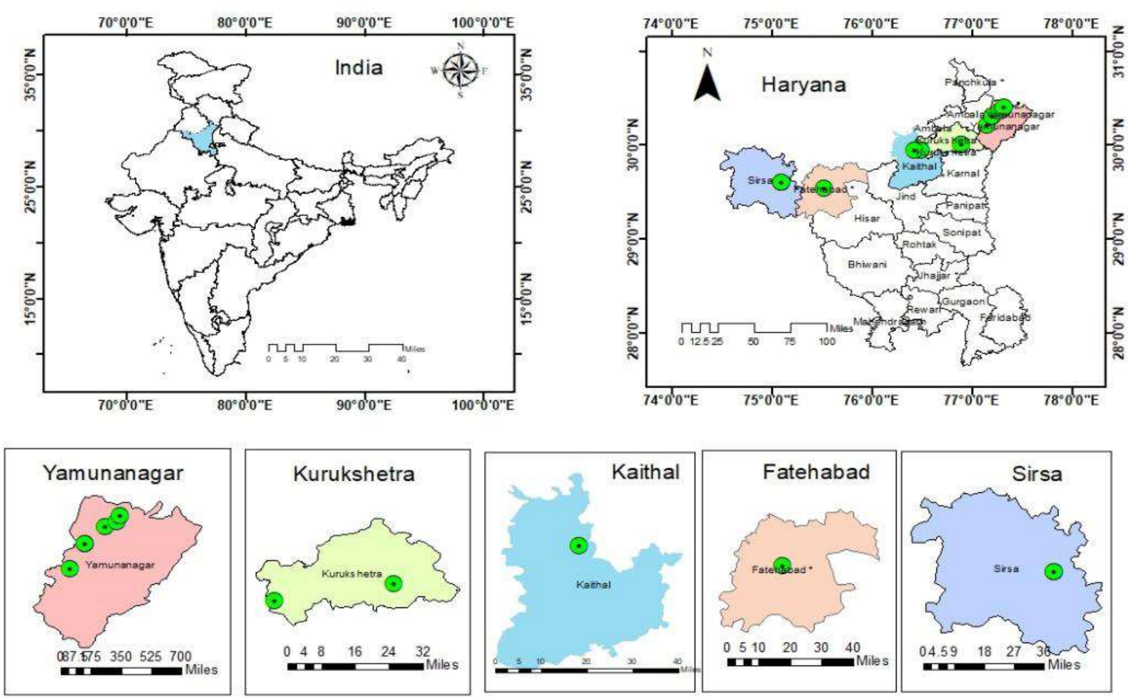

Fig. 1. Location of the studied area

Table 3. Relative horizon distinctness ratings

Profile No.	Horizon	Horizon boundary	Colour	Texture	Structure	Consistence	Cutans	Roots	Coarse fragment	CaCO3	EC	pH	RHD	RHD (Total)
P1 (Ranipur)	Ap1/Ap2	2	0	0	0	0	0	0	1	0	0	0	3	12
	Ap2/AC1	2	2	0	0	0	0	0	0	0	0	0	4	
	AC1/AC2	1	0	0	0	0	0	0	0	0	0	0	1	
	AC2/C1	1	0	0	0	1	0	0	0	0	0	0	2	
	C1/C2	1	2	0	0	0	0	0	0	0	0	0	3	
P2 (Mugalwali)	Ap/B1	2	3	1	0	1	0	1	0	0	0	1	9	20
	B1/B2	2	0	0	0	0	0	0	0	0	0	0	2	
	B2/B3	1	0	0	0	0	0	0	0	0	0	1	2	
	B3/C	1	2	1	1	1	0	0	0	0	0	1	7	
P3 (Bansewala)	Ap/B1	2	1	1	0	1	0	0	0	0	0	0	5	20
	B1/B2	1	2	0	2	0	0	0	0	0	0	1	6	
	B2/B3	1	1	0	0	1	0	0	0	0	0	0	3	
	B3/Cq	1	3	1	1	0	0	0	0	0	0	0	6	
P4 (Painsal)	A/AC	2	2	0	0	0	0	0	0	0	0	0	4	8
	AC/C1	1	1	0	0	0	0	1	0	0	0	0	3	
	C1/C2	1	0	0	0	0	0	0	0	0	0	0	1	
P5 (Painsal NB)	Ap/B1	2	2	0	0	0	0	0	0	0	0	1	5	17
	B1/B2	1	1	0	0	0	0	0	0	0	0	0	2	
	B2/BC	1	1	0	1	0	0	0	0	0	0	0	3	
	BC/C	1	2	1	1	1	0	0	0	0	0	1	7	
P6 (Mustafabad)	Ap/Bt	2	0	2	0	0	0	0	0	0	0	0	4	16
	Bt/B2	2	0	2	0	0	0	1	0	0	0	0	5	
	B2/B3	1	1	0	0	1	0	0	0	1	0	1	5	
	B3/C	1	0	0	1	0	0	0	0	0	0	0	2	
P7 (Ishargarh)	A/A/B	1	1	0	0	0	0	1	0	0	0	0	3	19
	A/B/B	1	1	2	2	2	0	0	0	0	0	0	8	
	B/Bq	1	3	0	0	0	0	0	0	0	0	0	4	
	Bq/Cq	2	2	0	0	0	0	0	0	0	0	0	4	
P8 (Mangna)	A/Bt1	2	0	1	0	0	0	0	0	0	0	0	3	14
	Bt1/Bt2	1	2	0	0	0	0	0	0	0	0	1	4	
	Bt2/B	1	1	0	1	0	0	0	0	0	0	1	4	
	B/C	1	1	0	1	0	0	0	0	0	0	0	3	
P9 (Kaekor)	A/B1	2	1	0	0	0	0	0	0	0	0	1	4	20
	B1/B2	1	1	3	1	0	0	0	0	0	0	0	6	
	B2/B3	1	4	1	1	0	0	0	0	0	0	0	7	
	B3/C	1	0	1	0	0	0	0	0	0	0	1	3	
P10 (Birdhana)	Ap/Bt1	3	1	1	1	0	0	0	0	1	0	0	7	16
	Bt2/Bt2	1	0	0	0	0	0	0	0	0	0	0	1	
	Bt2/C1	1	1	1	1	1	0	0	0	0	0	1	6	
	C1/C2	1	0	0	0	1	0	0	0	0	0	0	2	
P11 (Farwai)	A/AB	2	0	1	2	0	0	1	0	0	0	0	6	29
	AB/B1	1	1	2	0	1	0	1	0	0	0	0	6	
	B1/B2	1	0	1	2	1	0	0	0	0	0	0	5	
	B2/B3	1	2	0	0	0	0	0	0	0	0	0	3	
	B3/C	1	2	3	1	1	0	0	0	1	0	0	9	
P12 (Farwai NB)	Ap/Bu1	2	2	0	0	0	0	0	0	0	0	0	4	14
	Bu1/Bu2	1	0	0	0	0	0	0	0	0	0	0	1	
	Bu2/C1	1	2	1	1	0	0	0	0	0	0	0	5	
	C1/C2	1	0	1	0	1	0	0	0	0	0	1	4	

Zero value in a column means there is no difference in the attribute in question when compared to the below horizon

Table 4. Relative profile development ratings

Profile No.	Horizon	Horizon boundary	Colour	Texture	Structure	Consistence	Cutans	Roots	Coarse fragment	CaCO3	pH	EC	RPD	RPD (Total)
P1 (Ranipur)	Ap1/C2	2	0	0	0	1	0	0	1	0	0	0	4	19
	Ap2/C2	2	0	0	0	1	0	0	0	0	0	0	3	
	AC1/C2	1	2	0	0	1	0	0	0	0	0	0	4	
	AC2/C2	1	2	0	0	1	0	0	1	0	0	0	5	
	C1/C2	1	2	0	0	0	0	0	0	0	0	0	3	
P2 (Mugalwali)	Ap/C	2	1	2	1	1	0	1	0	0	0	1	9	32
	B1/C	2	2	1	1	1	0	1	0	0	0	0	8	
	B2/C	1	2	1	1	1	0	1	0	0	0	0	7	
	B3/C	1	2	1	1	1	0	1	0	0	0	1	8	
P3 (Bansewala)	Ap/Cq	2	1	3	1	2	0	0	0	0	0	1	10	31
	B1/Cq	2	0	2	1	1	0	0	0	0	0	1	7	
	B2/Cq	1	2	2	1	1	0	0	0	0	0	0	7	
	B3/Cq	1	4	1	1	0	0	0	0	0	0	0	7	
P4 (Painsal)	A/C2	2	1	0	0	0	0	0	0	0	0	4	7	13
	AC/C2	1	1	0	0	0	0	1	0	0	0	1	4	
	C1/C2	1	0	0	0	0	0	0	0	0	0	1	2	
P5 (Painsal NB)	Ap/C	2	2	1	1	1	0	1	0	0	0	2	10	33
	B1/C	1	2	1	1	1	0	1	0	0	0	1	8	
	B2/C	1	3	1	1	1	0	1	0	0	0	1	9	
	BC/C	1	2	1	0	1	0	1	0	0	0	0	6	
P6 (Mustafabad)	Ap/C	2	1	1	1	2	0	1	0	1	0	1	10	30
	Bt/C	2	1	1	1	2	0	1	0	1	0	1	10	
	B2/C	1	1	1	1	2	0	1	0	1	0	1	9	
	B3/C	1	0	0	0	0	0	0	0	0	0	0	1	
P7 (Ishargarh)	A/Cq	1	1	2	2	2	0	1	0	0	0	0	9	26
	A/B/ Cq	1	2	2	2	2	0	1	0	0	0	0	10	
	B/Cq	1	1	0	0	0	0	1	0	0	0	0	3	
	Bq/Cq	2	2	0	0	0	0	0	0	0	0	0	4	
P8 (Mangna)	A/C	2	2	1	1	0	0	0	0	0	0	0	6	16
	Bt1/C	1	2	0	1	0	0	0	0	0	0	0	4	
	Bt2/C	1	0	0	1	0	0	0	0	0	0	1	3	
	B/C	1	1	0	1	0	0	0	0	0	0	0	3	
P9 (Kaekor)	A/C	2	1	2	2	0	0	0	0	0	0	0	7	26
	B1/C	1	2	2	2	0	0	0	0	0	0	1	8	
	B2/C	1	4	1	2	0	0	0	0	0	0	1	9	
	B3/C	1	0	0	0	0	0	0	0	0	0	1	2	
P10 (Birdhana)	Ap/C2	3	2	0	0	1	0	0	0	1	0	1	8	22
	Bt2/C2	1	1	1	1	1	0	0	0	0	0	1	6	
	Bt2/C2	1	1	1	1	1	0	0	0	0	0	1	5	
	C1/C2	1	0	0	0	2	0	0	0	0	0	0	3	
P11 (Farwai)	A/C	2	0	1	2	0	0	1	0	1	0	0	7	33
	AB/C	1	1	2	0	1	0	1	0	1	0	0	7	
	B1/C	1	0	1	2	1	0	0	0	1	0	0	6	
	B2/C	1	2	0	0	0	0	0	0	1	0	0	4	
	B3/C	1	2	3	1	1	0	0	0	1	0	0	9	
P12 (Farwai NB)	Ap/ C2	2	2	1	1	1	0	0	0	0	0	3	10	28
	Bu1/ C2	1	2	1	1	1	0	0	0	0	0	1	7	
	Bu2/C2	1	2	1	1	1	0	0	0	0	0	1	7	
	C1/C2	1	0	1	0	1	0	0	0	0	0	1	4	

Zero value in a column means there is no difference in the attribute in question when compared to the below horizon

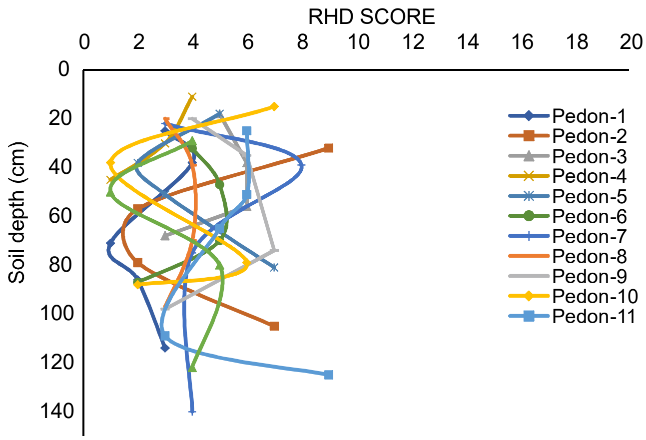

Fig. 2. Relative horizon distinctness

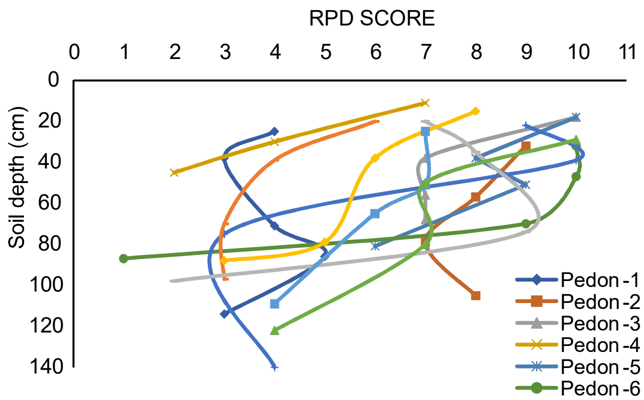

Fig. 3. Relative profile development

majority of the field morphological assessments were influenced by the horizon boundary's development as well as differences in colour, texture, structure, and consistency. The degree of pedological development is positively correlated with the size of the rating scale values for a certain horizon. The soils of pedon 2, 3, and 9 have a RHD (total) of 20 each, pedon 7, 5, 6 and 10 have 19, 17, 16 and 16 respectively and pedon 12 & 8 was 14 each. Maximum RHD (total) value is shown by pedon 11 i.e. 29 and hence this profile shows maximum profile development not generally; since the order is Inceptisol but comparatively. The distinctness of the horizon boundaries may be due to weathering as influenced by more precipitation in the recent alluvial plains (Gill et al., 2022). The lowest value of RHD (total) is shown by pedon 4 i.e., 8 which shows least profile development among the other profiles. According to the RHD values, the variations in the different profiles may be the result of pedological processes rather than changes in the structure or texture of the earth (Reza et al., 2010).

Relative profile development: When a landform is stable, the growth of the soil profile alters several soil morphological features, producing more RPD values (Zayed et al., 2021). Due to the greatest pedological development driven by weathering, the RPD values of all the profiles were highest in the B horizon (Dinesh et al., 2017). The RPD (Total) value of the pedons under investigation varied from 16 to 33 being minimum in pedon 8 and maximum in pedon 11 and 5 (Table 4). The horizon boundary, differences in moist colour, texture, structure, consistency, pH, and EC all had a role in the rating variation (Deka et al., 2009).

CONCLUSION

The RHD and RPD score of different pedon showed that the pedon 11 is comparatively more developed from other and pedon 4 is least developed. The soils of pedon 4 are very less developed since this part of palaeochannel has continuous accumulation of alluvio-fluvial material even now as represented by the presence of water within 50 cm depth. The horizon boundary, differences in moist colour, texture, structure, consistency, pH, and EC all had a role in the rating variation. The study also revealed significant difference in soil development when the soils are compared to in-situ soils which are well developed.

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Riparian Vegetation as Bioindicator of Heavy Metal Contamination and Soil Nutrient Dynamics in Vamanapuram River, Kerala

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Abstract: This study examines the relationship between riparian vegetation, soil nutrient dynamics, and heavy metal contamination along a 15 km stretch of the Vamanapuram River, Kerala. Six sites representing a gradient of anthropogenic disturbance were assessed through quadrat-based vegetation surveys and comprehensive soil chemical analysis, including macro- and micronutrients (K, Ca, Mg, B, Mn, Cu, Zn) and heavy metals (Hg, Pb, As, Ni, Cd, Cr, Bi, Ga) using ICP-MS. A total of 102 plant species belonging to 45 families were recorded, with Fabaceae and Poaceae dominating across the vegetated sites. Species diversity and abundance were highest at moderately disturbed sites Maveli Nagar (Site 2) and Vamanapuram Bridge (Site 3), which coincided with balanced soil nutrient profiles and lower levels of heavy metals. Conversely, Site 6 (Melattumoozhi), heavily impacted by soil dumping, exhibited minimal vegetation and the highest concentrations of mercury (0.41 mg/kg), arsenic (74.8 mg/kg), and gallium (133 mg/kg). Principal Component Analysis (PCA) revealed that PC1 (45.2% variance) was associated with heavy metal concentrations, while PC2 (27.6%) captured nutrient variability, particularly boron and calcium. Vegetated sites clustered distinctly from polluted sites in the PCA biplot, emphasizing a strong inverse relationship between vegetation cover and metal contamination. The study underscores the role of riparian vegetation as a bioindicator of soil quality and a regulator of contamination. Restoration and conservation of riparian buffers are essential for sustaining soil health and mitigating the ecological impacts of anthropogenic activities along tropical riverine systems.

Keywords: Riparian vegetation, Heavy metals, Soil nutrients, PCA, Vamanapuram River

Riparian zones are ecologically dynamic transition areas located along the margins of rivers, streams, and floodplains. These zones are characterized by unique combinations of vegetation, hydrological regimes, and soil properties that distinguish them from surrounding upland ecosystems (Okeke et al., 2022). This distinctiveness arises from variations in topographical gradients and increased moisture availability, fostering diverse habitats and biological productivity (Mikkelsen and Vesho 2000).

The soils in riparian zones are highly heterogeneous in their chemical composition, influenced by prolonged saturation, periodic inundation, and the bidirectional transfer of nutrients from terrestrial uplands and adjacent aquatic systems (Vidon et al., 2010). Frequent flooding and water level fluctuations slow the accumulation of organic and inorganic materials, often inhibiting the development of structured soil horizons (Mikkelsen and Vesho, 2000; Klemas, 2014). These processes contribute to the fertility and ecological complexity of riparian environments. Riparian zones serve as crucial ecological buffers that enhance water quality, stabilize stream banks, and support a rich diversity of aquatic and terrestrial species. Positioned at the interface between land and water, these zones regulate the flow of nutrients, intercept pollutants, and act as corridors for species migration and habitat connectivity (Liu et al., 2014).

The Vamanapuram River, locally known as “Attingalaru” or “Kollambuzhayaru”, originates from the Chemunji Mottai Hills (1,860 m above MSL) and flows westward through the Thiruvananthapuram district to the Anchuthengu backwaters. It serves as a vital freshwater source for the region. However, the river's catchment area is increasingly affected by anthropogenic activities, including rapid urbanization and agricultural expansion (notably rubber and oil palm plantations). These disturbances have led to growing concerns about heavy metal accumulation in the riparian zone, threatening both plant health and ecological stability.

Heavy metals such as mercury, arsenic, lead, and chromium enter riparian soils primarily through human activities including construction, agricultural runoff, and improper waste disposal (Vidon et al., 2010). These toxic elements impair plant growth, alter species composition, and reduce overall biodiversity. As the soil serves as a biologically active medium essential for supporting life, its contamination poses serious threats to ecological integrity and food security (Singh et al., 2010; Sarwar et al., 2017).

Riparian vegetation plays a pivotal role in maintaining river ecosystem health. Dense and fast-growing plant communities along water bodies enhance soil structure, increase porosity, and reduce erosion through their root systems, which anchor soil and buffer streambanks against water flow (Nielsen et al., 2014). Additionally, riparian plants filter surface runoff, intercept sediment, and prevent excessive deposition into watercourses (Fernandes et al., 2016, Hould-Gosselin et al., 2016). However, despite access to perennial groundwater, these plants may also experience water stress during periods of reduced availability or increased evapotranspiration demand (Singer et al., 2013). The objective of this study is to examine the relationship between riparian vegetation structure, soil nutrient dynamics and heavy metal contamination along the Vamanapuram River to underscore the role of riparian plant communities as a bioindicator of soil quality and a regulator of environmental contamination, thereby reinforcing the need for their conservation and restoration.

MATERIAL AND METHODS

Study area: The present study was carried out along the riparian corridor of the Vamanapuram River, situated in Thiruvananthapuram district, Kerala, India. The river originates from the Chemunji Mottai hills and flows westward through Vamanapuram Panchayat. Six sites were selected along 15 km stretch of the river (Table 1), encompassing varying degrees of anthropogenic influence, including agricultural runoff, soil dumping, and urban encroachment. Site 6, characterized by significant disturbance and minimal vegetation, was designated as the control site.

Table 1. Sampling locations

Site	Sampling points	Latitude	Longitude
Site 1	Perunthra	8° 43' 17" N	76° 53' 15"E
Site 2	Maveli Nagar	8° 43' 15" N	76° 53' 35" E
Site 3	Vamanapuram Bridge	8° 43' 35" N	76° 53' 50"E
Site 4	Balikkadavu	8° 43' 28" N	76° 53' 03" E
Site 5	Nedumparambu	8° 43' 20" N	76° 54' 35"E
Site 6	Melattumoozhi	8° 42' 43" N	76° 55' 26" E

Vegetation sampling: Vegetation analysis was conducted using the quadrat method. At each of the five vegetated sites, four quadrats measuring 10 m × 10 m were laid out perpendicular to the river channel, resulting in a total of 20 quadrats. Plant species within each quadrat were taxonomically identified, counted, and recorded. Site 6 lacked sufficient vegetation and was excluded from the floristic survey. Data on species richness, abundance, and family representation were compiled to assess the composition and structure of riparian plant communities.

Soil sampling and laboratory analysis: Soil samples were collected from the top 15 cm of the riverbank at each of the six study sites using standard composite sampling techniques. Five sub samples per site were homogenized, air dried, ground, and sieved (2 mm mesh) to produce one representative sample per location (500 g). Samples were stored in sealed polyethylene bags for chemical analysis (APHA 2005). Soil macro and micronutrients (potassium, calcium, magnesium, boron, manganese, copper, and zinc) as well as heavy metals (mercury, lead, arsenic, nickel, cadmium, chromium, bismuth, and gallium) were analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS; iCAP Q, Thermo Fisher Scientific). Calibration was performed using ICP Multielement Standard VI (Merck), and all measurements were reported in mg/kg dry weight.

Data analysis: Data analysis was performed using PAST 4.03 (Paleontological Statistics Software Package) and Microsoft Excel 2019. To identify patterns in soil nutrients and heavy metals to assess their relationship with riparian vegetation structure, Principal Component Analysis (PCA) was conducted using PAST 4.03. The input data matrix included standardized values of soil nutrients and heavy metals from the six sampling sites. PCA was based on a correlation matrix, and components with eigenvalues greater than 1 were retained for interpretation. Biplots were produced to visualize site clustering and variable loadings, thereby highlighting the dominant gradients influencing soil chemical variability.

RESULTS AND DISCUSSION

A total of 102 plant species were recorded across the riparian study sites, with species diversity increasing in the order: Site 1 (Perunthra) < Site 4 (Balikkadavu) < Site 5 (Nedumparambu) < Site 2 (Maveli Nagar) < Site 3 (Vamanapuram Bridge). The highest species density and abundance were observed in Sites 2 and 3, while Sites 1 and 4 exhibited the lowest. Fabaceae family was the most dominant, indicating its adaptability and ecological importance in riparian environments. The structure and composition of plant communities appeared to be closely linked to soil conditions, with nutrient-rich sites supporting a greater diversity of vegetation. The growth and health of riparian plants were influenced by the concentration and balance of essential nutrients such as nitrogen, phosphorus, and potassium, which play key roles in physiological processes like cell division, root development, and photosynthesis. Sites with well-balanced nutrient profiles exhibited more robust and diverse plant growth, while areas with nutrient imbalances showed reduced vegetation and signs of ecological stress (Liu et al., 2014, Hale et al., 2018)

Vegetation analysis: A total of 102 riparian plant species belonging to 45 families were recorded along the Vamanapuram River. The flora comprised a diverse mix of herbs, shrubs, trees, and climbers, with a predominance of herbaceous taxa. The most represented families were Fabaceae, Asteraceae, Poaceae, and Lamiaceae, highlighting their adaptability to riparian conditions. Frequently occurring species across the sites included *Mimosa pudica*, *Phragmites karka*, *Melastoma malabathrium*, *Hyptis suaveolens*, *Glyricidia sepium*, *Caryota urens*, *Tridax procumbens*, *Synedrella nodiflora*, and *Adiantum raddianum*, indicating wide ecological tolerance.

Site wise analysis revealed substantial variation in species composition influenced by anthropogenic pressure. Site 1, with 43 species from 22 families, showed reduced vegetation, dominated by grasses and a few woody plants due to disturbances such as grazing and bathing. Site 2, least disturbed, hosted 61 species from 32 families, including abundant riparian taxa like *Pandanus odoratissimus* and *Hyptis capitata*. Site 3 was the most floristically rich, with 64 species from 31 families and a high density of bamboos and ferns, notably *Bambusa bambos* and *Adiantum raddianum*. Site 4, under greater human impact, recorded only 44 species, dominated by disturbance-tolerant plants such as *Amaranthus spinosus* and *Hyptis capitata*. Site 5 supported 58 species across 28 families, marked by abundant grasses and bamboos (*Bambusa vulgaris*, *Phragmites karka*). Site 6, used as a control, was devoid of vegetation due to recent soil disturbances and hence excluded from vegetative analysis.

Analysis of the presence - absence matrix emphasized species specific spatial patterns. Certain species were restricted to single sites, such as *Barringtonia racemosa* and *Spondias pinnata*, suggesting localized habitat preferences (Table 2). In contrast, several taxa were consistently present across multiple sites, serving as indicators of general riparian adaptability. The vegetation structure and diversity were closely linked to underlying soil conditions and disturbance intensity, reinforcing the role of riparian zones as biodiversity hotspots and sensitive indicators of environmental change.

Soil nutrient analysis: The analysis of soil nutrients across the six sites along the Vamanapuram River demonstrated substantial variability in both macro- and micronutrient concentrations, which showed a strong correlation with the density and diversity of riparian vegetation. Potassium (K) levels were high across all sites, ranged from 12,721 mg/kg in Site 2 to 17,063 mg/kg in Site 1, indicating either naturally elevated levels or enrichment from agricultural runoff. Even the sparsely vegetated Site 6 recorded a high potassium concentration of 15,141 mg/kg. Calcium (Ca) levels varied more significantly, with the highest concentration observed in Site 6 (1,642 mg/kg), which had minimal vegetation, while Sites 2 and 3 characterized by rich plant diversity, recorded lower calcium levels of 389 mg/kg and 711 mg/kg, respectively. This suggests that excessive calcium may hinder plant nutrient uptake and root development, thereby restricting vegetation growth, whereas moderate calcium availability appears to support healthier and more diverse riparian plant communities. Radar chart (Fig. 1) illustrates the normalized average concentrations of soil nutrients across the study sites, highlighting major contributors like potassium, magnesium, and manganese, which dominate the soil nutrient profile.

Heavy metal analysis: Heavy metals like mercury, arsenic, lead, nickel, bismuth, gallium, cadmium, and chromium were analysed in this study. Heavy metals in the environment are problematic and pose a threat to human health, the heavy metals are increasing in the order mercury <cadmium <bismuth <lead <arsenic <gallium <nickel <chromium (Table 4).

The heavy metal analysis of soil samples from the riparian zones along the Vamanapuram River revealed significant spatial differences, largely driven by varying levels of human disturbance at each site. Site 6, which was most affected by activities such as soil dumping and construction, exhibited the highest concentrations of nearly all heavy metals measured. Specifically, mercury was recorded at 0.41 mg/kg, lead at 49.7 mg/kg, arsenic at 74.8 mg/kg, chromium at 319 mg/kg, and gallium at 133 mg/kg. These values were substantially higher than those at other sites and far exceeded typical background levels for uncontaminated soils. In contrast, Sites 2 and 3, which had richer vegetation and less disturbance, showed comparatively lower levels of these metals. The study indicate that anthropogenic pressures significantly contribute to heavy metal accumulation in riparian soils, with the highest contamination observed in areas lacking vegetative cover. Radar chart displayed the normalized average concentrations of heavy metals across the six study sites showing chromium, nickel, and gallium in the highest level (Fig. 2).

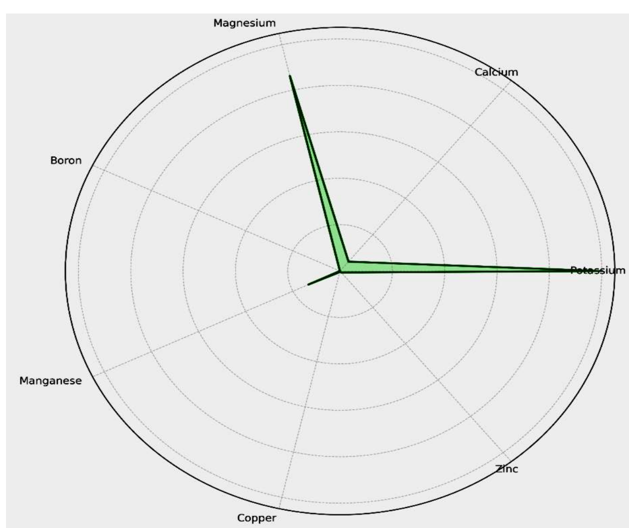

Fig. 1. Radar chart of soil nutrients

Table 2. Riparian plants along Vamanapuram River

Botanical name	Local name	Site 1	Site 2	Site 3	Site 4	Site 5
Adiantum raddianum	Maiden hair	√	√	√	√	√
Acacia caesia	Inja	√		√	√	
Adenanthera pavonina	Manjadi		√	√	√	√
Alstonia scholaris	Ezhilam paala		√			√
Anacardium occidentale	Kashumaavu		√			√
Orthosiphon aristatus	Poochameesa		√	√	√	
Curcuma amada	Mangayinji			√	√	
Areca catechu	Kavungu			√		
Artocarpus hetrophyllus	Plaavu	√			√	
A. hirsutus	Ayani maram		√		√	√
Argyreia hirsuta	Samudrappacha		√	√		
Synedrella nodiflora	Mudiyan pacha	√	√	√	√	√
Bambusa bambos	Mula	√		√	√	√
B. vulgaris	Manja mula	√			√	√
Bauhinia malabarica	Vellamandaram		√	√		
Tridax procumbens	Chiravanaakku	√	√	√	√	√
Biancaea sappan	Pathimukham		√			
Calophyllum calaba	Cherupunna				√	
Caryota urens	Olatti	√	√	√	√	√
Cinnamomum malabatrum	Vazhana	√				√
C. riparium	Aattuvayana	√	√			
Clerodendron infortunatum	Peringalam		√	√		√
Cassia fistula	Konnamaram		√	√		
Cyclea peltata	Paadathali		√	√		√
Acmella ciliata	Palluvedana chedi		√	√	√	√
Blumea axillaris	Kukkura		√	√		√
Cyanthillium cinereum	Poovamkurunila		√	√		
Emilia sonchifolia	Muyal cheviyan		√	√	√	√
Ficus hispida	Therakam	√				
Garcinia gummi-gutta	Kodampuli			√		
Stachytarpheta indica	Seemakongini		√	√		√
Glyrricedia maculata	Seemakkonna	√	√	√	√	√
Glycosmis pentaphylla	Kurumpannal			√		√
Heliconia rostrata	Vazhachedi		√			√
Hibiscus tiliaceus	Thaipparuthi			√		√
Holigarna arnottiana	Chaaru		√	√		
Amaranthus spinosus	Mullan cheera				√	√
Carica papaya	Papaya		√	√		
Humboldita vahliana	Aattuvanchi	√	√			√
Urena lobata	Uthiram		√		√	√
Hydnocarpus pentandra	Marotti			√		
Hyptis suaveolens	Naattappoochedi	√	√	√	√	√
Ixora coccinea	Vella Thetti		√	√		√
Justicia adhatoda	Aadalodakam	√	√	√		
Ludwigia perennis	Neerkarayambu	√			√	√
Lagerstroemia speciosa	Manimaruth			√		
Lawsenia inermis	Mylanchi		√			

Cont...

Table 2. Riparian plants along Vamanapuram River

Botanical name	Local name	Site 1	Site 2	Site 3	Site 4	Site 5
Leea indica	Choriyanthali	√				
Tragia involucrata	Kodithoova		√	√		√
Madhuca neriifolia	Aattilippa	√	√			
Pilea microphylla	Mathilppacha		√	√		
Desmodium triflorum	Nilamparanda	√	√	√		√
Clitoria ternata	Shankhupushpam		√	√		√
Melastoma malabathrium	Kalambatty	√	√	√	√	√
Mikania micrantha	Drutharashtrappacha	√		√	√	√
Mollotus philippensis var. philippensis	Kumkumam		√	√		
Mussaenda frondosa	Musanda		√	√		√
Myristica beddomei	Jaathi		√			
Crotalaria angulata	Kilukilukki			√	√	
Ochlandra travancoria	Eetta	√			√	
Ochlandra wightri	Eera	√			√	√
Senna alata	Aana thakara	√				√
Tinospora cordifolia	Chittamruth			√	√	
Palms	Pana		√	√		
Pandanus odoratissimus	Kaitha		√		√	√
Pennisetum polystachion	Poochavalan pullu	√			√	√
Persea macrantha	Uuraavu	√		√		√
Phragmites karka	Pullu	√	√	√	√	√
Piper nigrum	Kurumulaku		√			
Polycarpae corymbosa	Akkaramkolli			√		
Polyalthia longiflora	Aranamaram	√	√			√
Cynodon dactylon	Karuka					√
Crateva magna	Neermathalam			√		
Ricinus communis	Aavanakk	√		√	√	
Saccharum officinarum	Karimb				√	
Senna tora	Pon thakara		√	√	√	
Barringtonia racemosa	Samudrakkaya					√
Syzigium cumini	Njaval	√				√
Tiliacora acuminata	Valli kanjiram	√	√	√		
Solanum torvum	Chunda	√	√	√	√	
Spondias pinnata	Ambazham					√
Saccharum spontaneum	Choottapullu	√			√	√
Tabernaemontana divaricata	Paala	√	√	√		
Tamarindus indica	Pulimaram	√		√		
Pothos scandens	Paruvakkodi		√	√		√
Tectona grandis	Thekk		√	√		√
Terminalia bellinica	Thaanni					√
Tetracera akara	Nannalvalli			√		
Thespesia populnea	Cheelanthi	√				
Trema orientalis	Aamathaali		√	√	√	√
Trewia nudiflora	Thavalamaram			√		
Eclipta prostrata	Kayyonni	√	√	√		√
Colocasia esculenta	Thaalu		√	√	√	√
Panicum maximum	Kuthirappullu	√	√		√	√
Ziziphus oenopolia	Thodali		√	√	√	√
Terminalia catappa	Badaam	√	√		√	√
Sphagneticola calendulaceae	Manjakkanjunni	√		√	√	√
Memecylon umbellatum	Kaashaavu		√			
Mimosa diplotricha	Aanathottavadi	√		√	√	
Mimosa pudica	Thottavaadi	√	√	√	√	√
Lantana camara	Kongini	√	√			√
Hyptis capitata	Mittayi chedi		√	√	√	√

Principal component analysis: The first principal component (PC_1) accounted for 45.2% of the total variance and was strongly influenced by elevated concentrations of heavy metals, particularly mercury (0.82), arsenic (0.78), copper (0.75), and gallium (0.74), with zinc (0.65) contributing moderately. This axis reflects the pollution gradient among the sites. The second component (PC_2) contributed 27.6% of the variance and was driven predominantly by boron (0.88) and calcium (0.85), representing nutrient related variation. Zinc (0.48) also loaded moderately on PC_2, suggesting its overlapping association with both nutrient and pollution profiles. Combined, PC_1 and PC_2 explained 72.8% of the total variance, while PC_3 contributed an additional 12.1%, yielding a cumulative explanation of 84.9%.

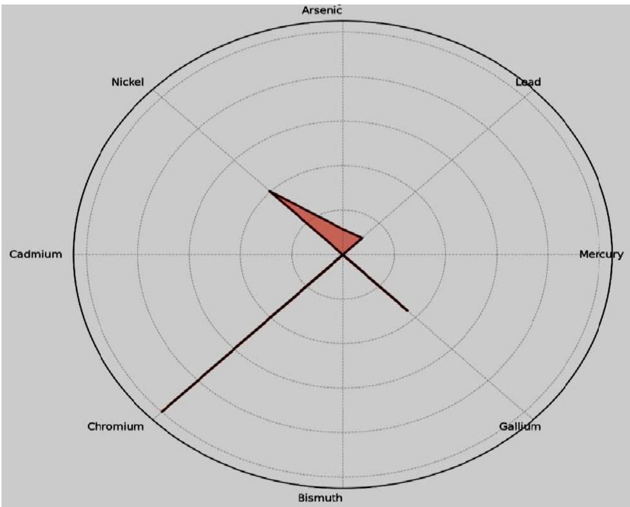

Fig. 2. Radar chart of heavy metals

The PCA biplot effectively separated the study sites based on their underlying soil chemistry (Fig. 3). Site 6 showed the highest score on PC_1, indicating pronounced heavy metal presence. Site 2 aligned positively with PC_2, corresponding to nutrient richness. Sites 3 and 5 formed a central cluster, reflecting balanced nutrient metal conditions, whereas Site 1 was positioned with low scores on both axes. The variable vectors revealed strong influences of gallium, manganese, and arsenic in differentiating the sites, emphasizing their role in driving spatial heterogeneity in riparian soil chemistry.

The findings from this study provide a comprehensive understanding of the interactions between riparian

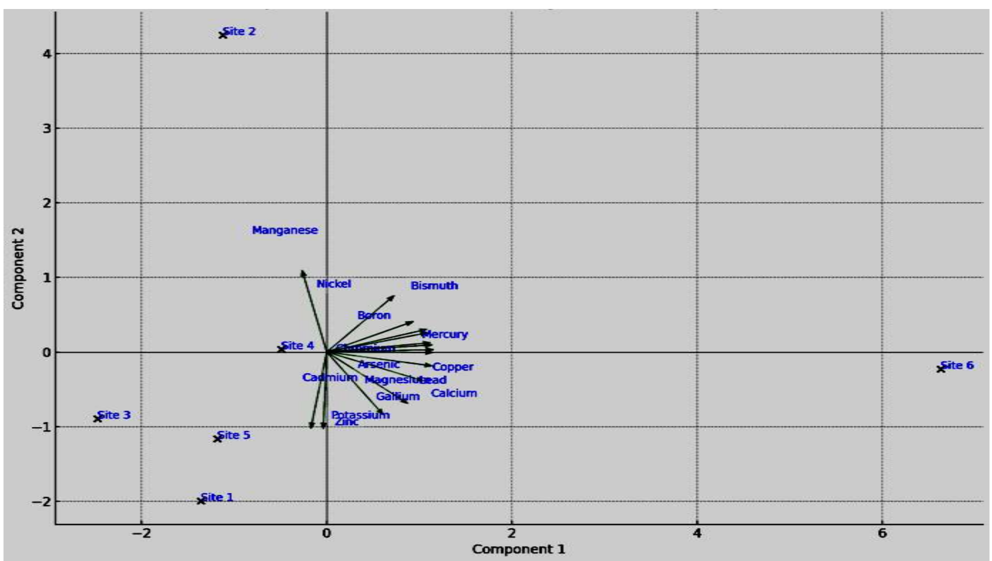

Fig. 3. Principal component analysis biplot of soil nutrients and heavy metals

Table 3. Status of soil nutrients

Nutrients (mg kg^{-1})	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Potassium	17063	12721	14783	14312	17052	15141
Calcium	849	389	711	616	514	1642
Magnesium	14929	9364	10754	13969	14151	15370
Boron	9.62	11.3	8.51	12.1	9.54	15.3
Manganese	1597	3610	1931	1630	1710	1636
Copper	64.1	67.9	61.9	62	62.4	97.0
Zinc	124.1	101	121	132	123	148

vegetation, soil nutrient dynamics, and heavy metal contamination along the Vamanapuram River. Sites with rich riparian vegetation, notably Sites 2 and 3, exhibited high species diversity and plant abundance, particularly from families such as Fabaceae and Poaceae, indicating their resilience and ecological adaptability (Baptista et al., 2020). These sites also showed comparatively lower concentrations of toxic heavy metals, suggesting that vegetation plays a vital regulatory role by acting as a natural buffer that reduces pollutant inflow and enhances soil health. In contrast, Site 6, which had minimal vegetation due to soil dumping and developmental disturbances, recorded the highest levels of heavy metals, including mercury (0.41 mg/kg), lead (49.7 mg/kg), arsenic (74.8 mg/kg), chromium (319 mg/kg), and gallium (133 mg/kg). This pattern indicates that in the absence of riparian plant cover, the soil becomes increasingly vulnerable to contamination, lacking the biological filtration and root systems that would otherwise intercept and stabilize pollutants (Sharma et al., 2018; Tchounwou et al., 2012).

Riparian plants also contribute to phytoremediation, a process through which certain species absorb and immobilize heavy metals, thereby improving soil conditions over time (Brdar et al., 2020). However, when concentrations of heavy metals exceed physiological thresholds, they become toxic to plants, inhibiting growth, altering species composition, and in severe cases, leading to mortality (Alloway 2013). This creates a feedback loop in which vegetation loss worsens contamination, while contamination itself further limits vegetation, ultimately degrading the ecological stability of the riparian zone.

Micronutrient patterns also correlated with vegetation health. Boron (B) levels, although below toxic thresholds, were highest in Site 6 (15.3 mg/kg) and lowest in Site 3 (8.51 mg/kg), suggesting accumulation in disturbed soils (Nable et al., 1997). Manganese (Mn) peaked in Site 2 (3,610 mg/kg), likely due to organic matter decomposition enhancing Mn solubility under acidic conditions. Despite these high levels, lush vegetation persisted in Site 2, possibly due to the presence of Mn tolerant species. Copper (Cu) and zinc (Zn) concentrations were notably high in Site 6 (97.0 mg/kg and 148 mg/kg, respectively), exceeding WHO's permissible limits. These elevated values suggest anthropogenic inputs such as construction debris and landfill materials as potential contamination sources.

The overall nutrient profile revealed that sites with moderate and balanced concentrations of macro and micronutrients (Sites 2 and 3) supported higher species richness and healthier plant communities. In contrast, nutrient imbalances and extreme values, especially in Site 6, correlated with reduced vegetation (Hale et al., 2018). This underscores the importance of maintaining nutrient equilibrium in riparian soils for sustaining vegetation structure and ecosystem services. The analysis of riparian soils revealed elevated concentrations of heavy metals particularly Pb, Cd, Cr, and Ni, indicative of pronounced anthropogenic influence stemming from agricultural inputs and unregulated waste discharge. Site-specific variations, such as the high Pb and Cr levels, highlight localized zones of contamination that can significantly disrupt riparian plant communities. These metals are known to impair physiological processes in plants, influencing growth, reproduction, and overall species composition. Notably, the elevated Ni concentration at Site 2 (130 mg/kg) did not correspond with reduced vegetation cover, suggesting a degree of metal tolerance or adaptive resilience among native flora, especially within the Fabaceae family. This points to their potential utility in phytoremediation strategies (Brdar et al., 2020).

Trace elements like Cu and Zn, while essential for plant metabolism, can exert toxic effects when present in excess, leading to enzymatic inhibition and photosynthetic disruption (Alloway 2013). The observed pattern of higher metal accumulation in sparsely vegetated zones and lower levels in areas with dense vegetation cover underscores a reciprocal relationship between metal pollution and plant community health. In this context, vegetation degradation appears not only as a consequence of contamination but also as a contributing factor, given the loss of natural buffering capacity. These dynamics emphasize the dual role of riparian vegetation in both mitigating and reflecting environmental stress, reinforcing the urgency of conservation-based

Table 4. Status of soil heavy metals

Heavy metals (mg kg^{-1})	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Mercury	0.04	0.07	0.04	0.05	0.04	0.41
Lead	21.8	28.8	27.1	27.7	28.2	49.7
Arsenic	23.9	15.2	15.6	35.4	22.8	74.8
Nickel	111	130	99.3	118	116	129
Cadmium	0.20	0.09	0.21	0.12	0.19	0.16
Chromium	289	294	272	287	281	319
Bismuth	0.36	0.74	0.43	0.41	0.71	1.03
Gallium	95.2	97.8	97.2	96.2	96.5	133

management (Liu et al., 2019, Selvi et al., 2019, Prodipto et al., 2024). Riparian vegetation provides vital ecological functions including riverbank stabilization, erosion control, runoff filtration, and habitat support. The dense root networks reduce sediment displacement and buffer pollutants, thus improving water quality and soil structure (Hould-Gosselin et al., 2016, Fernandes et al., 2016). In the context of the Vamanapuram River, areas with robust riparian vegetation were consistently associated with improved soil quality and reduced metal toxicity, reinforcing their importance in maintaining ecological balance and buffering against anthropogenic disturbances.

CONCLUSION

This study reveals an intricate link between riparian vegetation, soil chemistry, and contamination along the Vamanapuram River. Spatial analysis showed that sites with intact vegetation had more balanced nutrient profiles and lower concentrations of toxic heavy metals, while highly disturbed zones exhibited severe contamination and vegetation loss. Principal component analysis (PCA) successfully distinguished pollution-dominated sites Melattumoozhi (Site 6) from nutrient-rich, vegetated sites Maveli Nagar and Vamanapuram Bridge (Site 2 , 3), reinforcing the sensitivity of riparian zones to anthropogenic pressures. These findings underscore the necessity of conserving riparian buffers as ecological safeguards to mitigate contamination risks and ensure the long-term stability of tropical riverine ecosystems.

AUTHOR'S CONTRIBUTIONS

Sruthy Krishna M R was responsible for the core research activities, including field investigation, data collection, statistical analysis, and the initial drafting of the manuscript. Dr. Alexander T, as the corresponding author, provided the overall conceptualization of the study, supervised the project, and was responsible for the final manuscript review and submission. Dr. Anila George contributed by providing methodological guidance, aiding in data interpretation, and assisting with the manuscript review and editing processes**.**

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Nano Urea as Sustainable Nutrient Strategy for Improving Phenological Traits, Quality and Soil Fertility in Wheat in semi-arid Regions of Haryana

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Abstract: The field experiment was conducted during *Rabi* 2023-24 at CCS Haryana Agricultural University, Hisar, to assess the impact of nano urea on growth, physiology, grain quality, and soil properties in wheat. The study used a split-plot design with three varieties (WH 1270, DBW 222, DBW 303) and four nutrient levels: 100% RDN, 100% RDN + one foliar spray of nano urea @ 4 mL L^{-1} (45 DAS), 75% RDN + two sprays (45 and 65 DAS), and 50% RDN + three sprays (30, 60, and 90 DAS). Wheat phenology and physiology responded significantly to. WH 1270 with longest crop duration, while 75% RDN + two sprays extended grain filling. Maximum chlorophyll (53.88 SPAD) and relative water content (91.35%) were observed under 100% RDN + one spray and 50% RDN + three sprays, respectively. DBW 303 produced the best grain quality with highest protein (13.12%), sedimentation value (43.58 mL), and hectolitre weight (82.74 kg hL^{-1}). Post-harvest soil analysis showed maximum available nitrogen (148.64 kg ha^{-1}) and phosphorus (17.71 kg ha^{-1}) under 100% RDN + one spray. Soil pH, EC, and organic carbon remained stable. The study suggests nano urea enhances nitrogen efficiency and supports sustainable wheat production under semi-arid conditions.

Keywords: Foliar spray, Protein content, Chlorophyll Content, DBW 303, Soil fertility

Wheat (*Triticum aestivum* L.) is one of the most widely cultivated and consumed cereal crop globally, serving as a major source of carbohydrates and protein for more than a third of the world's population (FAO 2023). In India, wheat occupies a central role in food security, cultivated across over 30 million hectares with significant contributions from northwestern states like Punjab, Haryana, and Uttar Pradesh (Ministry of Agriculture & Farmers Welfare 2023). To meet rising food demands and sustain soil health under climate stress, there is a growing emphasis on optimizing crop nutrition through precise and efficient fertilizer management.

Nitrogen (N) is a vital macronutrient that influences multiple aspects of wheat growth, including leaf expansion, chlorophyll synthesis, grain protein content, and ultimately, yield (Chen et al., 2022). However, conventional nitrogen fertilizers such as urea suffer from low nitrogen use efficiency (30–50%), with a substantial fraction lost through volatilization, leaching, and denitrification (Zhang et al., 2021). This inefficiency not only increases production costs but also contributes to environmental degradation and soil quality decline. To address these limitations, nano fertilizers, particularly nano urea have emerged as an innovative solution. Nano urea is a liquid formulation developed using nanotechnology with particles typically less than 50 nm in size and high surface area to volume ratio enables rapid absorption through leaf stomata, reduces nitrogen losses, and allows for targeted delivery during critical crop growth stages (Tarafdar et al., 2021, Singh et al., 2023). These properties contribute to improved nitrogen use efficiency, better synchronization with plant demand, and enhanced physiological and phenological responses.

Phenological traits such as days to heading, anthesis, maturity, and grain filling duration are critical in determining wheat adaptability, resource use efficiency, and final yield potential. Timely and balanced nitrogen availability can influence the onset and duration of key growth stages, allowing for better grain filling and harvest index (Rathore et al., 2023). Foliar nano urea applied at booting or heading stage has accelerate crop maturity while extending grain filling under limited basal nitrogen input (Kumar et al., 2022). In parallel, physiological parameters such as chlorophyll content (SPAD value) and relative water content (RWC) serve as sensitive indicators of nitrogen availability and plant health. Nano urea has been reported to sustain higher chlorophyll levels during reproductive stages and improve RWC, thereby supporting photosynthesis and drought resilience (Gao et al., 2020, Meena et al., 2022). From a quality perspective, nitrogen fertilization plays a central role in determining grain protein content, sedimentation value (a proxy for gluten strength), and hectolitre weight, all of which are key determinants for market and processing suitability. Adequate and timely nitrogen supply especially via foliar nano urea can boost protein synthesis during grain filling, enhance gluten formation, and improve test weight, thereby elevating wheat quality (Al-Juthery et al., 2019, Burhan and Al-Hassan 2020). Traditional urea application often leads to nutrient depletion and organic carbon loss, contributing to long-term fertility issues. Nano urea, owing to its lower required dosage and higher efficiency, can maintain or even improve post-harvest levels of available nitrogen, phosphorus, potassium, and organic carbon while minimizing environmental risks (Lal et al., 2020, Singh and Ameta 2022). Despite growing evidence of the agronomic benefits of nano urea, comprehensive field studies assessing it impact on phenological, physiological, grain quality, and soil parameters in India remain limited. Additionally, varietal responses to nano urea under varying nitrogen regimes are not well understood. Therefore, this study aimed to evaluate the effects of nano urea on wheat phenology (physiological traits), grain quality and post-harvest soil properties

MATERIAL AND METHODS

Experimental site and climate: The field experiment was conducted during the *Rabi* season of 2023–24, Chaudhary Charan Singh Haryana Agricultural University), Hisar, Haryana, India (29°14′N latitude, 75°68′E longitude; 215.2 m above mean sea level). The region is characterized by a semi-arid subtropical climate with hot summers and cool winters. During the cropping period, weekly mean maximum temperatures ranged from 13.9°C to 37.4°C, and minimum temperatures ranged from 4.1°C to 19.1°C. Total rainfall during the growing season was negligible (0.25 mm), and irrigations were scheduled as per crop requirements (Fig. 1).

Soil characteristics: The experimental soil was sandy loam in texture, alkaline in reaction (pH 7.89), with low electrical conductivity (EC 0.25 dS m^{-1}). It had low organic carbon (0.42%) and was moderately fertile with available nitrogen (142.5 kg ha^{-1}), phosphorus (17.56 kg ha^{-1}), and potassium (254.32 kg ha^{-1}). Soil sampling was done before sowing and post-harvest (0–15 cm depth).

Experimental design and treatments

The experiment was laid out in a split-plot design with three replications and twelve treatments (Table 1). Each treatment plot measured 3 m × 5 m (15 m²). The recommended dose of NPK was 150: 60: 60 kg ha^{-1}, and $ZnSO_4$ @ 25 kg ha^{-1} was applied as basal. Urea was used as the nitrogen source. Nano urea used in this study was sourced from IFFCO (Indian Farmers Fertiliser Cooperative Limited). It is a patented liquid formulation containing 4% w/v nitrogen with nano-sized particles (20–50 nm) designed for foliar absorption.

Table 1. Treatment structure used in the field study

Designation	Varieties
Main plot (Wheat varieties)	
V_1	WH 1270
V_2	DBW 303
V_3	DBW 222
Sub-plot (Nutrient levels)	
T_1	100% Recommended Dose of Nitrogen (RDN)
T_2	100% RDN + 1 spray of nano urea @ 4 mL L^{-1} at 45 DAS
T_3	75% RDN + 2 sprays of nano urea @ 4 mL L^{-1} at 45 and 65 DAS
T_4	50% RDN + 3 sprays of nano urea @ 4 mL L^{-1} at 30, 60, and 90 DAS

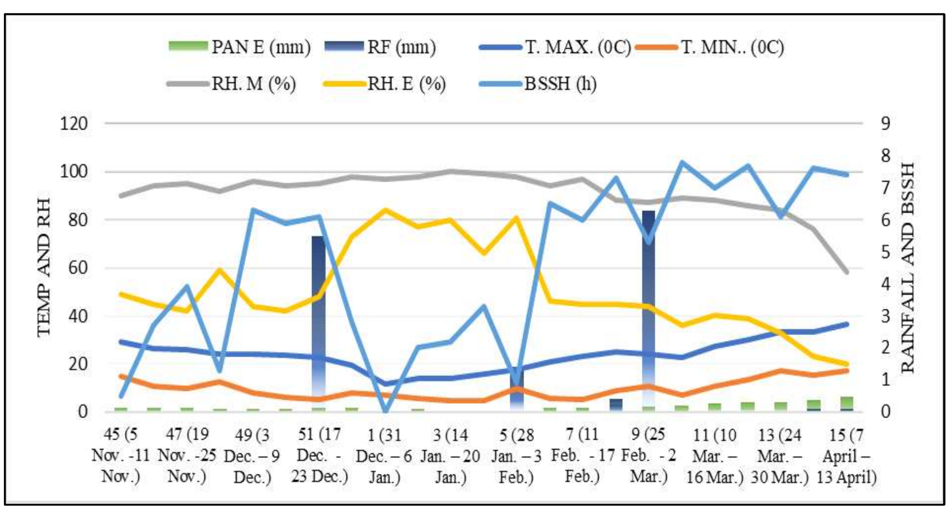

Fig. 1. Mean weekly meteorological data during the crop growing season 2022-23 at experimental area, CCS HAU, Hisar

Crop management: Wheat was sown on November 5, 2023, using behind-the-plough method at a row spacing of 22.5 cm. Half of the nitrogen and the full doses of phosphorus, potassium, and zinc were applied at sowing. The remaining nitrogen was top-dressed at the first irrigation (27 DAS). Foliar sprays were applied using a flat-fan nozzle at a concentration of 4 mL L^{-1} of water, as per treatment schedules. Sprays were conducted in the early morning or late afternoon to avoid rapid evaporation and ensure maximum leaf absorption. No other foliar fertilizers were mixed during application. Irrigations were applied at CRI, tillering, booting, heading, and dough stages. Standard agronomic practices were followed for weed, pest, and disease management. The crop was harvested on April 15, 2024.

Sample collection and analysis: Days to 50% heading, anthesis, and maturity were recorded y when 50% of the plants in each plot had reached the respective stage. Chlorophyll content was measured using a SPAD-502 chlorophyll meter on the flag leaf three days after each foliar spray of nano urea on the crop (30, 45, 60, 65, and 90 DAS). Relative Water Content (RWC) was measured using the formula:

$$RWC (\%) = (FW – DW)/ (TW – DW) \times 100$$

where FW = fresh weight, TW = turgid weight, and DW = dry weight of leaf samples .Samples for RWC were also collected three days after each foliar spray of nano urea at 30, 45, 60, 65 and 90 DAS. Protein content (%) was calculated by estimating nitrogen content using the Kjeldahl method and multiplying by the factor 6.25. Sedimentation value (mL) was determined using SDS-lactic acid test method. Hectolitre weight (kg hL^{-1}) was recorded using a standard hectolitre weight apparatus. pH (Jackson, 1973) and EC were measured from a 1: 2.5 soil-water suspension using digital meters (Richards, 1954). Organic carbon (OC) was analysed using the Walkley and Black method. Available N, P, and K were determined using alkaline $KMnO_4$, Olsen's, and flame photometry methods, respectively.

Statistical analysis: Data analysis was done by using “OPSTAT” software available at official website of CCSHAU, Hisar. (https://www.hau.ac.in/page/o-p-stat)

RESULTS AND DISCUSSION

Phenological parameters: Phenological stages were significantly influenced by both wheat varieties and fertility treatments (Table 2). Among varieties, V_1 took the maximum number of days to reach heading (84.3 days), anthesis (88.1 days), and maturity (138.3 days), indicating a longer growth period. In contrast, V_3 exhibited the earliest flowering (87.67 days) and maturity (147.33 days), making it more suitable for short-duration environments. Regarding nitrogen levels, T_4 had longstanding period for heading (74.56 days), anthesis (94.64 days), and maturity (157.22 days) due to extended vegetative growth induced by prolonged nitrogen availability. The T_1(100% RDF (150 kg N + 60 kg P_2O_5 + 60 kg K_2O + 25 kg $ZnSO_4$) and T_2 (100% RDN + one spray of nano urea at 45 DAS @ 4 ml/l of water) led to earlier phenological progression, promoting faster crop maturation and potentially avoiding terminal heat stress. Rathore et al. (2023) and Kumar et al. (2022), also observed that that foliar nano urea modulates hormonal and metabolic pathways associated with wheat phenology.

Physiological Parameters: Physiological responses were strongly impacted by nano urea applied through foliar feeding. The maximum SPAD value at 45 DAS (53.88) was in T_2 i.e. 100% RDN + one spray of nano urea at 45 DAS @ 4 ml/l of water, affirming enhanced chlorophyll biosynthesis due to improved foliar nitrogen absorption. However, at later

Table 2. Effect of different wheat varieties on phenological traits

Treatments	Phenological parameters		
Varieties	Days to heading	Days to anthesis	Days to maturity
V_1: WH 1270	73.10	92.72	158.08
V_2; DBW 303	72.83	93.13	157.50
V_3; DBW222	70.75	87.67	147.33
CD (p=0.05)	0.39	2.86	2.79
N levels			
N_1: 100% RDN	70.78	87.42	152.33
N_2: 100% RDN + 1 foliar spray of NU at 45 DAS	71.11	90.64	153.11
N_3: 75% RDN + 2 foliar sprays of NU at 45 and 65 DAS	73.00	91.98	154.56
N_4: 50% RDN + 3 foliar sprays of NU at 30, 60 and 90 DAS	74.56	94.64	157.22
CD (p=0.05)	0.71	1.59	1.75

stages (60–90 DAS), T_4 maintained higher chlorophyll content, (51.98) at 65 DAS and (41.94) at 90 DAS, reflecting a prolonged greening effect of nano urea and enhanced nitrogen uptake and sustained photosynthetic activity (Table 3). Gao et al. (2020) and Meena et al. (2022), also observed increased chlorophyll retention and delayed senescence in nano urea-treated wheat plants. Varietal effect on chlorophyll content was non-significant at all crop growth stages. However, numerically, higher values were exhibited by WH 1270 at all crop growth stages. In general, chlorophyll content decreased with increasing age of crop.

In terms of relative water content (RWC), WH 1270 consistently outperformed DBW 222 and DBW 303, likely due to better water retention and physiological adaptation. The highest RWC (97.07%) at 45 DAS was also observed in T_2, while T_4 sustained higher RWC during later growth stages (60–90 DAS), *viz.,* 86.87, 84.88 and 78.17% at 60, 65 and 90 DAS respectively, supporting prolonged metabolic activity under reduced nitrogen input (Table 4). Elevated RWC suggests improved cell turgor and water-use efficiency, likely due to better osmotic regulation facilitated by nitrogen-enhanced metabolism (Tarafdar et al., 2021).

Quality parameters: Nano urea treatments and varietal differences had significant impacts on grain quality traits (Table 5). Maximum protein content was in DBW 303 (13.12%) followed by WH 1270 (12.12%) which was at par with DBW 222. Among fertility treatments, T_3 produced the highest protein content (12.80%) and was statistically at par to T_4 . The variety DBW 303 showed significantly higher sedimentation value (43.58 ml) over WH 1270 and DBW 222 which were statistically at par to each other. T_3 showed highest sedimentation v (43.89 ml) and was statistically at par

Table 3. Effect of wheat varieties on chlorophyll content (SPAD value) at various crop stages

Treatments	Chlorophyll content (SPAD)				
Varieties	30 DAS	45 DAS	60 DAS	65 DAS	90 DAS
WH 1270	53.39	53.51	49.94	45.49	40.71
DBW 303	52.66	50.42	49.77	42.63	40.47
DBW222	52.92	47.91	49.66	41.99	39.42
CD (p=0.05)	NA	NA	NA	NA	NA
N levels					
N_1	53.69	47.41	47.31	40.98	38.32
N_2	53.63	53.88	50.86	42.93	39.78
N_3	52.23	51.58	49.02	45.28	40.77
N_4	52.42	49.58	51.98	44.29	41.94
CD (p=0.05)	0.98	4.65	0.56	2.61	1.36

See Table 2 for treatments details

Table 4. Effect of nano urea spray frequencies on relative water content at different crop growth stages

Treatments	Chlorophyll content (SPAD)				
Varieties	30 DAS	45 DAS	60 DAS	65 DAS	90 DAS
WH 1270	91.80	97.29	85.64	84.18	74.63
DBW 303	89.51	93.85	83.47	82.69	72.22
DBW222	91.28	95.04	84.62	82.20	73.44
CD (p=0.05)	0.47	1.24	1.37	0.81	1.79
N levels					
N_1	92.56	93.14	81.93	81.04	74.20
N_2	91.89	97.07	83.65	82.42	72.62
N_3	90.38	96.45	85.85	84.54	75.73
N_4	88.63	94.93	86.87	84.88	78.17
CD (p=0.05)	0.95	0.34	0.58	0.53	0.97

See Table 2 for treatments details

with T_2 (41.22 ml) and T_4 (42.22 ml). The improvement in protein content and gluten strength reflects efficient nitrogen assimilation during the grain filling period (Al-Juthery et al., 2019, Burhan and Al-Hassan 2020). Foliar nano urea, by directly supplying nitrogen to flag leaves, enhances amino acid synthesis and protein accumulation in grains (Ghafari and Razmjoo 2013). The analysis of data on hectolitre weight revealed significant difference as influenced by the spray of nano urea. Among varieties highest weight was r for DBW 303 (82.74 kg hL^{-1}), which was statistically at par with WH 1270). The highest nutrient response was observed in T_3 *i.e.* 82.94 kg hL^{-1} which was significantly higher than other nutrient levels. The lowest was recorded in T_1 (79.34 kg hL^{-1}). Higher hectolitre weight observed under nano urea treatments indicates better grain filling and density. Singh et al. (2023) also improved grain morphology and end-use quality with foliar nano nitrogen.

Soil parameters: Soil analysis post-harvest indicated that T_2 recorded the highest available nitrogen (148.64 kg ha^{-1}) and phosphorus (17.71 kg ha^{-1}), followed closely by other nano urea treatments (Table 6). Potassium levels remained unaffected across treatments. Soil pH, electrical conductivity, and organic carbon content showed no significant differences, indicating that nano urea did not adversely affect soil chemistry in a single season. Among varieties, DBW 222 maintained the highest soil nitrogen (145.59 kg ha^{-1}) and phosphorus (17.60 kg ha^{-1}), while all varieties showed comparable values for soil pH and EC. The enhanced residual nitrogen suggests reduced volatilization and better plant uptake efficiency (Tarafdar et al., 2021). Lal et al. (2020) and Singh & Ameta (2022), also emphasized that nano fertilizers reduce nutrient losses and contribute to long-term soil fertility sustainability. The stable pH and EC across treatments further demonstrate the environmental safety of

Table 5. Combined effect of wheat varieties and nano urea treatments on grain quality parameters

Treatments	Protein content (%)	Sedimentation value (ml)	Hectolitre weight (kg/hl)
Varieties			
WH 1270	11.63	40.67	78.97
DBW 303	13.12	43.58	82.74
DBW222	11.99	41.42	81.29
CD (p=0.05)	0.55	1.27	2.61
N levels			
N_1	11.57	40.22	79.34
N_2	11.86	41.22	80.71
N_3	12.80	43.89	82.94
N_4	12.76	42.22	81.00
CD (p=0.05)	0.46	1.95	1.75

See Table 2 for treatments details

Table 6. Post-harvest soil analysis showing the impact of wheat varieties and nano urea treatments on available N, P, K, soil pH, EC, and organic carbon

Treatments	Soil pH	Soil EC (dS/m)	Soil OC (%)	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)
Varieties						
WH 1270	7.87	0.24	0.43	139.47	17.59	256.46
DBW 303	7.76	0.24	0.42	142.49	17.44	253.79
DBW222	7.83	0.24	0.42	145.59	17.6	256.63
CD (p=0.05)	NS	NS	NS	4.13	0.05	NS
N levels						
N_1	7.81	0.24	0.41	144.06	17.63	255.78
N_2	7.82	0.25	0.44	148.64	17.71	258.56
N_3	7.83	0.25	0.42	143.68	17.46	254.83
N_4	7.81	0.24	0.42	137.68	17.37	253.33
CD (p=0.05)	NS	NS	NS	4.13	0.13	NS

See Table 2 for treatments details

nano urea, as no adverse effects on soil chemical properties were observed over the season.

CONCLUSION

The application of 75% + two foliar sprays of NU @ 4 mL L^{-1} at 45 and 65 DAS (T_3) emerged as the most effective strategy, achieving superior grain protein content (12.80%), sedimentation value (43.89 mL), and hectolitre weight (82.94 kg hL^{-1}), while also extending the grain filling duration (42.4 days). Physiologically, this treatment sustained high chlorophyll content and RWC, supporting prolonged photosynthetic activity. In terms of soil fertility, T_2 (100% RDN + 1 foliar spray of NU @ 4 mL L^{-1} at 45 DAS) maintained the highest available nitrogen and phosphorus levels, indicating the potential of nano urea to reduce nutrient losses and enhance residual fertility.

AUTHOR'S CONTRIBUTION

Data collection, data analysis, lab analysis, original draft preparation: Simran Sindhu, Supervision and Guidance: Rajesh Kathwal, Anita Kumari, Ram Prakash; Manuscript editing and revision: Ritika and Danveer Singh.

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Complementary Effect of Nano-Urea on the Performance of Geranium (*Pelargonium graveolens* L.) under Eastern Dry Zone of Karnataka

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Abstract: The present investigation was under taken to enhance the nitrogen use efficiency of applied conventional nitrogen fertilizer and to reduce the cost of cultivation so as to harness the better foliage yields of geranium. The recommended dose of nitrogen, 210 kg/ha is applied in six equal split doses at an interval of two months while, phosphorus and potassium as a single basal dose for geranium. The hypothesis of reducing the nitrogen dosage of conventional fertilizer through the complimentary spraying of nano-urea was tested during the *Rabi* season of 2022-23. Soil application of 50 per cent RDN (105 kg N/ha) through neem coated urea in three equal split doses and three sprays of nano-urea (150 g N/ha) at two months interval alternatively along with soil application of recommended doses of FYM (10 t/ha), P (35 kg P_2O_5/ha) and K (35 kg K_2O/ha) had produced superior growth attributes (plant height, number of leaves, branches and plant spread) at different stages of geranium growth in main as well as ratoon crops. The yield attributes such as fresh herbage yields of 22.30 and 32.89 t/ha, dry herbage yields of 5.33 and 8.21 t/ha and essential oil yields of 92.23 and 129.82 kg/ha in main and ratoon crops, respectively were significantly high in this treatment and realized highest net returns of Rs.14, 59, 453 per ha with a cost benefit ratio of 2.25.

Keywords: Nano-urea, Nitrogen levels, Geranium, Oil yield, Net returns

Deterioration of soil health has been reported to be the major cause for stagnation of the crop productivity in India. In India, about 82 per cent of the total fertilizer consumed is through urea. Around 30-40 per cent of nitrogen supplied through urea is utilized by plants and the rest gets wasted as a result of leaching, volatilization, denitrification and run off, indicating its low nitrogen use efficiency. The high N loss coupled with its low use efficiency forced the farmers to increase the amounts of applied N fertilizers in order to achieve better crop production (Rathnayaka et al., 2018), which resulted in escalated costs of the farming practice as well as increased environmental implications (Marchiol 2019). Therefore, there is a need to improve the N availability for plants, while reducing its harmful effects on the environment. One such possibility is the use of nano-materials of less than 100 nm size as they offer higher absorption rate, utilization efficacy, and minimum losses (Kumar et al., 2020).

Nitrogen is a basic component of purines and pyrimidine in nucleic acids (DNA and RNA), playing a pivotal role in biological inheritance, genetic variation and protein synthesis. The amino groups of peptides, proteins and enzymes primarily consist of nitrogen, thereby conferring its indispensable function in maintaining organismal activities. Furthermore, as an integral part of chlorophyll molecules, nitrogen facilitates light energy absorption during photosynthesis. It also significantly promotes root development and optimizes photosynthetic efficiency (Cao et al., 2025). It is responsible for greenness, vigorous growth and overall crop development; therefore, it must be available for plants in adequate amounts. Nano-fertilizers have particle size smaller than the pore size of plant leaves, allowing for greater penetration into plant tissues from the applied surface thereby improve the absorption and nutrient use efficiency. Foliar application of nano-urea liquid along with minimum quantity of conventional urea at critical crop growth stages of a plant effectively fulfils its nitrogen requirement and leads to higher crop productivity and quality in comparison to only conventional urea application (Oinam et al., 2025).

Rose scented geranium (*Pelargonium graveolens* L.) of genus *Pelargonium* L. Herit, belongs to the family Geraniaceae, is one of the economically important aromatic crops, from which highly prized geranium essential oil is extracted for flavour and fragrance. Monoterpenoids and acetate esters of monoterpenols are the main constituents of geranium essential oil with a rose fragrance. These include citronellol, geraniol, citronellyl acetate, geranyl acetate, isomenthone, menthone and linalool, *etc*. Geranium essential oil is basically used in perfumery, beauty and aroma therapy industries all around the world. It is one of the quality skin care oil as it is used for opening skin pores and cleansing complexions. In addition, geranium essential oil is used in treatment of infectious diseases, haemorrhoids, inflammation, serious menorrhea, internal organ ulcers, jaundice, liver issues, sterility, urinary stones and even cancer. Leaves are also utilized as herb drink to fight anxiety, relief strain, boost blood flow, and to treat inflammation (Cocos et al., 2023). Presently, it is being commercially cultivated mainly in the Nilgiris and Kodaikanal hills of Tamil Nadu and in and around Bengaluru in Karnataka in an area of about 2000 ha. The available nitrogen status of soils in Eastern dry zone of Karnataka is low because of higher temperature and semi-arid climate leading to more volatilization loss of applied conventional nitrogenous fertilizers. Application of nano-urea could reduce the volatilization loss of nitrogen and enhance the use efficiency of applied conventional nitrogenous fertilizers. The spraying of nano-urea to foliage may complement for the soil applied nitrogen in harnessing better yields (Acharjee et al., 2025). Therefore, the present investigation was under taken to study the effect of nitrogen levels and number of nano-urea sprays on performance of geranium as test crop at College of Horticulture, Bengaluru.

MATERIAL AND METHODS

The experiment was carried out during *Rabi* 2022-23 at College of Horticulture, Bengaluru, located at 12°58' N latitude and 77°35' E longitudes at an altitude of 930 m above MSL. Soil samples were collected from 0-15 cm depth randomly in a zig-zag manner to represent entire experimental site before initiation of the experiment and was analyzed for various physico-chemical properties by following standard procedures. The soil of the experimental site was sandy loam in texture, slightly acidic in reaction (pH: 6.26), low in soluble salts (EC: 0.09 dS/m), organic carbon (0.39%) and available N and K_2O (144.25 and 130.77 kg/ha, respectively) contents while medium in available P_2O_5 (30.00 kg/ha) status.

The geranium is an aromatic perennial crop and requires continuous supply of nitrogen throughout the crop growth period. The nitrogen is applied in six equal split doses at an interval of two months while, phosphorus and potassium as a single basal dose. The effect of reduction of nitrogen dosage through conventional urea by the complementary application of nano-urea sprays on the performance of geranium was observed using randomised complete block design with three replications consisting of eight treatments (Table 1).

The 1250 mL per hectare of IFFCO liquid nano-urea having 4% N was sprayed on to foliage @ 2 mL per litre of water which supplies only 50 grams of nitrogen per spray. Sixty days old rooted stem cuttings of geranium (*Pelargonium graveolens* L) cultivar 'Cim-Bio 171' were transplanted with a spacing of 60 cm x 60 cm in each plot in November 2022. The plots were kept completely free from weeds by regular hand weeding at 20 days interval till harvest of the crop. The plots were irrigated immediately after transplanting and later irrigation was done once in three to four days depending on soil moisture status and weather conditions besides, all the plant protection measures were taken.

Observations on growth parameters *viz.*, plant height (cm), number of leaves per plant (No.), number of branches per plant (No.) and the plant spread was measured in both N-S and E-W directions (cm^2). The first harvesting of foliage was done at 120 days after transplanting and subsequent harvests at 3 months interval. Fresh herb from each plot was harvested and weighed and expressed in kilogram to get the fresh herbage yield per plot. Fresh and dry herbage yield per plot was converted to per hectare on area basis and expressed in tonnes (t). The essential oil content was determined by hydro-distillation in a clevenger apparatus as described by Babu and Kaul (2005) and expressed in percentage. The essential oil yield was calculated based on the oil content and fresh herbage yield per plant, per plot and per hectare and is expressed in milliliters. The quality attributes of oil *viz*., acid value, saponification value, ester value, specific gravity and refractive index were calculated. The acid and saponification values of oil in terms of mg KOH per gram of oil were calculated as described by Pearson (1976). The ester value of oil was calculated by subtracting acid value from the saponification value while, specific gravity was calculated by dividing the weight of 1 mL of oil by

Table 1. Treatment details

Sl. No.	Treatment details
T_1	Control (Only recommended FYM @ 10 t/ha)
T_2	100% RDN (210 kg/ha) through NCU in six equal split doses at 2 months interval
T_3	83% RDN (175 kg/ha) through NCU in five equal split doses + 1 spray of nano-urea
T_4	67% RDN (140 kg/ha) through NCU in four equal split doses + 2 sprays of nano-urea
T_5	50% RDN (105 kg/ha) through NCU in three equal split doses + 3 sprays of nano-urea
T_6	33% RDN (70 kg/ha) through NCU in two equal split doses + 4 sprays of nano-urea
T_7	17% RDN (35 kg/ha) through NCU as basal dose + 5 sprays of nano-urea
T_8	Six sprays of nano-urea at 2 months interval

Note:
1. Recommended FYM 10 t/ha was applied to all treatments including control
2. Recommended P_2O_5 (35 kg/ha) and K_2O (35 kg/ha) were applied to all treatments except control
3. RDN: Recommended Dose of Nitrogen (210 kg/ha); NCU: Neem Coated Urea

the weight of 1 mL of water at room temperature and refractive index was measured using Abbe's refractometer at room temperature.

The cost incurred from land preparation to harvest of crop including cost of all the inputs, cost of all the operations carried out, supervision charge and pre farm to marketing charge were taken into consideration for working out the cost of cultivation and expressed as rupees per hectare. Gross returns per hectare was calculated by taking into consideration of economic yield obtained per hectare and the price of the essential oil prevailing in the market at the time of harvest as per price list of Fragrances and Flavours Association of India. The net returns per hectare was calculated by deducting cost of cultivation per hectare from the gross returns per hectare. The cost benefit ratio was worked out by dividing net returns (Rs./ha) by cost of cultivation (Rs./ha). The experimental data were analyzed using SPSS16 statistical software.

RESULTS AND DISCUSSION

Plant height: The plant height showed an increasing trend with the advancing stages of crop growth and was highest at harvest (Table 2). Significantly maximum plant height was at 30 and 60 DAT (19.40 and 30.87 cm, respectively) in main crop and at 30 DAFH (27.20 cm) in ratoon crop in the treatment which received alternate soil application of 50% RDN (105 kg/ha) through neem coated urea in three equal split doses and three sprays of nano-urea at two months interval (150 g N/ha) along with soil application of recommended doses of FYM, P and K (T_5). This might be attributed to improved nitrogen uptake by the plant due to synergetic effect of nano nitrogen through foliar penetration and conventional urea through roots uptake leading to improved photosynthesis, source and sink capacity (Benzon et al., 2015).

Number of leaves per plant: Significantly highest number of leaves per plant was observed in treatment which received alternate soil application of 50% RDN (105 kg/ha) through neem coated urea in three equal split doses and three sprays of nano-urea at two months interval (150 g N/ha) along with soil application of recommended doses of FYM, P and K (T_5) at 60 and 90 DAT (95.80 and 163.20, respectively) in main crop and at 90 DAFH (214.27) in ratoon crop (Table 2). Whereas, only six sprays of nano-urea at two months interval (300 g N/ha) along with soil application of recommended doses of FYM, P and K (T_8) had produced significantly a greater number of leaves per plant at 30 and 60 DAFH (79.27 and 150.13, respectively) in ratoon crop. However, both the treatments were *on par* with each other at all crop growth stages. This might be due to the fact that nitrogen delivered by foliar spray might have reached the cells more quickly through stomata or cuts and scrapes in the leaves, thereby helped to maintain continuity and speed of delivering the nutrients necessary for plant metabolic activities. Further, nitrogen increases the chlorophyll formation, rate of photosynthesis and overall growth of plant that might have resulted in formation of a greater number of leaves. These findings are in agreement with those findings of Rajasekar et al. (2017).

Number of branches per plant: The number of branches per plant of geranium at different growth stages differed due to nitrogen levels and number of nano-urea sprays (Table 3).

Table 2. Plant height and number of leaves per plant of geranium as influenced by nitrogen levels and number of nano-urea sprays

Treatments	Plant height (cm)							Number of leaves per plant						
	Main crop				Ratoon crop			Main crop				Ratoon crop		
	30 DAT	60 DAT	90 DAT	120 DAT	30 DAFH	60 DAFH	90 DAFH	30 DAT	60 DAT	90 DAT	120 DAT	30 DAFH	60 DAFH	90 DAFH
T_1	15.80	24.13	36.07	48.73	23.40	41.87	51.20	16.07	64.20	124.67	196.27	63.60	123.20	175.87
T_2	17.07	25.27	38.67	50.20	25.87	42.47	53.53	19.53	71.80	130.33	198.53	69.47	131.60	181.67
T_3	14.87	24.60	37.00	50.47	26.07	44.40	54.20	19.00	66.67	126.73	198.20	70.40	127.47	179.87
T_4	18.20	27.60	39.53	51.20	25.27	43.33	54.67	24.13	82.27	145.73	238.80	72.13	136.53	187.93
T_5	19.40	30.87	40.93	54.33	27.20	45.13	56.67	24.47	95.80	163.20	250.87	71.20	146.67	214.27
T_6	16.73	26.67	36.53	51.47	25.00	43.07	52.87	21.67	78.73	146.80	208.00	71.20	137.13	190.80
T_7	17.33	26.87	39.67	52.20	26.93	44.60	53.87	22.13	93.93	157.87	221.07	71.67	148.47	201.93
T_8	19.33	28.33	39.47	52.13	24.93	41.73	55.13	23.60	83.93	153.93	216.13	79.27	150.13	200.00
CD (p=0.05)	3.15	4.05	NS	NS	2.75	NS	NS	NS	21.40	28.18	NS	10.70	20.53	27.33

See Table 1 for treatment details
DAT: Days After Transplanting; DAFT: Days After First Harvest; NS: Non Significant

Maximum number of branches per plant were achieved with alternate soil application of 50% RDN (105 kg/ha) through neem coated urea in three equal split doses and three sprays of nano-urea at two months interval (150 g N/ha) along with soil application of recommended doses of FYM, P and K (T_5) at 60 and 90 DAT in main crop and at 60 and 90 DAFH in ratoon crop. This might be due to the fact that the plants received enough nitrogen through nano-urea as well as conventional urea at critical crop growth stages of geranium which would have maintained a constant supply of nitrogen thereby stimulated the cell elongation and meristematic activity in plants and ultimately resulted in a greater number of branches (Jassim et al., 2019).

Plant spread: Significantly maximum plant spread of 3177.67 cm^2 at 90 DAT in main crop was achieved with alternate soil application of 50% RDN (105 kg/ha) through neem coated urea in three equal split doses and three sprays of nano-urea at two months interval (150 g N/ha) along with soil application of recommended doses of FYM, P and K (Table 3). Significantly maximum plant spread of 2413.93 cm^2 at 30 DAFH in ratoon crop was noticed with soil application of 17% RDN (35 kg/ha) through neem coated urea as basal dose and five sprays of nano-urea at two months interval (150 g N/ha) along with soil application of recommended doses of FYM, P and K (T_7). However, both the treatments were *on par* with each other at 30 DAFH. The enhanced plant spread might be attributed to vigorous nature of plant growth at this level, as indicated by the greater number of branches and leaves. These findings are in agreement with findings of Sumathi et al. (2012) in *Pogostemon cablin*.

Fresh and dry herbage yields: The fresh and dry herbage yields data indicated that the treatment involving alternate soil application of 50% RDN (105 kg/ha) through neem coated urea in three equal split doses and three sprays of nano-urea at two months interval (150 g N/ha) along with soil application of recommended doses of FYM, P and K (T_5) had produced significantly maximum fresh herbage (22.30 and 32.89 t/ha) and dry herbage (5.33 and 8.21 t/ha) yields in both main and ratoon crops, respectively (Table 4). This might be attributed to the presence of greater number of leaves, branches, increased plant spread and optimum concentration of nitrogen which in turn might have enhanced the optimum uptake of P and K due to synergistic effect. The maximum fresh and dry herbage yields obtained due to combined soil application of conventional fertilizer in split doses and nano-urea which has been sprayed on plant surface that might have led to the storage of remaining nitrogen in plant cells which might have released slowly and prevented the plant biotic and abiotic stresses which in turn increased the vegetative growth (Khalil et al., 2019). Further, smaller particle size of nano-urea with greater specific surface area and number of particles per unit area of a fertilizer that might have provided greater opportunities for penetration and nutrient uptake, thus resulting in more vegetative growth and dry matter production (Fadhil et al., 2021). These findings are in line with findings of Mahmoodi et al. (2020) and Midde et al. (2022).

Essential oil yield: The essential oil yield in fresh herb of geranium differed significantly due to nitrogen levels and number of nano-urea sprays (Table 4). The highest essential oil yield in both main and ratoon crop of geranium (92.23 and 129.82 kg/ha, respectively) was obtained in treatment which received alternate soil application of 50% RDN (105 kg/ha) through neem coated urea in three equal split doses and

Table 3. Number of branches and plant spread of geranium as influenced by nitrogen levels and number of nano-urea sprays

Treatments	Number of branches per plant							Plant spread (cm^2)						
	Main crop				Ratoon crop			Main crop				Ratoon crop		
	30 DAT	60 DAT	90 DAT	120 DAT	30 DAFH	60 DAFH	90 DAFH	30 DAT	60 DAT	90 DAT	120 DAT	30 DAFH	60 DAFH	90 DAFH
T_1	1.67	6.00	12.00	22.73	10.80	15.27	22.73	308.28	1567.92	2113.04	4135.63	1940.48	3676.35	5936.45
T_2	2.27	5.60	12.20	23.80	11.67	15.67	24.07	372.85	1507.05	2139.67	5485.00	2154.75	3948.28	6234.87
T_3	2.20	6.53	12.93	22.87	12.20	16.93	24.80	336.09	1997.72	2655.63	4729.64	2181.19	4546.57	7152.03
T_4	3.33	6.67	12.93	23.13	12.47	17.00	25.33	441.04	1857.97	2512.65	5252.96	2235.77	4235.12	6875.60
T_5	3.07	7.60	14.47	25.47	12.13	18.40	27.53	483.41	2340.27	3177.67	5655.41	2314.43	4701.07	7296.48
T_6	2.40	7.20	13.87	25.13	11.93	16.53	25.60	389.29	2535.23	2948.73	5126.08	1795.83	3881.49	6524.63
T_7	2.67	6.87	13.73	24.80	13.20	17.67	26.60	375.84	2424.33	2746.45	4834.51	2413.93	3850.03	6415.41
T_8	2.93	6.80	13.53	24.60	12.67	18.00	26.20	484.88	2322.17	3016.21	5020.64	2265.88	4338.56	7276.13
CD (p=0.05)	NS	1.44	1.99	NS	1.63	2.02	2.76	NS	NS	831.08	NS	480.95	NS	NS

See Table 1 for treatment details
DAT: Days After Transplanting; DAFT: Days After First Harvest; NS: Non Significant

three sprays of nano-urea at two months interval (150 g N/ha) along with soil application of recommended doses of FYM, P and K (T_5). This was probably due to higher herbage yield and higher oil content. Rose-scented geranium had exhibited similar response to nitrogen levels (Bhaskar et al., 2001). The enhanced accumulation of essential oil in plants under the conditions of well supplied nitrogen might have resulted from the increased production of biomass and its direct impact on the biosynthesis of oil.

Quality parameters: The essential oil content in fresh herb of geranium differed significantly due to nitrogen levels and number of nano-urea sprays (Table 5). The essential oil content in both main and ratoon crops of geranium (0.43 and 0.42%, respectively) were significantly superior in plants treated with the six sprays of nano-urea at two months interval (300 g N/ha) along with soil application of recommended doses of FYM, P and K (T_8). In general, the essential oil content was more in main crop compared to ratoon crop. The response of volatile oil content to nano nitrogen fertilization might be attributed to *de novo* meristematic cell metabolism in building dry matter with essential oil production. Nitrogen being a part of three important coenzymes *viz*., ATP, NADPH and Co-A plays an important role in terpenoid biosynthesis (Sarab et al., 2008). However, other quality parameters *viz*., acid value, saponification value, ester value, specific gravity and refractive index of geranium essential oil in both main and ratoon crops were not influenced by nitrogen levels and number of nano-urea sprays (Table 5).

Economics: The highest gross returns (₹ 21,09,475 / ha), net returns (₹ 14,59,453 / ha) and cost benefit ratio (2.25) were realized with the alternate soil application of 50% RDN (105 kg/ha) through neem coated urea in three equal split doses and three sprays of nano-urea at two months interval (150 g N/ha) along with soil application of recommended doses of FYM, P and K (Table 6). This was due to reduced urea application and effect of nano-urea sprays at critical crop growth stages of geranium which resulted in higher

Table 4. Yield of geranium as influenced by nitrogen levels and number of nano-urea sprays

Treatments	Fresh herbage yield (t/ha)		Dry herbage yield (t/ha)		Essential oil yield (kg/ha)	
	Main crop	Ratoon crop	Main crop	Ratoon crop	Main crop	Ratoon crop
T_1	14.04	22.11	3.41	4.68	39.49	60.13
T_2	16.52	30.18	4.00	6.80	57.86	106.50
T_3	16.22	28.61	3.93	6.41	57.26	107.72
T_4	16.04	29.89	4.00	6.79	49.78	104.60
T_5	22.30	32.89	5.33	8.21	92.23	129.82
T_6	16.81	30.71	4.19	7.11	68.30	98.37
T_7	15.26	24.64	3.70	6.04	56.18	82.93
T_8	19.30	26.68	4.74	6.52	83.79	110.82
CD (p=0.05)	2.68	4.64	0.67	2.34	24.84	31.11

See Table 1 for treatment details

Table 5. Quality attributes of geranium oil as influenced by nitrogen levels and number of nano-urea sprays

Treatments	Oil content (% v/w)		Acid value		Saponification value		Ester value		Specific gravity		Refractive index	
	Main crop	Ratoon crop	Main crop	Ratoon crop	Main crop	Ratoon crop	Main crop	Ratoon crop	Main crop	Ratoon crop	Main crop	Ratoon crop
T_1	0.29	0.27	5.98	5.98	35.53	37.40	29.55	31.42	0.91	0.92	1.46	1.47
T_2	0.35	0.35	6.36	5.24	37.40	37.40	31.04	31.79	0.92	0.92	1.47	1.47
T_3	0.35	0.37	5.98	5.24	39.27	41.14	33.29	35.90	0.91	0.90	1.47	1.47
T_4	0.31	0.35	5.98	5.98	39.27	35.53	33.29	29.55	0.91	0.92	1.48	1.48
T_5	0.41	0.39	5.98	5.61	37.40	41.14	31.42	35.53	0.91	0.92	1.47	1.47
T_6	0.41	0.32	6.36	4.86	41.14	39.27	34.78	34.41	0.91	0.91	1.48	1.48
T_7	0.37	0.33	5.61	5.98	41.14	37.40	35.53	31.42	0.92	0.91	1.47	1.47
T_8	0.43	0.42	5.24	4.86	41.14	43.01	35.90	38.15	0.92	0.92	1.48	1.48
CD (p=0.05)	0.11	0.08	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

See Table 1 for treatment details

Table 6. Economics of geranium oil production as influenced by nitrogen levels and number of nano urea sprays

Treatments	Cost of cultivation (₹ ha^{-1})	Gross returns (₹ ha^{-1})	Net returns (₹ ha^{-1})	B : C ratio
T_1	585709	946390	360681	0.62
T_2	625326	1561420	936094	1.50
T_3	619330	1567310	947980	1.53
T_4	622244	1466610	844366	1.36
T_5	650022	2109475	1459453	2.25
T_6	626241	1583365	957124	1.53
T_7	602995	1321545	718550	1.19
T_8	620845	1848795	1227950	1.98

See Table 1 for treatment details

herbage and oil yields and as a result, higher net returns (Kumar et al., 2020).

CONCLUSION

The combined soil application of commercial nitrogen fertilizer and nano-urea spray had significant impact on growth, yield and quality of geranium. The treatment involving alternate soil application of 50% RDN (105 kg/ha) through neem coated urea in three equal split doses and three sprays of nano-urea at two months interval (150 g N/ha) along with soil application of recommended doses of FYM, P and K was most effective. This treatment not only provided optimal growth and yield but also had the highest cost benefit ratio, indicating favourable economic returns on investment.

AUTHOR'S CONTRIBUTION

It is the collaborative work of all the authors. ML Chaithra carried out the experiment and prepared the initial draft of the manuscript, author BN Dhananjaya gave technical guidance and supervised the experiment, BN Maruthi Prasad supplied the inputs and other resources, BS Harish conceptualized and formulated the hypothesis, TH Shankarappa prepared the manuscript, BR Premalatha recorded all the agronomic observations and J Jayappa analysed the data. All the authors have read and approved the final manuscript.

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Growth and Yield Response of Broccoli (*Brassica oleracea* var. *italica*) to Application of Calcium, Boron and Zinc in Alkaline Soils of Indo-Gangetic Plains of India

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Abstract: The field experiment was conducted during winter (*rabi*) season of 2022-23 and 2023-24 at Shri Guru Granth Sahib World University, Fatehgarh Sahib, Punjab (India) to evaluate the effect of soil application of calcium (gypsum) and soil or foliar application of boron (B) and zinc (Zn) on growth and yield of broccoli on alkaline soils under sub-tropical conditions of central Punjab. Soil application of boron (1.5 kg B/ha) or zinc (5 kg Zn/ha) as well as foliar application of B (0.3% borax) or Zn (0.5% zinc sulphate), alone or in combination, increased the growth (plant height, number of leaves plant^{-1}, leaf length, leaf width and plant spread), yield attributing characters (number of secondary heads plant^{-1}, weight of main head plant^{-1}, weight of secondary heads plant^{-1} and total weight of heads plant^{-1}) and total marketable head yield (t ha^{-1}) of broccoli significantly over their no application (control) during both the years. Foliar application of both B and Zn was better than their soil application in increasing the crop growth and productivity. Combined application of B and Zn was better than their sole application, irrespective of their mode of application. The highest value of growth parameters, yield attributes and marketable head yield was obtained with combined foliar application of B and Zn. Foliar application of B or Zn alone were at par with each other in terms of growth, yield attributes and the yield, proving significantly better than soil application of B or Zn alone or combined while their combined application in soil was significantly better than their sole application in soil. However, soil application of Zn alone performed better than soil application of B alone. Application of calcium (100 kg ha^{-1}), however, could not increase the growth and yield of the crop over its no application. The combined application of B and Zn, preferably through foliar spray (B as 0.3% borax and Zn as 0.5% zinc sulphate), may be suggested for obtaining higher marketable yield of broccoli in micronutrient (B and Zn) deficient alkaline soils of central Punjab.

Keywords: Broccoli, Calcium, Boron, Foliar spray, Marketable yield

Broccoli (*Brassica oleracea* var. *italica*) is a cruciferous vegetable of the *Brassicaceae* family and is one of the under-exploited vegetable crops in India. Plant nutrition is an important factor for increasing productivity of a crop. Broccoli requires not only major plant nutrients such as nitrogen, phosphorus, potassium and calcium and but also vital micronutrients such as boron and zinc to produce a bountiful harvest. Hence there is need to supply these nutrients for the plants if these are not available in sufficient quantities in soil. Calcium is an important secondary macro-nutrient which is not only a structural component of cell wall and membranes but also serves as a second messenger in many developmental and physiological processes (Thor 2019) and hence helps in overall plant growth and development. Broccoli has as much Ca as milk (Dhotra et al., 2018) and hence is expected to have higher Ca requirement. Although available in plenty in most cultivated soils, availability of Ca is reduced due to its conversion into insoluble form by reacting with other applied nutrients like phosphorous, particularly at high soil pH, and also due to Ca adsorption on clay particles in fine textured soils (Prasad and Shivay 2020), necessitating its application for promoting plant growth and yield. Boron is development of reproductive tissues in plants and its deficiency is associated with poor quality seeds and fruits (Prasad et al., 2014). Zinc is another important micronutrient which is a component of different enzymes catalysing many metabolic reactions in plants and plays a significant role in enhancing disease resistance, photosynthesis, protein synthesis, pollen formation and chlorophyll formation in plants (Hussain et al., 2015).

Alkaline soils in many areas of Indo Gangetic Plains (IGP) in India, including the state of Punjab situated in western part of IGP, are prone to deficiency of micronutrients like zinc and boron due to several reasons such as poor solubility of these micronutrients at higher soil pH, low organic carbon in soil, intensive cultivation practices, adoption of exhaustive cropping systems like rice-wheat, growing of high yielding varieties of crops and excessive use of micronutrient-free high analysis fertilizers (Shukla et al., 2021, Khurana 2022). Therefore, it is essential to apply sufficient quantities of the micronutrients, particularly B and Zn, for the crop plants as these two are the most deficient micronutrients in such soils (Thapa et al., 2016, Mondal and Ghosh 2023). Moreover, the research work on B and Zn application in broccoli is scanty, particularly in intensively cultivated alkaline fine textured soils of Punjab under sub-tropical climate. The present field investigations were, therefore, carried out to assess the response of broccoli to application of calcium and micronutrients (B and Zn) in alkaline soils of central Punjab in order to find out their optimum combination and method of application to obtain higher yield of the crop under sub-tropical conditions.

MATERIAL AND METHODS

The field experiment on broccoli was conducted at the Farm of Department of Agriculture, Sri Guru Granth Sahib World University, Fatehgarh Sahib, Punjab (India) during winter season of 2022-23 and 2023-24 with variety Palam Samridhi. The soil of experimental field was clay loam in texture, alkaline in reaction (pH 7.9) and low in organic carbon (0.54%), available nitrogen (160 kg N ha^{-1}), boron (0.34 mg kg^{-1}) and zinc (0.60 mg kg^{-1}) but medium in available phosphorus (14 kg P ha^{-1}) and potassium (280 kg K ha^{-1}). The experimental treatments consisted of two levels of calcium viz. C_1: soil application of calcium @ 100 kg ha^{-1} and C_0: no calcium and seven different treatments of B and Zn viz. B_1: soil application of boron @ 1.5 kg ha^{-1}; B_2: foliar spray of B (0.3% borax); Z_1: soil application of Zn @ 5 kg ha^{-1}; Z_2: foliar spray of Zn (0.5% zinc sulphate); B_1+Z_1: soil application of B @ 1.5 kg ha^{-1} + Zn @ 5 kg ha^{-1}; B_2Z_2: foliar spray of B (0.3% borax) + Zn (0.5% zinc sulphate) and B_0Zn_0 (Control). The treatments were arranged in factorial randomized block design with three replications on plots of size 3.6 x 3.6 m for each replication of a treatment. Calcium was applied in soil through agricultural grade gypsum (22.5% Ca) a week before transplanting of broccoli seedlings and mixed well in soil. Boron and zinc in soil were applied through disodium tetraborate (20% B) and zinc sulphate (21% Zn), respectively one day before transplanting and mixed in the soil. Foliar spray of B was done using 0.3% borax (11% B) whereas 0.5% zinc sulphate (21% Zn) was used for foliar spray of Zn. Foliar spray of both B and Zn was done at 20, 35 and 50 days after transplanting. Recommended dose of NPK used for crop was 125: 75: 75 kg ha^{-1} with N, P and K supplied through urea, diammonium phosphate and muriate of potash, respectively in all the treatments. Full dose of P and K was applied at transplanting whereas N was applied in three equal splits at 0, 20 and 40 days after transplanting. The crop was sown in nursery beds on 15 September, 2022 and 10 September, 2023 and the seedlings (28 days old) were transplanted in the main field at 45 x 45 cm spacing. The other recommended cultural practices were followed for raising the crop successfully. The data on various growth parameters (plant height, number of leaves $plant^{-1}$, leaf length, leaf width and plant spread), yield attributes and yield were recorded from five randomly selected plants in each plot. The growth observations were recorded at 60 days after transplanting. The commercially matured primary heads were separated from each plant to record average primary head weight $plant^{-1}$. The secondary heads or sprouts arising from leaf axils of each plant, after removal of its primary head, were also harvested later on (as and when commercially matured), counted and weighed to record their total number and weight $plant^{-1}$. Total head weight (weight of primary + secondary heads) $plant^{-1}$ as well as total yield (marketable yield) in tonnes per hectare (t ha^{-1}) were also worked out. The data were subjected to statistical analysis at 5% level of significance using online statistical analysis tool (OPSTAT) accessible at the website of Chaudhary Charan Singh Haryana Agricultural University, Hisar (www. hau.ac.in).

RESULTS AND DISCUSSION

Growth parameters: Soil application of calcium @ 100 kg ha^{-1} (through gypsum) could not increase any of the crop growth parameters (plant height, number of leaves $plant^{-1}$, leaf length and leaf width and plant spread) significantly over no calcium application (Table 1). But all these growth parameters registered significant increase with the application of both B and Zn alone or combined, irrespective of mode of their application (soil or foliar), over the control (B_0Z_0). Among various treatments comprising micronutrients, maximum plant height, number of leaves $plant^{-1}$, leaf size (length and width) and plant spread were observed in plants treated with foliar spray of both B and Zn (B_2Z_2) and the values were significantly higher than that obtained with all other treatments. Foliar application of either Zn (B_2) or B (Z_2), though at par with each other, proved to be significantly better than combined application of B and Zn in soil (B_1+Z_1). The combined application of B and Zn in soil (B_1+Z_1) also registered significantly higher value of all these growth parameters than their sole application in soil (B_1 or Z_1). Sole application of Zn in soil (Z_1) recorded significantly higher plant height and number of leaves $plant^{-1}$ than the sole application of B in soil (B_1) but these two treatments were at par in respect of their effect on leaf width and plant spread. Response of boron can be attributed to the fact that it is required in structure of cell wall and is also essential for translocation of carbohydrates to developing organs in plants (Prasad et al., 2014). Zinc might have promoted the crop growth because of the fact that it assists in formation of carbohydrates and chlorophyll (Mahmoud et al., 2019). Therefore, comparatively higher vegetative growth of the crop with combined application of both B and Zn might be due to their dual positive effect. The results are in agreement with that of Ain et al. (2016), Patel et al. (2017) and Mondal and Ghosh (2023).

Yield attributes and yield: Application of calcium in soil did not increase the yield attributes and head yield (marketable) of broccoli over no application, indicating that availability of Ca in the soil was sufficient to meet the crop requirement. But application of both of B and Zn, alone or in combination, increased the yield attributes and marketable yield of the crop significantly over their no application (control), irrespective of their mode of application (Table 2). Various treatments of micronutrients also differed significantly in respect of their effect on the yield attributes and yield. The highest primary head weight plant^{-1}, number of secondary heads plant^{-1} (7.97 to 8.13), weight of secondary heads plant^{-1} (119.8 to 126.2 g), total head weight plant^{-1} (495.8 to 520.3 g) and consequently the marketable yield (23.33 to 24.81 t ha^{-1}) was obtained with combined foliar application of B and Zn (B_2Z_2), which proved significantly better than all other treatments. Combined application of B and Zn in soil (B_1Z_1) was significantly better than sole application of B (B_1) or Zn (Z_1) in soil, suggesting that the B x Zn interaction was synergistic on plant growth and ultimately the yield as also observed by Halim et al. (2023). Foliar application of B alone (B_2) or Zn alone (Z_2) were, however, statistically at par with each other but both proved significantly better than their sole (B_1 or Z_1) or

Table 1. Effect of application of calcium and micronutrients on plant height, number of leaves per plant, leaf length, leaf width and plant spread in broccoli

Treatments	Plant height (cm)		Number of leaves		Leaf length (cm)		Leaf width (cm)		Plant spread (cm)	
	22-23	23-24	22-23	23-24	22-23	23-24	22-23	23-24	22-23	23-24
Calcium levels										
C_1	44.1	47.2	19.8	21.3	24.8	27.4	17.1	17.5	40.7	44.5
C_0	42.7	45.5	19.2	20.7	24.7	26.6	16.6	17.0	39.0	43.5
CD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Micronutrients										
B_1	39.1	41.2	17.1	18.2	21.9	23.9	15.6	16.1	34.7	39.2
B_2	46.8	50.3	21.4	22.9	27.1	29.5	18.2	18.4	44.7	48.3
Z_1	41.8	44.0	18.6	19.8	23.5	25.8	15.7	16.1	35.5	39.8
Z_2	46.9	50.5	21.4	23.4	27.2	29.6	18.2	18.5	44.8	48.5
$B_1 + Z_1$	44.3	47.6	20.1	21.4	25.2	27.6	16.9	17.2	39.8	44.3
$B_2 + Z_2$	49.4	53.2	22.7	24.9	29.2	31.6	19.3	19.7	48.7	52.7
B_0Zn_0	35.4	37.8	15.0	16.4	19.2	21.0	14.0	14.9	30.8	35.1
CD (p=0.05)	2.3	2.5	1.2	1.3	1.6	1.7	1.0	1.1	3.3	3.8

C_1: Ca @ 100 kg ha^{-1}; C_0: without Ca; B_1: soil application of B @ 1.5 kg ha^{-1}; B_2: foliar spray of B (0.3% borax); Z_1: soil application of Zn @ 5 kg ha^{-1}; Z_2: foliar spray of Zn (0.5% zinc sulphate) and B_0Z_0: control; NS: not significant

Table 2. Effect of application of calcium and micronutrients on weight of primary head, number of secondary heads, weight of secondary heads, total head yield plant^{-1} and marketable yield of broccoli

Treatments	Primary head weight (g)		Number of secondary heads (g)		Weight of secondary head (g)		Total head yield plant^{-1}		Marketable yield (t ha^{-1})		
	22-23	23-24	22-23	23-24	22-23	23-24	22-23	23-24	22-23	23-24	Mean
Calcium levels											
C_1	327.9	340.5	5.76	6.04	103.9	111.5	431.8	452.0	20.46	21.59	21.03
C_0	317.8	330.0	5.51	5.81	100.7	108.3	418.5	438.3	19.97	20.96	20.47
CD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
Micronutrients											
B_1	288.6	296.2	4.09	4.35	91.7	99.5	380.3	395.7	18.15	18.83	18.49
B_2	349.9	362.5	6.82	7.20	110.3	118.1	460.2	480.6	21.94	23.09	22.52
Z_1	308.3	318.4	4.78	5.13	98.4	105.8	406.7	424.2	19.41	20.26	19.84
Z_2	352.3	372.5	6.94	7.31	111.0	119.5	463.3	492.0	22.04	23.39	22.72
$B_1 + Z_1$	330.2	340.1	5.52	5.95	103.7	111.8	433.9	451.9	20.66	21.71	21.19
$B_2 + Z_2$	376.0	394.1	7.97	8.13	119.8	126.2	495.8	520.3	23.33	24.81	24.07
B_0Zn_0	255.1	263.2	3.29	3.41	81.4	88.5	336.5	351.7	16.01	16.90	16.46
CD (p=0.05)	18.3	19.8	0.53	0.68	5.1	5.7	23.1	24.5	1.21	1.31	

See Table 1 for details

combined application in soil (B_1+Z_1). Tundu et al. (2020) also obtained significantly higher response of broccoli with combined application of B and Zn, irrespective of their mode of application. However, soil application of Zn alone (Z_1) was more effective in increasing the growth and yield of the crop than the soil application of B alone (B_1). There was no significant interaction between calcium and micronutrients in respect of any parameter of the crop. The crop response was of similar nature during both the years. Favourable response of B and Zn is attributable to their role improving production and translocation of assimilates to the storage organs (heads) of the plants which improved the growth (Table 1) and ultimately the yield attributes and yield of the crop (Table 2). Favourable response of B and Zn on yield attributes and yield of broccoli has also been documented earlier (Islam et al., 2015, Patel et al., 2017, Mondal and Ghosh 2023). The response to foliar application of the micronutrients was significantly better than their soil application which may be attributed to the fact that the nutrients applied on plant foliage are easily available for absorption by plant system, avoiding soil constraints to availability and absorption of the micronutrients applied in soil (Alshaal and El-Ramady 2017).

CONCLUSION

Broccoli responded significantly to soil or foliar application of both B and Zn but foliar application was better than their soil application in increasing the crop growth and productivity. Combined application of B and Zn was better than their sole application, irrespective of their mode of application. However, combined application of B (0.3% borax) and Zn (0.5% zinc sulphate) as foliar spray was found to be the best treatment in increasing the growth and yield of broccoli and hence can be suggested for obtaining higher yield of broccoli in micronutrient (B and Zn) deficient alkaline fine textured soils of Punjab.

AUTHORS CONTRIBUTION

Prabhjot Kaur: Conducting the experiment, collection of the data and statistical analysis and writing of initial manuscript. Abhishek: Literature contribution, providing critical feedback and shaping the analysis and manuscript communication with editor and incorporation of comments. Dr. C. P. Mehla: Conceptualization, planning of the experiment and guiding the research trial. Dr. Jaspreet Kaur: Supervision of the research project, agricultural inputs and involvement in planning and supervision of the work. Dr. Mangat Ram: Contribution to the final version of the manuscript and supplementing the literature and references required for the manuscript.

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Optimizing Sowing Window and Cultivar Selection for Sustainable Foxtail Millet (*Setaria italica* L.) Cultivation in Punjab, India

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Abstract: Heat use efficiency plays a vital role in determining the growth and yield performance of foxtail millet under varying thermal regimes. The field investigation was carried out to evaluate the growth, phenology, yield and heat use efficiency of three foxtail millet cultivars (SiA-3156, Punjab Foxtail millet-3, and PFM-2) under six sowing dates (starting end April to mid-July) to expose the crop to a range of temperature conditions. The significant variation in HUE among cultivars and sowing dates, with early-sown crops generally exhibiting higher efficiency due to optimal thermal accumulation during critical growth phases was observed. Early sowing (S_1-26th April) significantly improved growth and yield, with the highest grain yield of 1.66 t ha^{-1} while late sowing (S_{6}-11th July) showed reduced grain yield despite higher biological yield. The delayed sowings resulted in shortening grain filling duration, and lowering both biomass accumulation and yield. The S_1-26th April sown crop accumulated 1738.8 growing degree days, which was lower than that other treatments, yet exhibited the highest heat use efficiency and grain yield. Among the cultivars, Punjab Foxtail Millet-3 outperformed the others, showing the highest HUE and producing the maximum grain and biological yields, followed by PFM-2. These results highlight the critical role of timely sowing and cultivar selection in optimizing foxtail millet productivity.

Keywords: Growth, Yield, Punjab foxtail millet-3, Sowing date, Phenology

Since the green revolution, Punjab's agriculture has become increasingly dominated by wheat and rice, leading to a significant decline in the cultivation of pulses, oilseeds, fibre crops, and millets (Statistical Abstracts of Punjab, 1970-2024). The rice–wheat cropping system has also led to several problems, including a declining water table, deteriorating soil health, and the imbalanced use of fertilizers and agrochemicals (Kang et al., 2015, Singh et al., 2016). Thus, to address the problems associated with the rice–wheat monoculture and the challenges posed by global climate change, crop diversification is necessity (Kaur et al., 2020). Millets are C4 plants that can efficiently utilize elevated atmospheric CO_2, enabling more biomass production (Brahmachari et al., 2018), may help in mitigating the impact of global warming. Millets, particularly foxtail millet (*Setaria italica*), are nutrient-dense and highly adaptable to low-fertility soils, requiring minimal inputs (Cui et al., 2022). Despite being the third most important millet after pearl millet (*Pennisetum glaucum*) and finger millet (*Eleusine coracana*), foxtail millet remains underutilized (Zheng et al., 2024). Under conditions of insufficient rainfall or water stressed conditions, foxtail millet (*Setaria italica*) is frequently cultivated as a substitute for sorghum (*Sorghum bicolor*), owing to its superior drought resilience (Jyothi et al., 2016). Typically grown in semi-arid regions, foxtail millet requires minimal water and benefits from a short growth cycle, making it well-suited to areas with limited rainfall. Its resilience and adaptability allow it to be sown in environments where most other crops fail to thrive. The increasingly unpredictable climatic patterns such as delayed monsoons and early cessation of rainfall, optimal sowing time has become critical. Timely planting promotes better root development and efficient resource utilization, leading to higher yields, whereas delayed sowing shortens the growth duration and significantly reduces productivity (Xing et al., 2023, Sharma et al., 2018).

Crop growth and phenology analyses conducted in specific environments are often insufficient for determining the optimal combinations of crop cultivars, environmental conditions, and cultivation practices (Sahoo et al., 2020). Understanding the thermal time requirement of a crop can help estimate both the harvest date and key developmental stages. In addition, the field performance of crop cultivars is largely influenced by interaction effects and random variability among genotypes, environmental conditions, and management practices (Nandini and Sridhara 2019). The interaction and environmental effects account for more than 50% of the variability in varietal performance across different regions and years (Ning et al., 2015, Nandini and Sridhara 2019, Sahoo et al., 2020). Therefore, it is essential to evaluate the performance of various cultivars under different sowing times to ensure sustainable yields. Hence, the present investigation was conducted to find the best sowing time and cultivar for sustainable yield by evaluation their effect on the growth, phenology, heat use efficiency and yield of foxtail millet.

MATERIAL AND METHODS

Experimental location: The study was conducted during 2023 and 2024 at the Regional Research Station of Punjab Agricultural University, Gurdaspur, Punjab. The experimental site is located in the sub-mountainous undulating zone of Punjab, at a latitude of 32.02°N, longitude of 75.22°E, and an elevation of 265.2 meters above sea level. The region receives an average annual rainfall of approximately 1325 mm, 80% of which is usually received during the south western monsoon season and remaining during the winter season. The soil was classified as fine loamy, non-calcareous, mixed, hyperthermic typic Haplustalfs with silt loam texture (41 % sand, 40.5 % silt and 18.5% clay) (Singh and Sharma 2021). The soil has 7.5 pH (1:2 soil), electrical conductivity 0.13 dSm^{-1} (1:2 soil, superannuated solution), organic carbon 0.52 %, available phosphorus 37.5 kg ha^{-1}, and available potassium 62.5 kg ha^{-1}.

Three cultivars namely SiA-3156, Punjab Foxtail millet-3, and PFM-2 (coded cultivars under testing by Punjab Agricultural University) were sown on six sowing dates namely S_1: 26th April, S_2: 11th May, S_3: 26th May, S_4: 10th June, S_5: 25th June, S_6: 11th July. The experiment was conducted using a factorial randomized complete block design with two factors: date of sowing and cultivar, each replicated three times. The crop management was done as per recommended practices by PAU, Ludhiana.

Observations recorded: The maximum plant height, number of tillers, number of days taken to 100% flowering and maturity were recorded from the randomly selected five plants from each plot. The harvesting was done once the crop reached physiological maturity i.e. when grains turned brown. The crop was harvested and left in the field for drying. After 2-3 days of drying the crop was threshed using multicrop thrasher. The grain and straw yield were recorded and expressed in quintals per hectare.

The growing degree days as a measure of thermal time, were calculated by simple arithmetic accumulation of daily mean temperature above the base temperature. The growing degree days for each stage were calculated (Nuttonson 1955):

$$\text{Growing degree days (°C days)} = \sum_{i=1}^{n} T_i - T_b \qquad (1)$$

Where, i is the i^{th} day from sowing, T_i is mean temperature for that day, n is the number of days in the growing season (Ellis et al 1990) and T_b is Base temperature and was taken equal to 10°C. The heat use efficiency was calculated using the following formula:

$$\text{Heat use efficiency (Kg/ha/°C days)} = \frac{\text{Grain or dry matter yield (Kg/ha)}}{\text{AGDD (°C day)}} \qquad (2)$$

AGDD is Accumulated growing degree days (°C Day) is sum of growing degree days acquired by crop during life cycle.

The daily maximum and minimum temperatures were recorded at Agrometeorological Observatory, Punjab Agricultural University Regional Research Station, Gurdaspur situated near the experimental site.

Statistical analysis: The analysis of variance for factorial randomised complete design was performed using RStudio (R Core Team). The least significant difference (LSD) was used to compare treatment means at 5% level of significance.

RESULTS AND DISCUSSION

Plant height significantly varies due to change in date of sowing windows (Table 1). Maximum plant height was recorded in S_1 (131.4 cm) date of sowing which was at par with S_3 and S_4 sowing date. Plant height also influenced by different cultivars, the maximum plant height was observed under Pb Foxtail millet-3 (130.7 cm), that was statistically similar to the PFM-2 (130.1 cm). The early sowing i.e. S_1 typically offer more favourable environmental conditions such as optimal temperature and photoperiod, which promote better vegetative growth and lead to greater plant height. Delayed sowing (S_6) exposes crops to suboptimal growing conditions or stress (e.g., heat, excess water), resulting in shorter plants. Additionally, the delayed sowing date of S_6 coincides with monsoon rainfalls, may results in crop lodging due to water logging or storms. The differences in plant height among cultivars are genetically controlled. The cultivars like, Pb Foxtail millet-3 possess traits such as greater internode elongation or longer growth duration, contributing to taller stature.

Number of tiller/plants: Among the date of sowings, the highest number of tillers was observed under S_1 and among cultivars the maximum number of tillers was observed under Pb Foxtail millet-3 (Table 1). The early sown (26th April) crop resulted in maximum number of tillers per plant. Tillering is sensitive to sowing time; early sowing (S_1, S_2) allows longer vegetative phases, supporting the formation of more tillers. Late sowing (S_6) compresses the vegetative phase and coincide with higher temperatures or water stress, reducing tiller formation. Varietal differences in tillering capacity are typically linked to genetics. The Pb Foxtail millet-3 have higher tillering potential, might be due to better tiller initiation and survival, efficient nutrient use, or more vigorous early growth.

Phenological stages: The maximum number of days to attain flowering were acquired taken by S_1 sowing date (70.2 days), while S_2 and S_4 took minimum days to flowering as compare to S_3 sowing date. Among the cultivars, SiA-3156 took the longest time to flower, which was significantly higher compared to the other cultivars. The Pb Foxtail-3 variety reached flowering in 67.2 days. Revathi et al. (2017) also reported that with delay in sowing induces earlier flowering, resulting in less vegetative growth and earliness in maturity.

The early sown (26th April) (87.6 days) took the highest number of days to reach maturity, whereas other sowing dates did not significantly affect the time taken to reach physiological maturity (Table1). The SiA-3156 (88.3 days) required significantly higher number of days to reach physiological as compared to Pb Foxtail millet-3 (85.5 days), and PFM-2 (85.1 days). Navya *et al* (2015) also reported that crop variety characteristics directly affect the physiological maturity.

Grain yield: The highest grain yields were in the crop sown on S_1 (1.66 t ha^{-1}) date of sowing that was statistically at par with S_2 (1.65 t ha^{-1}) (Table 2). Minimum grain yield was observed under S_6 sowing dates (1.10 t ha^{-1}). Among cultivars, the maximum grain yield was observed under Pb Foxtail millet-3 (1.47 t ha^{-1}) foxtail variety, while minimum grain yield was in SiA-3156 variety (1.27 t ha^{-1}). The increase in grain yield is mainly attributed to increase in plant height and leaf area, increases the assimilatory surface area per plant might have led to the accumulation of a large quantity of photo assimilates directly affecting the grain yield. The results are in accordance with Munirathnam *et al* (2006).

Biological yield: Maximum biological yield of foxtail millet was in S_6 (6.78 t ha^{-1}), that was statistically at par with sowing date of S_5 (6.74 t ha^{-1}) (Table 2). The sowing dates of S_2, and

Table 1. Effect of date of sowing and different cultivars on Growth and yield attributes of foxtail millet

Treatments	Plant height (cm)	No. of tillers per plant	Days to flowering	Days to physiological maturity
S_1: 26th April	131.4	5.40	70.22	87.6
S_2: 11th May	129.4	5.27	68.44	87.0
S_3: 26th May	130.6	5.01	69.60	86.9
S_4: 10th June	130.4	4.81	68.05	85.9
S_5: 25th June	127.9	4.45	67.07	85.6
S_6: 11th July	126.6	3.92	66.40	84.7
LSD (p=0.05)	NS	0.30	1.98	NS
SiA-3156	127.5	4.66	70.65	88.3
Pb Foxtail millet-3	130.7	4.94	67.16	85.5
PFM-2	130.1	4.84	67.08	85.1
LSD (p=0.05)	NS	0.17	1.66	1.6
Interactions (S×V)	NS	NS	NS	NS

Table 2. Effect of date of sowing and different cultivars on growth and yield attributes of foxtail millet

Treatments	Grain yield (t ha^{-1})	Biological yield (t ha^{-1})	AGDD (°C days)	Heat use efficiency (kg ha^{-1} °C days^{-1})
S_1: 26th April	1.66	6.11	1,738.8	0.959
S_2: 11th May	1.65	5.90	1,763.7	0.936
S_3: 26th May	1.48	5.92	1,761.7	0.843
S_4: 10th June	1.21	6.37	1,775.0	0.682
S_5: 25th June	1.10	6.74	1,724.7	0.637
S_6: 11th July	1.09	6.78	1,673.8	0.65
LSD (p=0.05)	0.16	0.38	45.9	0.076
SiA-3156	1.27	5.76	1,778.6	0.711
Pb Foxtail millet-3	1.47	6.60	1,724.6	0.853
PFM-2	1.35	6.54	1,715.7	0.789
LSD (p=0.05)	0.08	0.33	32.5	0.054
Interactions (S×V)	NS	NS	NS	NS

S_3 recorded minimum biological yield compare to all other treatments, whereas produced maximum grain yield indicating more harvest index. The foxtail variety influence the biological yield, where Pb Foxtail millet-3 and PFM-2 recorded maximum biological yield of 6.60 and 6.54 t ha^{-1}, respectively that was significantly higher than the biological yield observed under SiA-3156 (5.76 t ha^{-1}). Early sowing often leads to higher biological yields due to favourable weather conditions that promotes key growth traits (taller plants, larger leaf area, and increased dry-matter accumulation), which in turn enhance biomass production.

Growing degree days: The accumulated growing degree days (AGDD) based on date of sowing to maturity under different sowing times and cultivars (Table 2). Different phenological stages of both the cultivars require different heat units till maturity under all dates of sowing. The highest requirement of GDD was d at maturity for all the cultivars. The requirement of heat unit was less for April sown crop and this increased up to June 10 sowing. GDD requirement was maximum in June 10 sown crop followed S_3 and S_2 sowings. Further delay in sowing beyond June 10 reduced the growing degree day requirement of foxtail cultivars. Among the cultivars, Pb Foxtail millet-3 recorded significantly more heat units as compared to other two cultivars and it was at par with the heat units recorded by PFM-2 cultivar. than late sown crop due to longer period for all the phenological stages in the timely sown crop. The April and late June sowing accumulated lesser heat units due to lower temperatures as compared to May and early June sown crops. The delay in sowing also decreased the duration of maturity period which forced the crop to attain early maturity. The high temperature during vegetative phases and low temperature during reproductive phases lead to lesser heat unit accumulation.

Heat use efficiency: The effect of sowing times on heat use efficiency was significant with highest heat use efficiency was obtained in April 26 sowing, which was statistically at par with May 11 sown crop but significantly higher than other sowing dates (Table 2). The normal growing plants produced higher grain yield by using accumulated heat units efficiently. Due to consistently favourable temperatures during the normal growing season, the crop was able to utilize heat more efficiently, which in turn stimulated physiological processes and resulted in higher grain yield. Pb Foxtail millet-3 exhibited significantly higher heat use efficiency as compared to other two cultivars. The interaction effect was not significant among sowing dates and cultivars.

The regression relationship between the grain yield and heat use efficiency shows the high value for coefficient of determination which means 97 percent variation in grain yield is determined by the heat use efficiency (Fig. 1). With

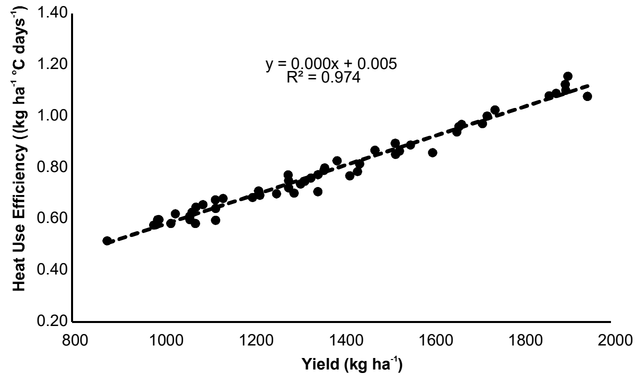

Fig. 1. Relationship of foxtail millet yield and heat use efficiency under different sowing times

progressive delay in sowing, HUE declined markedly. Crops sown in later windows (e.g., S_6) experienced not only reduced grain yields but also lower heat use efficiency, despite sometimes showing higher biological (vegetative) yield. This reduction arises primarily from shortened grain filling duration and the exposure of sensitive growth stages to higher temperatures, which limits the efficient conversion of heat units into grain due to thermal and potential water stress, resulting in poor grain set and lower harvest indices (Nandini and Sridhara 2019).

CONCLUSION

The study highlights the significant influence of sowing dates on the accumulation and utilization of heat units, ultimately affecting the growth duration, phenological development, and yield of foxtail millet cultivars. Early sowing (S_1-26th April) resulted in better synchronization with favorable thermal regimes, allowing the crop to accumulate optimal growing degree days) and achieve higher heat use efficiency and the highest grain yield) and better growth parameters in foxtail millet. Delayed sowing (S_{6}-11th july) reduced GDD accumulation during critical stages leading to reduced grain yield despite higher biological yield. The Pb foxtail millet-3 variety, performed best with the highest grain and biological yield. And required more days to mature but had lower yield performance. Interaction between sowing date and variety was non-significant for all traits.

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Performance of Rice Crop under Direct Seeded And Transplanted Method in Central Punjab

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Abstract: Field experiments were conducted at Punjab Agricultural University Ludhiana, Punjab to study the effect of planting methods on yield and yield contributing characteristics of rice crop during *kharif* 2021 and 2022. The experiments comprise of two dates of sowing (20th May and 30th May), two rice varieties (PR 126 and PR 128) and two planting methods, puddled transplanted rice (PTR) and direct seeded rice (DSR). The highest LAI and tiller number was in PTR method compared to DSR method. The variety PR 128 attained highest LAI and tiller numbers compared to variety PR 126 in both the seasons. Significant difference in grain yield was observed with delay in sowing between 20th May and 30th May. Significantly higher grain yield (8.1 t/ha) was recorded in variety PR 128 as compared to variety PR 126 (6.8 t/ha). The yield contributing characteristics were also significantly higher in 30th May sown crop (variety PR 128), under PTR as compared to DSR. The crop sown in PTR method gave significantly higher grain yield (8.0 t/ha) as compared to DSR method (6.9 t/ha).

Keywords: Puddled transplanted rice, Direct seeded rice, Sowing method, Sowing time

Rice stands as one of the primary cereal crop in northwestern India, particularly in Punjab. The rice cultivation has been increased significantly in Punjab, owing to the accessibility of high-yielding varieties, improved irrigation infrastructure, and government-backed support prices. Rice occupied approximately 31.45 lakh hectares with a total production of about 203.71 lakh tons (PAU Package and Practice 2023). An average of over 4000 liters of water is utilized to yield one kilogram of rice (IRRI 2008). But in Punjab, due to decline in ground water table in most of the places, the cost of pumping the groundwater was increased and water quality also deteriorate over the years (Singh et al., 2022). Along with increasing labour challenges it is very hard to maintain the sustainability of the rice ecosystem. Transplantation into puddled fields was a conventional agricultural technique that often leads to the formation of hard pan, consequently ruin the soil structure. Moreover, the consistently inundation of water, enhances the deep percolation and sometime excessive irrigation, surpassing the actual water demands of the crop. Hence, other practices for rice growing need to be explored to solve this problem such as direct-seeded rice, which need less water in comparison to conventional transplanting method (Kamboj et al., 2022). Direct-seeded rice offers several advantages over transplanted rice, including systematic water utilization, mechanization compatibility, enhanced profitability, increased the quality and early maturity. Additionally, it facilitates an optimal sowing window for subsequent wheat crops, thereby contributing to the sustainability of the rice-wheat cropping system (Ishfaq et al., 2018, Anjum et al., 2019). Given the pressing necessity to enhance the water productivity of rice cultivation in Punjab to mitigate economic losses and ecological deterioration, field experiments were undertaken to investigate the impact of different planting methods on rice yield and yield contributing characteristics.

MATERIAL AND METHODS

The field experiment was carried out at Punjab Agricultural University, Ludhiana, during the 2021 and 2022 *kharif* season. It is situated at 30°54'N latitude and 75°48'E longitude and is 245 m above mean sea level. Two rice varieties, PR 126 (V_1) and PR 128 (V_2) were transplanted (M_1) and directly seeded (M_2) on two different dates 20th May and 30th May for direct seeded rice and 20th June and 30th June for transplanted rice. The experiment was laid out in the split-split plot design with four replication. In the context of direct-seeded rice (DSR) method, urea was given at a rate of 321 kg per ha, distributed evenly across three applications occurring at four, six, and nine weeks after sowing. Conversely, for puddled transplanted rice (PTR) method, urea was applied at a rate of 222.3 kg per hectare, while phosphorus was applied as diammonium phosphate (DAP) at 66.7 kg per hectare, and potash was provided as muriate of potash (MOP) at 49.4 kg per hectare (PAU Package and Practice 2023). Biometric parameters *viz*. leaf area index (LAI) and tiller numbers were recorded at 15 days interval. Yield and yield contributing characteristics viz. number of grains per plant, number of effective tillers per plant, 1000-grain weight, biological yield, grain yield and straw yield were recorded at harvesting. The meteorological data was recorded at the Agro meteorological Observatory located 150m away from the experimental site.

Statistical analysis: The data collected on different yield and yield contributing characteristics was statistically analyzed by using split-split plot design by using SPSS software and OPStat software.

RESULTS AND DISCUSSION

Weather during *kharif* 2021 and 2022: Throughout the *kharif* of 2021, the maximum temperature ranged between 26.0 and 38.0°C, whereas in the corresponding period of 2022, it varied from 25.6 to 43.7°C (Fig. 1, 2). Similarly, the minimum temperature during the *kharif* 2021 ranged between 8.5 to 28.5°C, while in *kharif* 2022, it spanned from 28.7 to 8.7°C. During the crop growing season of *kharif* 2021, the morning relative humidity ranged between 49.5 to 93.0 per cent and the evening relative humidity ranged between 23.1 to 75.0 per cent. But in *kharif* 2022 the morning relative humidity ranged between 39.4 to 95 per cent and the evening relative humidity ranged between 16.0 to 71.5 per cent. The total sunshine hours ranged between 3.4 to 10.8 hours during

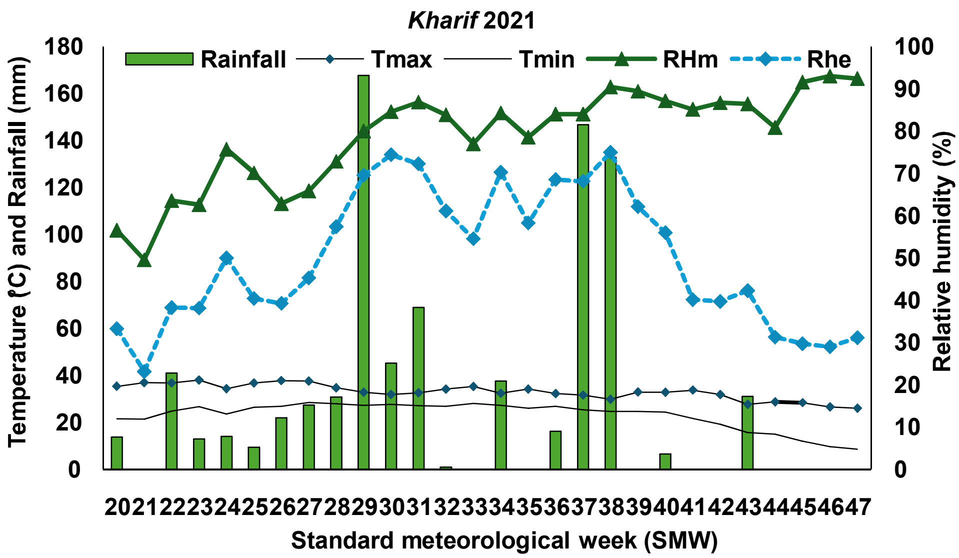

Fig. 1. Variation of meteorological parameters during *kharif* 2021

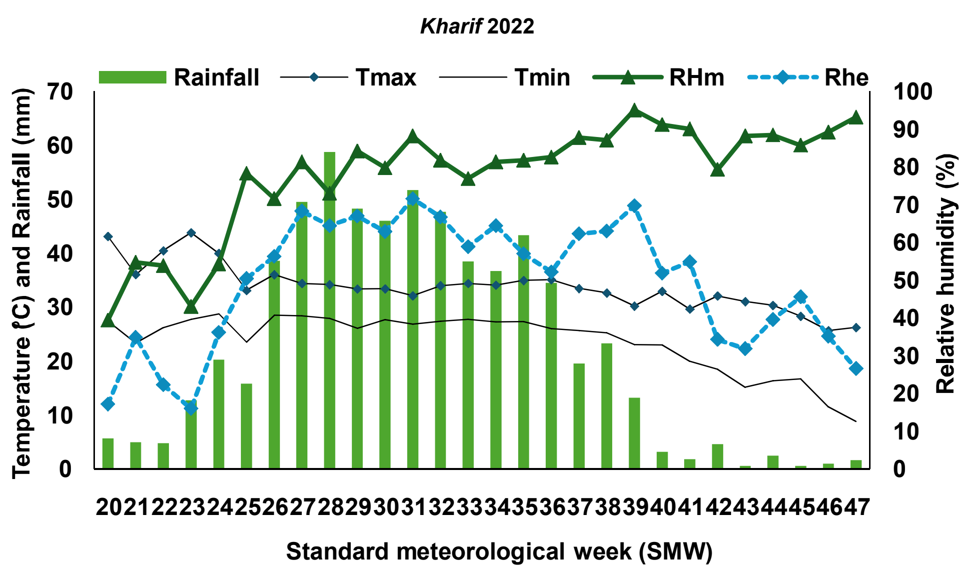

Fig. 2. Variation of meteorological parameters during *kharif* 2022

the crop growing season of *kharif* 2021. But in *kharif* 2022 the total sunshine hours ranged between 1.6 to 10.3 hours. The total rainfall was 759.4 mm during the *kharif* 2021 and 643.7 mm in *kharif* 2022.

Biometric Parameters

Leaf area index - Leaf area index (LAI) was increased up to 90 days after sowing and later decreased up to physiological maturity in both the years (Table 1). The LAI was higher during 30th June transplanting / 30th May sown crop as compared to 20th June transplanting / 20th May sown crop. For 20th June transplanting / 20th May sowing the highest LAI was recorded during 90 days after sowing (DAS), which was 3.62, and for 30th June transplanting / 30th May sowing it was 3.65. Significant difference in LAI were found at all physiological stages (except 45 DAS) between two dates of sowing. There was significant difference between two varieties at every physiological stages. In variety PR 126, the highest LAI was at 90 DAS (3.43), however in variety PR 128, the highest LAI was at 90 DAS which was 3.84. Inherent variances in leaf arrangement, morphology, developmental behaviour and genetic diversity play an important role in varietal expression of biophysical characters in rice. Temperature, the availability of water, and the concentration of nutrients all have an impact on leaf development and overall plant growth, which in turn affects LAI (Hour et al., 2020). In case of method of sowing, LAI was at higher side in PTR compared to DSR. But there was significant difference in LAI at all physiological stages (except 45 DAS). The enhanced growth observed in rice cultivated using the PTR method can be attributed to increased accessibility to photosynthetically active radiation (PAR) and an improved supply of light conducive to photosynthesis. These favorable conditions likely facilitated improved physiological processes, including enhanced carbohydrate metabolism and respiration, thereby promoting the overall development and growth of rice plants in PTR method. Similar results were observed by Gill et al. (2011).

Periodic number of tillers: The highest number of tillers per square meter for both the varieties (PR 126 and PR 128) was at 105 days after sowing (DAS), which was highest in 30th June transplanted/30th May direct sown crop and lowest in 20th June transplanted/20th May direct sown crop. The number of tillers per meter square showed significant response to different transplanting dates (Akbar et al., 2010). This was due to the low temperature during the pollen development stage, which may have led to fewer tillers (Gill et al., 2006). Overall variety PR 128 produced more number of tillers compared to variety PR 126 in both PTR and DSR method. However, at 105 DAS, 30th June transplanted variety PR 128 had highest number of tillers (338 tillersper square meter), followed by 20th June transplanted variety PR 128 (331 tillers per square meter), 30th June transplanted variety PR 126 (297 tillers per square meter), 20th June transplanted variety PR 126 (290 tillers per square meter). Similarly, at 105 DAS, 30th May direct sown variety PR 128 had highest number of tillers followed by 20th May direct sown variety PR 128. The number of tillers in PTR was more as compared to DSR. The competition for nutrients and space between plants was more in DSR due to improper thinning, which was absent in PTR (Choudhary et al., 2016 , Luo 2022). the number of tillers in rice plants decreases significantly under high plant populations, whereas it can be significantly increased with sufficient nutrient supply with proper plant population. This highlights the importance of plant population in regulating tiller development (Chen et al., 2020).

Table 1. Effect of different treatments on leaf area index of rice (Pooled data of 2 years)

Treatments	45 DAS	60 DAS	75 DAS	90 DAS	105 DAS	120 DAS
Date of sowing (D)						
20th May	0.33	0.99	2.09	3.62	2.92	2.22
30th May	0.34	0.90	2.14	3.65	2.97	2.27
CD (p=0.05)	NS	0.04	0.03	0.01	0.02	0.03
Variety (V)						
PR 126	0.32	0.97	2.07	3.43	2.84	2.12
PR 128	0.35	0.92	2.16	3.84	3.05	2.37
CD (p=0.05)	0.02	0.04	0.06	0.15	0.14	0.11
Method of sowing (M)						
PTR	0.33	0.96	2.14	3.65	2.97	2.27
DSR	0.33	0.94	2.10	3.62	2.93	2.22
CD (p=0.05)	NS	0.01	0.03	0.02	0.03	0.02

PTR – Puddled transplanted rice; DSR - Direct seeded rice

Yield and Yield Contributing Characters

Effective tillers: The time of sowing influenced the number of effective tillers per plant (at harvest) in both the varieties and significant differences were observed in varieties under different dates of sowing during both the crop seasons (Table 2). The number of effective tillers per plant was higher in 30th June transplanting/30th May direct sowing (9.6) compared to 20th June transplanting/20th May direct sowing (9.3) and are significantly different from each other (Table 2). There may be an influence of sowing timing on tiller development in rice crop. The rice crop enters the initial phase of development slowly when sown early and contrarily, passes quickly when sown late (Urazmetov et al 2023). The variety PR 128 produced more number of tillers per plant (9.7) compared to

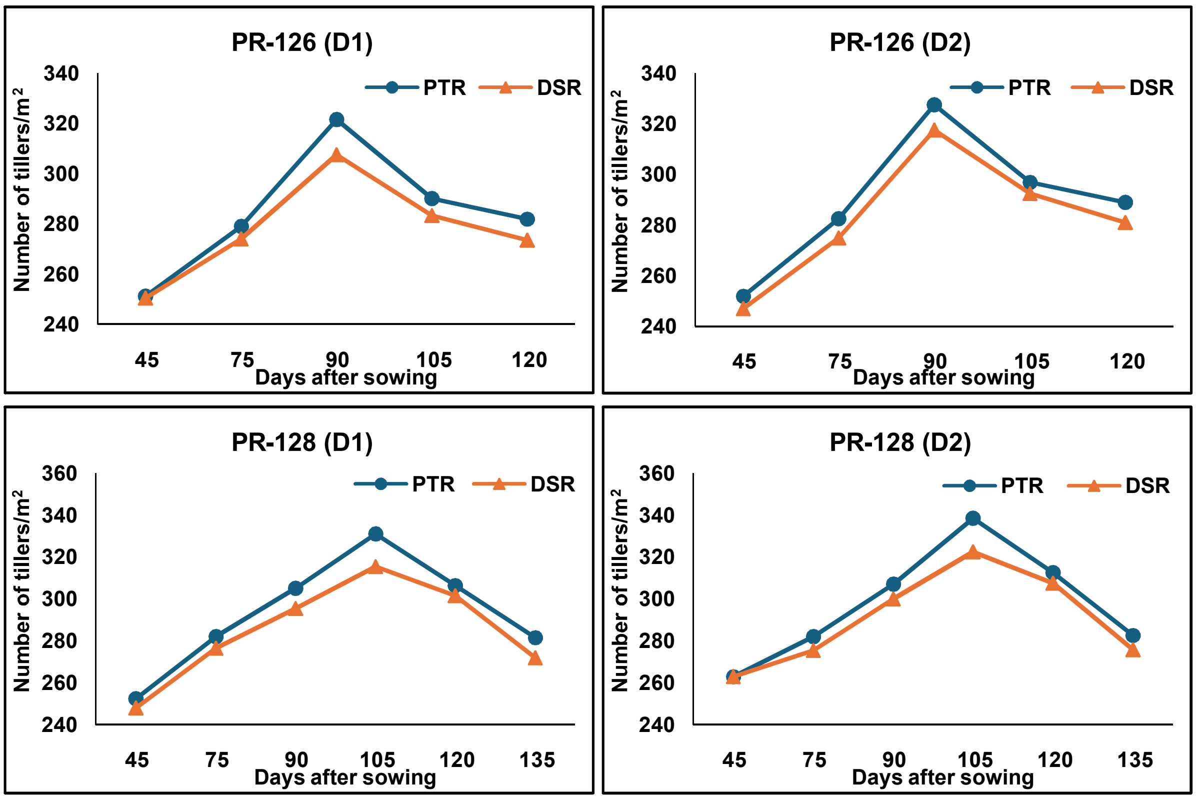

Fig. 3. Periodic number of tillers under different methods of sowing (Pooled data of 2021 and 2022)

Table 2. Effect of different treatments on yield and yield attributes of rice (Pooled data of 2 years)

Treatments	No of effective tillers/plant	No of grains per panicle	1000 grain weight (g)	Biological yield (t/ha)	Grain yield (t/ha)	Straw yield (t/ha)
Date of sowing (D)						
20th May	9.3	123.4	25.2	21.5	7.5	14.0
30th May	9.6	146.0	25.8	24.5	7.4	17.1
CD (p=0.05)	0.2	9.3	NS	1.5	NS	1.3
Variety (V)						
PR 126	9.1	130.8	25.6	21.6	6.8	14.8
PR 128	9.7	138.6	25.7	22.4	8.1	14.3
CD (p=0.05)	0.3	NS	NS	NS	0.8	NS
Method of sowing (M)						
PTR	9.7	147.6	25.9	21.8	8.0	13.8
DSR	9.2	123.3	25.2	22.3	6.9	15.4
CD (p=0.05)	0.4	13.9	NS	NS	0.7	NS

variety PR 126 (9.1). They are significantly different from each other. PTR method produced more number of effective tillers (9.7) compared to DSR method and are significantly different from each other. The PTR method involving transplanting seedlings into puddled and flooded fields, creates conducive conditions for tiller initiation and development. Chaudhary et al (2023) also highlighted the more tiller production in transplanted rice systems due to enhanced nutrient availability and controlled seedling establishment in puddled fields. The PTR method may promote faster planting and maturing, which can contribute to better tiller production.

Number of grains per panicle: The number of grains per panicle were more (146.0) in 30th June transplanting/30th May direct sowing compared to 20th June transplanting/20th May direct sowing (123.4) and there are significant difference in both date of sowing (Table 2). For variety PR 128 the number of grains per panicle (138.6) was more compare to variety PR 126 (130.8) but they were statistically at per. But there was a significant differences in number of grains per panicle between transplanting method and direct seeded method. Transplanting method produced more number of grains (147.6) compared to directly sown crop (123.3). This aligns with the research conducted by Gavric and Omerbegovic (2021), emphasized the potential for increased grain yield in transplanted rice systems due to enhanced tillering and panicle formation. The observed higher grain count in the transplanting method reflects its efficiency in creating favourable conditions for rice crop growth, thereby contributing to increase in overall grain yield compared to the direct seeding approach.

1000-grain weight: The 1000-grain weight was higher in 30th June transplanting/30th May direct sowing (25.8 g) compared to 20th June transplanting/20th May direct sowing (25.2 g), with no significant difference between them (Table 2). The increase in 1000-grain weight highlighted the sensitivity of this key yield-contributing factor to variations in environmental conditions associated with different sowing dates. The 30th May sowing date likely provided more favourable conditions for grain filling and maturation, resulting in larger and heavier grains (Soleymani and Shahrajabian 2011). There was no significant difference in 1000-grain weight for two varieties and two method of sowing.

Grain yield: Grain yield was higher in variety PR 128 (8.1 t/ha) compared to PR 126 (6.8 t/ha) with significant difference between them (Table 2). But there was no significant difference in grain yield between two dates of sowing. However, the highest grain yield was observed in PTR method (8.0 t/ha), but grain yield obtained in DSR method was 6.9 t/ha and they are significantly different from each other. The higher grain yield in the PTR method can be attributed to factors such as enhanced tiller development, controlled seedling establishment and favorable conditions provided by puddling and transplanting. Khush (2013) also demonstrated the potential for increased grain yield in transplanted rice systems. Additionally, a meta-analysis by Xu et al (2019) also reported that the yield of transplanted rice was higher than that of directly seeded rice.

Biological and straw yield: The other yield contributing characters viz. biological yield and straw yield are significantly different during both date of sowing. The biological yield was higher in 30th June transplanting/30th May direct sowing (24.5 t/ha) compared to 20th June transplanting/20th May direct sowing (21.5 t/ha) and they are significantly different (Table 2). Similarly, 30th June transplanting/30th May direct sowing produced more straw yield (17.1 t/ha) compared to 20th June transplanting/20th May direct sowing (14.0 t/ha) and there was a significant difference between them (Table 2). This may be due to availability of favourable temperature during panicle and grain development period in 30th June (D_2) transplanted crop. These findings were supported by Urazmetov et al (2023) and Chaudhary et al (2023). Similarly, varieties sown directly produced higher biological yield compared to puddle transplanted rice varieties with no significant differences. However, in DSR method, straw production was higher (15.4 t/ha) compared to PTR method (13.8 t/ha). This may be due to the fact that,in direct seeded rice method, seeds are sown directly into the field rather than being transplanted from a nursery, plants typically experience less disturbance to their root systems. This reduced disturbance allows for more extensive root development, which in turn promotes greater nutrient uptake and biomass production, including the production of straw (Tian et al 2022).

CONCLUSION

The biometric parameters in rice as well as yield and yield contributing characters were markedly influenced by varying dates of sowing, varieties and methods of sowing. In general the yield and yield contributing characteristics were comparatively higher in 30th June transplanted/30th May direct sown crop compared to 20th June transplanted/20th May direct sown crop. Similarly, yield and yield contributing characters were higher in variety PR 128 compared to variety PR 126. Overall PTR method provide better grain yield compared to DSR method in both the crop growing seasons. Therefore, adjustments in sowing dates and sowing methods represent a cost-effective strategy for improvingthe rice yield.

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Performance of Planting Methods of *Gobhi Sarson* in Pigeonpea-*Gobhi Sarson* Cropping System in Sub-tropical Zone

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Abstract: The study evaluated the performance of different planting methods of *Gobhi sarson* in the pigeonpea-*Gobhi sarson* cropping system to identify the most effective establishment technique. The study was conducted during *Rabi* 2023-24 and 2024-25 at PAU, Ludhiana, using a randomized complete block design with three planting methods- relay cropping, direct seeding and transplanting. Planting methods significantly influenced growth and yield. Relay cropping performed best due to early establishment, better plant stand and efficient use of residual moisture, resulting in higher growth vigour and superior yield attributes. Direct seeding showed moderate performance, while transplanting exhibited comparatively lower growth and yield. Overall, relay cropping proved most suitable for maximizing productivity. The growth, yield attributes and yield of *Gobhi sarson* were significantly affected by pigeonpea-based cropping systems. Relay cropping systems (pigeonpea + cowpea - *Gobhi sarson* - summer moong) recorded highest growth traits (plant height, dry matter accumulation and LAI), owing to better moisture conservation, improved soil structure and residual fertility from preceding legumes. Relay cropping also enhanced yield attributes, including number of siliquae plant^{-1} and seeds siliquae^{-1}, while test weight remained non-significant. The highest seed yield was obtained in pigeonpea (AL 882) + cowpea (F) - *Gobhi sarson* (relay cropping) - summer Moong (24.26 q ha^{-1}), statistically at par with T1 (24.13 q ha^{-1}), but significantly higher than direct-seeded and transplanted treatments. Improved yield under relay cropping resulted from timely establishment, better plant stand, efficient resource use and an extended effective growth period. Overall, relay cropping proved to be the most suitable, efficient and sustainable establishment method for maximizing productivity of *Gobhi sarson* in pigeonpea-based cropping systems, followed by direct seeding and transplanting.

Keywords: Relay cropping, *Gobhi sarson*, Direct seeding, Transplanting, Pigeonpea-*Gobhi sarson* system

Gobhi sarson (*Brassica napus* L.) is an important *Rabi* oilseed crop grown extensively in northern India due to its high oil content, wider adaptability and stability under diverse agro-climatic conditions (Rathore et al., 2018). India is one of the leading producers of rapeseed-mustard, contributing nearly 40% of the country's total edible oilseed production, with an average productivity of 1.3-1.5 t ha^{-1} in recent years (DES 2023). Enhancing productivity of oilseed crops like *Gobhi sarson* is essential for reducing the national oil import burden and strengthening domestic oilseed security. Pigeonpea (*Cajanus cajan* L.) is a key *Kharif* pulse crop in India, cultivated on about 5.0 million hectares, with an average productivity of 850-900 kg ha^{-1} (DAC & FW 2023). Being a deep-rooted legume, pigeonpea improves soil fertility through biological nitrogen fixation, enhances soil structure and leaves behind considerable residual moisture after harvest (Paroda 2022). This makes it a suitable preceding crop for *Gobhi sarson* in sequential cropping systems. The pigeonpea-*Gobhi sarson* sequence is therefore highly complementary, where pigeonpea enriches the soil and *Gobhi sarson* efficiently utilizes the conserved moisture and nutrients, contributing to improved system productivity and sustainability (Deshmukh et al., 2023). However, in this system, the major challenge is timely sowing of *Gobhi sarson*, as pigeonpea matures late. Delayed sowing often leads to poor crop establishment, reduced branching, fewer siliquae and ultimately lower seed yield (Singh et al., 2024). This challenge is aggravated under climate variability, where erratic rainfall and temperature fluctuations can delay pigeonpea harvest and adversely affect sowing windows for the succeeding oilseed crop. Climate-related constraints such as moisture deficit at sowing, lower temperatures during early growth and fluctuating humidity levels can further limit the productivity of late-sown *Gobhi sarson*. To address these constraints, alternative planting methods such as relay cropping, direct seeding and transplanting are used. Relay cropping allows *Gobhi sarson* to be sown before the harvest of pigeonpea, ensuring timely establishment and better moisture utilization. Direct seeding is simpler but depends heavily on available soil moisture, while transplanting helps manage delays but may cause transplanting shock and reduced early vigour (Chourasiya et al., 2024). Relay cropping has been recognized as a climate-smart and resource-efficient technique that enhances cropping intensity, improves soil health, minimizes the fallow period between crops and ensures more effective use of sunlight, nutrients and moisture. Considering the national need for enhanced oilseed productivity and the agronomic benefits of pigeonpea-*Gobhi sarson* sequence, evaluating suitable establishment techniques becomes essential. Therefore, the present study was undertaken to compare relay cropping, direct seeding and transplanting to identifying the most effective planting method for maximizing growth, yield and overall system productivity of *Gobhi sarson* in pigeonpea-based cropping systems.

MATERIAL AND METHODS

The field experiment was conducted during *Rabi* 2023-24 and 2024-25 at Punjab Agricultural University, Ludhiana, to assess the performance of planting methods of *Gobhi sarson* in the pigeonpea-*Gobhi sarson* cropping system in Sub-tropical zone. Prior to initiating the experiment, representative soil samples were collected from 0-15 and 15-30 cm depths and analyzed for physico-chemical characteristics. The soil of the experimental field was sandy loam in texture, slightly alkaline in reaction (pH 7.6-7.8), low in electrical conductivity (0.44-0.46 dS m^{-1}), and low to medium in organic carbon (0.41-0.44%). Available nitrogen content was low (135.4-145.2 kg ha^{-1}), whereas available phosphorus (15.2-18.3 kg ha^{-1}) and potassium (180.6-200.5 kg ha^{-1}) were in the medium range. Bulk density ranged from 1.41 to 1.44 g cm^{-3}. The experiment was laid out in a randomized complete block design with three replications, comprising three planting methods of *Gobhi sarson*; relay cropping, direct seeding and transplanting in standing pigeonpea and after uprooting of Pigeonpea in respect two different genotypes (PAU 881 and AL 882). In the relay cropping of *Gobhi sarson*, sowing was done in standing pigeonpea before harvest. Then under direct seeding method, crop was sown immediately after harvest of pigeonpea and in transplanting, seedlings raised in a nursery were transplanted (30 days old nursery) after pigeonpea harvest. Standard agronomic practices for raised bed technology have been followed in *Gobhi sarson* were uniformly followed across treatments during both the crop seasons (Anonymous 2025-26). Growth parameters such as plant height, number of branches $plant^{-1}$, leaf area index and dry matter accumulation were recorded at regular intervals. Yield attributes including siliquae $plant^{-1}$, siliqua length, seeds $siliqua^{-1}$ and 1000-seed weight were measured at maturity. Seed, stover and biological yields were recorded from the net plot area. The data on growth and yield attributes were statistically analyzed using analysis of variance (ANOVA), and treatment means were compared using the critical difference at 5% probability level using SAS statistical software (SAS Institute Inc. 2014).

RESULTS AND DISCUSSION

Growth parameters: Growth parameters *viz.* plant height, dry matter accumulation, leaf area index (LAI) were significantly influenced by pigeonpea-based different cropping systems (Table 1). Among the treatments, pigeonpea (AL 882) + cowpea (F) - *Gobhi sarson* (relay cropping) - summer moong (T4) recorded the highest plant height (100.9 cm), which was statistically at par with pigeonpea (PAU 881) + cowpea (F) - *Gobhi sarson* (Relay cropping) - summer moong (100.7 cm) but significantly highest than the direct-seeded and transplanted treatments. The lowest plant height was observed under transplanted *Gobhi sarson* following pigeonpea in both cultivars (T3 and T6). The plant height showed that higher growth under relay cropping may be attributed to better soil moisture retention, improved soil structure and residual fertility due to the preceding pigeonpea-cowpea system, which provided a favourable microenvironment for vegetative development (Morya et al., 2023). The, dry matter accumulation exhibited a pattern closely associated with plant height. The highest dry matter accumulation was recorded under pigeonpea (AL 882) + cowpea (F) - *Gobhi sarson* (relay cropping) - summer moong T4 (452.9 g m^{-2}), which was closely followed by T3 (416.9 g m^{-2}). This improvement may be ascribed to better canopy development, greater leaf area index and enhanced photosynthetic activity due to improved soil fertility and moisture conditions left by the preceding pigeonpea-cowpea system. The residual nitrogen and organic matter from legume crop likely promoted higher photosynthetic rate and assimilate production, resulting in increased biomass. These observations are supported by the findings of Pandey et al. (2021). The leaf area index (LAI), indicated non-significant differences among treatments. But numerically highest LAI was in T4 (2.65) whereas the lowest LAI was in T3 (2.24) and T6 (2.28). The LAI across treatments (T1-T6) remained non-significant because *Gobhi sarson* exhibits strong compensatory leaf growth, resulting in a uniform canopy under similar plant population, moisture and nutrient conditions (Table 1). Singh et al. (2019) also observed the same trend. Among the cultivars, the differences in weed population and dry matter accumulation were non-significant. Shergill et al. (2012) also reported that Hyola PAC 401 and GSC 6 have no difference in competitive ability for control of weeds, irrespective to their yield potential.

Yield attributes and yield: Yield attributes and yield *viz.* number of branches $plant^{-1}$, number of siliquae $plant^{-1}$, number of seeds $siliquae^{-1}$, test weight and seed yield were significantly influenced by pigeonpea-based different cropping systems demonstrate in (Table 1). The number of primary branches, showed non-significant differences among treatments. The numerically highest number of primary branches was in pigeonpea (AL 882) + cowpea (F) - *Gobhi sarson* (relay cropping) - summer moong (T4) (6.3).

Table 1. Growth, yield attributes and yield of *Gobhi sarson* under varied sowing/transplanting methods (Pooled data of two years)

Treatments	Plant height (cm)	Dry matter accumulation ($g\ m^{-2}$)	Leaf area index	Number of primary branches	Number of secondary branches	Number of siliquae $plant^{-1}$	Number of seeds $siliquae^{-1}$	Test weight (g)	Seed yield ($q\ ha^{-1}$)
Days after sowing (DAS) and Transplanted (T)									
T1: Pigeonpea (PAU 881) + Cowpea (F) - *Gobhi sarson* (Relay cropping) - Summer Moong	100.7	451.2	2.54	6.2	288.0	22.8	8.65	4.64	24.13
T2: Pigeonpea (PAU 881) + Cowpea (F) - *Gobhi sarson* (DS) - Summer Moong	99.0	431.2	2.33	5.9	269.1	21.5	7.85	4.06	22.14
T3: Pigeonpea (PAU 881) + Cowpea (F) - *Gobhi sarson* (T) - Summer Moong	97.0	416.9	2.24	5.6	253.1	21.2	6.70	3.91	21.22
T4: Pigeonpea (AL 882) + Cowpea (F) - *Gobhi sarson* (Relay cropping) - Summer Moong	100.9	452.9	2.65	6.3	288.9	23.1	8.75	4.71	24.26
T5: Pigeonpea (AL 882) + Cowpea (F) - *Gobhi sarson* (DS) - Summer Moong	100.3	430.2	2.36	6.0	270.3	22.1	8.05	4.08	22.27
T6: Pigeonpea (AL 882) + Cowpea (F) - *Gobhi sarson* (T) - Summer Moong	98.2	416.6	2.28	6.1	254.2	21.4	6.90	3.95	21.32
CD (p=0.05)	1.86	34.47	NS	NS	34.83	1.75	1.68	NS	1.63

The lowest number of primary branches was observed in T3 (5.6). The primary branches remained non-significant because all treatments followed uniform sowing time, plant density and nutrient management, leading to similar early vegetative growth. However, secondary branches varied significantly as relay cropping (T1 and T4) created a more favourable micro-environment with better soil moisture, and improved residual fertility, resulting in greater assimilate availability and enhanced axillary bud development. Additionally, the superior varietal vigor of AL 882 in T4 contributed to increased secondary branching compared to other treatments. Similar observations were reported by Vibhanshu and Sarlach (2023). The number of secondary branches also indicated significant differences among treatments. The highest number of secondary branches was under relay cropping in T4 (288.9), which was statistically at par with T1 (288.0) and significantly superior to direct-seeded and transplanted treatments. The lowest number of secondary branches was observed in T3 (253.1), followed by T6 (254.2). The siliquae $plant^{-1}$ were higher in relay-cropped treatments (T1 and T4) because relay sowing improved early establishment, conserved soil moisture and provided a longer effective growth period, creating a favourable microclimate for reproductive branching, siliqua formation and seed set compared to direct seeding and transplanting (Table 1). The results are in agreement with the findings of Kumar (2020). The number of siliquae $plant^{-1}$ was highest in T4 (23.1), which remained at par with T1 (22.8) and significantly highest to transplanted treatments. The minimum number of siliquae $plant^{-1}$ was observed in T3 (21.2). The relay cropping systems (T1 and T4) provided a more favourable microenvironment with better light interception and reduced intra-plant competition, leading to higher siliquae formation and seed set. The genotypic differences, as seen between PAU 881 and AL 882, could influence reproductive efficiency, resulting in higher seeds per siliqua in AL 882 under relay cropping. These findings are in agreement with the results reported by Sharma et al. (2025). The test weight did not differ significantly among treatments in the pooled analysis.

The numerically highest test weight was observed in T4 (4.71 g) and T1 (4.64 g), while the lowest test weight was in T3 (3.91 g). The lack of significant variation suggests that seed size was comparatively less sensitive to differences in establishment methods. Seed yield exhibited significant variation among treatments. The highest seed yield was in T4 (24.26 q ha^{-1}), which was statistically at par with T1 (24.13 q ha^{-1}) and significantly superior to direct-seeded and transplanted treatments. The lowest seed yield was recorded in T3 (21.22 q ha^{-1}), followed closely by T6 (21.32 q ha^{-1}). The enhanced seed yield under relay cropping can be attributed to better growth conditions, extended period for vegetative and reproductive development and improved partitioning of assimilates towards seeds. Relay cropping likely facilitated efficient use of available nutrients, moisture, and light, reducing interspecific competition during the critical growth stages of *Gobhi sarson*. The double sowing and timely sown systems exhibited lower seed yields, possibly due to restricted growth duration and greater competition for resources. These findings are in agreement with previous studies, Kaparwan et al. (2020) reported that relay cropping in pulse-based intercropping systems significantly improves seed yield of mustard by optimizing crop growth and resource utilization.

CONCLUSION

Planting methods significantly influenced the growth and yield of *Gobhi sarson* in the pigeonpea-based cropping system. Relay cropping pigeonpea (AL 882) + cowpea (F) - Gobhi sarson (relay cropping) - summer Moong recorded the highest plant height, dry matter accumulation, leaf area index, number of branches, siliquae plant^{-1}, seeds siliqua^{-1}, test weight and seed yield. Enhanced performance under relay cropping was due to timely establishment, better light interception, efficient moisture and nutrient use and minimal interspecific competition. Direct seeding showed moderate performance, while transplanting resulted in comparatively less productive. Therefore, relay cropping may be recommended as an efficient and sustainable planting method for enhancing *Gobhi sarson* productivity in pigeonpea-based cropping systems.

AUTHOR'S CONTRIBUTION

This study was conducted as part of the Doctoral Research Project under Punjab Agricultural University. Coauthor GK contributed to the conduct of experiment, data collection, analysis of data and manuscript writing. KSS planned the experiment, time to time field operation guidelines, conceptualization of the study and provided theoretical, academic inputs and interpretation of results. JK contributed in data analysis and writing of paper. Shreya assisted in data collection and helped in data analysis.

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Effect of Mycorrhiza, Alga Al-Zuhoor and Foliar Spraying with Bio-Fertilizer on *Eriobotrya aponica* Seedling

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Abstract: The study was conducted in Alsiyahii region of Babylon province to study the effect of Mycorrhiza, seaweed extract (ALGA AL-Zuhoor) and foliar spray with biofertilizer EM1 on vegetative traits and nutrients content in leaf. The experiment included soil application of Mycorrhiza and ALGA (5 ml L^{-1}) and EM1 at 5 and 10 ml L^{-1}. The Mycorrhizae application significantly increased the number of leaves, the leaf area dry matter and chlorophyll and nitrogen content of leaf. The Mycorrhiza and EM1 10 ml L^{-1} also indicated the same trend. The interaction between the Mycorrhiza, ALGA and foliar spray with EM1 10 ml L^{-1} caused significant effect in improving all studied traits compared to the control.

Keywords: Loquat seedling, *Eriobotrya japonica* L., Mycorrhiza, ALGA AL-Zuhoor

In recent years in many countries of the world emphasis is on environmentally friendly processes and technologies to manage the pollution with use of organic fertilizers, biological fertilizers, bacteria, fungi, yeasts, etc. The bio organic improves the physical and chemical properties of soil and increases the activity of microorganisms in the soil that increase the nutrient readiness to plant, which reflects positively on growth (Zaki and Mohamed 2007). Among these Mycorrhiza improves the properties of the soil and increases the readiness of nutrients by forming a symbiotic relationship between the plant roots and soil, which facilitates the transport of nutrients to the plant. It also contributes to the adhesion of soil particles through its secretion of polysaccharide compounds, which increases the susceptibility of the soil to water retention (Driver 2005). Mycorrhiza is also a fungus that spreads rapidly (Badawi 2008). These fungi protect plants from various pathogens (Smith and Read 2008). The seaweed extract also play an important role in plant growth and development as these contain many macro and microelements in addition to growth regulators of auxin, cytokinins and gibberellins. When added to the soil and absorbed by the root system, improve the vegetative growth of the plant as a result of cell division (Abd - EL-Mawgoud et al 2010). The organic fertilizers, EM1 is an effective microorganism natural biological fertilizer produced by the Japanese company. EMRO contains many microorganisms, including lactic acid, actinomycetes bacteria, yeasts and fungi (Anonymous 2005). These fertilizers secrete many growth harmons that accelerates plant growth by supplying with important nutrients (Al-Saidi 2005). Allawi (2013) observed that the adding of Mycorrhiza gave a significant increase in vegetative growth. Ismail and Abdel Sattar (2012) showed when treating olive seedlings with seaweed extract (marine fruit) there was significant increase in vegetative growth and. Strik (2003) concluded that adding seaweed extract to olive trees led to a significant increase in the vegetative growth and chlorophyll content of leaf. Abd-Rahman and Mansoure (2015) concluded that adding EM1 to banana bushes gave a significant increase in vegetative growth traits and also increased the NPK content of leaf. Ahmed et al. (2013) observed that treating Valencia orange cultivar with bio-fertilizer, resulted in significant increase in leaf area, content of chlorophyll and nutrients NPK. The current research aims to observe the effect of ground adding of mycorrhiza and seaweed extract (ALGA) and foliar spraying with EM1 seedlings of *Eriobotrya aponica* on growth and nutrient content in leaf.

MATERIAL AND METHODS

The study was conducted in Alsiyahii region in the Babylon province during 2016 to study the effect of mycorrhiza, ALGA and foliar spray with biofertilizer EM1 on vegetative traits and nutrients content leaf The experiment included three factors, Mycorrhiza seaweed extract (ALGA AL-Zuhoor) at 5 ml $L^{-1}$and foliar spraying with EM1 at 5 and 10 ml L^{-1}. Random soil sample was taken to a depth of 30 cm to study the physical and chemical properties of soil before conducting the research (Table 1). Mycorrhiza was added after digging a hole on the sides of the roots with a depth of 3 to 5 cm, and then covered with soil. ALGA was added to the soil at 5 ml L^{-1} (Table 2). The biofertilizer spraying, was done on two dates: the first spray was sprayed on May 4, 2016 and the second sprayed on May 21, 2016. The spraying was done on the total vegetative of seedlings to complete wetness of the seedlings. Spraying was conducted early in the morning, and untreated seedlings (control) were sprayed with distilled water only. The experiment was conducted according to the randomized complete block design with 12 treatments (2 x 2 x 3) each replicated three times. The results were analyzed according to the variance analysis and averages were compared using the Genstat 2010 test at 5% probability level.

Vegetative growth: The leaf area (cm^2) was calculated by taking 10 fully-grown leaves from different parts and weighing, and leaf area calculated. The number of leaves was also calculated form10 plant. The chlorophyll content of leaves were determined for ultra-wide leaves by Chlorophyl meter Spade-502 (Minolta Co. LTD Japanese Ltd). The percentage of dry matter was estimated after calculating the fresh weight of the leaves and then dried in the oven at a temperature of 70C°. The weight was recorded when achieved constant weight. The nitrogen was estimated by collecting the leaves from the main fruiting branches that reached full width. It was washed with water and then distilled water and put in perforated paper bags and placed in an electric oven (Oven) at a temperature of 70°C. After drying the leaves forms sample and necks were milled using an electric mill and 0.5 g of each was taken and digested using sulfuric acid and perchloric and get colorless extracts ready.

RESULTS AND DISCUSSION

Number of leaves (leaf plant^{-1}): The treatment with Mycorrhiza significantly gave the highest average in the number of leaves (41.3 leaf plant^{-1}) (Table 3). The bi-interaction between the treatment of Mycorrhizas and foliar

Table 1. Physical and chemical properties of soil

Traits	K	P	N	Soil texture	Sand	Silt	Clay	EC	pH
Percentage	0.91	0.41	1.05	Clay loam	112	321	477	3.72	7.45
Units	%	%	%	....	gk^{-1}	gk^{-1}	gk^{-1}	dsm^{-1}	....

Table 2. Components of seaweed extract (ALGA AL-Zuhoor)

Contains auxins, cytokinins, gibberellins, amino acids and carbohydrates	Cu	Zn	Mn	Fe	Mg	K_2O	P_2O_5	N
	12.6	17.5	31	30	32	4	4	4
	ppm	%	ppm	ppm	ppm	%	%	%

Table 3. Effect of mycorrhiza, ALGA AL-Zuhoor, and foliar spraying with bio-fertilizer (EM1) on loquat seedling on average leaf number

Mycorrhiza	ALGA	EM1 (ml.L^{-1})			Average (A*B)
		0	5	10	
Without adding	Without adding	28.1	30.4	31.4	30.0
	Adding	33.7	39.3	40.2	37.7
Adding Mycorrhiza	Without adding	35.1	42.4	41.6	39.7
	Adding	38.1	43.7	47.0	42.9
Average (C)		33.8	39.0	40.0	
LSD (p=0.05)		C	A*B	A*B*C	
		5.33	6.16	10.67	
A*C					Average (A)
		Without adding	30.9	34.9	33.9
		Adding	36.6	43.1	41.3
LSD (p=0.05)			AC=7.54		A=4.35
B*C					Average (B)
		Without adding	31.6	36.4	34.8
		Adding	35.9	41.5	40.3
LSD (p=0.05)			B C=7.54		B=4.35

Mycorrhiza, ALGA and EMI indicate factor A, B and C respectively

Mycorrhiza, ALGA and EMI indicate factor A, B and C respectively

spraying with EM1 at a concentration of 10 ml L^{-1} indicated significant highest average (44.3 leaf $plant^{-1}$) while the control treatment gave the lowest average (30.9 leaf $plant^{-1}$). The interaction between Mycorrhiza and ALGA and foliar spray with EM1 gave significant higher number of leaves reached (47.0 leaf $plant^{-1}$) compared to control (28.1 leaf $plant^{-1}$).

Leaf area (cm^2): The ground application of Mycorrhiza gave a significant higher leaf area (53.9 cm^2) than in control (Table 4). The interaction between Mycorrhiza and foliar spray with EM1 at a concentration of 10 ml L^{-1} recorded a significant higher leaf area (66.3 cm^2) compared to the control (50.7 cm^2). The interaction treatment between Mycorrhiza, ALGA

Table 4. Effect of Mycorrhiza, ALGA AL-Zuhoor, and foliar spraying with bio-fertilizer (EM1) on loquat seedling on leaf area (cm^2)

Mycorrhiza	ALGA	EM1 ml.L^{-1}			Average (A*B)
Without adding	Without adding	64.2	68.0	71.3	30.0
	Adding	65.8	75.2	73.1	37.7
Adding Mycorrhiza	Without adding	66.8	76.3	79.5	39.7
	Adding	63.5	69.9	71.6	42.9
Average (C)		63.5	69.9	71.6	
LSD (p=0.05)		C	A*B	A*B*C	
		5.29	6.11	10.58	
A*C					Average (A)
		Without adding	60.7	64.1	66.8
		Adding	66.3	75.8	76.3
LSD (p=0.05)			AC=7.48		A= 4.32
B*C					Average (B)
		Without adding	61.5	67.6	67.7
		Adding	65.5	72.2	75.4
LSD (p=0.05)			B C=7.48		B = 4.32

Mycorrhiza, ALGA and EMI indicate factor A, B and C respectively

Table 5. Effect of Mycorrhiza, ALGA AL-Zuhoor, and foliar spraying with bio-fertilizer (EM1) in Loquat seedling on The leaf content of chlorophyll (SPAD)

Mycorrhiza	ALGA	EM1 ml.L^{-1}			Average (A*B)
Without adding	Without adding	23.30	25.22	25.93	24.82
	Adding	28.31	33.43	34.00	31.91
Adding Mycorrhiza	Without adding	29.77	36.33	35.92	34.01
	Adding	32.13	37.00	39.00	36.04
Average (C)		28.38	32.99	33.71	
LSD (p=0.05)		C	A*B	A*B*C	
		4.07	4.70	8.14	
A*C					Average (A)
		Without adding	25.80	29.32	29.96
		Adding	30.95	36.66	37.46
LSD (p=0.05)			AC=5.76		A=3.32
B*C					Average (B)
		Without adding	26.54	30.78	30.93
		Adding	30.22	35.21	36.50
LSD (p=0.05)			B C=5.76		B=3.32

Mycorrhiza, ALGA and EMI indicate factor A, B and C respectively and EM1 foliar spray resulted in significant higher average leaf area (69.5 cm^2) compared to the control (47.2 cm^2).

Chlorophyll content (SPAD) in leaf: Mycorrhiza application significantly increased chlorophyll of leaf (35.02 SPAD) compared to the control treatment (28.36 SPAD). The application of Mycorrhiza +EMT at 10 ml L^{-1} also recorded higher chlorophyll content (37.46 SPAD) compared to the control treatment that gave (25.80 SPAD) There was significant increase in chlorophyl content with Mycorrhiza in combination with ALGA and foliar spray with EM1 (39.00 SPAD) compared to control (23.30 SPAD).

Dry matter in leaves (%): The results in (Table 6) indicate that the treatment with Mycorrhiza significantly affected raising the average percentage of dry matter in the leaves amounted to (38.16%) compared to the control treatment that gave the lowest average amounted to (29.57%).It is also noted from the same table that there is a significant excelled of the same traits with bi-interaction between the treatment of Mycorrhiza and foliar spray with EM1 at a concentration of 10 ml L^{-1} reached (38.16%) compared to the control treatment amounted to (29.57%), It is also noticed that the triple interaction treatment between Mycorrhiza , ALGA and foliar spray with EM1 at a concentration of 10 ml L^{-1} has a significant excelled effect in the percentage of dry matter in the leaves amounted to (40.21%), while control treatment gave (27.14%)

Nitrogen content of leaf (%): There was significant increase with Mycorrhiza in nitrogen content of leaf (1.34%) compared to the control (1.08%) (Table 7). The bi-interaction between Mycorrhiza and foliar spray by EM1 at 10 ml L^{-1} significantly increased the nitrogen contents (1.41%) as compared to the control (0.95%). The interaction between Mycorrhiza, ALGA and EM1 foliar spray at a concentration of 10 ml L^{-1} also indicated significant increase in nitrogen content of leaf (1.47%) as compared to control (0.76%).

The increase in the vegetative traits and nutrients content of leaves of the as a result of the ground adding of Mycorrhizas can be due to the role that these fungi play in the formation of fungal strands (hypha) and the surface increase due to the complexity of these hyphae which increases the absorption traits of the roots and thus prepares the plant in quantities large nutrients and improve the nutritional status of the plant thus increasing the vegetative growth of the (Al-Tamimi 2000) The increase in the studied traits as a result of adding the seaweed extracts (alga), because the extract contains such regulators as auxin and cytokinins, which play an effective and essential role in cell division and amplitude. It also increases the efficiency of the roots in absorbing nutrients from the soil which in turn contributes to improving the nutritional status of the seedlings and increasing the leaves content of the nutrients (Davies 2004). (Moncuso et al (2006) observed that increase resulting from spraying with fertilizer EM1, may be due to an increase in the efficiency of photosynthesis and increase in the nutrients, reflected positively in the increase in the studied vegetative traits and nutrients content of leaf(Sashi and Rubini 2011). The

Table 6. Effect of Mycorrhiza, ALGA AL-Zuhoor, and foliar spraying with bio-fertilizer (EM1) in Loquat seedling on the percentage of dry matter in leaves (%)

Mycorrhiza	ALGA	EM1 ml.L^{-1}			Average (A*B)
		57.2	60.1	62.3	
Without adding	Without adding	27.14	30.09	36.42	31.22
	adding	32.00	34.74	35.31	34.02
Adding Mycorrhiza	Without adding	33.24	37.63	36.11	35.66
	adding	34.01	38.30	40.21	37.51
Average (C)		31.60	35.19	37.01	
LSD 0.05		C	A*B	A*B*C	
		4.59	5.31	9.19	
A*C					Average (A)
		Without adding	29.57	32.41	35.86
		adding	33.63	37.97	38.16
LSD 0.05			AC= 6.50		A=3.75
B*C					Average (B)
		Without adding	30.19	33.86	36.27
		adding	33.01	36.52	37.76
LSD 0.05			B C= 6.50		B = 3.75

Table 7. Effect of Mycorrhiza, ALGA AL-Zuhoor, and foliar spraying with bio-fertilizer (EM1) in loquat seedling on nitrogen content of leaf (%)

Mycorrhiza	ALGA	EM1(ml.L^{-1})			Average (A*B)
		0	5	10	
Without adding	Without adding	0.76	0.94	1.07	0.92
	Adding	1.14	1.28	1.31	1.24
Adding Mycorrhiza	Without adding	1.21	1.40	1.35	1.32
	Adding	1.24	1.42	1.47	1.37
Average (C)		1.08	1.26	1.30	
LSD (p=0.05)		C	A*B	A*B*C	
		0.12	0.13	0.24	
A*C					Average (A)
		Without adding	0.95	1.11	1.19
		Adding	1.22	1.41	1.41
LSD (p=0.05)			AC=0.17		A=0.09
B*C					Average (B)
		Without adding	0.98	1.17	1.21
		Adding	1.19	1.35	1.39
LSD (p=0.05)			B C= 0.17		B =0.09

interaction treatment, the results showed a positive effect in may be due to the important roles played by the Mycorrhiza, ALGO and EM1 in combination.

CONCLUSION

The interaction between Mycorrhiza and ALGA and EM1 foliar spray combined gave the best results in improving the vegetative traits, and nitrogen and chlorophyll contents in comparison with other treatments and the control treatment, Therefore, recommend treatment with these treatments because these are natural materials and not harmful to the environment compared to chemical compounds, and they also contribute widely to improving the nutritional status of the plant.

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Genetic Variability and Character Association Studies in Anola (*Phyllanthus emblica* L.), Germplasm from South Western Punjab

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***Abstract*:** Aonla (*Phyllanthus emblica* L.), holds significant medicinal and nutritional benefits. The present investigation was carried out at Punjab Agricultural University, Regional Research Station, during 2019-20 to assess the genetic variability of 30 accessions of *Phyllanthus emblica* L. The results showed a wide range of diversity among the germplasm. Mean sum of squares owing to genotypes (GCV) was highly significant for all of the characters under study. The phenotypic coefficient of variation (PCV) for all traits was greater than the genotypic coefficient of variation, indicating the presence of environmental factors that can have impact on the the phenotypic expression of some traits. The high estimates of PCV and GCV were for fruit weight and plant girth and the lowest estimates of PCV and GCV were observed for pH and genetic advance as percent of mean was observed for fruit yield, stone weight, and plant girth. The significant positive correlation between fruit yield was with fruit breadth, fruit diameter, and fruit weight Smilarly, the fruit weight exhibited a significant correlation with fruit breadth and diameter. The path correlation analysis indicated that girth has the greatest positive direct effect on yield per plant, followed by fruit length, stone weight, and pH. The PCA showed a considerable amount of the total variability, accounting for 80.15% of the cumulative variance contribution rate across the first 5 PCs identified. The PC was highly contributed by positive loading with fruit characters viz. fruit weight, fruit breadth, fruit diameter, fruit yield, plant girth, fruit length, plant height, TSS, pH indicating that these characters should be considered as selection criteria for increasing fruit yields in breeding programs.

***Keywords*:** Anola, *Phyllanthus emblica* L., South Western Punjab, Genetic variability

Aonla (*Phyllanthus emblica* L), is a hardy and extremely nutrient-dense fruit crop of immense medicinal and economic value. It is the richest source of vitamin C, antioxidants, and phyto chemicals .Tropical Southeast Asia is its native habitat, especially central and southern India, which is thought to be the origin of aonla. In terms of area (99000 ha) and production (1216000 MT), India is leading country (Anonymous 2021-22). The fruit has multiple medicinal uses and can be turned into a variety of goods with added value. *Phyllanthus* species offer a variety of therapeutic qualities, including antiviral, antibacterial, antipyretic, anti-inflammatory, anti-hepatotoxic, antioxidant, and analgesic activity. It includes numerous bioactive substances like as flavonoids, phenolics, tannins, alkaloids, kaempferol, gallic acid, and quercetin. The plant is modest to medium in height, with a spreading crown. It is the perfect crop for arid and semi-arid areas like South-Western Punjab, where soil salinity and water scarcity frequently restrict the development of traditional fruit crops. This is due to its versatility under a variety of agro-climatic conditions. Since there are no standard commercial methods of vegetative propagation, farmers have been propagating the plant from seeds, and as a result, there is a large variability among the existing populations in morphology, plant shape, fruit color, fruit size, and chemical constituents of the fruits. However, no systematic research has been undertaken to yet on the genetic diversity of wild populations in order to identify elite varieties with horticultural essential fruit attributes. Exploration of available germplasm and identification of acceptable genotypes are critical components of every breeding program. High heterozygosity and frequent cross-pollination resulted in the current heterogeneity in seedling populations, from which promising genotypes were chosen (Dinesh and Vasugi 2010). Genetic variability studies using leaf and fruit metrical features may reveal genetically diverse wild genotypes with crop improvement attributes (Nogueira et al., 2012).The evaluation of genetic characteristics such as genotypic coefficient of variation, phenotypic coefficient of variation, heritability, and genetic advancement is necessary for effective selection and improvement in the base population. Path analysis using the phenotypic correlation coefficient reveals additional information about the contribution of several variables to yield. The current study was aimed to elucidate the phenotypic diversity among the natural populations and to select the elite types that may have very important horticultural characteristics.

MATERIAL AND METHODS

The experiment was carried out in 2019-20 on ten-year-old trees of 30 desi Aonla seedlings grown and maintained at the experimental farm PAU, Regional Research Station, (30º11'23.0"N 74º57'18.6"E) Bathinda, Punjab, using a randomized block design with three replications (one plant per replication). The plantation was done according to the square system and the plants were kept under uniform cultural operations in accordance with the PAU's package of practices for Aonla production (Anonymous 2020). The climate is arid with lowest and maximum temperatures ranging from 16 to 32ºC and an average annual rainfall of 480 mm. The soil was sandy loam with an 8.31 pH, 0.32% organic carbon, 0.24 dS/m electrical conductivity, 216 kg/ha available N, 22.7 kg/ha available P, and 368.0 kg/ha available potassium. The data was recorded on various vegeative, fruits, quality and yield parameter (Table 1, 2).

Total soluble solids in fruits were conducted at ambient temperature using a handheld refractometer with a 0-32 range (ERMA). The determination of titratable acidity was performed according to the method suggested in A.O.A.C (2010).The measurement of juice pH was taken by pH metre by placing the rod into the juice beaker and the value was taken as displayed by the pH metre.

Statistical analyses: The data from 30 desi Aonla seedlings involving 14 traits were analyzed by R software version 4.3.1. The correlation analyses used parametric Pearson correlations to analyze the quantitative and qualitative traits. Heritability in broad sense and estimates of appropriate genetic variance components were substituted for the parameters to predict expected genetic gain. Genotypic coefficient of correlation (rg) and phenotypic coefficient of correlation (rp) were computed as per Robinson et al. (1949). Path coefficient was measure of direct and indirect effects of each character on fruit yield was estimated using a partial regression coefficient (Dewey and Lu 1959). The PCA biplot was generated using the R packages ggplot 2, factoextra, grid extra, corrplot, and facto mine R, along with path coefficient analysis (Dewey and Lu 1959).

RESULTS AND DISCUSSION

Analysis of variance for characters studied during the experiment was significant among treatments (Table 1 and 2). The mean values of the characters, ranges, genotypic mean sums of squares, standard error (SE) of means, and coefficients of variation also showed sufficient amounts of variation for morphological and biochemical components of aonla genotypes. The genotypic and phenotypic coefficients of variation are reliable parameters for measuring the degree of variability demonstrated by different traits. Higher PCV than GCV was in all the parameters indicating the role of environments on the expression of characters but differences were very minor. The slight variation between the genotypic and phenotypic coefficients of variation indicates that expressions of traits are predominately governed by genetic factors. The selections based on these traits are likely to be effective with equal chances of success. The high PCV and GCV were found for the fruit weight plant girth, stone weight, stone breadth, yield and height offers a good scope of improvement with the selection of these traits. The findings corroborate with findings of Singh et al. (2012) in aonla.

High heritability (>97%) for all the traits studied in this research suggested that genetic factors rather than the environment mainly controlled the expression of traits. The yield, girth and stone weight were the most heritable traits (97%), whereas, the plant height (90.87%) were the least

Table 1. Analysis of variance of different characteristics in Aonla

Source of variation	d.f.	Mean sum of squares						
		Height (m)	Girth (cm)	Fruit Dia	Fruit breadth	Fruit length	Stone Dia	Stone breadth
Replication	2	77.38	1.39	1358.51	306.46	253.79	279.51	26.27
Treatment	29	192.48*	11.36*	1538.98*	362.76*	319.21*	235.21*	140.14*
Error	58	12.47	0.22	42.34	10.22	7.22	8.05	3.06

* Significant at 1.0 percent level

Table 2. Analysis of variance of different characteristics in Aonla

Source of variation	d.f.	Mean sum of squares						
		Stone length	Stone (Wt)	Fruit (Wt)	Acidity	pH	TSS	Yield
Replication	2	30.82	13.22	5916.29	6.51	7.85	376.75	9631.74
Treatment	29	146.56*	75.56*	54775.94*	8.61*	6.48*	615.38*	26696.59*
Error	58	3.18	1.48	1122.89	0.23	0.16	16.59	514.33

* Significant at 1.0 percent level

heritable (Table 3). Traits such as fruit weight yield and fruit diameter exhibited high genetic advance, indicating that their heritability is mainly due to additive gene effects and direct selection based on these traits in genetically diverse genotypes could be effective for desired improvement. The phenotypic correlation coefficients were lower than the genotypic correlation coefficients for traits studied, indicating a strong genetic link. Plant height, plant girth, fruit breadth, fruit diameter, fruit length and fruit weight were showed positive relationship with yield/plant. Hence, selecting genotypes with higher values of these traits may improve crop yield.

Singh et al (2012) reported similar results for these traits in aonla. Genotypic and phenotypic coefficient of variance was used to assess the nature and level of relationships between traits for strategic improvement programs through selection or hybridization. The observations are in accordance with the findings of Patel et al. (2015).These characters also exhibited high GCV, therefore, selection based on phenotypic performance for these traits would be effective in improving these characters directly in the population.

Correlation analysis: Significant positive associations were found between fruit diameter, and yield, fruit width and yield, pH and yield, and girth and yield (Fig. 1). Stone length and breadth were shown to be negatively correlated with fruit weight. There was a positive association between fruit weight and both fruit diameter and width. Hazarika et al. (2025) also observed strong positive relationship between fruit weight and TSS, fruit length, and fruit diameter. Ganopoulos et al. (2018), Khadvi et al. (2019), and Srivastava et al. (2019) have published similar results, showing a strong positive association between fruit weight and fruit length and diameter.

Path coefficient analysis: Path correlation analysis revealed girth exhibits the highest positive direct effect on yield per plant, followed by fruit length, stone weight and pH (Table 4). The negative direct effects were observed on yield per plant for, stone length, fruit breadth and stone diameter .This indicates that there is a real correlation between these traits and yield, and selection for this characteristic directly will increase strawberry yield. Maximum indirect positive effect was found for fruit yield with fruit weight) via stone length followed by fruit breadth via stone length, fruit diameter via fruit length. The highest negative indirect effects were for stone breadth via stone length followed by fruit weight via fruit breadth, acidity via stone length

Principal component Analysis of various parameters: To ascertain the relationship between genotypes and the associations between various attributes within the subsets,

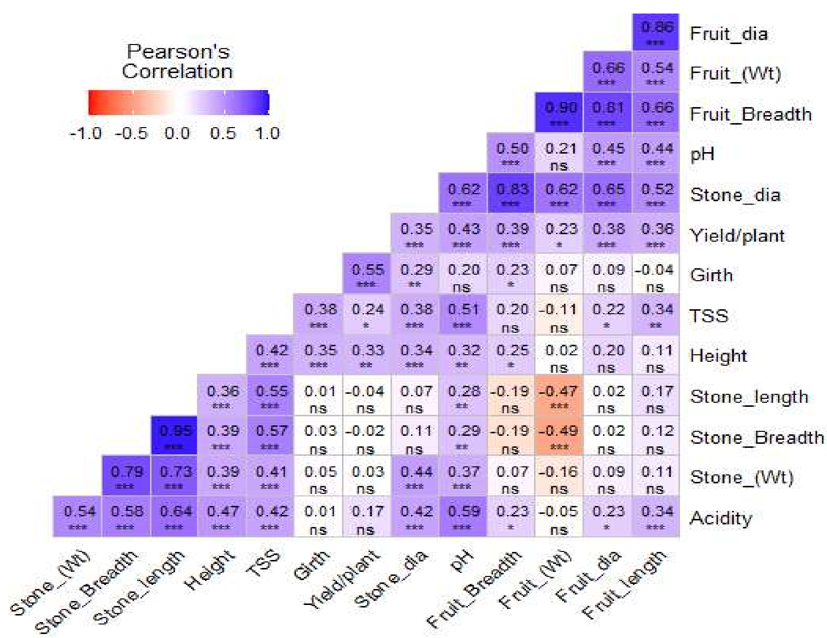

Fig. 1. Linear correlation among the various attributes such as vegetative, fruit, quality and yield attributes calculated using Pearson correlation. The $p \geq 0.05$: non-significant, $^{*}p<0.05$ (significant at 5 % level of significance); $^{**}p<0.01$ (significant at 1 % level of significance); $^{***}p<0.001$ (significant at 0.1 % level of significance)

Table 3. Genetic variability among different characters in Aonla

Name of character	GM	Minimum	Maximum	CV	PCV	GCV	H² (%)	GA	GAM
Height (m)	6.88	4.10	9.50	6.74	22.32	21.28	90.87	2.87	41.78
Girth (m)	0.93	0.30	2.00	6.68	39.41	38.84	97.12	0.73	78.84
Fruit diameter (mm)	28.60	22.00	35.00	2.98	14.90	14.61	96.10	8.44	29.50
Fruit breadth (mm)	13.63	10.40	17.30	3.08	15.21	14.87	95.64	4.08	29.96
Fruit length (mm)	12.46	9.30	15.20	2.83	15.53	15.28	96.75	3.86	30.95
Stone diameter (mm)	13.10	0.25	1.37	2.84	12.78	12.47	95.14	3.28	25.05
Stone breadth (mm)	4.02	2.30	6.40	5.70	31.89	31.41	97.07	2.57	63.76
Stone length (mm)	4.31	2.10	6.60	5.43	30.41	29.94	96.92	2.62	60.72
Stone (Wt)	2.84	1.00	5.15	5.63	33.17	32.69	97.12	1.88	66.36
Fruit (Wt)	59.80	7.17	14.20	7.35	42.40	41.75	96.98	50.65	84.70
Acidity	1.98	1.00	2.43	3.19	16.11	15.79	96.04	0.63	31.88
pH	2.19	1.80	2.80	2.38	12.50	12.30	96.83	0.55	24.93
TSS (°Brix)	15.11	10.40	21.40	3.53	17.84	17.48	96.06	5.33	35.30
Yield (kg/plant)	76.90	35.00	100.00	3.87	23.00	22.67	97.17	35.40	46.03

Table 4. Path correlation among differeent traits with yield of the plants

Attributes	Height	Girth	Fruit_D	Fruit_Br	Fruit_L	Stone_D	Stone_Br	Stone_L	Ston_Wt	Fruit_Wt	Acidity	pH	TSS
Height	0.124	0.136	0.028	0.072	-0.169	0.020	0.014	-0.126	0.085	-0.037	-0.007	-0.021	-0.032
Girth	0.027	0.621	0.022	-0.010	-0.165	-0.023	-0.006	0.100	-0.031	-0.008	0.010	-0.014	-0.052
Fruit_D	-0.030	-0.118	-0.116	-0.299	0.343	-0.086	-0.022	0.269	-0.086	0.137	0.014	-0.020	0.073
Fruit_Br	-0.020	0.013	-0.075	-0.458	0.177	-0.191	-0.039	0.471	-0.097	0.206	0.014	-0.001	0.076
Fruit_L	-0.046	-0.224	-0.086	-0.177	0.458	-0.019	-0.012	0.102	-0.072	0.098	0.006	-0.018	0.025
Stone_D	-0.009	0.050	-0.035	-0.304	0.030	-0.287	-0.017	0.251	0.089	0.128	0.004	0.036	0.027
Stone_Br	0.029	-0.068	0.043	0.307	-0.095	0.083	0.058	-0.645	0.266	-0.147	-0.017	-0.001	-0.097
Stone_L	0.023	-0.091	0.045	0.315	-0.068	0.105	0.055	-0.686	0.240	-0.144	-0.020	-0.007	-0.089
Stone_Wt	0.030	-0.054	0.028	0.126	-0.093	-0.072	0.044	-0.466	0.354	-0.067	-0.015	0.027	-0.050
Fruit_Wt	-0.022	-0.023	-0.075	-0.448	0.214	-0.176	-0.040	0.471	-0.113	0.210	0.013	-0.007	0.085
Acidity	0.025	-0.173	0.043	0.172	-0.070	0.029	0.027	-0.372	0.145	-0.074	-0.036	0.042	-0.007
pH	-0.014	-0.045	0.012	0.001	-0.043	-0.052	0.000	0.024	0.050	-0.008	-0.008	0.195	-0.025
TSS	0.018	0.151	0.039	0.162	-0.053	0.036	0.026	-0.285	0.083	-0.083	-0.001	0.023	-0.215

Fruit_D: Fruit diameter; Fruit_Br: Fruit diameter; Fruit_L: Fruit diameter: Stone_D: Stone diameter: Stone_Br: Stone Breadth: Stone_L: Stone length; Stone_Wt: Stone weight; Fruit_Wt: Fruit WeighT

Table 5. Eigen values, variance and cumulative variance (%) with principle components

Variables	PC1	PC2	PC3	PC4	PC5
Height	-0.15	0.21	-0.28	0.21	-0.46
Girth	0.01	0.53	-0.23	0.29	0.13
Fruit diameter	0.32	-0.28	0.08	0.31	0.10
Fruit breadth	0.39	-0.10	-0.27	0.09	-0.07
Fruit length	0.22	-0.38	0.26	0.29	0.29
Stone diameter	0.21	-0.15	-0.61	0.03	0.02
Stone breadth	-0.39	-0.20	-0.06	0.26	0.07
Stone length	-0.38	-0.23	0.01	0.21	0.05
Stone weight	-0.26	-0.26	-0.44	0.20	0.02
Fruit weight	0.40	-0.13	-0.21	0.07	-0.09
Acidity	-0.25	-0.25	-0.14	-0.34	-0.16
pH	-0.03	-0.05	-0.30	-0.54	0.57
TSS	-0.22	0.10	-0.09	0.34	0.47
Yield plant	0.11	0.41	-0.02	0.07	0.28
Eigen values	5.15	2.12	1.65	1.21	1.09
Variance (%)	36.79	15.14	11.78	8.65	7.80
Cumulative variance (%)	36.79	51.93	63.71	72.35	80.15

principal component analysis or PCA was done utilized (Ruiz and Egea 2008). PCA results indicated that since the eigen values (λi) were greater than 1, the first five PCs may be used to examine the relationship between the qualities assessed in our study. The combined variance proportion of the first two PCs was 51.93%. (Table 5).The first PC accounted for 36.79% of the total variance, while the second PC explained 15.14% (Figs. 2, and 3). After analyzing the qualities'

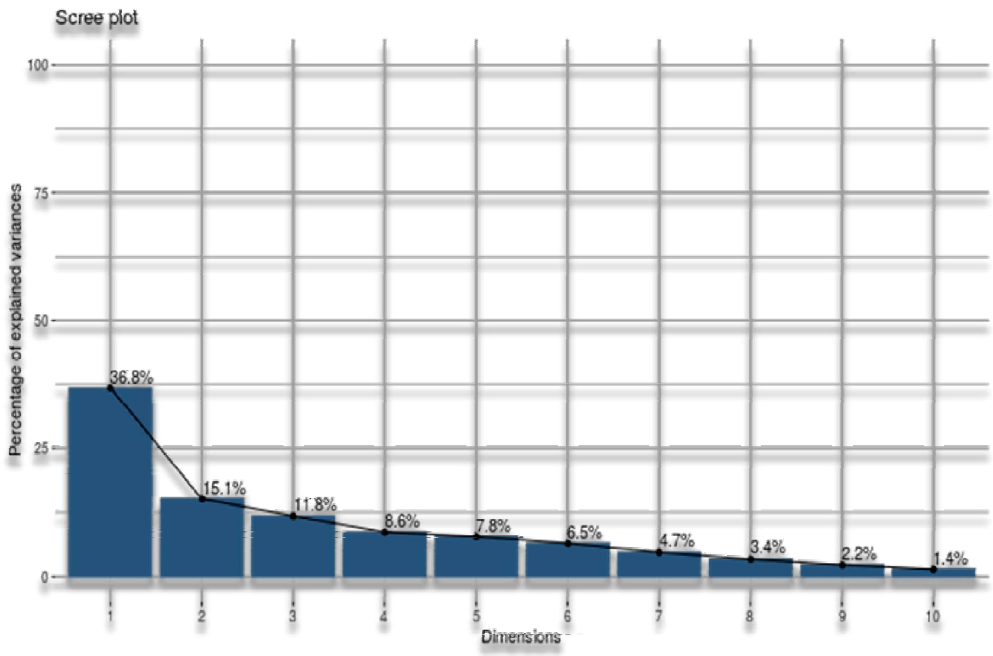

Fig. 2. Scree plot depicting the variability explained by each Principal component of the various vegetative, fruit, quality and yield attributes of amla

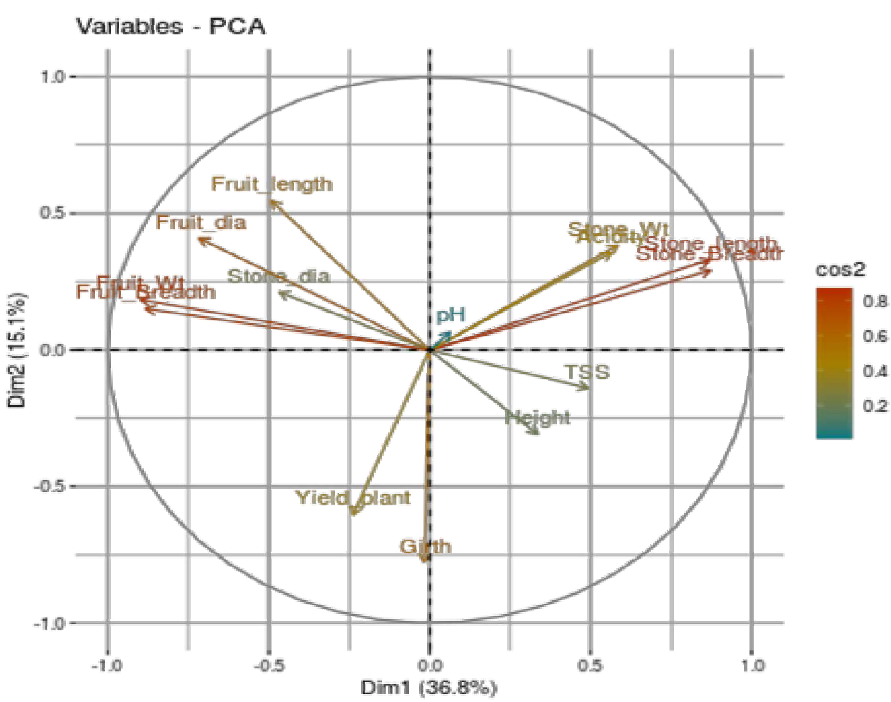

Fig. 3. Principal component analysis with the contribution percentage of various vegetative, fruit, quality and yield attributes to variability

contributions, that PC1 and PC2 benefited more from the levels of fruit weight and yield, respectively. Hazarika et al*.* (2025), also observed variation among aonla accessions, as demonstrated by scatter plots and scree plots along PCA biplots, which explained 86.66% of the total variation. Fruit length, diameter, weight, yield, TSS, acidity, and other attributes that are represented in the PCs are integrated to emphasize the significance of these crucial quantitative factors.

CONCLUSION

The present investigation on Aonla accessions exhibited significant variability in the studied in the vegetative , fruiting and biochemical characters offering a valuable resource for including them in breeding programmes and mainstreaming them. The existence of wide ranges of variability for most of the characters among the aonla germplasm provides opportunities for genetic gain through selection or hybridization. The fruit weight and girth showed the high estimates of PCV and GCV, and genetic advance as percent of mean was observed highest for fruit yield stone weight and plant girth. Fruit yield showed strong positive and significant correlations with fruit breadth, fruit diameter, and fruit weight. Thus, selection may be possible for these characters for improving yield.

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Evaluation of Quality Protein Maize Genotypes for Yield and Profitability under Varying Fertility Levels

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***Abstract*:** A field experiment was conducted during *Kharif,* 2024 to evaluate the performance of Quality Protein Maize (QPM) hybrids under varying fertility regimes. The trial was laid out in a split-plot design with three fertility levels in the main plots 100% Recommended Dose of Fertilizers (RDF) (200:60:50 N:P_2O_5:K_2O kg ha^{-1}), 125% RDF (250:75:62.5 N:P_2O_5:K_2O kg ha^{-1}), and 150% RDF (300:90:75 N:P_2O_5:K_2O kg ha^{-1})] and nine QPM hybrids (IQPMH 2205, IQPMH 2204, APQWH8, APH6, AQWH5, IQMH 203, HM 8, HQPM 5 and APH1) in the sub-plots, replicated thrice. Application of 150% RDF significantly enhanced plant height, hastened flowering, improved cob traits and increased grain (6620 kg ha^{-1}) and stover yields (8293 kg ha^{-1}) as well as economic returns (₹73,727 ha^{-1} net returns with B:C ratio of 2.05). Among the hybrids, IQPMH 2204 was found superior recording the highest grain yield (7398 kg ha^{-1}), stover yield (9189 kg ha^{-1}) and profitability (₹93,507 ha^{-1} net returns with B:C ratio of 2.31). It is therefore concluded that cultivation of QPM hybrid *viz*. IQPMH 2204 under 150% RDF can be recommended for achieving higher productivity and profitability during *Kharif* season in Andhra Pradesh.

Keywords: Quality protein maize, Fertility levels, Grain yield, Economic returns, Andhra Pradesh

Maize (*Zea mays* L.) is one of the world's most important cereals, cultivated for food, feed and industrial uses. Globally, it ranks third after rice and wheat in terms of area and production and in India it occupies over 10 million hectares with an annual output of nearly 30 million tonnes. Beyond direct consumption, maize supports a wide range of industries including starch, ethanol, poultry feed and dairy nutrition, thereby contributing substantially to food, nutrition and livelihood security.

Despite its economic and dietary importance, conventional maize protein is deficient in lysine and tryptophan, two essential amino acids vital for human growth and efficient feed utilization in monogastric animals. Diets dominated by normal maize therefore lacks balanced protein quality and may contribute to malnutrition, particularly in regions where alternative protein sources such as pulses, milk and meat are scarce or unaffordable (Laskowski et al., 2019).

To overcome this limitation, researchers developed Quality Protein Maize (QPM) through conventional breeding. The improvement was achieved by incorporating the *opaque-2* (o2) gene, which elevates lysine and tryptophan content along with modifier genes that restore kernel hardness and agronomic desirability. QPM contains nearly twice the lysine and tryptophan levels of normal maize, offering a cost-effective solution to protein-energy malnutrition. Since it is a product of traditional breeding rather than genetic engineering, QPM represents a sustainable and widely acceptable form of biofortification (Milan-Carrillo et al., 2004, Denic et al., 2012). Adoption of QPM has been shown to improve child growth, enhance livestock productivity and strengthen food and nutritional security in several developing countries.

However, the success of QPM depends not only on its nutritional superiority but also on its agronomic performance and economic viability. Maize productivity is strongly influenced by fertilizer management, as adequate nutrient supply promotes vegetative growth, cob development, kernel filling and grain yield. Fertilizer dosage is therefore a critical determinant of yield and profitability. Insufficient application restricts productivity, whereas excessive use reduces economic efficiency and may lead to environmental problems.

Research has shown that fertilizer dosage influences important yield-contributing traits such as cob length, kernel row number and grain weight, which ultimately determine grain and stover yield (Asghar et al., 2010, Gul et al., 2015). At the same time, maize hybrids differ in their responsiveness to fertilizer levels due to genetic variation in nutrient-use efficiency and adaptability. Studying genotype × fertilizer interactions is thus essential for developing location-specific recommendations that maximize returns while ensuring sustainability.

Although QPM has been widely tested in different agro-ecological regions, information on its performance under variable fertilizer dosages in India remains limited. This knowledge gap is particularly evident in the sandy-loam soils of Andhra Pradesh, where farmers often apply fertilizers without precise guidance on hybrid-specific requirements. Identifying suitable QPM hybrids and optimal fertilizer regimes in such environments is important for enhancing both productivity and profitability while ensuring better nutritional outcomes.

MATERIAL AND METHODS

Experimental site and soil characteristics: The field experiment was conducted during the *kharif*, 2024 at the Agricultural Research Station (ARS), Peddapuram, Andhra Pradesh, India (17.08° N latitude, 82.13° E longitude, 35 m above mean sea level). The experimental field had sandy loam soils, slightly acidic in reaction (pH 6.92), with an electrical conductivity of 0.12 dS m^{-1} and organic carbon content of 0.47%. The available nutrient status was low in nitrogen (218 kg ha^{-1}) and potassium (106 kg K_2O ha^{-1}), medium in phosphorus (38 kg P_2O_5 ha^{-1}).

Weather during the crop season: Weather data recorded at the ARS meteorological observatory during the crop season from June to October, 2024 showed a total rainfall of 894.6 mm received over 59 rainy days (Table 1). The mean maximum temperature ranged from 31.5°C to 35.6°C, while mean minimum temperature varied between 24.8°C and 28.2°C. Relative humidity remained high, (85**%**) during the crop period.

Experimental design and treatments: The experiment was conducted in a Split Plot Design with three replications. The main plots were assigned to different fertility levels, while the sub-plots consisted of nine pre-release QPM hybrids. The fertility treatments included three levels (100 % RDF-200:60:50 kg NPK /ha, 125 % RDF- 250:75:62.5 kg NPK /ha and 150 % RDF -300:90:75 kg NPK /ha). The sub-plots were allotted to nine different QPM hybrids (IQPMH 2205, IQPMH 2204, APQWH8, APH6, AQWH5, IQMH 203 [(C)], HM 8 [(C)], HQPM 5 [(C)], APH1).

Crop management and data collection: Fertilizers were applied as per the respective treatments, with nitrogen supplied through urea in three equal splits at the basal, knee-high, and flowering stages; the entire phosphorus dose was applied through single superphosphate (SSP) at basal; and potassium was applied through muriate of potash (MOP) in two equal splits at basal and flowering. The crop was sown on 08 July 2024, with a spacing of 60 cm × 20 cm, ensuring a uniform plant population across treatments. Recommended agronomic practices were followed for timely weeding, earthing up and intercultural operations. Irrigations were provided at critical stages when rainfall was inadequate. Plant protection measures were undertaken as per ANGRAU recommendations to minimize pest and disease incidence. Five plants were selected at random and tagged for recording growth parameters, yield and yield attributes. Grain and stover yield from net plot area was converted into per hectare basis. Economic returns were worked out based on the prevailing market prices of inputs, cost of fertilizers and outputs. Returns per rupee invested were worked out by considering net returns and cost of cultivation.

Statistical analysis: The data were statistically analyzed using OPSTAT (online statistical analysis tools).

RESULTS AND DISCUSSION

Growth and phenology: Fertility management significantly influenced the plant height and phenological traits of QPM hybrids (Table 2). Increasing the nutrient supply from 100% to 150% RDF led to rise in plant height from 175.8 cm to 191.7 cm, indicating the strong vegetative response of maize to enhanced nutrient availability. Similar increases in vegetative growth with increased fertilizer application were earlier observed by Asghar *et al*. (2010) and Manea *et al*. (2015). The QPM hybrids exhibited pronounced variability in plant height, with IQPMH 2204 attaining the maximum height (194.1 cm), while APH6 recorded the shortest stature (170.7 cm) (Table 2). These differences likely arise from inherent genotypic variation in nutrient uptake and utilization efficiency, particularly nitrogen, which is a key determinant of vegetative growth and internode elongation. Sanchez et al. (2023) also highlighted that genotypic differences in nitrogen assimilation pathways significantly influence maize plant architecture.

Table 1. Weather data during the cropping season

Month	Rainfall (mm)	No. of rainy days	Temperature (°C)		Relative humidity (%)	
			Maximum	Minimum	Maximum	Minimum
June, 2024	182.6	13	34.6	28.0	77.8	62.9
July, 2024	134.2	12	32.8	28.2	81.3	75.4
August, 2024	328.3	12	35.6	24.8	88.8	77.8
September, 2024	170	14	31.5	25.8	91.3	73.5
October, 2024	79.5	8	33.4	25.4	86.6	73.6
Total/ Average	894.6	59	33.6	26.4	85.2	72.6

Plant and cob population were unaffected by fertility, but hybrids differed significantly, likely due to inherent differences in seed vigour, emergence rate and early stand establishment capacity among genotypes. Fertility levels affected flowering behavior of QPM hybrids. Increasing fertility hastened phenological events, as tasseling advanced from 51.1 days at 100% RDF to 49.5 days at 150% RDF, while silking was reduced from 52.9 to 51.5 days. This advancement of flowering under higher nutrient supply can be attributed to improved crop vigor and earlier attainment of reproductive competence. Among hybrids, APH1 was the earliest to flower, while IQPMH 2204 and HM 8 were comparatively late, indicating inherent genotypic differences. Similar trends of nutrient-induced earliness in maize phenology were reported by Murugudu et al. (2023).

Yield attributes: Yield attributing characters were significantly influenced by fertility levels (Table 3). Application of 150% RDF resulted in higher values of cob length (15.7 cm), cob girth (15.0 cm), kernel rows per cob (15.2) and kernels per row (32.2) compared to 100% RDF. This improvement may be attributed to better nutrient availability, which enhances root activity, reproductive development and assimilate translocation to developing cobs. Narayanaswamy and Siddaraju (2011) and Gul et al. (2015) also reported similar improvements in cob traits with balanced NPK application. Significant variation was also observed among hybrids. IQPMH 2204 recorded greater cob length (16.6 cm), girth (16.0 cm), kernel rows (17.1) and kernels per row (33.0) compared to other entries. These differences may be due to the inherent genetic potential of hybrids to utilize available nutrients effectively. Ullah et al. (2025) also emphasized the role of genotype-specific responses in determining yield attributes under variable nitrogen regimes.

Grain and stover yield: Grain and stover yields were positively influenced by increasing fertility levels (Table 3) Application of 150% RDF recorded significantly higher grain yield of 6620 kg/ha than observed at 100% RDF (5442 kg/ha) and 125% RDF (6097 kg/ha)**.** Stover yield also followed a similar trend, increasing from 6870 kg/ha (100% RDF) to 8293 kg/ha (150% RDF). The increase in biomass and grain production under higher fertility levels may be attributed to improved vegetative growth, better cob development and enhanced source–sink relationship. Hargilas (2012), Owla et al. (2015) and Ghosh et al. (2009) also 0bserved that appropriate nutrient application supports yield improvements in maize. Hybrids differed noticeably in productivity. IQPMH 2204 recorded a grain yield of 7398 kg/ha and stover yield of 9189 kg/ha, outperforming the check and other hybrids. The enhanced performance of

Table 2. Growth and phenology of quality protein maize genotypes as affected by varying nutrient levels during *Kharif,* 2024

Treatments	Plant height (cm)	Plants ('000/ha)	Cobs ('000/ha)	Days to 50% tasselling	Days to 50% silking
Main plot: Fertility levels					
100 % RDF	175.8	77.9	72.2	51.1	52.9
125 % RDF	185.9	79.3	71.9	50.5	52.3
150 % RDF	191.7	80.5	73.1	49.5	51.5
CD (p = 0.05)	3.68	NS	NS	0.67	0.42
CV (%)	5.6	4.8	5.2	1.7	1.0
Sub - plot: QPM hybrids					
IQPMH 2205	183.4	79.2	72.5	49.7	51.7
IQPMH 2204	194.1	81.7	75.9	52.8	54.6
APQWH8	190.0	78.0	72.3	50.5	52.7
APH6	170.7	78.5	70.9	49.1	51.2
AQWH5	185.9	79.1	75.1	51.1	52.8
IQMH 203 (C)	188.2	80.2	74.1	49.8	51.2
HM 8 (C)	181.0	80.5	72.2	52.3	54.3
HQPM 5 (C)	185.6	77.0	65.8	51.3	53.1
APH 1	180.9	79.1	72.9	46.6	48.4
CD (p = 0.05)	6.15	1.63	3.00	0.46	0.57
CV (%)	5.5	2.1	4.3	0.9	1.1
Interaction	NS	NS	NS	NS	NS

Table 3. Yield attributes and yield of quality protein maize as affected by varying nutrient levels

Treatments	Cob length (cm)	Cob girth (cm)	No. of kernel rows/cob	No. of kernels /row	100-seed weight (g)	Grain yield (kg/ha)	Stover yield (kg/ha)
Main plot: Fertility levels							
100 % RDF	14.4	13.9	14.0	28.1	25.1	5441.6	6870.3
125 % RDF	15.1	14.4	14.5	29.8	26.2	6096.7	7358.1
150 % RDF	15.7	15.0	15.2	32.2	27.6	6620.1	8292.7
CD (P = 0.05)	0.49	0.5	0.36	1.09	0.61	417.9	739.2
CV (%)	4.3	4.6	3.3	4.8	3.1	9.1	12.7
Sub - plot: QPM hybrids							
IQPMH 2205	15.3	14.2	14.2	27.9	26.4	6083.6	7655.9
IQPMH 2204	16.6	16.0	17.1	33.0	32.5	7397.6	9189.3
APQWH8	15.2	13.7	13.1	29.8	26.4	5940.5	7275.1
APH6	13.9	13.9	13.6	27.4	21.3	5026.9	6062.0
AQWH5	15.9	15.3	16.5	31.7	28.2	6671.1	8314.2
IQMH 203(C)	15.7	14.7	15.2	31.3	27.3	6425.4	8092.2
HM 8 (C)	14.4	14.4	13.4	30.5	25.3	5256.6	6428.1
HQPM 5 (C)	15.1	14.0	14.0	30.0	26.5	5499.4	6921.2
APH 1	13.5	14.1	14.3	28.5	22.5	6174.1	7625.2
CD (P = 0.05)	0.73	0.78	0.65	1.23	1.72	491.7	708.7
CV (%)	5.1	5.7	4.7	4.3	6.9	8.5	9.9
Interaction	NS	NS	NS	NS	NS	NS	NS

Table 4. Economics of quality protein maize genotypes as affected by varying nutrient levels

Treatments	Gross returns (Rs./ha)	COC (Rs./ha)	Net returns (Rs./ha)	B: C Ratio
Main plot: Fertility levels				
100 % RDF	121086	68615	52471	1.77
125 % RDF	135663	71099	64564	1.91
150 % RDF	147311	73584	73727	2.05
CD (P = 0.05)	9554		9549.6	0.13
CV (%)	9.1		19.3	9.0
Sub - plot: QPM hybrids				
IQPMH 2205	135375	71099	64275	1.90
IQPMH 2204	164606	71099	93507	2.31
APQWH8	132189	71099	61089	1.86
APH6	111860	71099	40761	1.57
AQWH5	148443	71099	77344	2.08
IQMH 203(C)	142972	71099	71873	2.01
HM 8 (C)	116974	71099	45874	1.64
HQPM 5 (C)	122377	71099	51278	1.72
APH 1	137386	71099	66287	1.93
CD (P = 0.05)	10978.2		10978.3	0.15
CV (%)	8.6		18.1	8.5
Interaction	NS		NS	NS

IQPMH 2204 may be attributed to its prolonged vegetative growth, better cob development and efficient nutrient use. Khan et al. (2011) and Ullah et al. (2025 also emphasized the importance of genotype × nutrient interactions in determining yield potential under variable fertility conditions.

Economics: Economic returns were strongly influenced by both fertility levels and hybrid performance (Table 4). The 150% RDF treatment recorded net returns of ₹73,727/ha with a benefit–cost ratio of 2.05, reflecting improved profitability compared to lower fertility levels. Inadequate fertilizer application not only limited yield potential but also reduced economic efficiency. Kumar *et al*. (2015) observed that increased nutrient input enhances profitability, despite higher cultivation costs. Hybrids also differed in their economic performance. IQPMH 2204 achieved net returns of ₹93,507/ha with a B:C ratio of 2.31, followed by AQWH5 (₹77,344/ha; 2.08) respectively and IQMH 203 (₹71,873/ha, 2.01) respectively. These results emphasize that both genotype selection and nutrient management play a pivotal role in optimizing profitability, supporting the conclusions of Pal and Bhatnagar (2012) and Murugudu et al. (2023).

CONCLUSION

Fertility levels significantly influenced the growth, phenology, yield attributes, and productivity of QPM hybrids on sandy loam soils during *Kharif*, 2024. Application of 150% RDF (300:90:75 kg $N:P_2O_5:K_2O$/ha) promoted earlier flowering, improved cob traits and increased both grain and stover yields. Among the tested hybrids, IQPMH 2204 was the most productive and profitable, followed by AQWH5 and IQMH 203. Thus, cultivating IQPMH 2204 under 150% RDF is recommended for achieving high productivity and profitability of QPM under the agro-climatic conditions of Andhra Pradesh.

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Screening of Muskmelon Genotypes (*Cucumis melo* L.) for Drought Tolerance

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Abstract: Drought is one of the major environmental stress (abiotic) which interrupts growth and development of plant and its productivity, so screening of genotypes that exhibit drought tolerance in muskmelon crop may help to develop resilient that can withstand the period of water scarcity. Fifteen muskmelon genotypes collected from different sources sown in polybags containing potting mixture of soil: sand: farm yard manure in the ratio of 2:1:1 and were maintained under polyhouse condition. As treatment, water stress was imposed after 30 days after sowing by withholding irrigation for next 15 days and growth parameters are recorded. Among genotypes, Chamarajnagar Local, Muskmelon Round and Kerala 2 gave better response to drought and these genotypes can be further used to test under field conditions and can also be used in breeding programmes for drought tolerance.

Keywords: Drought, Genotypes, Muskmelon, Stress tolerance

Muskmelon botanically called as *Cucumis melo* L. belongs to the family Cucurbitaceae with diploid chromosome number of 24 and Asia is considered as the center of origin of melons. Melons are rich source of antioxidants, protein, lipid, carbohydrate, vitamins and minerals. Additionally, they contain beneficial omega-3 and omega-6 fatty acids. As they are source of minerals, they are crucial for proper body functioning and a healthy immune system and also contain vitamin K, which is essential for blood clotting, making melons beneficial in preventing cardiovascular diseases (Manchali and Murthy 2020). Seeds of melon are also rich source of nutrients which include carbohydrate, protein, fat and dietary fiber (Mehra et al., 2015). Melons are cultivated worldwide and which have wider adaptability and in India they are cultivated in an area of 68.7 thousand hectares with a production of 1518.5 metric tons (Anonymous 2023).

Understanding the response of plants to changing climate conditions is crucial for mitigating global warming concerns, as drought has been the most deleterious aspects of environmental stress that is responsible for the low crop yield which affects agricultural production. It rapidly induces an osmotic imbalance in crops, resulting in several physiological and biochemical dysfunction (Chaves and Oliveira 2004). Depleted water sources or lack of sufficient moisture affects healthy plant development and fruit production. During the vegetative stage, melons require consistent moisture to support leaf growth, root development and overall plant establishment. Insufficient water during this phase can lead to stunted growth and poor plant vigor. As the plants enter the reproductive stage, which includes flowering and fruit formation, water requirements increase further. Inadequate watering during this phase can result in reduced fruit size, blossom end rot or even fruit drop (Sharma et al., 2014). This study favors to select genotypes that exhibit promising drought tolerant traits. Selected genotypes as drought tolerant through this screening can be introduced in drought prone areas and also can be used in breeding Programme to develop drought tolerant varieties/hybrids.

MATERIAL AND METHODS

The experiment was carried out at Department of Horticulture, University of Agricultural Sciences, Bangalore. The experimental material consists of fifteen muskmelon genotypes collected from various sources; Puttikaayi, Dharwad 1, Gadag Local, Kerala 1, Kashi Madhu, Alpur Green, Giriyal Green, Chamarajnagar Local, Banaspathre, Sirsi Local, Bagalkot Local, Muskmelon Round, Muskmelon Local, Kekkarike and Kerala 2. Seeds of fifteen genotypes were sown in polybags containing potting mixture of soil: sand: farm yard manure (2:1:1) and were kept under polyhouse condition. Three replications for both control and treatment were maintained. The observations like shoot length, root length, number of leaves, plant height stress tolerant index, root length stress tolerant index, fresh weight, dry weight and chlorophyll content were recorded. Shoot and root lengths were measured in centimeters at 45 days after sowing (DAS) using a measuring scale. Number of leaves per plant was counted under both control and stress conditions. Fresh and dry weights of seedlings were recorded using a digital balance, with dry weight taken after oven-drying at 60 °C for 72 hours. Chlorophyll content was estimated using a SPAD meter. Stress tolerance indices for plant height and root length were calculated as per Ashraf et al. (2006). The experiment was laid out in a factorial completely randomized design (FCRD), and data were analyzed using ANOVA at 1% significance level.

$$\text{Plant height stress tolerance index} = \frac{\text{Plant height of stressed plants (cm)}}{\text{Plant height of control plants (cm)}} \times 100$$

$$\text{Root height stress tolerance index} = \frac{\text{Root height of stressed plants (cm)}}{\text{Root height of control plants (cm)}} \times 100$$

RESULTS AND DISCUSSION

The shoot length of fifteen selected muskmelon genotypes was measured under control and water stress conditions at 45 days after sowing. Among the treatments, shoot length under water stress conditions was lower than that of control (Table 2). Among the genotypes, Dharward 1 recorded maximum shoot length of 88.83 cm and minimum was recorded in Puttikaayi (34.75 cm). The lower mean reduction in shoot length was observed in Dharward 1 (15.57 %), which in on par with Chamarajnagar Local (17.75 %) and Muskmelon Round (18.85 %), while highest reduction noticed in the genotype Muskmelon Local (53.61 %). In the study, reduction in shoot length was observed under moisture stress condition, Drought stress decline water potential and turgor in plant leaves, which leads to a slowed growth rate and impacting various biological processes within the plant (Hussain et al., 2018). Moisture stress also impacts the availability of stem reserves, reduces internodal length and lowers water potential, all of which contribute to decreased plant height. This aligns with the findings, of Salvadora et al. (2017) in tomato and Ansari et al. (2019) and Rehman et al. (2023) in melons, where decreased plant height was observed under stress condition. The effect water stress on root length of selected genotypes was recorded at 45^{th} day after sowing. In moisture stress condition, root length got decreased as compared to control (Table 1). An increase in the root length can be seen in genotypes like Puttikaayi, Kerala 1, Bagalkot Local and Muskmelon Round compared to control, in which highest percentage increase was recorded in genotype Muskmelon Round (22.76 %). All other genotypes showed a decrease in root length under water stress conditions. Where, Alpur Green had maximum root length (32.17 cm) with 5.06 per cent reduction, while Giriyal Green shown minimum root length of 23.19 cm with only 1.06 per cent reduction of root length compared to control and genotype Kekkarike showed highest percentage reduction (26.75 %) under water stress condition.

In this study, drought stress is imposed in polybags which restricts the growth of roots, here both increase and decrease in root length is recorded. Drought stress often leads to changes in soil structure, such as increased compaction or reduced moisture, which can inhibit root growth. Under water deficit plant adopt themselves to preside over water stress condition by changing in physiological traits, i.e. root and shoot length (Kusvuran and Dasgan 2017) in tomato (Pandey et al., 2018) muskmelon. Number of leaves per plant of genotypes was recorded (Table 1). Under moisture stress conditions, the number of leaves per plant was consistently lowered compared to the control. Among genotypes, the higher number of leave s was recorded in Giriyal Green (18.67) and lower in Alpur Green (10.17) and the lower mean reduction in number of leaves observed in Kerala 2 (2.32 %), which in on par with Kashi Madhu (8.33 %) and highest reduction were noticed in the genotype Puttikaayi (36.74 %). Water stress conditions lead to fewer leaves as the plant adapts by reducing its surface area to limit water evaporation and optimize survival. Munne-Bosch and Alegre (2004) reported that decrease in leaf number under moisture stress is a controlled physiological adaptation that helps the plant survives in adverse climatic conditions. Water scarcity reduces leaf size, longevity and the number of leaves per plant in tomato (Shao et al., 2008).

The Plant height stress tolerance index (PHSTI) values for fifteen genotypes assessed were analyzed (Table 2). Among genotypes, Dharward 1 (92.21) obtained maximum PHSTI which was on par with Chamarajanagar Local (91.13) and Muskmelon Round (90.57), while minimum was obtained at Muskmelon Local (73.20) and Banaspathre (74.71). The plant height stress tolerance index can indicate adaptation of genotypes to drought conditions based on their shoot growth responses. In the present study, the shoot length found to be decreased under water stress there by reduced PHSTI compared to control. Saensee *et al* (2012) in sunflower and Faizan, (2020) in brinjal reported that stress tolerance index can be used effectively for selecting genotypes which are tolerant to moisture stress. The Root length stress tolerance index (RLSTI) of fifteen genotypes was computed analysed data were presented in Table 2. Among genotypes, Muskmelon Round (111.40) showed maximum RLSTI, followed by Kerala 1 (108.06), whereas Kekkarike (86 .63) and Banaspathre (88.43) recorded minimum. The root length stress tolerance index can indicate adaptation of genotypes to drought conditions based on their root growth responses. Genotypes have a higher root stress tolerance index, indicating their greater resilience to stress, whereas lower index, reflecting their susceptibility to stress. It can be used effectively used for selecting genotypes which are tolerant to moisture stress (Saensee et al., 2012) in sunflower and (Gobu et al., 2014) brinjal.

Plant fresh weights of genotypes were measured at 45th day after sowing. The recorded data is illustrated in Figure 1. Among the genotypes, the minimum mean reduction was found in Chamarajnagar Local (38.50 %) followed by Puttikaayi (42.28 %). The highest mean reduction percentage was recorded in Kashi Madhu (79.86 %), which is on par with Giriyal Green (74.39 %). While the Giriyal Green recorded maximum plant fresh weight (33.92 g), which also had higher mean reduction of 74.39 per cent. Muskmelon Round recorded minimum fresh weight (16.05), while interaction effect showed maximum fresh weight in Kerala 2 (18.00 g) and minimum was in Kashi Madhu (7.97 g) under water stress condition (Fig. 2). Higher plant fresh weights under moisture deficit environments are desirable characters with respective to drought tolerance. A common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production Zhao *et al.* (2006) and Farooq *et al.* (2009). Under moisture deficit conditions, plant height, leaf number, size and stem diameter typically decrease, which finally lead to decreased biomass production causing reduced fresh weight of the seedlings.

The dry weights of genotypes were taken at 45th day after sowing. The data is illustrated in Figure 3. The genotype Giriyal Green recorded maximum dry weight (10.70 g), which also shown highest mean reduction of 70.54 per cent. While Banaspathre recorded minimum dry weight of plant (3.52 g) with lowest mean reduction of 11.53 per cent. Among genotype and treatment interaction, maximum dry weight is

Table 1. Effect of water stress on shoot length, root length and number of leaves of muskmelon genotypes at 45th day after sowing

Genotypes	Shoot length (cm)				Root length (cm)				Number of leaves			
	Control	Treatment	Mean	Mean reduction (%)	Control	Treatment	Mean	Mean reduction (%)	Control	Treatment	Mean	Mean reduction (%)
Puttikaayi	43.67	25.83	34.75	40.85	23.67	25.67	24.67	-8.45	16.33	10.33	13.33	36.74
Dharwad 1	96.33	81.33	88.83	15.57	29.27	27.17	28.22	7.17	18.67	12.33	15.50	33.96
Gadag Local	86.00	51.67	68.83	39.92	29.93	25.00	27.47	16.47	17.33	12.00	14.67	30.76
Kerala 1	72.33	45.33	58.83	37.33	26.98	31.33	29.16	-16.12	13.33	9.33	11.33	30.01
Kashi Madhu	96.67	57.33	77.00	40.70	32.47	27.95	30.21	13.92	12.00	11.00	11.50	8.33
Alpur Green	75.67	49.67	62.67	34.36	33.00	31.33	32.17	5.06	12.00	8.33	10.17	30.58
Giriyal Green	67.33	50.17	58.75	25.49	23.38	23.00	23.19	1.63	22.00	15.33	18.67	30.32
Chamarajnagar Local	77.00	63.33	70.17	17.75	26.67	25.00	25.83	6.26	13.33	11.00	12.17	17.48
Banaspathre	57.33	28.33	42.83	50.58	30.37	23.33	26.85	23.18	13.00	10.33	11.67	20.54
Sirsi Local	65.67	40.33	53.00	38.59	31.33	27.00	29.17	13.82	17.00	11.33	14.17	33.35
Bagalkot Local	62.67	49.33	56.00	21.29	28.83	30.33	29.58	-5.20	19.67	13.00	16.33	33.91
Muskmelon Round	61.00	49.50	55.25	18.85	22.67	27.83	25.25	-22.76	13.33	10.67	12.00	19.95
Muskmelon Local	74.00	34.33	54.17	53.61	30.73	24.00	27.37	21.90	15.00	10.67	12.83	28.87
Kekkarike	84.00	60.50	72.25	27.98	28.67	21.00	24.83	26.75	14.33	11.67	13.00	18.56
Kerala 2	47.33	39.33	43.33	16.90	27.33	23.67	25.50	13.39	14.67	14.33	14.50	2.32
Mean	71.13	48.42	59.77	31.98	28.35	26.24	27.29	7.44	15.47	11.44	13.45	26.05
		CD (1%)				CD (1%)				CD (1%)		
Genotype (G)		14.42 *				3.92*				2.52*		
Treatment (T)		5.27 *				1.43*				0.92*		
G×T		NS				5.55*				3.56*		

* Significant; NS: Non-significant, CD- Critical difference

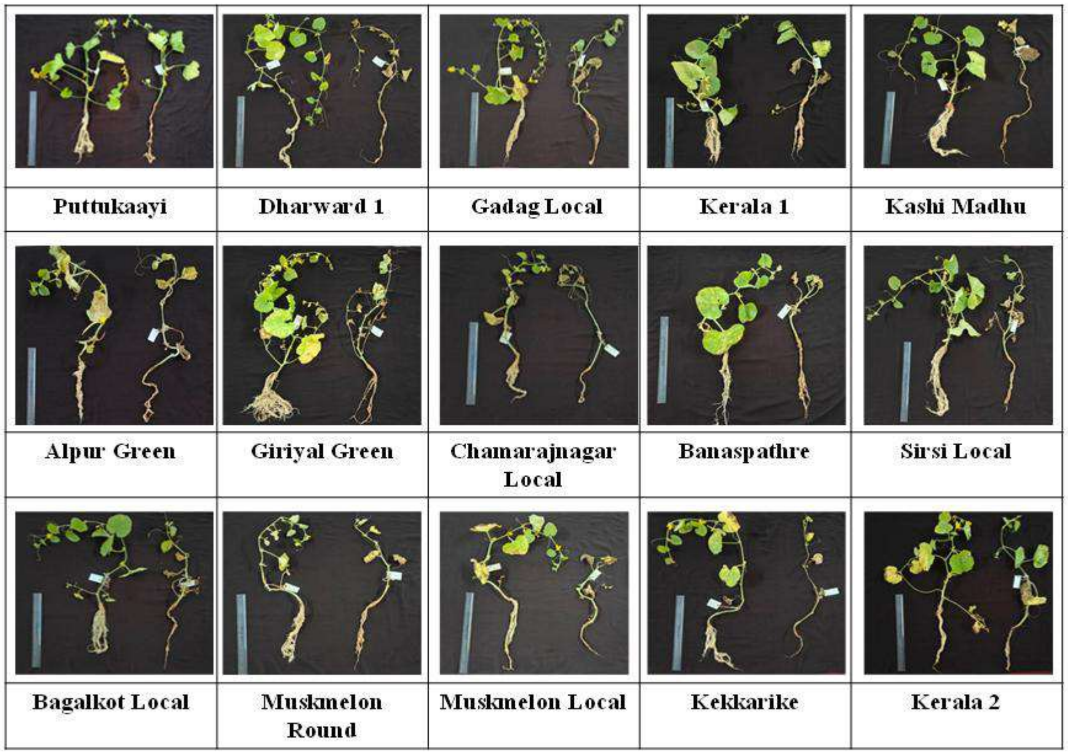

Fig. 1. Effect of water stress on the shoot and root growth of fifteen muskmelon genotypes under control and treatment at 45[th] day after sowing

Table 2. Effect of water stress on PHSTI and RLSTI of muskmelon genotypes at 45[th] day after sowing

Genotypes	PHSTI			RLSTI		
	Control	Treatment	Mean	Control	Treatment	Mean
Puttikaayi	100.00	59.16	79.58	100.00	108.45	104.23
Dharwad 1	100.00	84.43	92.21	100.00	92.83	96.41
Gadag Local	100.00	60.08	80.04	100.00	83.52	91.76
Kerala 1	100.00	62.67	81.34	100.00	116.12	108.06
Kashi Madhu	100.00	59.31	79.66	100.00	86.09	93.04
Alpur Green	100.00	65.64	82.82	100.00	94.95	97.48
Giriyal Green	100.00	74.51	87.25	100.00	98.36	99.18
Chamarajnagar Local	100.00	82.25	91.13	100.00	93.75	96.88
Banaspathre	100.00	49.42	74.71	100.00	76.84	88.42
Sirsi Local	100.00	61.42	80.71	100.00	86.17	93.09
Bagalkot Local	100.00	78.73	89.36	100.00	105.20	102.60
Muskmelon Round	100.00	81.15	90.57	100.00	122.79	111.40
Muskmelon Local	100.00	46.40	73.20	100.00	78.09	89.05
Kekkarike	100.00	72.02	86.01	100.00	73.26	86.63
Kerala 2	100.00	83.10	91.55	100.00	86.59	93.29
Mean	100.00	68.02	84.01	100.00	93.53	96.77
		CD (1%)			CD (1%)	
Genotype (G)		10.91*			8.75*	
Treatment (T)		3.98*			3.20*	
G×T		15.43*			12.38*	

*Significant; PHSTI: Plant Height Stress Tolerant Index; RLSTI: Root Length Stress Tolerant

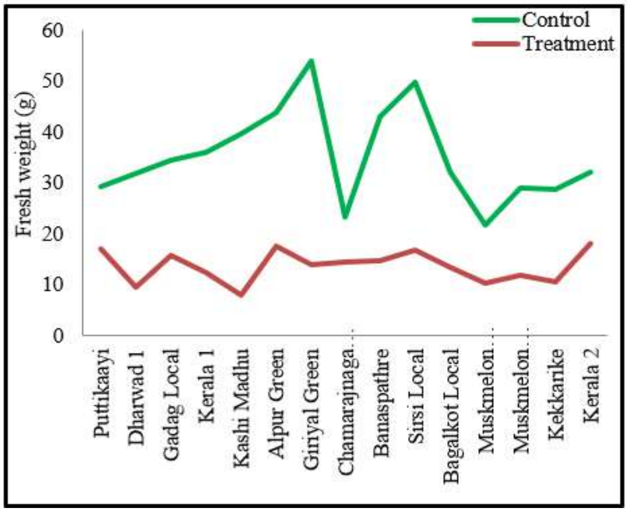

Fig. 2. Effect of water stress on fresh weight (g) of muskmelon genotypes at 45th day after sowing

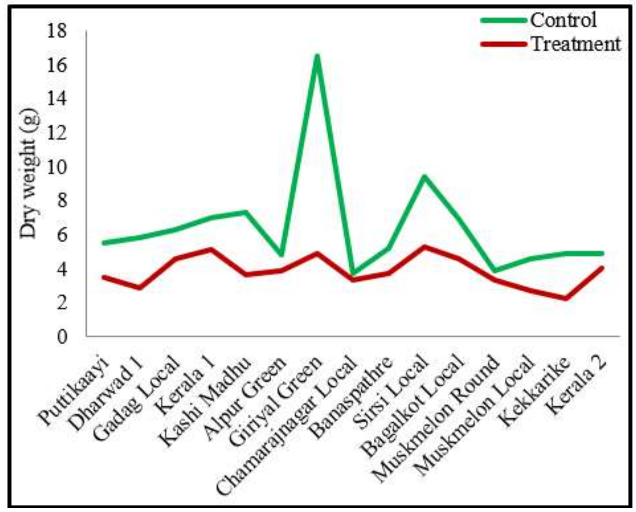

Fig. 3. Effect of water stress on dry weight (g) of muskmelon genotypes at 45th day after sowing

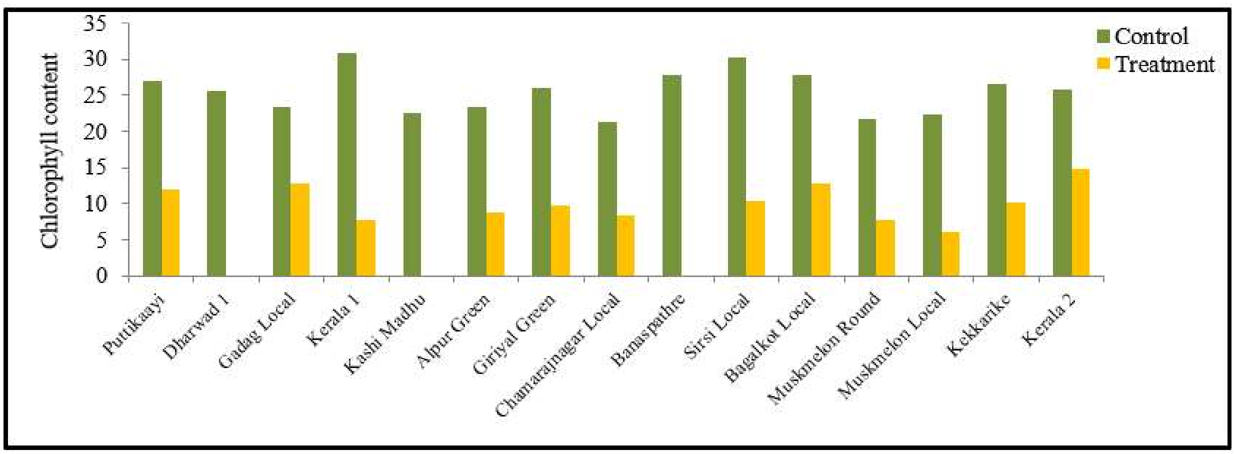

Fig. 4. Effect of water stress on chlorophyll content (SPAD units) of muskmelon genotypes at 45th day after sowing

recorded in Sirsi Local (5.27 g), while minimum was recorded in Kekkarike (2.25 g) followed by Muskmelon Local (2.73 g) in water stress condition (Fig. 3). Due to moisture stress condition, plant growth and development processes gets affected by which leads in reduction in dry matter accumulation. In the present study, total dry weight of muskmelon plants declined under moisture stress. Similar results were observed by Abdalla et al. (2018) and Gonzalez-Orenga et al. (2023) in brinjal; Rezaei et al. (2012) and Mahpara et al. (2018) in tomato.

The chlorophyll content of genotypes was measured using SPAD meter. The chlorophyll content found to be drastically reduced under water stress condition (Fig. 4). Among the genotypes maximum chlorophyll content was recorded in Kerala 2 (20.28) with minimum mean reduction of 42.57. Whereas, plants which are wilted due to water stress (Dharward 1, Kashi Madhu and Banaspathre) showed no chlorophyll content (Fig. 4). Drought stress limits water availability, which affects the plants ability to carry out photosynthesis. As it is crucial for conversion of light energy and into chemical energy, reduced photosynthetic activity often leads to a decrease in chlorophyll content. Rehman *et al* (2023) studies reported the decrease in chlorophyll under drought stress in muskmelon plants. Reduction or no-change in chlorophyll content of plant under drought stress has been observed in different plant species and its intensity depends on stress rate and duration. Drought stress imposed during vegetative growth or anthesis significantly decreased total chlorophyll content in chickpea (Mafakheri et al., 2010).

CONCLUSION

The present study was performed to screen muskmelon genotypes for drought tolerance, where the results showed that there is significant tolerance to drought in the muskmelon genotypes like Chamarajnagar Local, Muskmelon Round and Kerala 2. While, genotype Muskmelon Local along with

Banaspathre and Kekkarike showed susceptible to drought conditions. Therefore, the tolerant genotypes can be further study under field conditions and can be used to develop varieties for drought conditions.

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AUTHOR'S CONTRIBUTION

Kavya S conducted the research and collected the data. Suneetha C was responsible for the initiation and conceptualization of the study. Shivapriya M provided the seed material for the study. Venkatesha Murthy conceptualized the experiment and supervised the overall research work. Bhavani P and Sunitha T.R supervised the experimental work. Yuvaraj S and Praveenakumar R wrote and edited the manuscript. All authors read and approved the final manuscript.

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Nutritional Profiling of Different Maturity Stages in Jackfruit

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***Abstract*:** The investigation was undertaken to evaluate the variations in the nutritional composition of jackfruit during its progressive stages of maturity. Its nutritional attributes vary from immature to fully ripe stages, influencing its suitability for different value-added applications. The fruits were categorized into four maturity stages; immature, mature, ripe and over-ripe, analyzed for proximate composition (moisture, protein, fat, fibre, ash, and carbohydrate), mineral content (calcium, magnesium, copper, potassium, and iron), and bioactive compounds (vitamin C, total phenols, carotenoids, and antioxidants). There was significant increase in total soluble solids, sugars, and carotenoids with advancement in maturity, whereas moisture, fibre and vitamin C contents showed a gradual decline. The mature stage exhibited balanced nutritional composition, making it suitable for culinary and process into various food products. The study highlights the dynamic changes in nutritional and biochemical parameters of jackfruit across maturity stages and provides valuable insights for optimizing harvest time, postharvest utilization and product development strategies.

***Keywords*:** Jackfruit, Maturity stages, Nutritional composition, Proximate analysis, Bioactive compounds, Antioxidants

Jackfruit (*Artocarpus heterophyllus* Lam.) is one of the largest edible fruits in the world and a member of the family Moraceae. It is widely cultivated in tropical and subtropical regions of Asia, particularly in India, Bangladesh, Sri Lanka, Thailand, and Indonesia. Known for its versatility, jackfruit is consumed at various stages of maturity-tender, mature unripe, ripe and fully ripe-each possessing distinct sensory attributes, nutritional composition, and potential uses in food and processing industries (Chai et al., 2021). The fruit serves as a rich source of carbohydrates, dietary fibre, vitamins, minerals, and phytonutrients, while its seeds also contribute appreciable quantities of protein and starch (Ranasinghe et al., 2019). Jackfruit has recently gained global attention as a functional food owing to its health-promoting properties and its potential as a plant-based meat alternative. However, the nutritional and biochemical composition of the fruit varies considerably across different stages of maturity due to physiological and biochemical changes that occur during fruit development and ripening (Ranasinghe et al., 2019). Understanding these variations is essential for determining the optimal harvest stage for different end uses such as culinary consumption, processed food products, or industrial applications.

In India, despite being widely cultivated and recognized as a “poor man's fruit,” systematic studies on the nutritional dynamics of jackfruit across its maturity stages are limited. Characterizing these variations can help in the value addition, postharvest management, and product diversification of jackfruit, contributing to its commercialization and nutritional security. Therefore, the present study was undertaken to evaluate the nutritional profile of jackfruit at different stages of maturity. The research aims to assess the changes in proximate composition, mineral content, and biochemical parameters during fruit development, thereby providing insights into its nutritional potential and suitable utilization at each stage of maturity.

MATERIAL AND METHODS

The experiment was conducted at the Department of Postharvest Management**,** College of Horticulture, Bengaluru during the years 2023-2024. Jackfruits representing different stages of maturity were obtained from Bioversity International, 3HV8+788, College of Horticulture, Bengaluru. Fruits were harvested at four distinct maturity stages based on the number of days after fruit emergence (DAFE): Tender (40±2 days), Mature (80±2 days), Ripe (120±2 days), and Overripe (135±2 days). For the study five jackfruit varieties-Byra Chandra, Gumless, Royal Jack, Rudrakshi, and Thailand Pink-were selected. All the biochemical analysis were carried out using standard methods (Table 1).

RESULTS AND DISCUSSION

The nutritional and biochemical composition of jackfruit varies significantly across different varieties and stages of maturity, reflecting the dynamic physiological and metabolic changes occurring during fruit development. Carbohydrate content was increased markedly with ripening, reaching the highest level of 24.40% in the ripe stage of the Thailand Pink variety, while the tender stage of Rudrakshi exhibited the lowest carbohydrate concentration at 4.35%. This upward trend can be explained by the enzymatic breakdown of complex starch molecules into simpler, soluble sugars, which contribute to the characteristic sweetness and enhanced palatability of ripe jackfruit. Rahman et al. (1999), also documented carbohydrate contents ranging from 9.4 to 11.5% in young jackfruit and from 16.0 to 25.4% in ripe fruit, suggesting a conserved ripening-associated increase in sugar content across different genotypes.

Protein content exhibited an opposite trend, being highest (2.56%) in the tender stage of Byra Chandra and declining progressively to the lowest of 1.23% in the over-ripe stage of Thailand Pink. This reduction can be primarily ascribed to the intensified activity of proteolytic enzymes during maturation, which degrade storage and structural proteins into amino acids, peptides, and other nitrogenous compounds. Such enzymatic conversion results in a net loss of measurable total protein, a phenomenon well documented across multiple fruit species and Vazhacharickal et al. (2015). The initially higher protein content in younger fruits likely reflects the requirement for structural and metabolic proteins during active growth phases, whereas, the relative carbohydrate accumulation in later stages results in a dilution effect of protein concentration relative to the fruit mass, as described by Azeez et al. (2015).

Ascorbic acid, a vital antioxidant known for its health benefits, varied substantially among varieties and maturity stages. The Gumless variety at the tender stage exhibited the highest ascorbic acid content (12.08 mg/100g), whereas the lowest levels (3.76 mg/100g) were in the over-ripe stage of the Royal Jack variety. During ripening, biochemical pathways facilitate the breakdown of precursor molecules, releasing ascorbic acid and thereby increasing its concentration. However, prolonged maturation and senescence may lead to oxidative degradation and reduction of ascorbic acid levels. This pattern agrees with observations by Baliga et al. (2011) reported similar trends in jackfruit and other horticultural crops.

Titratable acidity, an important organoleptic parameter influencing fruit taste and preservation potential, was highest at 0.39% in the tender stages of Rudrakshi and Thailand Pink and declined to 0.21% in their over-ripe stages. The progressive decrease in acidity is linked to the metabolic conversion of organic acids, such as citric and malic acids, into sugars during ripening, thereby increasing sweetness and altering flavor profiles (Jagadeesh et al. 2010, Shafiq et al. 2017). This acid-sugar interconversion is a broadly recognized phenomenon in fruit physiology.

Dietary fiber content peaked at 4.57% in the tender stage of Rudrakshi and diminished considerably to 0.97% in the over-ripe stage of Gumless. The decline in fiber is related to the breakdown of cellulose, hemicellulose, and lignin components of the cell wall during fruit softening and maturation (Kaur et al., 2023). These structural changes reduce the fibrous texture of the pulp, as also observed by Amadi et al. (2018) and Ong et al. (2006), indicating a trade-off between textural firmness and edibility during ripening.

Ash content, indicative of total mineral matter, decreased from 3.40% in the tender Rudrakshi to 1.11% in the over-ripe Byra Chandra. The reduction in ash content reflects the dilution of minerals as the fruit accumulates water and carbohydrates in later stages of development and ripening. Similar trends have been previously reported by Amadi et al. (2018) and Ojwang et al. (2018). This suggests a shift in

Table 1.

Parameter	Method	Reference
Antioxidant capacity (mg AAE 100 g^{-1})	Ferrous ion-reducing antioxidant power (FRAP) assay	Benzie and Strain, 1996
Ascorbic acid (mg 100 g^{-1})	2,6-dichlorophenol indophenol (DCPIP) dye method	Sadasivam and Manickam, 1992
Ash content (%)	Muffle furnace	AOAC (1995).
Total carbohydrates (%)	Phenol-sulphuric acid method	AOAC, 1995
Carotene (mg 100 g^{-1})		Ranganna (2008)
Fat content (%)	Soxhlet extraction using petroleum ether	AOAC, 2006
Protein (%)	Lowry's method	Lowry et al., 1951
Mineral elements (Ca, Mg, Zn, and Fe) (mg 100 g^{-1})	Wet digestion using HNO_3–$HClO_4$ di-acid mixture	Piper, 1966
Crude fiber (%)	Double digestion	Sadasivam and Manickam, 1996
Total flavonoids (mg QE 100 g^{-1})	Aluminium chloride colorimetry	Chun et al., 2003
Total phenolics (mg GAE 100 g^{-1})	Folin–Ciocalteu method	Singleton and Rossi, 1965
Titratable acidity (%)	Titration against 0.1 N NaOH using phenolphthalein as an indicator	AOAC, 1995

metabolic priorities from mineral accumulation towards the synthesis and storage of organic compounds.

Fat content showed an incremental increase over the course of maturation, with the highest (0.82%) in ripe Thailand Pink, contrasting with the lowest fat content (0.22%) in its tender stage. The accumulation of lipids during later developmental stages may be attributed to the formation of oil bodies and lipid storage compounds within the fruit pulp. Ranasinghe et al. (2018), also highlighted a low baseline fat content in young fruits with a modest increase upon ripening.

Total carotenoid content was significantly influenced by both maturity and genotype. The ripe stage of Thailand Pink amassed the highest carotenoid concentration (0.734 mg/100g), whereas, tender Byra Chandra contained the lowest (0.129 mg/100g). Red flaked varieties consistently demonstrated elevated carotenoid levels relative to yellow-flaked genotypes. This variation is likely due to differential expression of carotenoid biosynthesis genes and pigment accumulation (Jagadeesh et al. 2010, Shyamalamma et al. 2015).

Antioxidant activity assessed using the ferric reducing antioxidant power (FRAP) assay peaked in ripe Thailand Pink (193.02 mg AAE/100g) and was lowest in tender Rudrakshi (18.84 mg AAE/100g). The total phenolic content followed a similar pattern, with maximum in ripe Thailand Pink (0.34 mg GAE/100g) and minimum in tender Gumless (0.09 mg GAE/100g). These differences underscore the relationship between phenolic accumulation and antioxidant potential and reflect genotype-dependent biosynthetic capacity and maturity-related metabolic changes, as reported by Jagtap et al. (2010) and Bakar et al. (2009). Phenolics play a crucial role in plant defence mechanisms and contribute beneficially to human health.

The total flavonoids, a subclass of phenolic compounds, showed significant increase with ripening, exemplified by the highest content found in ripe Thailand Pink (3.26 mg QE/100g) compared to the lowest in tender Rudrakshi (0.36 mg QE/100g). Such elevated flavonoid levels in mature fruits suggest enhanced secondary metabolic activity possibly related to the fruit's defence against environmental stresses (Saeed et al., 2019, Hossain et al., 2020).

The mineral composition variation was observed among genotypes and maturity stages. Potassium content was highest in ripe Rudrakshi (309.60 mg/100g), calcium peaked in tender Rudrakshi (37.88 mg/100g), magnesium was maximal in ripe Rudrakshi (21.50 mg/100g), manganese in ripe Rudrakshi (2.39 mg/100g). Copper was highest in ripe Royal Jack (0.38 mg/100g), and iron content was l in ripe Byra Chandra (3.63 mg/100g). These mineral profiles are consistent with previous studies by Amadi et al. (2018), Shyamalamma et al. (2016), and Tiwari et al. (2015), which emphasize the strong influence of genetic factors and stage

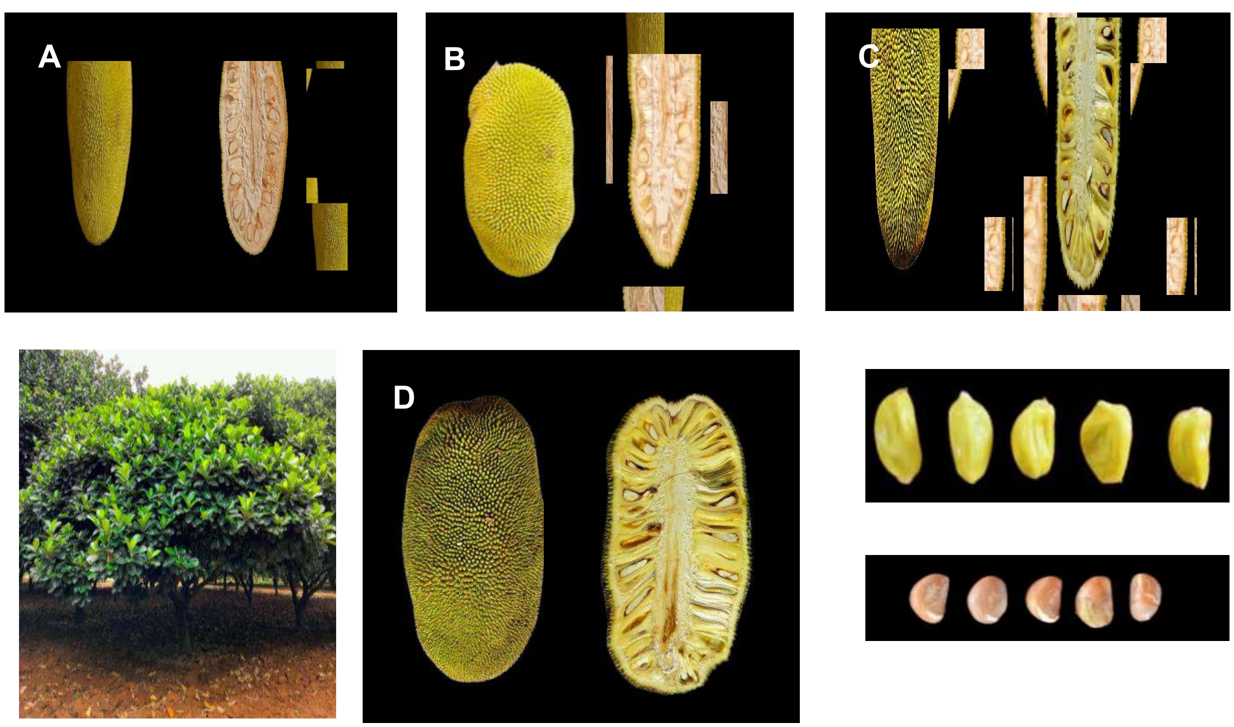

Plate 1. Different maturity stages of jackfruit (Byra Chandra) (A) Tender, (B) mature, (C) ripe and (D) over-ripe stages

Table 2. Chemical parameters of jackfruit varieties at different maturity

Variety	Maturity stages	Moisture content (%)	Titratable acidity (%)	Protein content (%)	Ash content (%)	Fibre content (%)	Total Phenol content (mg GAE 100g^{-1})	Ascorbic acid content (mg100g^{-1})	Total Carotenoid content (mg 100g^{-1})	Total carbohydrate (%)	Total Flavonoid content (mg QE 100g^{-1})	Antioxidant activity (mg AAE 100g^{-1})
Byra Chandra	Tender	85.35	0.36	2.56	2.42	3.77	0.10	8.26	0.129	6.41	0.63	37.31
	Mature	77.60	0.31	2.15	1.99	2.84	0.12	6.73	0.262	5.25	0.87	112.65
	Ripe	66.25	0.28	1.74	1.61	1.37	0.27	5.29	0.379	23.28	1.80	169.45
	Over-ripe	58.18	0.26	1.35	1.11	1.21	0.22	4.88	0.362	19.64	1.53	165.48
Gumless	Tender	84.59	0.35	2.48	2.51	3.91	0.09	12.08	0.176	6.01	0.41	28.67
	Mature	75.32	0.31	2.07	2.07	2.22	0.13	7.25	0.299	9.77	0.82	109.56
	Ripe	68.4	0.27	1.41	1.66	1.75	0.22	6.27	0.495	18.55	1.94	163.67
	Over-ripe	57.51	0.24	1.25	1.19	0.97	0.19	4.55	0.427	14.06	1.42	161.06
Rudrakshi	Tender	74.43	0.39	2.14	3.40	4.57	0.12	8.98	0.147	4.35	0.36	18.84
	Mature	68.46	0.34	2.01	2.75	2.79	0.16	5.44	0.364	9.36	1.16	97.45
	Ripe	61.35	0.26	1.77	1.85	1.53	0.26	4.32	0.541	15.53	2.02	162.92
	Over-ripe	57.83	0.21	1.50	1.47	1.25	0.21	3.88	0.505	12.39	1.54	161.21
Royal Jack	Tender	81.37	0.37	2.48	2.39	3.66	0.07	9.49	0.134	5.28	0.49	21.67
	Mature	74.36	0.33	2.25	1.79	2.47	0.17	6.43	0.379	12.06	1.11	97.54
	Ripe	63.06	0.28	1.71	1.95	1.54	0.22	6.12	0.636	17.60	2.28	172.19
	Over-ripe	52.31	0.23	1.39	1.62	1.25	0.18	3.76	0.612	14.03	1.48	168.45
Thailand Pink	Tender	83.8	0.39	2.55	3.02	3.81	0.13	11.68	0.142	4.36	0.68	39.52
	Mature	79.84	0.31	2.19	2.40	3.28	0.18	5.6	0.389	14.12	1.76	119.65
	Ripe	70.86	0.25	1.52	1.44	1.62	0.34	6.38	0.734	24.4	3.26	193.02
	Over-ripe	61.06	0.21	1.23	1.32	1.57	0.3	4.72	0.632	21.8	2.74	189.64

of maturity on mineral uptake and accumulation. The mineral nutrients analyzed are essential for both plant physiological processes and human nutrition.

Collectively, these results highlight the dynamic and complex nutritional landscape of jackfruit as influenced by variety and ripening. Such variability has critical implications for fruit utilization in fresh consumption and processing, as well as for breeding programs aimed at improving nutritional quality.

CONCLUSION

The study demonstrates that jackfruit's nutritional content varies greatly with variety and maturity. The ripe Thailand Pink variety had the highest carbohydrates, antioxidants, phenols, and carotenoids, making it a good energy-rich and healthful choice. The tender Rudrakshi variety was rich in minerals, protein, fiber, and firmness, making it ideal for early harvest and consumption. These differences highlight the importance of choosing the right variety and harvesting stage to get the desired nutritional and quality traits. Overall, these findings help in selecting jackfruit varieties and maturity stages for improved nutrition and processing.

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Enhancing Germination and Seedling Vigor in Papaya (*Carica papaya* L.) Through Herbal Extract-Based Seed Priming

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Abstract: The present study was conducted at Lovely Professional University, Phagwara, Punjab, to evaluate the efficacy of herbal seed priming in enhancing germination, seedling vigor, biochemical attributes, and early growth performance of papaya seedlings. The experiment consisted of six treatments incorporating various combinations of aloe vera gel, coconut water, moringa leaf extract, neem leaf extract, turmeric extract, and garlic extract, with distilled water as the control. Papaya seeds were soaked in the respective herbal solutions for 12 hours at a 10% concentration, followed by germination under controlled conditions. Significant variations were observed across treatments, with (neem leaf extract + turmeric extract + aloe vera gel exhibited the highest germination percentage, seedling vigor, and chlorophyll content. Similarly, aloe vera gel + coconut water + moringa leaf extract showed notable improvements in root and shoot length, as well as seedling biomass accumulation. The increased germination speed and seedling vigor index in treated seeds suggest that herbal bioactive compounds play a crucial role in stimulating early seedling growth and metabolic activity. The findings indicate that herbal seed priming can serve as an eco-friendly and sustainable alternative to synthetic growth stimulants, improving seed performance while reducing reliance on chemical inputs.

Keywords: Papaya (*Carica papaya* L.) herbal, Neem leaf extract, Turmeric extract, Aloe vera gel, Ginger extract, Coconut water, Moringa leaf extract

Papaya (*Carica papaya* L.) is a tropical fruit crop widely cultivated for its high nutritional and economic value. It plays a vital role in global fruit production due to its rapid growth, short juvenile period, and high fruit yield (Yang et al., 2025). Among various agronomic practices that influence the germination and vigor of papaya seeds, seed priming has emerged as a promising technique to enhance seed performance under varying environmental conditions (Khajjak et al., 2022). Seed priming is a pre-sowing treatment that improves seed germination, accelerates seedling emergence, and enhances overall plant establishment. The extensive use of synthetic priming agents, such as chemical osmopriming and hormone treatments, has raised concerns due to their potential environmental impact and inconsistent effects on seed quality (Cañizares et al., 2025). In contrast, natural plant-derived bioactive compounds, including herbal extracts, offer a sustainable and eco-friendly alternative for improving seed germination and seedling vigor. Various studies have demonstrated the efficacy of herbal extracts such as Aloe vera, neem, moringa, turmeric, garlic, and coconut water in enhancing seed metabolism, boosting antioxidant activity, and improving stress tolerance in plants (Ansari et al., 2024). However, limited research has been conducted on the effect of herbal extract-based seed priming in papaya seeds, necessitating further exploration of this approach. Modern agricultural systems rely heavily on chemical interventions, which often degrade soil health, reduce microbial diversity, and contribute to environmental pollution. To address these challenges, herbal-based seed priming offers a novel strategy to enhance crop resilience while reducing reliance on synthetic agrochemicals. The present study aims to evaluate the effect of herbal extract combinations on papaya seed germination, seedling vigor, biochemical responses, and early growth performance (Pareek et al., 2025). By integrating plant-based priming treatments, this research seeks to identify cost-effective and sustainable alternatives for improving seedling establishment in papaya cultivation.

MATERIAL AND METHODS

The present investigation was undertaken at Lovely Professional University Research Farm, Punjab, during 2024–25 to examine the impact of herbal extract-based seed priming on the germination, growth, and biochemical attributes of papaya (*Carica papaya L.*). The study was carried out under controlled greenhouse conditions to ensure uniform environmental influences and minimize external variability.

The experiment was arranged in a completely randomized design (crd) with six treatments, including a control, and was replicated three times. The seeds underwent priming with herbal extracts, and subsequent growth observations were recorded at predefined intervals. The herbal solutions were prepared using fresh plant materials and standardized extraction procedures. The seed priming process was conducted in multiple phases (Table 1).

Initially, fresh herbal materials were homogenized with distilled water in a 1:10 w/v ratio, filtered, and stored for immediate use. Papaya seeds were immersed in respective extract solutions for 12 hours at room temperature (25 ± 2°C), ensuring adequate absorption of bioactive compounds. Post-treatment, the seeds were air-dried in shade for 24 hours to restore moisture balance before sowing. The seeds were then sown in plastic trays containing a sterilized soil-sand mixture (2:1 ratio), and the trays were kept in the greenhouse under optimal conditions for germination and seedling growth. Six seed priming treatments were evaluated, including a control (T_1) with no priming using distilled water. The other treatments involved soaking seeds for 12 hours in different herbal extract combinations at 10% concentration: T_2 consisted of aloe vera gel, coconut water, and moringa leaf extract; T_3 included neem leaf extract, turmeric extract, and aloe vera gel; T_4 combined garlic extract, moringa leaf extract, turmeric extract, and coconut water; T_5 comprised aloe vera gel, neem leaf extract, and turmeric extract; and T_6 included garlic extract, moringa leaf extract, and coconut water.

Observations and Parameters Recorded

Seed germination and vigor attributes: Germination percentage was calculated at 7 and 14 days after sowing (DAS) by counting the number of emerged seedlings. Mean Germination Time (MGT) was determined to assess the average duration required for seed sprouting, while the Germination Index (GI) was computed to evaluate germination speed. Seedling vigor was assessed using the Seedling Vigour Index (SVI), which was derived by multiplying the germination percentage with the total seedling length.

Seedling growth and morphological parameters: Seedling growth was evaluated at 14 DAS by measuring shoot and root lengths. Fresh and dry biomass of seedlings was recorded, with drying carried out at 60°C for 48 hours to obtain accurate dry weight values. Leaf area was determined using a leaf area meter (LI-COR 3000) to quantify differences in foliage expansion across treatments.

Chlorophyll content and biochemical assays: Total chlorophyll content in the leaves was estimated using the DMSO extraction method (Hiscox and Israelstam, 1979). Absorbance was measured at 645 nm and 663 nm using a UV-VIS spectrophotometer, and chlorophyll content was expressed as mg g^{-1} fresh weight. Total soluble sugars were determined using the Anthrone reagent method, and proline accumulation was assessed following the Bates et al. (1973) protocol. Membrane stability was evaluated by estimating the Membrane Stability Index (MSI) using standard procedures.

Statistical analysis: Duncan's Multiple Range Test (DMRT) with SPSS software (version 25.0)

RESULTS AND DISCUSSION

Seed germination and vigor attributes: Germination percentage varied significantly among different treatments at 7 and 14 DAS. The highest germination percentage (94.0%) at 14 DAS was in T_2 (neem leaf extract + turmeric extract + aloe vera gel), followed by T_1 (aloe vera gel + coconut water + moringa leaf extract) and T_3 (garlic extract + moringa leaf extract + turmeric extract + coconut water). The lowest germination percentage (85.1%) was observed in the control (T_6). Mean germination time (MGT) was lowest in T_2 (4.1 days), indicating a faster emergence rate, while the control (T_6) longest MGT (5.1 days). Germination indexwas highest in T_2 (19.2), followed by T_1 and T_3, suggesting that herbal seed priming treatments improved germination speed. Seedling vigour index (SVI) was significantly higher in T_2 (1702), followed by T_1 and T_3, while the lowest SVI was in T_6. The improved germination and vigor in treated seeds may be due to the presence of bioactive compounds in plant extracts, which stimulate enzyme activity, enhance water absorption, and provide essential nutrients (Table 1). Singh et al. (2024) also reported that organic seed treatments enhance early seedling establishment.

Seedling growth and morphological parameters: Seedling growth parameters, including shoot length, root length, and biomass accumulation, showed significant variations among treatments. The tallest seedlings were observed in T_2 (neem leaf extract + turmeric extract + aloe vera gel), followed by T_1 and T_3, whereas the shortest seedlings were in the control (T_6).

Root length was also maximum in T_2, which was statistically at par with T_1 and T_3. Fresh and dry biomass accumulation followed a similar trend, with T_2 exhibiting the highest values, indicating improved nutrient assimilation and seedling robustness. Leaf area was significantly greater in T_2 compared to other treatments, suggesting enhanced photosynthetic efficiency (Table 2). The superior seedling growth observed in herbal priming treatments may be attributed to the presence of growth-promoting compounds, antioxidants, and antimicrobial properties that protect emerging seedlings from stress conditions. Abir et al (2022) and Arraf and Al-madhagi (2025) found that organic formulations enhance seedling establishment by promoting root development and nutrient uptake.

Chlorophyll content and biochemical assays: Total chlorophyll content was highest in T_3 followed by T_2 and T_1. The control (T_6) had the lowest chlorophyll content, indicating that herbal priming treatments positively influenced photosynthetic efficiency. Total soluble sugars were significantly higher in T_2, reflecting better carbohydrate accumulation, which is crucial for early seedling

Table 1. Effect of seed priming on germination and vigor attributes

Treatment	Germination (%)		Mean germination time (days)	Germination index	Seedling vigor index
	(7 DAS)	(14 DAS)			
T_1 (Control)	78.5	92.3	4.2	18.6	1650
T_2	80.2	94	4.1	19.2	1702
T_3	77	91.5	4.4	17.8	1623
T_4	75.8	89.7	4.5	17.2	1580
T_5	72.6	86.9	4.8	16.4	1503
T_6	70.3	85.1	5.1	15.7	1438
CD (p=0.05)	2.5	2.8	0.3	1.2	55

Table 2. Impact of seed priming on seedling growth and morphological parameters

Treatment	Shoot length (cm)	Root length (cm)	Leaf area (cm²)	Fresh biomass (g)	Dry biomass (g)
T_1 (Control)	12.4	7.3	42.27	1.92	0.68
T_2	13.1	7.9	45.42	2.08	0.72
T_3	12.0	7.1	43.75	1.87	0.66
T_4	11.7	6.8	44.08	1.82	0.64
T_5	11.2	6.5	43.98	1.74	0.61
T_6	10.8	6.1	44.42	1.68	0.59
CD (p=0.05)	0.9	0.5	NS	0.12	0.05

Table 3. Effect of seed priming on chlorophyll content and biochemical parameters

Treatment	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total soluble sugars (mg/g)	Proline content (µg/g)	Membrane stability index (%)
T_1 (Control)	1.45	0.86	18.9	4.3	72.4
T_2	1.52	0.91	19.5	4.7	74.1
T_3	1.39	0.83	18.2	4.1	71.2
T_4	1.35	0.80	17.8	3.9	70.6
T_5	1.28	0.76	17.2	3.7	69.5
T_6	1.21	0.72	16.5	3.4	67.8
CD (p=0.05)	0.08	0.05	0.9	0.4	2.1

establishment. Proline accumulation was also higher in T_2, suggesting enhanced stress tolerance due to the antioxidant properties of herbal extracts (Table 3). Membrane Stability Index (MSI) was highest in T_2, followed by T_1 and T_3, while the control exhibited the lowest MSI, indicating greater susceptibility to oxidative damage. The enhanced biochemical attributes in herbal priming treatments may be linked to the bioactive compounds that improve membrane integrity and physiological efficiency. Hassan et al. (2020) and Megbowon et al. (2024), also highlighted the role of organic formulations in improving stress tolerance and seedling quality.

CONCLUSION

The integration of herbal seed priming treatments demonstrated a significant impact on seed germination, seedling vigor, biochemical composition, and overall plant growth. Among the different treatments, the combination of neem leaf extract, turmeric extract, and aloe vera gel consistently outperformed other treatments by enhancing germination speed, seedling vigor, root development, and chlorophyll accumulation. The improved physiological and biochemical responses observed in treated seedlings highlight the potential of plant-derived bioactive compounds in stimulating early growth and stress tolerance. The herbal seed priming could serve as a sustainable and eco-friendly alternative to conventional seed treatments, reducing dependency on synthetic chemicals while promoting robust seedling establishment. The enhanced seedling vigor and biochemical efficiency observed in primed seeds could lead to improved crop resilience under varying environmental conditions. Future research could explore the molecular mechanisms behind these effects, as well as the potential of herbal priming for large-scale agricultural applications.

AUTHORS CONTRIBUTION

Vikanksha: Experimentation, data collection, Arun Kumar: Conceptualization, supervision, manuscript writing, Ankit: – Statistical analysis, literature review, Jatinder Singh: Guidance, critical review, editing.

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Impact of Sowing Time on Sandalwood (*Santalum album*) Seedling Growth in Sub-Tropical Zone

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Abstract: The aim of the study was to determine the appropriate sowing time for sandalwood seeds at Punjab Agricultural University, Ludhiana, India. Dried sandalwood seeds collected from Marayoor, Kerala were used in the study. Sandalwood seeds were treated for 16 to 24 hours with 0.05 % gibberellic acid and sown at 24 different dates throughout the year. Seeds sown in April showed superiority in all germination and growth parameters followed by August, 1st September and 15th March. The highest germination percentage (77.5), germination value (2.56), survival percentage (63), plant height (22.5 cm) and collar diameter (0.22 cm) were witnessed in 15 April sown seeds. Lowest plant height (4 cm) and collar diameter (0.07 cm) were in the December sown seeds which were at par with November and January. Sturdiness quotient and volume index was also higher in April sown seeds. Extreme temperatures, whether winter or summer affected germination and growth of sandalwood. The study concludes that period from 15th March to end April as well as August and September is suitable for sandalwood seed sowing.

Keywords: Climatic conditions, Germination behavior, Month of sowing, Sandalwood growth

Santalum album L., often known as East Indian White Sandalwood is widely recognised across the world for its aromatic heartwood and immense therapeutic value. The genus, Indian sandalwood has the highest oil (6-7%) and santalol content. It is one of the slow-growing tropical trees that are being cultivated on a large scale for plantations. Sandalwood cultivation poses a significant challenge in obtaining high-quality stock. The essential oil market is predicted to reach USD 197 million by the end of 2026 (Global Sandalwood Oil Markets Report, 2020). Unfortunately, its production in its natural habitat (Karnataka and Tamil Nadu) has declined dramatically in recent years, due to indiscriminate exploitation, especially for its high export value, coupled with poor regeneration, disease, and changes in land-use patterns and is listed as a "vulnerable" tree species by the IUCN (Kumar et al., 2019). Sandalwood heartwood is prized for carving, while its oil is used extensively in attars, perfumes, cosmetics, medicinal products, and flavored tobacco (Chavan et al., 2024). The natural distribution of *S. album* spans an estimated 9,000 km², with about 90% of this area concentrated in Karnataka, Tamil Nadu, Madhya Pradesh, and select regions of Kerala. Smaller populations also exist in semi-arid areas of Maharashtra, Telangana, and parts of Uttar Pradesh (Madhuvanthi et al., 2024). Recently, commercial plantations have been expanding into non-traditional areas such as Andhra Pradesh, Telangana, Maharashtra, Gujarat, Assam, and Punjab (Durai et al., 2022). Therefore, to expand cultivation and bridge the gap between demand and supply that can reduce pressure on the wild population, it is now proposed to introduce sandalwood cultivation in northern India.

Optimizing the sowing time is important because every seed needs a specific climate or season to take out maximum resources from the soil (Sumathi and Srimathi 2013) and varies from species to species. The ability of a viable seed to germinate is controlled by a series of factors, including inherent causes of germination and external conditions. Sandalwood seeds take around 4–8 weeks to complete germination or reach the transplanting stage and it is an erratic, low-yield and time-consuming process. Seasonal temperature fluctuations is an important factor in determining seed germination and species in different locations exhibit different germination behaviors in response to temperature fluctuations (Liu et al*.,* 2013). Extreme temperatures give rise to oxidative damage in seeds; low temperatures reduce seed germination percentage, extend the first germination time and inhibit early seedlings growth (Fu et al*.,* 2017). High temperatures up-regulate abscisic acid biosynthesis genes and down-regulate catabolism genes. When seeds are exposed to favourable temperature conditions, changes in this phytohormone favour and seed germination (Sumathi and Srimathi 2013). Due to the constant change in climatic conditions in Punjab, it is very difficult to choose an appropriate time for sowing sandalwood seeds because these are vulnerable to climate change. Sowing and transplanting times affect not only seedling growth after germination but also the germination process itself. The success of a sandalwood plantation is directly influenced by raising quality seedlings, which further depends on optimum timing.

Information on the seasonal germination of sandalwood seeds in northern conditions is lacking as this has been introduced in north India recently. Keeping in view the economic potential of the species as well as the tremendous increase in the number of farmers willing to take up sandalwood cultivation for diversification, the study was planned to study the germination behaviour of *Santalum album* seeds during different months.

MATERIAL AND METHODS

The experiment was conducted at Punjab Agricultural University, Ludhiana, India during the year 2021 (January 21-December 21). The study area is situated at 247 m above mean sea level and lies at 30° 45` N latitude and 75° 40` E longitude.

Climate: The experimental area is situated in the central part of Punjab. Generally, weather conditions are sub-tropical to tropical, with long dry spells from late September to early June and an effective wet season from July to September. May and June are the hottest months, whereas December and January are the coldest months. Frost occurrences are not common. On average, the site receives an annual rainfall of 704 mm, which is not evenly distributed and most of which is received from July to September (75–80%). From meteorological data (Fig. 1), mean monthly minimum and maximum temperatures as well as relative humidity were calculated in the study region.

Pre sowing seed treatment: Sandalwood seeds were soaked in 500 ppm gibberellic acid (GA_3) to break the dormancy and enhance seed germination. To prepare a 500 ppm GA_3 solution, 1 g of GA_3 was weighed in a glass beaker and dissolved in a few drops of alcohol. Water was then added gradually, and the mixture was stirred thoroughly to make a final volume of 2 liters.

Germination bed preparation: Germination beds were prepared with a 2:1:1 mixture of sand, FYM, and soil. More proportion of sand provides good drainage, aeration, and disinfection against microorganisms, which protects the viability of seeds. This same mixture of materials was used to create four different beds, each with 50 seeds.

Transplanting stage: Transplanting was done in the evenings when the temperature was low. The ideal stage for pricking was observed to be a 2-4 leaf stage. Seedlings were pricked out and planted in 8 x 12 inch polybags after being treated with Bavistin. Then, plants were kept in the shade for 5–6 days to avoid transplanting shock. Host plants (pulses: gram, moong, arhar) were sown along with sandalwood seedlings in every polybag after one week of transplanting.

Germination Parameters

Germination percentage: The germination percentage of the seeds was calculated as the ratio between the total number of sown seeds and the number of germinated seeds after the germination period had passed.

Germination energy (GE): The per cent germination energy was number of total seeds those had germinated when the peak of germination was achieved that means number of highest seed germinated in a period of 24 hours.

$$GE\ (\%) = \frac{\text{Number of seeds germinated up to the time of peak germination}}{\text{Total number of seeds sown}} \times 100$$

Germination value (GV): Seed germination is a measure of speed and completeness of seed germination with a single

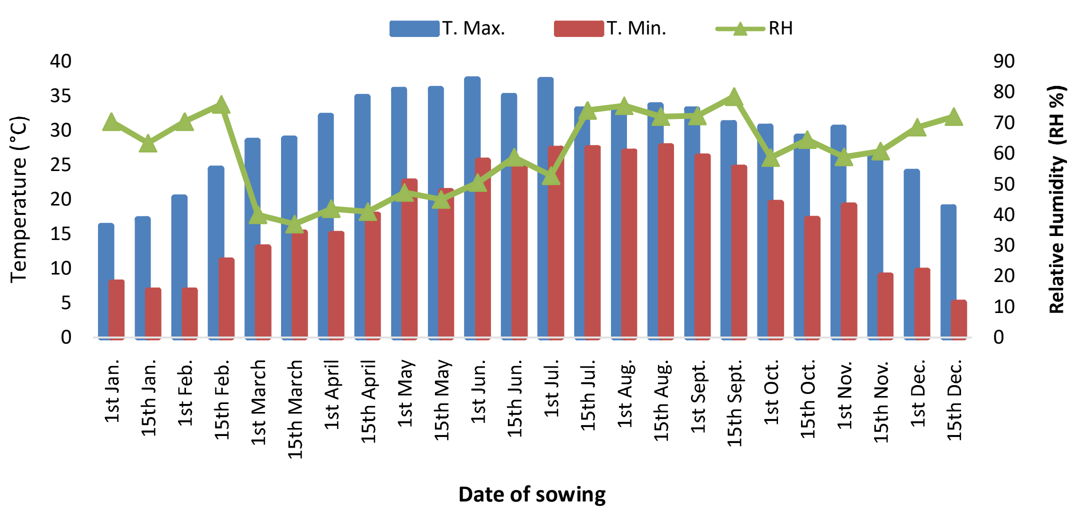

Fig. 1. Climatic conditions during different sowing times (January 21 to December 21)

figure which was calculated (Czabator 1962).

$$GV = PV \times MDG$$

Where, GV = Germination value

PV = Peak value of germination is the highest value of the cumulative germination per cent divided by the number of days since the start of the experiment.

Or

$$PV = \frac{\text{Cumulative germination per cent}}{\text{Days since sowing}}$$

MDG = Mean daily germination

Or

$$MDG = \frac{\text{Cumulative germination per cent at the end of the test}}{\text{Days since sowing to the end of test}}$$

Survival percentage:

$$\text{Survival percentage} = \frac{\text{No. of seedlings survived after 180 days}}{\text{No. of seeds sown}}$$

Growth Parameters

Seedling height (cm): The height of the seedling was recorded with the help of scale from the upper surface of soil filled in polythene bag to the shoot tip of the seedling.

Seedling collar diameter (mm): The seedling diameter at collar region of seedlings was measure from the collar region with the help of vernier caliper and expressed in millimeters.

Number of leaves: Fully opened leaves were considered as matured leaves and were counted visually in each seedling.

Quality Parameters

Sturdiness Quotient (SQ): SQ of the seedling was derived (Roller 1997).

$$SQ = \frac{\text{Height of seedling (cm)}}{\text{Collar diameter (mm)}}$$

Volume index (VI): VI was derived (Manavalan 1990).

$$VI = \text{Collar diameter}^2 \times \text{Height (cm)}$$

Statistical analysis: The data obtained from different observations were analyzed using the procedures of completely randomized design with CPCS1 software.

RESULTS AND DISCUSSION

Germination parameters: The effect of time of sowing on germination of sandalwood revealed highly significant variations in all parameters, *viz*. days taken to initiate germination, days taken to complete germination, germination percentage, germination value, germination energy and survival percent (Table 1). The seeds sown on 15th March, 1st April, 15th April, 1st May, 1st August, 15th August and 1st September recorded seed emergence (initial germination), 7-8 days earlier than that in 1st March and July months (27 to 31 days). Seeds took around 33 to 40 days to initiate germination in June and October, whereas more than 45 days in November, December and January. Similarly, fewer days (40 to 43) required to complete the germination of seeds in the period from 15th March to end April and in August and 1st September. In March and July, sown seeds took around 44 to 49 days to complete germination or reach the 2-4 leaf stage. Lastly, seeds sown in November, December and January took more than 60 days to complete germination or reach the transplanting stage. This may be due to varied temperature and weather conditions throughout the year as the availability of food reserves at an optimum temperature favours early initiation and completion of seed emergence. Extreme temperatures, whether high or low, both adversely affected the initiation and completion of germination, which explains the sensitivity of sandalwood seeds to temperature and weather conditions. At optimal conditions, the time taken for the initiation of sandalwood germination can only be influenced by the pretreatment of dormant seeds, but the variation in time taken for the completion of germination entirely depends upon environmental conditions. From March 15 to end April, and again in August and September, the average temperature in Punjab ranged from 24 to 30°C which suits germination, when it takes around 20 days to initiate and 43 days to complete germination. Batabyal et al. (2014) reported that in the Bankura district of West Bengal, where temperatures ranged from 10-48°C, sandalwood seeds took 15.66 days to initiate germination and 57.33 days to complete germination. More than 70% germination rate was recorded in sowing dates of 15th March, 1st April, 15th April, 1st May and September, whereas in November, December and January seeds germinated at a rate of less than 15%. With an increase in temperature, the rate of germination percentage increased from February to till 1st May and then again from July to September. Germination declined from September 15th to February as temperatures began to fall. The seeds sown on April 15th had the highest germination percentage followed by those in 1st September .The lowest germination percentage was t 0.5% in December, which might be due to low temperature. Baskin and Baskin (2014) predicted that the low germination of some species at low temperatures might be due to physiological dormancy. However, Mohapatra et al. (2019) recorded maximum germination percentage (55%) in May in Bubaneashwar, Orissa. Batabyal et al. (2014) recorded 44.66% germination in December month sown seeds in West Bengal. The findings indicate that sandalwood seeds prefer optimum temperatures for germination, which vary due to diverse climatic conditions across the country.

Maximum germination energy was obtained in April and

1st September, sown seeds which were statistically at par. Germination energy and germination value showed similar results to germination percentage. Among different months, a statistically significant germination percent was on 15th April and in 1st September, and the minimum germination value was obtained in the months of January, February, November and December and was due to the optimum temperature during seed germination, which enabled the accumulation of higher photosynthates, which affected germination energy and germination value. Mohapatra et al. (2019) reported that seeds sown without seed coat in the month of May in sand recorded higher germination capacity (70%), and germination energy (41.50%) in Bhubanashewar, Orissa, than sowing seeds in April, May, and June.

The highest plant percentage was obtained from sowing dates in April, 15th August and 1st September, and the minimum in November, December and January. Survival percent was highest in April and 1st September, closely followed by 15 March and 15 August respectively. A portion of these early-germinating juvenile seedlings were observed damaged or killed by severe frost and cold in December and January. The extreme summer also recorded the lowest plant percent, which might be due to higher temperatures that could have caused the mortality of weaker seedlings due to heat shock. Gulcu et al. (2010) observed a reduction in the germination rate, retardation of growth, and heterogeneous physical structure of the surviving seedlings due to environmental variations.

Overall, highest significant values were obtained for seeds sown on March 15, April 1, April 15, May 1, and both August and September in almost all germination parameters. This may be due to the high ambient temperature and relative humidity, as well as the seeds' better adaptation to the April climatic conditions in Punjab. Moreover, better performance in these months can be credited to better internal and external conditions of seed. On contrary, southern part of the

Table 1. Germination parameters of sandalwood sown at twenty four dates of sowing (January 21 to December 21)

Treatments (Date of sowing)	Days taken to initiate germination	Days taken to complete germination	Germination percent	Germination value	Germination energy	Survival percent
1.1.21	59.01	64.20	10.50	0.03	19.34	2.50
15.1.21	47.02	61.03	10.50	0.03	18.39	1.50
1.2.21	44.02	58.10	30.01	0.24	32.55	10.00
15.2.21	40.04	56.20	46.02	0.51	42.69	17.50
1.3.21	31.02	53.10	51.50	0.88	44.70	48.50
15.3.21	20.01	43.46	72.01	2.09	52.56	61.00
1.4.21	21.01	41.21	71.50	2.27	55.53	63.00
15.4.21	21.41	43.04	77.50	2.56	56.17	63.00
1.5.21	20.09	43.10	75.50	1.98	51.36	59.50
15.5.21	27.02	49.05	65.03	1.52	51.67	58.00
1.6.21	38.00	54.20	25.51	0.17	30.31	20.75
15.6.21	33.01	53.01	44.52	0.80	38.92	37.50
1.7.21	28.00	46.20	45.50	0.87	39.78	38.50
15.7.21	27.08	44.53	59.54	1.65	46.99	56.00
1.8.21	22.00	41.06	61.52	1.72	50.46	60.50
15.8.21	20.05	40.08	65.04	2.15	48.14	61.00
1.9.21	23.00	45.07	74.03	2.48	54.37	63.00
15.9.21	24.00	48.01	54.52	1.61	44.98	42.00
1.10.21	37.02	52.31	51.51	0.68	46.99	40.00
15.10.21	40.01	57.12	38.50	0.23	39.49	13.50
1.11.21	54.04	60.67	26.05	0.16	30.64	7.00
15.11.21	55.04	61.23	13.08	0.04	20.26	5.00
1.12.21	61.91	63.09	6.51	0.01	14.37	1.20
15.12.21	64.90	65.21	0.56	0.00	8.13	1.00
CD (p=0.05)	1.71	2.71	1.82	0.16	19.34	2.07

country favors December and January months for sowing sandalwood nursery due to existing vast difference in the weather and climatic conditions. The internal conditions include food reserve, completion of the ripening process, less dormancy, and the external conditions include congenial temperature and climatic conditions. Kamondo et al. (2014) also suggested that sandalwood seeds should be sown in a mixture of sand and fertile soil at temperatures between 25°C and 40°C, and high humidity. The results are in conformity with Mohapatra et al. (2019) and Madhuvanthi et al. (2024).

Growth parameters: Different seedling growth parameters, *viz*. seedling height, collar diameter, and number of leaves, were significantly influenced by the date of sowing. Data for seedling height, collar diameter, and number of leaves were recorded at 90 and 180 days after transplanting (DAT) (Table 2). The plant height in *Santalum album* was found to increase as the number of days increased. Among the 24 sowing dates evaluated, significant differences were observed for this parameter. Plant height at 150 DAT, the plant height ranged from 3.6 cm (15th December) to 17.6 cm (1st April). At 180 DAT, the plant height ranged from 4.0 cm (15 December) to 22.5 cm (15 April). April-sown seeds had a significantly higher value, which could be attributed to the growing season as the temperature and relative humidity remained favourable for growth due to the increased photosynthetic surface, whereas December-sown seeds performed poorly due to the dormant period. Higher and lower temperatures during the vegetative period reduced plant height. The adverse effect of summers on plant height was also studied by Sumathi and Srimathi (2013).

The collar diameter also differed significantly at different periods of observation. Higher collar diameter can be attributed to the availability of starch and stored material under optimal conditions. At 90 DAT, collar diameter ranged from 0.064 cm (1 January) to 0.120 cm (15th April). The collar diameter at 120 DAT ranged between 0. 071 cm (15th

Table 2. Growth parameters of sandalwood sown at different dates of sowing

Treatments	Height (cm)		Collar diameter (mm)		Number of leaves		Sturdiness quotient (180 DAT)	Volume index (cm^3) (180 DAT)
Date of sowings	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT		
1.1.21	4.13	9.20	0.64	1.21	7.00	14.0	7.60	0.01
15.1.21	5.38	10.00	0.71	1.19	5.00	14.0	8.30	0.15
1.2.21	6.50	12.80	0.74	1.37	6.00	13.0	9.40	0.24
15.2.21	6.38	15.50	1.00	1.63	9.00	13.1	9.50	0.41
1.3.21	6.88	19.50	1.08	1.77	10.0	14.2	11.00	0.62
15.3.21	9.63	20.10	1.13	1.83	16.0	13.1	11.10	0.67
1.4.21	11.38	21.40	1.15	2.09	10.0	19.4	10.40	0.95
15.4.21	11.13	22.50	1.20	2.23	13.0	15.1	10.30	1.15
1.5.21	9.75	19.50	1.08	1.95	12.0	17.0	10.30	0.76
15.5.21	8.88	19.40	1.04	1.82	10.0	14.0	10.60	0.65
1.6.21	5.00	11.40	0.74	1.50	5.00	14.0	7.60	0.26
15.6.21	6.00	12.30	1.02	1.38	12.0	12.0	8.90	0.23
1.7.21	8.75	13.40	1.04	1.53	10.0	13.2	8.80	0.32
15.7.21	10.13	15.00	1.04	1.67	14.00	14.1	9.00	0.42
1.8.21	9.00	16.00	1.09	1.74	11.00	14.1	9.20	0.49
15.8.21	10.00	19.00	1.07	1.89	11.00	14.0	10.1	0.69
1.9.21	9.38	21.00	1.06	1.94	12.00	13.3	10.8	0.80
15.9.21	8.75	18.50	1.03	1.70	10.00	13.1	10.9	0.54
1.10.21	7.50	13.80	1.08	1.51	6.00	13.4	9.10	0.32
15.10.21	6.25	10.50	1.02	1.60	11.00	10.2	6.60	0.27
1.11.21	5.75	9.50	0.92	0.99	10.00	10.1	9.60	0.09
15.11.21	4.75	7.10	0.68	0.93	5.00	9.00	7.70	0.06
1.12.21	3.75	6.00	0.68	0.74	5.00	10.2	8.10	0.03
15.12.21	2.38	4.00	0.67	0.73	5.00	9.00	5.50	0.02
CD (p=0.05)	0.74	1.30	0.06	0.17	1.12	2.48	1.00	0.16

December) and 0.163 cm (15 March) followed by 150 and 90 DAT. The collar diameter was significantly low at 180 DAT, ranging from 0.07 cm (15 December) to 0.22 cm (15 April). Collar diameter results showed conformity with Chakarborty et al. (2021). The number of leaves also showed a statistically significant difference, fully opened leaves were counted as one, The number of leaves at were maximum at 180 DAT and ranged from 9 (November and December) to 19 (1st April) followed by 120 and 150 DAT.

The sowing period from 15th March to 1st May, August and September showed higher plant height, collar diameter and number of leaves compared to other months. Overall, superior plant heights of 22.5 cm and collar diameters of 0.22 cm were recorded for 15 April sown seeds at 180 days after transplanting, and lowest plant heights of 4 cm and collar diameters of 0.07 cm were observed in the month of December. Observations revealed that winter seed sowings produce low-quality seedlings and require more maintenance and protection during the harsh winter. Likewise, Krishnakumar et al. (2018) reported that the existence of variability in growth parameters was due to variability in weather and climatic conditions. The length of the growing season might be the core factor that affects maximum vegetative growth because a longer growing season provides more time for nutrient accumulation than a shorter growing season. The best sowing period is one that produces the healthiest nursery within the constraints of the local environment (Doddabasawa et al., 2021). It is evident from the data that all the germination parameters were statistically significant across different dates of sowing. This may be due to variations in weather and climate throughout the year.

Plant quality is a challenging parameter to characterize, and can be measured by morphological, physiological, and performance factors. The SQ and VI were used to evaluate seedling survival rate and growth performance Statistically significant results were obtained among treatments, at 180 days after transplanting, the highest SQ (11.1) was in March, which is statistically at par with April , May , 15th August, and September sown seeds. Large SQ ratios are often found in densely planted seeds on beds, which usually grow tall and slender (Huang et al., 2012). However, a smaller SQ ratio indicates a sturdy plant of better quality among container-grown seedlings. Apart from this, at 180 days after transplanting, the VI ranged from 0.013 to 1.153 cm^3. The highest volume index (1.153 cm^3) was also observed on April 15, which was statistically equal to April 1 (0.948 cm^3), followed by September and March sown seeds. SQ and VI measurements of seedlings in nurseries have a positive bearing on establishment in plantations (Krishnakumar et al., 2018).

CONCLUSIONS

Sowing period 15th March to end April, August and September were the best with regards to germination and growth parameters. The environmental conditions that prevailed during these months provided optimal conditions for reserve nutrient availability, which further helped in seedling establishment. The months of October to February, as well as June and July, were not ideal for seedling growth and germination behavior. However, the 15th of April sowing date outperformed the others.

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AUTHORS CONTRIBUTION

All authors made substantial contributions to the work: conception and design of the study .RIS Gill & B Singh: acquisition, analysis and interpretation of data; N Kaur & H Kaur: drafting of the manuscript; B Singh & A Singh: critical revision and improvement of the draft .

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Marketing of Non-Timber Forest Products: Comparative Analysis of Organized Large-sized Agricultural Multipurpose Societies and Unorganized Channels in Karnataka

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Abstract: Non-Timber Forest Products (NTFPs) play a crucial role in rural livelihoods by providing food and nutritional security and supplementary income, particularly for tribal and forest-dependent households which accounts for nearly 71.23% of total forest sector revenue in India and contribute significantly to the subsistence economy in tribal regions. The three NTFPs reveal that honey the consumer price was highest under large-sized agricultural multipurpose societies (LAMPs) being ₹633.60 and lowest under UO-I (₹399.00). The PSCR was highest in UO-I (72.68 %) and lowest in LAMPs (45.77%). The marketing efficiency declined from 2.66 in UO-I to 0.84 in LAMPs. In tamarind, the producer share in consumer rupee (PSCR) decreased from 71.43% in UO-I (Unorganised Channel-I) to 40.23% in UO-IV with marketing efficiency from 3.68 to 0.94 respectively. Similarly the lichens, PSCR declined from 91.07% in UO-I to 56.54% in Unorganised Channel IV UO-IV with marketing efficiency from 21.25 to 1.72, respectively. The findings suggest that organized channels enhance market outreach; unorganized channels remain more profitable for collectors. Thus strengthening the LAMPS through transparency, timely payments, and reduction in bureaucratic delays is essential to make organized channels more attractive and to ensure equitable benefit-sharing among forest-dependent communities in the study area.

Keywords: LAMPs, Organised, Unorganised, Marketing, NTFPs

The non-timber forest products (NTFPs) have been used since time immemorial for food, fodder, fiber, medicine, fuel, and cultural purposes, forming an integral part of rural subsistence and livelihood security moreover the households involved in NTFP collection and processing reported enhanced income stability and reduced dependence on agricultural or wage labour and NTFPs are not only ecological assets but also critical economic and social capital for forest-dependent communities (Akomaning 2023, Basavaraj and Akash 2025). These are rich in nutritional value and act as vital supplements during food scarcity (Khan et al., 2024). The sector contributes significantly to employment, with 7.5 million people particularly women engaged annually (TERI 2018). Importantly, NTFPs can substantially contribute to household incomes and poverty alleviation in forest-fringe communities and also extracted sustainably, ensuring biodiversity conservation and ecological balance (Asamoah et al., 2025).

The forestry sector contributes 1.90% to India's GDP (2022–23), of which NTFPs account for 71.23% of total forest sector revenue (Planning Commission, 2011) with annual trade of ₹6,000–10,000 crores (UNDP 2019). At disaggregated level, NTFPs constitute about 20 to 40 per cent of the rural GDP in tribal regions (World Bank, 2006) moreover they are significant for 1/3rd of the tribal population. The Karnataka state accounts the total number of tribal people is about 42, 48,978 which is 6.95 per cent of the total population of the State.

Recent studies show that commercialization of non-timber forest products often leads to inequitable benefit sharing tribal or marginal collectors tend to receive disproportionately low returns due to market intermediaries, lack of bargaining power, poor access to infrastructure, and absence of fair price mechanisms (Abhishek and Parayil 2024, Sahu et al., 2025).

In India, the marketing channels critically determine NTFPs collector's benefits and their livelihood. Accordingly, the government initiative to provide institutional support for tribal development and NTFP marketing. The Large-sized Agricultural Multipurpose Societies (LAMPS) were established in 1970s to streamline NTFPs collection, aggregation, and value addition, ensuring fair returns to tribal collectors. They were designed to function as large cooperative societies at the block level, with the objectives of providing credit, marketing facilities, supply of essential commodities, and ensuring fair price procurement of NTFPs from tribal gatherers. In the same line, Karnataka state, LAMPS have established in the tribal dominated districts such as Chamarajanagar, Kodagu, and Uttar Kannada during the 1970s, under the supervision of the Department of Cooperation and Tribal Welfare. However, the issues like bureaucratic delays, lack of transparency, and weak institutional capacity reduce their effectiveness. As consequences of this, many collectors in the region often prefer unorganized channels due to immediate cash payments, absence of quality grading, low transaction costs, and easy accessibility. Therefore, thus assessing the comparative assessment of organized market (LAMPS) unorganized marketing channels for NTFPs in terms of price spread, marketing efficiency, and collector's share in the consumer's rupee. The systematic evaluation of both channels is necessary to understand which one provides greater economic benefits to collectors the insights of the study helps the policymakers and development agencies design interventions to strengthen organized channels and make them more attractive to collectors, thereby improving livelihoods and reducing market exploitation. Nevertheless, the previous studies have mainly focused on ecological, nutritional, and livelihood roles of NTFPs however the limited work has been carried out on marketing efficiency between the organized (LAMPS) and the unorganized channels, particularly in tribal dominated regions. This study considered three important NTFPs namely tamarind, honey, and lichens in Chamarajanagar district where forests form a critical part of tribal and rural livelihoods. The honey is one of the most important NTFPs in this region with a production of about 20 tonnes in a season. The lichens, locally known as *Paasi*, form another important NTFP, have documented as many as 97 species of lichens across 47 genera and 25 families, indicating their ecological richness and livelihood potential. The tamarind (*Tamarindus indica*) is equally significant in the district's forest economy collected both from wild stands and from naturally growing trees in forested and semi-forested areas. These NTFPs (tamarind, honey, and lichens) represent the nutritional, ecological, and economic dimensions of NTFPs in Chamarajanagar district.

MATERIAL AND METHODS

Study area: The present study is confined to Chamarajanagara districts with territorial jurisdiction of BR Hills and Male Mahadeshwara forest divisions. The Chamarajanagara district has been purposefully selected for the study because of good concentrations of the forest cover with 2791.60sq. km accounts of about 49.10 percent to the total geographical area of the district.

Selection of market functionaries: The preliminary inquiry has made to prepare a list of marketing functionaries including collectors, village trader, wholesaler, retailers and processors who were operating in the marketing of NTFPs particularly honey, tamarind and lichens. Thus, a total 60 marketing functionaries under the unorganized marketing channel and 60 functionaries who area depends on organized marketing channel governed by LAMPs totally 120 marketing functionaries were selected for the present study. In order to obtain the relevant information on marketing of NTFPs for both organised and four unorganised marketing channel. The price spread, total marketing cost, marketing margin, marketing efficiency, and collectors share in consumer rupee were all computed with help of collected information. Out of these the best channel was identified and based on collector's shares in consumer rupees, market efficiency, market margin and price spread in marketing of NTFPs. The following marketing channels (Unorganised and organised) were identified in the study.

1. Unorganised Channel-I (UO –I): NTFPs collectors - consumers
2. Unorganised Channel II (UO-II): NTFPs Collector - wholesaler - processors –consumers
3. Unorganised Channel III (UO-III): NTFPs collector - Wholesaler - processors - retailers – consumer
4. Unorganised Channel IV (UO-IV): NTFPs Collector – Village agents – Wholesalers- Processor- Retailer – consumer
5. Organised Marketing channel (LAMPS)
6. NTFPs Collector – Registered agents – LAMPs- Traders- Processor – Retailer – Consumers

Analytical Tools

Marketing cost: The total cost incurred on marketing by the collector's and of the various intermediaries involved in marketing of NTFPs.

TC = PC + ∑MCi

Where,

TC = Total Cost

PC = Cost incurred on marketing of the NTFPs by the collector

MCi = Cost incurred by the ith middlemen

Price spread: The difference between the price paid by consumer and price received by the collector's is the marketing margin or price spread. Generally, the economic efficiency of marketing system is measured in terms of price spread. Smaller the price spread greater is the efficiency of the market system.

$$Price\ spread = \frac{(Consumer\ price - Net\ price\ recieved\ by\ product)}{Consumer\ price} X\ 100$$

Collector's share in consumer's rupee (CSCR)

$$\mathrm{PS} = \frac{FP}{RP}\ X\ 100$$

Where,

CS= Collector's share in consumer rupee

FP= price received by the farmer per unit of output

RP= Retail price per unit of output

Marketing efficiency (ME) of market channel: The marketing efficiency of the market channels in the present study was computed by using Acharya's method (Acharya and Agarwal 2020).

$$ME = \frac{RP}{MC + MM} - 1$$

Where;

ME= Marketing efficiency

RP= Retailer's Price

MC= Total marketing cost

MM= Total marketing margins

RESULTS AND DISCUSSION

Marketing channel-wise performance indicators of honey: The consumer price was highest in the LAMPs channel (₹633.60), followed by UO-IV, while the lowest was in UO-I (₹399.00) (Table 1). The price spread varied considerably across channels, ranging from ₹109.00 in UO-I to ₹343.60 in LAMPs. The producer's share in consumer rupee (PSCR) was relatively higher in UO-I (72.68%), indicating that collectors received a greater share in the direct unorganised channel, whereas the lowest PSCR was in LAMPs (45.77%). Consequently, marketing efficiency (ME) was highest in UO-I (2.66) and the lowest in LAMPs (0.84). These findings suggest that unorganised Channel-I was more efficient in terms of collectors' returns, while organised marketing through LAMPs ensured higher consumer prices however lowest share for the NTFPs collectors. LAMPs enhanced consumer access and market penetration, were unable to translate benefits into higher profitability for collectors because of higher price spread with Rs 343.60 per kg as against of 109.00 in the UO-I.

Marketing Channel–wise performance of Tamarind: The consumer price was highest in the LAMPs channel (₹92.00), followed by UO-IV (₹87.00), while the lowest was observed in UO-I (₹49.00) (Table 2). The price spread varied considerably across channels, ranged from ₹9.50 in UO-I to ₹38.45 in UO-III. The Producer's Share in Consumer Rupee (PSCR) was relatively higher in UO-I (71.43%), indicating that collectors received a greater share in the direct unorganised channel, whereas the lowest PSCR was in UO-IV (40.23%). Consequently, marketing efficiency (ME) was highest in UO-I (3.68) and the lowest in UO-III (0.94). These findings suggest that unorganised Channel-I was more efficient in terms of collectors' returns, while organised marketing through LAMPs ensured higher consumer prices, however with a lower share for the NTFPs collectors. LAMPs enhanced consumer access and market penetration were unable to translate benefits into higher profitability for collectors because of a higher price spread of ₹36.88 per kg as against ₹9.50 in UO-I.

The performance of lichens marketing channels is presented in Table 3. The consumer price was found to be the highest in the LAMPs channel (₹490.00), followed by UO-IV, while the lowest was in UO-I (₹280.00). The price spread varied considerably across channels, ranging from ₹12.00 in UO-I to ₹148.25 in UO-IV. The Producer's Share in Consumer Rupee (PSCR) was relatively higher in UO-I (91.07%), indicating that collectors received a greater share in the direct unorganised channel, whereas the lowest PSCR was in UO-IV (56.54%). Consequently, Average Absolute Marketing Efficiency (AAME) was found to be the highest in UO-I (21.25) and the lowest in UO-IV (1.72). These findings suggest that unorganised Channel-I was more efficient in

Table 1. Marketing channel-wise performance indicators of honey

Channel	Consumer price (₹)	Price spread (₹)	PSCR (%)	Total MC (₹)	ME
UO–I	399.00	109.00	72.68	22.00	2.66
UO–II	527.74	237.74	54.95	83.80	1.22
UO–III	609.31	319.31	47.59	103.80	0.91
UO–IV	617.80	327.80	46.94	103.81	0.88
LAMPs	633.60	343.60	45.77	111.70	0.84

Table 2. Marketing channel-wise performance of Tamarind

Channel	Consumer price (₹)	Price spread (₹)	PSCR (%)	Total MC (₹)	ME
UO–I	49.00	9.50	71.43	4.50	3.68
UO–III	85.00	38.45	42.35	10.55	0.94
UO–II	79.00	32.25	46.84	9.75	1.15
UO–IV	87.00	36.36	40.23	10.24	0.96
LAMPs	92.00	36.88	43.48	10.82	1.08

Table 3. Marketing Channel-wise performance of Lichens

Channel	Consumer price (₹)	Price spread (₹)	PSCR (%)	Total MC (₹)	ME
UO−I	280.00	12.00	91.07	13.00	21.25
UO−III	440.00	138.98	57.95	46.02	1.83
UO−II	424.00	136.50	60.14	32.50	1.87
UO−IV	451.00	148.25	56.54	47.75	1.72
LAMPs	490.00	79.50	59.18	63.50	3.65

terms of collectors' returns, while organised marketing through LAMPs ensured higher consumer prices, however with a reduced share for the NTFPs collectors. LAMPs enhanced consumer access and market penetration, but were unable to translate benefits into higher profitability for collectors because of a higher price spread of ₹79.50 per kg as against ₹12.00 in UO-I.

The UO-I (direct unorganised channel) was the most beneficial to NTFPs collectors across selected commodities, ensuring both a higher producer's share and marketing efficiency. In the flip side, the LAMPs enhanced the consumer access and organised trade**,** but the benefits did not fully reach collectors because of disproportionately high price spreads. Thus, LAMPs serve as an important organised marketing platform for Non-Timber Forest Products in terms of market penetration and consumer outreach however fails to reduce marketing margins and strengthen collectors' share in consumer rupee.

The direct unorganised channels (UO-I) consistently ensured a higher share of the consumer rupee and greater marketing efficiency for collectors, while organised channels such as LAMPs, higher consumer prices and market penetration, translated into reduced profitability for the primary collectors due to wider price spreads. However, descriptive statistics alone cannot confirm whether these differences are statistically meaningful.

CONCLUSION

The study highlights the contrasting outcomes of organized and unorganized marketing channels of NTFPs in Chamarajanagar district of the selected commodities tamarind, honey, and lichens. The study assessed the marketing performance of honey, tamarind, and lichens across organised and unorganised marketing channels. The analysis revealed that the direct unorganised marketing channel provided highest producer share in consumer rupee and marketing efficiency for collectors as compared to LAMPs. Study concludes that reforms in organized marketing structures are dire need policy interventions should focus on reducing transaction costs, introducing fair grading practices, ensuring prompt payment, and promoting value addition at the local level.

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Ecosystem Services and Financial Benefits from Urban Green Spaces using The i-Tree Eco Model: A Case Study of *Anandabana*, Bhubaneswar

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***Abstract*:** This study evaluated ecosystem services offered by *Anandabana* (Urban green space), a curated urban forest in Bhubaneswar, Odisha, using the i-Tree Eco model developed by the U.S. Forest Service. The study aimed to quantify carbon storage and sequestration provided by prominent tree species within the park and to assess the applicability of the i-Tree Eco model in an Indian urban context. Field data were collected from 484 individual trees of *Acacia auriculiformis, Adenathera pavonina, Alstonia scholaris, Bombax ceiba, Delonix regia, Lagerstroemia speciose, Simarouba glauca* and *Sterculia foetida*. The data on diameter at breast height (DBH), total height, crown spread, and health indicators were processed using the i-Tree Eco model. Study revealed that *Anandabana* stored approximately 44.48 tons of carbon, valued at ₹16.38 lakhs, and sequestered about 8.63 tons of carbon annually, equating to ₹3.18 lakhs per year. Avoided stormwater runoff was also quantified and it was 123.76 l/tree/year with financial gain of Rs. 11,235.81 from the all species. This study highlights both the strengths and limitations of using i-Tree Eco model in urban ecosystems. This offers a rapid and replicable approach for ecosystem service valuation, limitations arise owing to its default parameters, necessitating local calibration for greater accuracy. Despite challenges, this tool could be used to provide baseline data for urban forest management, climate resilience planning, and policy advocacy.

Keywords: Ecosystem services, i-Tree Eco Model, Urban green spaces, Carbon sequestration

Quantifying the value of ecosystem services is vital for highlighting the concrete benefits derived from natural ecosystems and providing justification for financial investments in their establishment and management. This approach is widely acknowledged as critical in environmental economics and policy development, especially when ecosystem services such as carbon storage, air and water filtration, climate regulation, and recreational opportunities lack direct market pricing (Thakur et al., 2011, Attar et al., 2016, Panwar et al., 2022). By assigning monetary value to these services, decision-makers and stakeholders can more effectively evaluate the returns on investments in conserving natural ecosystems and creating protected areas (TEEB 2010). This rationale extends equally to semi –natural ecosystems and curated green spaces in urban settings, such as parks, botanical gardens, green roofs, and landscaped avenues, where significant public and private resources are invested. Although these spaces are not strictly natural, they provide substantial ecosystem services that contribute to human well-being, urban climate resilience, and biodiversity enhancement (Gomez-Baggethun and Barton 2013, Bhusara et al., 2016, Panchal et al., 2017).

According to Millennium Ecosystem Assessment (2005) Ecosystem services are classified into four categories of provisioning, regulating, cultural and supporting services. Though some researchers opine that this classification denotes ecosystem processes for achieving services and the services themselves (Wallace 2007), this is most popularly used classification of ecosystem services. Climate regulation is a regulating service which encompasses several ecological processes to atmospheric composition, the greenhouse effect, the ozone layer, precipitation, air quality, moderation of temperature and weather patterns both at both global and local scales (Costanza et al., 1997). Air pollution mitigation, carbonation, storm water management, urban cooling and water retention remains the most discussed topic among the regulating services of urban ecosystems. There has been a surge in literature on service of the habitat in providing shelter, protection, and nutritional needs of organisms, cultural services and health benefits resulting in overall improvement in the quality of life of citizens in the recent past (Luo and Patuano 2023)

Urban green spaces play a pivotal role in enhancing climate resilience by mitigating the impacts of climate change such as heatwaves and flooding (Kabisch et al., 2016, Hanna et al., 2023). They mitigate the urban heat island effect, reduce local temperatures, and lower the energy costs for cooling (Oberndorfer et al., 2007). They also improve air quality by filtering pollutants and sequestering carbon dioxide (Bolund and Hunhammar 1999, Singkran 2022). They often act as lungs of urban areas and mitigates air pollution (Agbelade and Onyekwelu 2020, Song et al., 2020a,b, Lopez-Lopez et al., 2018). Green roofs and permeable surfaces enhance stormwater management by reducing runoff and improving water quality (Mentens et al., 2006). They contribute to climate resilience by mitigating urban heat islands, with cooling effects up to 7.7°C on surfaces with reductions in energy consumption through insulation and evapotranspiration along with other benefits such as improved air quality (Veerkamp et al., 2021).

Parks and botanical gardens provide recreational opportunities, improve mental health, and foster social cohesion (Chiesura 2004). Access to green spaces has been linked to reduced stress and improved quality of life (Tzoulas et al., 2007). They also support biodiversity by providing habitats for birds, insects, and other species, thereby contributing to ecological connectivity in fragmented urban landscapes (Kong et al., 2010). Enhancing understorey vegetation cover and incorporating native plants increased the species occupancy of birds, bats, and insects in urban green spaces (Threlfall et al., 2017). Carbon sequestration by trees depends on their type, size, health, where they grow, and the biomass generated through physiological transformation. Unlike its natural habitats, carbon sequestration may not be as predictable in cities where site factors vary greatly because of space constraints, poor soil conditions mainly due to compaction with hard pavements, air pollution, and anthropogenic interferences (Nowak et al., 2008). However, there is lack of standardised simple methods to quantify these services and deficiency of data that could be effectively put to use by policy makers and urban ecosystem managers.

There are direct and indirect methods to quantify these valuable services. The i-Tree Eco model, is a scientifically validated and widely used tool for quantifying the structure of urban forests and ecosystem services, such as carbon storage and sequestration (Nowak and Crane 2000, Nowak et al., 2016, Zhou et al., 2021, Davies et al., 2023, Zhang et al., 2024). Systematic evaluations of curated green spaces through methods such as modelling tools (e.g., i-Tree Eco) can substantiate their value and promote their strategic integration into urban planning frameworks. The present study focused on evaluating the ecosystem services, particularly carbon storage and sequestration, provided by trees in a green space within the urban area of Bhubaneswar, using the i-Tree tool application.

MATERIAL AND METHODS

Study area: *Anandabana*, is a planned semi-natural green area created in 2020 as part of the "Nagar Van Yojana" initiative of Ministry of Environment, Forest and Climate Change, Government of India, to promote a healthy and wholesome living environment and to help the nation's cities become cleaner, greener, healthier, and more sustainable. It is located in the western part of the Bhubaneswar city, in the east coastal plain of Odisha, India. It has tropical monsoon type of climate with average annual temperature of 27 °C and annual rainfall of 1450 mm. *Anandabana* is a lush green space spanning over an area of 89.50 acres, located to the North-west of the city (Fig. 1).

Species selection and data collection: The area under study has several species of various ages, planted while curating the space and several others that were already present as the area was earlier a part of an arboraceous

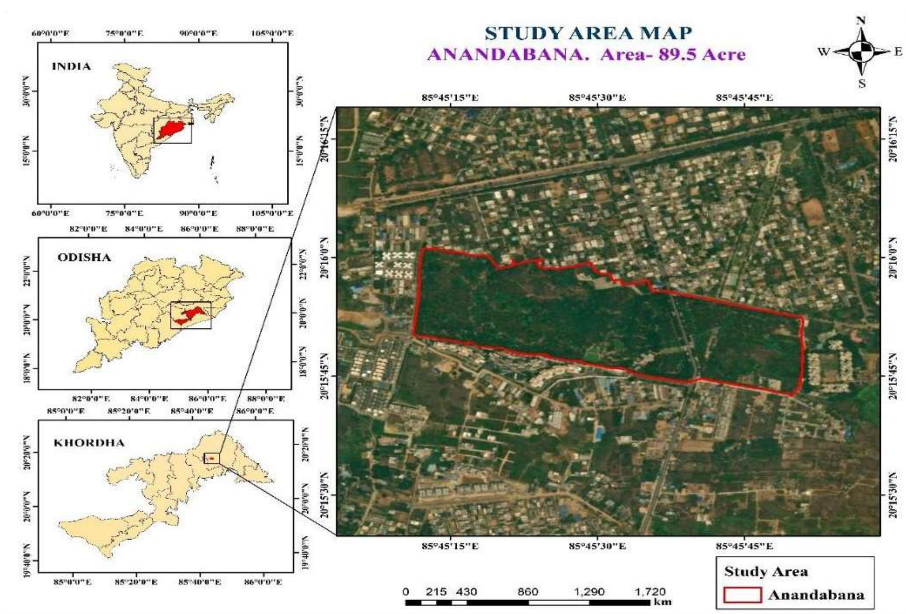

Fig. 1. Study site *Ananadabana*

vegetation. A transect survey was undertaken to document all the tree species. Eight tree species whose scientific names could be validated against the tree data set in the i-tree database were selected for the study. These species were among the ten most prominent tree species in the study area and were available in the presets of the i-Tree Eco software. The species selected and the number of trees considered for assessment are shown in Figure 2.

A total of 484 trees of the eight species were present in the area under study. The parameters (default for the software) that were required for the assessment of carbon and avoided runoff *viz*., Girth at Breast Height (GBH)/Diameter at Breast Height (DBH), height, crown width, crown length, crown light exposure, health of the trees of the eight species tallied were measured using standard methods and recorded.

Software model used for assessment: The software used was i-Tree eco v6.0.38, a peer-reviewed software suite of USDA Forest Service that provides urban and community forestry analysis and assessments (*itreetools.org*).

RESULTS AND DISCUSSION

The studied species showed considerable variation in their C storage and C sequestration potential, indicating that the tool could not only be employed for a quick understanding of the value of ecosystem services, but also for a deeper understanding of the C sequestration and storage potential of the species. A total of 44.48 tons of carbon**,** generating over ₹16.37 lakhs was assessed to have been sequestered from the data provided (Table 1). S*imarouba glauca* contributed roughly 40% of the total carbon and approximately 40% of the total revenue**,** despite being less than 20% of the trees in number. S. *glauca* trees of diameter 10–20 cm reportedly produced ~ 57.80 kg of biomass per tree, more than five-year-old tree plantation which stored 2.73 t of C/ha (Mohamed et al., 2016, Anil 2009). Although per-ton profitability seems uniform (₹/ton is constant), species with higher total carbon yielded more revenue (Table 1). *B. ceiba***,** stored only 1.06 t, bringing in proportionally less financial gain (₹39,195). *A. auriculiformis* also showed low carbon performance (~0.02 t/tree) in this study. The annual financial gain amounted to ₹3,17,904, and the total C sequestered by 484 trees was 8.63 tonnes /year (Table 2). *S. glauca,* despite being just 20% of the total trees, contributed to approximately 32% of the total carbon sequestered and 32% of the total estimated revenue.

The collected data also provided estimates of the avoided runoff in litres and the resultant financial gains from the avoided runoff (Table 3). The economic evaluation of storm water runoff avoidance provided by various tree species in an urban green space summed up to ₹11,235 per year. The trees in all avoided runoff of 59897.97 litres per year, equivalent to approximately 60 cubic meters of water saved annually (Table 3). *A. pavonina* contributed the most (15,297.57 l/yr), despite having only 57 trees, owing to its high per-tree efficiency. *D. regia* had the highest per-tree value (284.99 l/tree/year), indicating that it is highly effective for in front of runoff prevention on a per-tree basis. *A. auriculiformis* had a lower per-tree rate (31.03 l/tree/year), but its large numbers made it a significant contributor overall. The total average across all trees was 123.76 l/tree/year as indicated by the results of this study.

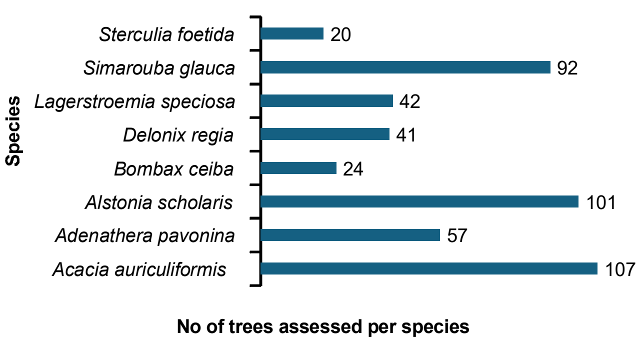

Fig. 2. Species and number of trees assessed

Table 1. Total carbon stored and estimated financial gain

Species	No of trees	Total C stored (tons)	Stored C/tree (Kg)	Estimated financial gain (₹)*
Acacia auriculiformis	107	2.03	18.97	74,784
Adenathera pavonina	57	6.75	118.42	2,48,532
Alstonia scholaris	101	5.35	52.97	1,96,837
Bombax ceiba	24	1.06	44.17	39,195
Delonix regia	41	4.82	117.56	1,77,384
Lagerstroemia speciosa	42	5.32	126.67	1,95,843
Simarouba glauca	92	17.64	191.74	6,49,707
Sterculia foetida	20	1.51	75.50	55,522
Total	484	44.48		16,37,808

*The values have been provided by the i-Tree tool, the exchange value for C is estimated at ₹200/ton, which is the international standard as taken for assessment by the i -Tree model

Several studies have indicated the use of i-tree tools coupled with other remote sensing methods for holistic and reliable estimation of both natural and curated spaces (Prigioniero et al., 2022, Sharma et al., 2024, Sharma et al., 2025). Analysis of the available data revealed comparable carbon sequestration rates for *A. auriculiformis*, but notable variations were observed for *A. scholaris*, *Bombax ceiba*, and *D. regia* (Table 4). *L. speciosa* demonstrated consistently

Table 2. Total C sequestered and estimated financial gain per year

Species	No. of trees	Total C sequestered (tons/year)	Sequestered C/tree/Year (Kg)	Estimated financial gain (₹/yr)*
Acacia auriculiformis	107	0.59	5.514	21,814
Adenathera pavonina	57	1.66	29.12	61,293
Alstonia scholaris	101	1.12	11.09	41,145
Bombax ceiba	24	0.25	10.42	9,110
Delonix regia	41	0.87	21.22	31,954
Lagerstroemia speciosa	42	1.12	26.67	41,097
Simarouba glauca	92	2.76	30.00	1,01,557
Sterculia foetida	20	0.27	13.50	9,930
Total	484	8.63		3,17,904

*The values have been provided by the i-Tree tool, the exchange value for C is estimated at Rs. 200/ton, which is the international standard as taken for assessment by the i -Tree model

Table 3. Avoided run-off by species under study and the estimated financial gain

Species	No. of trees	Avoided runoff (l/yr)	Avoided runoff (l/tree/year)	Estimated financial gain (Rs/Yr)
Acacia auriculiformis	107	3319.82	31.03	622.74
Adenathera pavonina	57	15297.57	268.38	2869.55
Alstonia scholaris	101	10736.23	106.30	2013.93
Bombax ceiba	24	2107.60	87.82	395.35
Delonix regia	41	11684.41	284.99	2191.79
Lagerstroemia speciosa	42	4088.97	97.36	767.02
Simarouba glauca	92	9705.08	105.49	1820.5
Sterculia foetida	20	2958.32	147.92	554.93
Total	484	59897.97	123.76	11,235.81

Table 4. Comparative review on carbon sequestered reported earlier case studies and present study

Name of the species	DBH (cm)	Height (m)	Carbon stored kg/tree	Carbon Sequestered kg/tree/year	Reference
Acacia auriculiformis	14.83	4.84	18.97	5.51	Current study
Acacia auriculiformis	Not specified	Not specified	18.60	7.77	Sharma et al. (2021)
Alstonia scholaris	17.14	6.36	52.97	11.09	Current study
Alstonia scholaris	Not specified	Not specified	55.36	55.27	Dadhich et al. (2023)
Bombax ceiba	18.11	5.82	44.17	10.42	Current study
Bombax ceiba	241.15	14.26	436.2	1599.00	Korra Simhadri et al. (2016)
Delonix regia	23.31	9.67	117.56	21.22	Current study
Delonix regia	270	16.09	520.41	1908.00	Korra Simhadri et al. (2016)
Lagerstroemia speciosa	19.73	6.25	126.67	26.67	Current study
Lagerstroemia speciosa	Not specified	Not specified	19.10	19.07	Sharma et al. (2021)
Sterculia foetida	19.17	8.32	75.50	13.50	Current study
Sterculia foetida	80.89	Not specified	71.00	5.92	Amir et al. (2024)

high carbon sequestration across different locations in India, with rates of 26.67 kg/tree/year and 19.07 kg/tree/year elsewhere, suggesting its reliability in urban planting initiatives. *S. foetida* exhibited moderate sequestration of 13.5 kg/tree/year in *Anandabana*. Based on the findings of this study, both *L. speciosa* and *A. scholaris* emerged as dependable and effective choices for urban carbon sequestration programs.

The comparisons presented here serve as illustrative examples of carbon sequestration estimates for the studied species and should not be used to evaluate the superiority of any estimation method, as site-specific conditions significantly influence tree growth and sequestration outcomes. Reports of avenue trees storing approximately 1016.15 metric tons of carbon, with an annual carbon sequestration of 25.69 tons using i-tree tools (Watson and Bhai 2025) indicates an acceptance of this tool as a feasible option for qualification of ecosystem services in Indian context and its use in air pollution management strategies in urbanized regions (Vashist et al., 2024).

CONCLUSION

The i-Tree Eco tool offers a rapid and effective approach for assessing carbon sequestration and storage in urban and managed forests, delivering both biophysical estimates and economic value of these services with limited inputs. Study indicated that *Anandabana* stored approximately 44.48 tons of carbon, valued at ₹16.38 lakhs, and sequestered about 8.63 tons of carbon annually, equating to ₹3.18 lakhs per year. Avoided storm water runoff was also quantified and it was 123.76 l/tree/year with financial gain of ₹11,235.81 from the all species. While i-Tree Eco model offers a rapid and replicable approach for ecosystem service valuation, limitations arise owing to its default parameters, necessitating local calibration for greater accuracy. Despite challenges, this tool could be used to provide baseline data for urban forest management, climate resilience planning, and policy advocacy. This study has examined only the explorative aspect where the feasibility of an available information was analysed in a local context and would reiterate the tools wider applicability for several other services.

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Sanchit Mohanty, Keshaba Sahoo, Satyajit Nayak, Krishanlal Paramanik, were involved in the project work. T. L. Mohanty, H. Nayak, M. C. Behera, Smitha G Nair provided research inputs for formulation of the project.

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Shrub Diversity and Distribution in High Altitude Forested Zone of Indian Western Himalaya

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***Abstract*:** The high altitudes of the Himalayas are the hotspots of the biodiversity. The forests of these zones are having the unique elements of species and vast varieties of ecologically, socially and economically important vegetation. The present study was conducted in the high altitude forested zone of Kedarnath Wildlife Sanctuary Western Himalaya. The altitude range from 2000 m asl. to 3500 m asl. was covered for the extensive sampling of shrub species diversity. The present study recorded 55 species (50 dicots and 05 monocots) from the study site. The species belongs to 18 families and 31 genera in which 43 species recorded as native and 17 species as endemic to the Himalaya. Considering the good numbers of shrub species richness, nativity and endemism into this zone these high altitude forests should be conserved and manage to maintain the unique identity of this zone.

***Keywords*:** High altitude zone, Shrub diversity, Nativity, Endemism, Species richness

Western Himalaya is known for its rugged topography and steep vertical gradient and includes three states of India namely, Jammu and Kashmir, Himachal Pradesh and Uttarakhand and is known for diversity of its forests (Rawal 2021). Due to enchanting, picturesque landscapes this region has attracted ecologists, naturalists and pilgrims since time immemorial, and it has remained a centre of attraction for floristic and ecological studies from centuries. In the context of floristic diversity, Jammu and Kashmir supports about 4000 plant species, of which about 280 are trees, 573 are shrubs and > 3000 are herbs. Himachal Pradesh supports 3400 plant species, of which 323 are trees, 543 shrubs, 2534 are herbs and >500 species of medicinal plants (Samant et al., 2013), while Uttarakhand supports over 5000 species of Angiosperms, of which 538 are trees, 900 are shrubs and > 4500 are herbs (Samant 2015).

Trees at the high altitudes make a conspicuous vegetation boundary and because of heat deficiency (mainly), fail to grow beyond a certain elevation, resulting in a “physiognomic discontinnum”, characterized by the separation of forests from treeless alpine meadows (Singh 2018). These transition zones have vast bio-geographic importance with a wide climatic, ecological and socio-economic relevance (Callaghan et al., 2002). These zones are the rich pockets of native and endemic species because of sharing the biomes of two distinct physiognomies i.e. forests and alpine grasslands (Dhar 2000). The high alitutde forests have their own specialized elements, as they share the elements of low temperate zones and high alpine zones (Barman et al., 2021). Along the altitudinal gradient in the Western Himalaya, various changes in vegetation compositions are apparent. The sub-tropical sal (*Shorea robusta)* and pine (*Pinus roxburghii*) forests are replaced by broadleaf (Oak–Quercus spp. and mixed broadleaf) and coniferous (*Cedrus deodara, Cupressus torulosa*, etc.) forests in temperate zone. In sub-alpine zone, birch (*Betula utilis*) and fir (*Abies pindrow, A. spectabilis*) forests along with the various combinations of broadleaf species exhibit dominance, which finally give way to the vast areas of alpine meadows (Gairola et al., 2008). The most prominent of these changes along the altitudinal range is represented by the subalpine transition between temperate forests and alpine grassland ecosystems, termed as timberline zone (Dhar 2000). Till date 1471 species of vascular plants have been reported from the high altitude zone (2000-3500 m asl.) of Uttrakhand, which includes 106 trees, 233 shrubs and 1132 herbs. This zone represents 14.0% species, 31.5% genera, and 59.6% family diversity of total reported plant diversity of Himalaya (Rawal et al., 2018). These forests are recognized for their unique conservation values and richness of economically important biodiversity (Gairola et al., 2008). The aim of current study was to assess the species richness of shrubs as provide the current status of the shrub diversity in these high altitude zones.

MATERIAL AND METHODS

Study site: The present study was conducted in the high altitude forested zone of Kedarnath Wildlife Sanctuary (30°30'03.0" to 30°29'23.8" N and 79°09'52.8" to 79°12'42.3" E) covering the altitudinal range from 2000 m asl. to 3500 m asl. in Uttarakhand, Western Himalaya. The forests of the area fall from close canopy forests (temperate) to sub-alpine/timberline and tree line ecotone which latter give way to the alpine meadows (Rawal et al., 2023).

Methods: The extensive survey method was used covering all the seasons from 2016 to 2020 to collect the information from the study site. The plant specimens collected in field were brought to the GBP-NIHE for herbarium preparation and further examination and identification. The herbarium specimens were prepared following standard methods of herbarium preparation (Jain and Rao 1977). The specimens were identified with the help of published flora (Brandis 1906, Hooker 1906; Chowdhery and Wadhwa 1984; Osmaston 1927; Naithani 1984; Gaur 1999 and Rai et al., 2017) and Northern regional centre of Botanical Survey of India (Dehradun). The checklist of study site flora was prepared and voucher specimens were deposited at herbarium of G.B. Pant National Institute of Himalayan Environment (GBP-NIHE). Further, for the nomenclature of the species i.e. updated botanical names and other details, online websites were consulted i.e. Tropicos, The Plant List, eFlora of Pakistan, eFlora of China and Plants of the World Online [POWO]. Information on habit, altitude zones etc. were taken and list of species was updated.

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

RESULTS AND DISCUSSION

The present study recorded 55 shrub species (Table 1) in the high altitude forested zone, which is 23.6% of previously reported species 233 (Rawal et al., 2018) and greater than the reported number 40 (Sekar et al., 2024). Out of total recorded 55 species, 50 were dicots and 5 species were monocots. Total 18 families were recorded in which family Rosaceae was the dominant family with 7 genera and 17 species and Berberidaceae was the co-dominant family with 1 genera and 8 species (Fig. 1). In a study from western Himalaya Mehta et al. (2019) reported Rosacease as a second dominant family. Total 31 genera were recorded in which *Berberis* and *Rubus* were the dominant genera with 7 species, similar observations for genera *Berberis* was also made by Sekar et al. (2024). Considering the nativity and endemism 43 (86%) species were recorded native and 17 (34%) species as endemic. This represents the high nativity and endemism of shrub species in the representative study site, which might be governed by difficult terrain present in the region (Rawal and Tewari 2022). The present study reported a good proportion of shrub species numbers as well as native and endemic species is indication of good ecosystem and health of the forests in such high altitude regions.

CONCLUSION

The high altitude forested zones are the rich pockets of native and endemic species which have high conservation values. The transition from lower close canopy forests to timberline treeline and then the alpine meadows provide the suitable environmental condition and habitat to unique species for their growth and nourishment. The present study highlights shrub species richness, diversity and distribution in this high altitude zone and provide the baseline information for future. Having a good number of shrub species this forests zone need to be regularly monitor and maintain for conservation.

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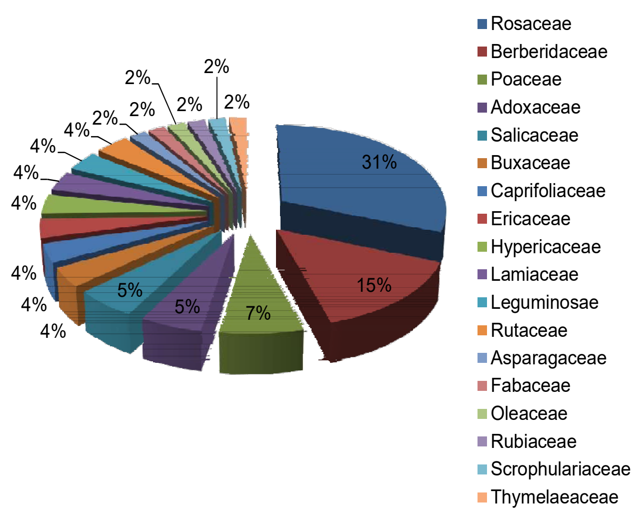

Fig. 1. Species distribution into recorded families of shrubs in the high altitude forested zone

Table 1. Checklist of shrub species recorded in the high altitude forested zone (NE-near endemic, E-endemic).

Botanical name	Family	Altitude zone (m asl.)	Endemism	Nativity	Distribution
Aruncus dioicus subsp. triternatus (Maxim.) Hara	Rosaceae	2800	-	Reg Bor Temp	North America, China, India, Japan
Asparagus filicinus Buch.-Ham ex D.Don.	Asparagaceae	2400	-	Reg Himal Burma	Bhutan, India, Myanmar, Thailand, China
Berberis lycium Royle	Berberidaceae	1600-2400	-	Reg Himal	Kashmir, Pakistan and N. W. Himalayas.
Berberis pseudumbellata R. Parker	Berberidaceae	2100	NE	Reg Himal	Pakistan, West Himalaya
Berberis aristata DC.	Berberidaceae	2600-3500	-	Reg Himal	China South-Central, East Himalaya, India, Nepal, West Himalaya
Berberis asiatica Roxb. ex DC.	Berberidaceae	1200-2400	-	Reg Himal	Afghanistan, Pakistan, Nepal, Bhutan, India
Berberis chitria Buch.-Ham. ex Lindl.	Berberidaceae	2000-3000	-	Nepal	Pakistan, India, Nepal, Bhutan
Berberis jaeschkeana C.K.Schneid.	Berberidaceae	3100	NE	Reg Himal	Pakistan, Tibet, West Himalaya
Berberis kumaonensis C.K.Schneid.	Berberidaceae	3100	NE	Reg Himal	India, Nepal
Buddleja paniculata Wall.	Scrophulariaceae	1700-2700	NE	Reg Himal Burma	Assam, China South-Central, China Southeast, East Himalaya, Myanmar, Nepal, Vietnam
Caragana versicolor Benth.	Leguminosae	2600	-	Reg Himal As Bor	Afghanistan, Pakistan, Nepal, Bhutan, China
Cotoneaster affinis Lindl.	Rosaceae	1500-3500	-	Reg Himal	Bhutan, India, Nepal, China
Cotoneaster bacillaris Wall. ex Lindl.	Rosaceae	2200	NE	Reg Himal	Afghanistan, India, Nepal, Pakistan, China, Bhutan
Cotoneaster microphyllus Wall. ex Lindl.	Rosaceae	2500-3400	-	Reg Himal	Afghanistan, Pakistan, Nepal, Bhutan, India, China
Daphne papyracea Wall. ex Steud.	Thymelaeaceae	1800-3000	NE	Reg Himal	W. Himalayas, Western Nepal, N. Uttar Pradesh, Simla, W. Pakistan
Drepanostachyum falcatum (Nees) Keng f.	Poaceae	1500-2600	-	Reg Himal	India, Nepal
Elsholtzia fruticosa (D.Don) Rehder	Lamiaceae	1500-2500	-	Reg Himal China	Bhutan, India, Nepal, China
Himalayacalamus falconeri (Hook.f. ex Munro) Keng f.	Poaceae	2000-2800	-	Reg Himal	East Himalaya, Nepal, Tibet, West Himalaya
Hypericum choisianum Wall. Ex N. Robson.	Hypericaceae	1200-2500	-	India China	Pakistan, Nepal, Bhutan, China
Hypericum oblongifolium Choisy	Hypericaceae	1500-2500	NE	Reg Himal	Western Himalayas from Kurram to Nepal, Pakistan
Indegofera heterantha Wall. ex Brandis	Fabaceae	1200-2500	-	Reg Himal	Afghanistan, China, Bhutan, India, Nepal, Pakistan, Sri lanka
Jasminum humile L.	Oleaceae	1500	-	As Trop	Afghanistan, China, Tajikistan, Pakistan, Nepal, India
Juniperus communis L.	Cupressaceae	3000-4000	-	Reg Bor Temp et Arct	Afghanistan, Alabama, Alaska, Albania, Algeria, Altay, Amur, Arizona, Austria, Baltic States, Belarus, Belgium, British Columbia, Yakutskiya, Yugoslavia, Yukon etc.
Leptodermis lanceolata Wall.	Rubiaceae	1500-2000	NE	Reg Himal	Afghanistan, Nepal, Pakistan, West Himalaya
Leycesteria Formosa Wall.	Caprifoliaceae	2000-3000	-	Reg Himal	East Himalaya, Myanmar, West Himalaya
Mahonia napaulensis DC.	Berberidaceae	1200-3000	E	Reg Himal China	Bhutan, India, Myanmar, Nepal, China

Cont...

Table 1. Checklist of shrub species recorded in the high altitude forested zone (NE-near endemic, E-endemic).

Botanical name	Family	Altitude zone (m asl.)	Endemism	Nativity	Distribution
Perilla frutescens (L.) Britton	Lamiaceae	800-2400	-	India Or	Bhutan, Cambodia, India, Indonesia (Java), Japan,
Piptanthus nepalensis (Hook.) D.Don	Leguminosae	1600-4000	NE	Reg Himal	Bhutan, India, Kashmir, Nepal, China
Potentilla rigida Wall. ex Lehm.	Rosaceae	2500-4000	-	Reg Bor Temp Ind Or	Widespread
Prinsepia utilis Royle	Rosaceae	1500	-	Reg Himal	Bhutan, N India, Nepal, Pakistan, China
Pyracantha crenulata (Roxb. ex D.Don) M.Roem.	Rosaceae	1500-2500	-	Reg Himal	Bhutan, India, Kashmir, Myanmar, Nepal, China
Rhododendron campanulatum D. Don	Ericaceae	2900-3800	NE	Reg Himal	Bhutan, N India, Kashmir, Nepal, Sikkim, China
Rhododendron lepidotum Wall. ex G. Don	Ericaceae	3000-4000	NE	Reg Himal	Nepal, Bhutan, China, Myanmar
Rosa macrophylla Lindl.	Rosaceae	1500-3500	-	Reg Himal China	Afghanistan, Bhutan, China, Nepal, Pakistan, India
Rosa moschata Herrm.	Rosaceae	2500	-	Oriens	Pakistan, Kashmir, Himalayas to Bhutan, India, Burma, China, SE Asia, Japan. Introduced-naturalized in N. America.
Rosa sericea Wall. ex Lindl.	Rosaceae	3000-4500	-	Reg Himal	Pakistan, India, Bhutan, Myanmar, China
Rubus ellipticus Sm.	Rosaceae	1500	-	India Or	Bhutan, India, Laos, Myanmar, Nepal, Pakistan, Philippines, Sikkim, Sri Lanka, Thailand, Vietnam
Rubus foliolosus D.Don	Rosaceae	1500	-	Reg Himal	India, Nepal
Rubus foliolosus var. *racemosus* (Hook.f.) B.D. Naithani/ Rubus niveus Thunb.	Rosaceae	2200	-	Reg Himal	Western Himalaya
Rubus nepalensis hort.	Rosaceae	2600	NE	Reg Himal	India, Nepal, Bhutan
Rubus niveus Thunb.	Rosaceae	1700	-	Reg Himal	China, Afghanistan, Bhutan, India, Indonesia, Kashmir, Laos, Malaysia, Myanmar, Nepal, Philippines, Sikkim, Sri Lanka, Thailand, Vietnam
Rubus paniculatus Sm.	Rosaceae	1500	-	Reg Himal	Bhutan, N India, Kashmir, Nepal, Sikkim
Rubus macilentus Jacquem. ex Cambess.	Rosaceae	2000-3500	-	Reg Himal	China South-Central, East Himalaya, Nepal, Tibet, West Himalaya
Salix denticulata Andersson	Salicaceae	2500-4000	NE	Reg Himal	Afghanistan, India, Kashmir, Nepal, Pakistan, China
Salix karelinii Turcz. ex Stschegl.	Salicaceae	3500	-	As Centr	Afghanistan, China, Kyrgyzstan, Nepal, Pakistan, Tajikistan
Salix lindleyana Wall. ex Andersson	Salicaceae	3000-4000	-	Reg Himal Amer Bor As et Amer Temp	East Himalaya
Sarcococca coriacea Müll. Arg.	Buxaceae	2100-2900	-	S E As	Assam, China South-Central, East Himalaya, India, Myanmar, Nepal, Sri Lanka, Thailand, Vietnam, West Himalaya
Sarcococca pruniformis Lindl.	Buxaceae	1600-3000	-	Ind Or Malaya	Afghanistan, India, Nepal, Pakistan, China
Skimmia anquetilia N.P. Taylor & Airy Shaw.	Rutaceae	1800-3500	-	Reg Himal	Afghanistan, Pakistan, Nepal, India

Cont...

Table 1. Checklist of shrub species recorded in the high altitude forested zone (NE-near endemic, E-endemic).

Botanical name	Family	Altitude zone (m asl.)	Endemism	Nativity	Distribution
Thamnocalamus spathiflorus (Trin.) Munro	Poaceae	2500-3500	NE	Reg Himal	East Himalaya, Nepal, Tibet, West Himalaya
Viburnum cotinifolium D.Don	Adoxaceae	2800	-	Reg Himal Amer Bor	Afghanistan, China, India, Nepal
Viburnum grandiflorum Wall. ex DC.	Adoxaceae	3050	NE	Reg Himal	Bhutan, China, India, Nepal, Pakistan
Viburnum mullaha Buch.-Ham ex D.Don	Adoxaceae	2500	-	Reg Himal	Bhutan, China, India, Nepal
Yushania anceps (Mitford)W.C. Lin.	Poaceae	2400-2800	E	Reg Himal	E. Asia - N.W. Himalayas
Zanthoxylum armatum DC.	Rutaceae	1500	-	Reg Himal China	Himalayas, from Swat to Bhutan, Khasia Hills; Japan, Korea, China, Pakistan

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Genotypic and Phenotypic Association Studies in *Melia composita* Benth. Progenies Grown in Different Agroclimatic Conditions in Punjab

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***Abstract*:** The present study was conducted on 5-year-old progenies of *Melia composita* Benth. in 2022 to assess the association among traits under two different climatic conditions in Punjab, where the central plain zone (Ludhiana) is an irrigated plain and the western plain zone (Bathinda) is a semiarid region. Significant variation among progenies was observed for all growth parameters. The genotypic correlation coefficients for all traits were higher than the phenotypic correlation coefficients at both sites, indicating that there were inherent associations between the traits. Volume showed a highly significant positive correlation with almost all traits at both genotypic and phenotypic levels, with the exception of the number of branches per meter of crown length and crown length. Path analysis revealed that diameter at breast height was the most important trait due to its highest positive direct and indirect effects on tree volume. The study also revealed that the magnitude of relationships among traits changed with environmental conditions, reflecting significant genotype × environment interactions.

***Keywords*:** *Melia composita* Benth., Genotypic correlation, Phenotypic correlation, Path analysis

India is under tremendous pressure to meet the rising demand for wood and wood-based products. With supplies of high-quality timber from conventional, long-rotation species declining, industries are increasingly turning to fast-growing tree species as a sustainable source of raw material (Sharma 2019). Plantation-grown wood is therefore becoming indispensable for meeting future needs, particularly for industrial and structural applications. Among these species, Burma dek (*Melia composita*) has emerged as a promising agroforestry tree owing to its fast growth, straight bole, and adaptability to diverse climatic conditions. Its favourable physical and mechanical properties make it suitable for furniture, joinery, and other value-added applications, thereby serving as a viable alternative to traditional timbers such as shisham, mango, and teak (Kumar et al., 2018). Moreover, *Melia composita* is increasingly recognized as a potential diversifier in the poplar eucalyptus dominated agroforestry systems of north-western India. The multipurpose utility including pulp and paper production, bioenergy generation, construction, furniture, and even musical instruments has further increased its popularity and commercial demand (Parthiban et al., 2009, Chinnaraj et al., 2011). The species also thrives in a wide range of soil types and requires relatively low water input, which enhances its suitability for large-scale cultivation.

The improvement of such plantation species depends heavily on the availability of genotypic variability for yield-related traits across agro-climatic conditions (Ahmad et al., 2018). In this context, trait association studies provide essential insights for breeding and selection. Correlation analysis is widely used to evaluate relationships among yield attributes and to identify traits contributing directly or indirectly to productivity (Ali et al., 2003). However, correlation alone does not partition the magnitude of direct and indirect effects of traits (Silva et al., 2009). Path coefficient analysis, first proposed by Wright and later applied in forestry and crop breeding (Larik 1979, Baye et al., 2020), overcomes this limitation by decomposing correlations into direct and indirect effects, thereby offering greater reliability in identifying causal relationships and formulating effective selection strategies. Despite the increasing importance of *Melia composita*, very limited research has been conducted in India on trait associations and their temporal or spatial variations. This knowledge gap restricts efforts toward systematic improvement and genetic enhancement of the species. The present study was therefore undertaken to evaluate different progenies of *M. composita*, with the objectives of assessing the relationships between timber volume and its contributing traits, and determining the direct and indirect effects of these traits on volume production through path analysis.

MATERIAL AND METHODS

Experimental site: The study was conducted in the year 2022 at two different sites in the Indian state of Punjab. The first location was situated at Research farm of department of Forestry & Natural Resources (30°54'30.6"N, 75°48'43.8"E), Punjab Agricultural University, Ludhiana (Fig. 1) and second at Ruldu Singh Wala Farm (30°01'15.22"N, 74°78'19.27"E), Regional Research Station**,** Punjab Agricultural University, Bathinda (Fig. 2). These sites lie in two different agroclimatic zones of Punjab in which Ludhiana (First site) comes in central plain zone (irrigated plain) and the Bathinda (second site) in the western plain zone (semi-arid zone).

Cultural practices and experimental design: The progeny trial was established in the year 2016 using 14 progenies of *Melia composita* collected from different parts of northern Punjab and Uttarakhand region. The same cultural and management practices i.e. irrigation, ploughing, pruning etc. were performed as and when needed at both the sites. The tree species at both the sites were exposed to same treatment and cultural operations during the entire study duration. The experiment was laid out in completely randomised block design with four replications at both the sites. The progenies were planted with five plants per progeny with the line to line spacing of 4m and the plant to plant spacing of 3m.

Fig. 1. Overview of *Melia composita* progeny trial at PAU, Ludhiana

Fig. 2. Overview of *Melia composita* progeny trial at RRS, Bathinda

The measurements for traits, tree height (m), diameter (cm), clear bole height (m), stem straightness, crown length (m), crown width (m), number of branches per meter crown length and tree volume were recorded at 5 years age. The volume was calculated by using the regression equation.

Volume per tree (VPT) = $0.000137 * D^{2.48}$ (Choudhary et al., 2024)

Where, D is the diameter at breast height in cm.

Statistical analysis: Statistical analysis was carried out using the R software (version 4.2.1). Analysis of variance for completely randomized block design was carried out by following the method given by Panse and Sukhatme (1989).

Phenotypic correlation coefficient (PCC)

$$\text{PCC } (rp_{12}) = \frac{\sigma p_{12}}{\sqrt{\sigma^2 p1.\sigma^2 p2}}$$

Where,

$\sigma^2 p1$ = Phenotypic Variance of observation X_1.

$\sigma^2 p2$ = Phenotypic Variance of observation X_2.

$\sigma p12$ = Phenotypic Covariance between the observations, X_1 and X_2

Genotypic correlation coefficient (GCC)

$$\text{GCC } (rg_{12}) = \frac{\sigma g_{12}}{\sqrt{\sigma^2 g1.\sigma^2 g2}}$$

Where,

$\sigma^2 g1$ = Genotypic Variance of observation X_1.

$\sigma^2 g2$ = Genotypic Variance of observation X_2.

$\sigma g12$ = Genotypic Covariance between the observations, X_1 and X_2

To test the level of significance, the phenotypic correlation co-efficient was compared by the 'r' value given by Fisher and Yates (1963) at (n-2) degrees of freedom at 1% and 5% level of significance simultaneously.

Path coefficients analysis: The path coefficient analysis was carried out as suggested by Wright (1921) and demonstrated by Dewey and Lu (1959). The path analysis was calculated by solving the following set of n simultaneous equations:

$Py_1 + Py_2 r_{12} + \ldots\ldots + P_{yn} r_{in} = ry_1$

$Py_1 r_{12} + Py_2 + \ldots\ldots + P_{yn} r_{2n} = ry_2$

$Py_1 r_{1n} + P_{y2} r_{2n} + \ldots + P_{yn} r_{2n} = ry_n$

Where,

Py, $Py_{2 \ldots\ldots} \ldots Py_n$, are the direct path effects of 1, 2....n independent variables affecting dependent variable 'y'.

$r_{12}, r_{13} \ldots\ldots r_{in} \ldots\ldots r_n$ (n-1) are the correlation coefficients between various independent variables. The indirect effects of the i^{th} variables via j^{th} variables were worked out as (Pyj + rij). From the simultaneous equations, it is clear that the correlation coefficient is the sum of direct and indirect path coefficient. The residual effect was calculated as follows:

Degree of determination $R^2 = P^2_{y1} + 2Py_1, Py_2, r_{12} + 2Py_1, Py_3, r_{13} + ... P^2_{y2} + 2Py_2, Py_3, r_{23} + ... P_{yn}$

Residual variation = $1-R^2$

Residual = $\sqrt{1-R^2}$

RESULTS AND DISCUSSION

Significant variations were observed in all tree growth parameters at both sites among 14 progenies of *Melia composite*. Tree volume ranged from 0.055 to 0.166 m³ at Ludhiana and from 0.080 to 0.240 m³ at Bathinda, with slightly higher mean volume at Bathinda (0.141 m³) than Ludhiana (0.124 m³) as mentioned in Table 1. Mean DBH and total height followed a similar pattern, being marginally higher at Bathinda than at Ludhiana. Crown-related traits showed a contrasting site response. Mean crown width was greater at Ludhiana (4.40 m) than at Bathinda (3.47 m), whereas, crown length was higher at Bathinda (9.48 m) than at Ludhiana (8.67 m). Number of branches was also higher at Ludhiana (1.78) than Bathinda (1.42), while mean stem straightness scores were comparable between sites (3.54 at Ludhiana and 3.51 at Bathinda). The ranges (minimum–maximum) for all traits were relatively wide at both locations, confirming marked within-progeny and among-progeny variability in volume, diameter, height, crown attributes (CL & CW), and number of branches. Significant variation in *Melia composita* was also reported by Kaur et al. (2023). Similar finding was also reported by Chauhan et al. (2018) in *Melia azedarach.*

Genetic and phenotypic correlation studies: The genotypic correlation coefficients for all the characters were higher than the phenotypic correlation coefficient at both the sites (Fig. 3 & 4), this indicates that there was inherent association between the characters (Al-Tabbal and Al-Fraihat 2012); its phenotypic expression may deflect by the influence of environment (Shahid 2002). The higher magnitude of genotypic correlations than phenotypic correlations were also reported in earlier studies on various crops i.e. in rice by Nithya et al., (2020) and Karim et al., (2014), in barley by Al-Tabbal and Al-Fraihat (2012), in Egyptian bread wheat by Abd El-Mohsen (2012) and in poplar by Jha (2012).

Volume had a highly significant positive correlation with almost all of the characters at both the genotypic and phenotypic levels, with the exception of number of branches per meter crown length, and the crown length, out of these two characters the NOB has non-significant correlation at both the sites but the crown length had showed variation in correlation among the two sites, at Ludhiana site the CL has a non-significant positive correlation with volume but at Bathinda the correlation was reported positive and highly significant, these variation can be attributed to genotypic environmental interaction because the climate and soil conditions of the both the regions are different. At Bathinda, where water stress due to longer duration of dry spell and low average rainfall (Singh et al., 2022), is anticipated to be higher, trees with longer crowns (Table 1) might capture more light/use water more effectively, yielding a strong positive CL–volume correlation. At Ludhiana, higher water availability might weaken this dependency. A study on *Pinus contorta*

Fig. 3. Genotypic (above diagonal) and phenotypic (below diagonal) correlation among different characters of *Melia composita* progenies grown at Ludhiana (** significant at 1%, * significant at 5%)

and *Picea glauca* across moisture-limited forest landscapes in western Canada reported that tree radial growth became strongly influenced by precipitation, especially in water−stressed sites (Lopez et al., 2019). This assumption could also be true for similar other different relationships at both different sites. Jha (2012) in his study on poplar also reported highly significant positive correlation of juvenile wood volume with DBH, CBH and plant hight. Indirect selection for these traits will also be rewarding for volume, which is our main commercially important trait.

Strong correlation of tree height with DBH, CBH, crown width and crown length at genotypic level revealed the existence of pleiotropy or linkage or both between the correlated traits. Therefore, these characters must be given proper emphasis during selection programme and can be exploited for indirect selection. Similar findings were also supported by Thakur and Thakur (2015) in *Melia azedarach*. Parthiban et al. (2019) and Kundal et al. (2020) in *Toona ciliata*, Singh et al. (2015) in *Populus deltoides*. But NOB and stem straightness has shown contrasting correlations with tree hight at both the sites and also genotypically and phenotypically. Stem straightness has showed positive significant correlation genotypically at Ludhiana and positive highly significant genetic and phenotypic correlation at Bathinda but non-significant positive correlation at Ludhiana, whereas NOB has shown non-significant negative genetic

Fig. 4. Genotypic (above diagonal) and phenotypic (below diagonal) correlation among different characters of *Melia composita* progenies grown at Bathinda (**significant at 1%, * significant at 5%)

Table 1. Preliminary information on tree growth parameters among 14 progenies of *Melia composita* planted at two different sites

Parameters	Minimum		Maximum		Mean ± SD	
	Ludhiana	Bathinda	Ludhiana	Bathinda	Ludhiana	Bathinda
Volume (m^3)	0.055	0.080	0.166	0.240	0.124 ± 0.032	0.141±0.049
DBH (cm)	11.13	12.60	17.47	20.08	15.40 ±1.78	16.06±2.31
TH (m)	13.00	12.35	15.24	16.85	14.18±0.63	14.84±1.14
CBH (m)	4.69	4.64	6.15	6.08	5.50 ±0.46	5.44 ±0.43
CW (m)	3.09	2.82	4.96	4.29	4.40±0.50	3.47 ±0.42
CL (m)	7.53	8.27	9.24	10.77	8.67 ±0.47	9.48±0.72
NOB	1.24	1.06	2.44	1.75	1.78 ±0.32	1.42±0.19
ST	2.85	2.58	4.23	4.09	3.54 ±0.38	3.51±0.43

DBH: Diameter at breast height, TH: Total height, ST: Stem straightness CBH: Clear bole height, CS: Crown spread, NOB: Number of branches per meter crown length, CL: Crown length and phenotypic correlation at Ludhiana, whereas at Bathinda it has shown significant and non-significant positive genotypic and phenotypic correlation with tree height, respectively. Number of branches per meter crown length has reported non-significant association with most of the traits at both the sites. Number of branches per meter crown length at PAU Ludhiana trial indicated highly significant negative correlation with crown length and significant correlation with clear bole height. The number of branches per meter crown length at RRS, Bathinda showed non-significant association with crown length and positive phenotypic association with total height, straightness, and crown spread. Non-significant association of number of branches with any of the morphological traits was also reported by Kumar et al. (2022) in *Melia dubia*. Dhillon et al. (2000) in *Dalbergia sissoo* Roxb observed that seedling height, leaf weight, and height up to first branch correlated more directly to collar diameter, a key trait in forest trees at the seedling stage.

Path coefficient analysis: In the present study, at the Research Farm, PAU, Ludhiana, diameter at breast height (DBH), total height, exhibited a positive direct effect on timber volume both genotypically and phenotypically (Table 2). Additionally, DBH showed a positive indirect effect through total height, straightness, and the number of branches per meter of crown length. Traits such as total height, straightness, clear bole height (CBH), crown spread, and crown length also exerted a positive indirect effect on tree volume via DBH. Parthiban et al. (2017) in *Melia dubia* reported the positive direct and indirect of effect (via tree height) of DBH on tree volume. Jha (2012) in similar study on poplar also reported the positive direct effect of DBH and tree height on the juvenile wood volume and also reported positive indirect effect of DBH on juvenile wood volume via plant height, diameter of branches, crown diameter, and survival.

At the Regional Research Station, Bathinda, DBH was the only trait that exhibited a significant positive direct effect on timber volume at both the genotypic and phenotypic levels (Table 3). However, other traits including total height, straightness, CBH, crown spread, number of branches, and crown length contributed positively to volume indirectly through DBH. Espahbodi et al. (2018) in *Sorbus tarminalis* (L.) Crantz concluded that collar diameter had strong positive direct effect on plant height at phenotypic level. Similar findings were also reported by Sharma et al. (2024) in which high positive direct effect of DBH on volume and negative direct effect of tree height on volume. Parthiban et al. (2017) also reported negative direct effect of tree height on tree volume in *Melia dubia*. DBH exerted positive indirect effect via tree height whereas; tree height expressed negative indirect effect via DBH on tree volume.

The highly significant positive genotypic and phenotypic correlation between volume and DBH is due to high positive direct effect of DBH, which reveals true relationship between them and therefore direct selection for this trait would be

Table 2. Direct and indirect effect of all the independent components on volume (m^3 $tree^{-1}$) of *Melia composita* progenies grown at Ludhiana

		DBH	TH	ST	CBH	CW	NOB	CL	GC and PC with volume
DBH (cm)	G	0.841	0.011	-0.024	-0.012	0.254	-0.060	-0.018	0.995 **
	P	1.057	0.163	0.003	-0.118	-0.052	-0.004	-0.060	0.9914 **
TH (m)	G	0.542	0.018	-0.007	-0.012	0.126	0.063	-0.060	0.671 **
	P	0.610	0.283	0.002	-0.127	-0.029	0.002	-0.147	0.5835 **
ST	G	0.592	0.004	-0.034	-0.011	0.193	-0.085	0.025	0.6865 **
	P	0.468	0.095	0.006	-0.183	-0.028	-0.006	0.079	0.4247 **
CBH (m)	G	0.722	0.015	-0.025	-0.014	0.251	-0.069	-0.023	0.8579 **
	P	0.432	0.125	0.004	-0.288	-0.023	-0.007	0.119	0.385 **
CW (m)	G	0.813	0.009	-0.025	-0.013	0.262	-0.119	0.007	0.9345 **
	P	0.836	0.122	0.002	-0.101	-0.066	-0.007	-0.018	0.781 **
NOB	G	0.251	-0.006	-0.014	-0.005	0.156	-0.200	0.058	0.2408 NS
	P	0.260	-0.026	0.002	-0.111	-0.028	-0.017	0.132	0.2042 NS
CL (m)	G	0.221	0.016	0.013	-0.005	-0.026	0.174	-0.067	0.327 NS
	P	0.225	0.149	-0.002	0.122	-0.004	0.008	-0.281	0.2377 NS

Genotypic residual effect = 0.0133; Phenotypic residual effect = 0.0039. *G & P in the table indicate the genotypic and phenotypic path coefficient respectively. GC-Genotypic correlation; PC- Phenotypic correlation

Table 3. Direct and indirect effect of all the independent components on volume (m^3 $tree^{-1}$) of *Melia composita* progenies grown at Bathinda

		DBH	TH	ST	CBH	CW	NOB	CL	GC and PC with volume
DBH (cm)	G	1.1050	0.1145	-0.0665	-0.0654	-0.0123	-0.0046	-0.0734	0.9968**
	P	0.9250	-0.0264	0.0876	-0.0531	0.0801	-0.0186	-0.0079	0.9911**
TH (m)	G	1.0066	0.1256	-0.0775	-0.0698	-0.0091	-0.0059	-0.0859	0.8835**
	P	0.7084	-0.0344	0.1036	-0.0518	0.0533	-0.0215	-0.0092	0.7541**
ST	G	0.9255	0.1226	-0.0794	-0.0616	-0.0081	-0.0058	-0.0804	0.8121**
	P	0.6650	-0.0293	0.1216	-0.0502	0.0436	-0.0210	-0.0089	0.7105**
CBH (m)	G	0.8608	0.1046	-0.0583	-0.0839	-0.0079	-0.0051	-0.0456	0.7640**
	P	0.5363	-0.0195	0.0668	-0.0914	0.0506	-0.0192	-0.0036	0.5443**
CW (m)	G	1.0857	0.0914	-0.0516	-0.0533	-0.0125	-0.0055	-0.0574	0.9962**
	P	0.7120	-0.0176	0.0511	-0.0445	0.1041	-0.0210	-0.0050	0.7701**
NOB	G	0.3339	0.0485	-0.0303	-0.0281	-0.0044	-0.0154	-0.0196	0.2845
	P	0.2295	-0.0098	0.0341	-0.0234	0.0292	-0.0748	-0.0024	0.2272
CL (m)	G	0.9331	0.1241	-0.0734	-0.0440	-0.0083	-0.0034	-0.0869	0.8409**
	P	0.6080	-0.0264	0.0903	-0.0277	0.0435	-0.0154	-0.0120	0.6528**

Genotypic residual effect = 0.006, Phenotypic residual effect = 0.0132

rewarding for volume improvement. It also shows positive indirect effect through total height on tree volume. Other traits such as total height, straightness, CBH, crown spread and crown length showed significant indirect effect so these traits would be considered for selection. Kumar and Dhillon (2016) in *Eucalyptus* clones reported the highest positive phenotypic and genotypic direct effect of collar diameter on volume index followed by plant height. He also reported that plant height exerted positive indirect effect via collar diameter. Number of branches showed negative direct effect on volume.

CONCLUSIONS

The present study provides important insights about significant variability and correlations among growth and volume characteristics in *Melia composita* progenies grown in two different climatic conditions. The study concluded that DBH and tree height was the most important trait due to its highest positive direct and indirect effects on tree volume. The study also revealed that relationships among traits change with environmental conditions, as evident by the differing correlation coefficients at the two sites, reflecting significant genotype × environment interactions.

AUTHOR'S CONTRIBUTIONS

RC: Data curation, Formal analysis and Interpretation, Writing - original draft, Writing - review & editing; RISG: Conceptualization, Methodology, Investigation, Resources, Writing - review & editing; AKD: Data curation, Formal analysis and Interpretation, Methodology, Supervision, Validation, Writing - original draft, Writing - review & editing; GPSD: Conceptualization, Investigation, Resources, Writing - review & editing .

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Rapid Multiplication of *Dendrocalamus hamiltonii* in *vitro* Regeneration Techniques from Nodal Explants

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Abstract: The present study reported an advanced, effective and reproducible *in vitro* propagation protocol for *Dendrocalamus hamiltonii* using nodal explants. Secondary branches of the mother plant of *D. hamiltonii* were used to collect nodal explants of length 12-15 mm. Explant sterilization was carried out with 70% ethyl alcohol for 30s followed by mercuric chloride (0.1%) for 5 min and then inoculated on MS basal media for establishment. For multiplication, established shoots were implanted on MS media supplemented with 4.0 mg/l BAP, which resulted in a greater number of shoots multiplication after 21 days of incubation. Clumps of excised propagules transplanted on ½ MS media supplemented with 3 mg/l IBA + 3 mg/l IAA and 2 % sucrose induced rooting after 25 days and profuse rooting of shoots after 40 days of incubation. A two-step acclimatization process was done, in which soil: sand: vermicompost (1:1:1) media showed 80 per cent survival in *in vitro* raised shoots of *D. hamiltonii*. Field-transplanted plants exhibit prolific growth and development. This study was carried out to standardise multiplication of *Dendrocalamus hamiltonii through In vitro* regeneration.

Keywords: Acclimatization, Bamboo*, In vitro* regeneration, Multiplication, Shoot establishment

Dendrocalamus hamiltonii Nees et Arn. Ex Munro, family *Poaceae*, commonly called Maggar, is a versatile bamboo with numerous prominent uses, and a source of nutritive green fodder for the cattle. *D. hamiltonii* is an economically important species of bamboo that is distributed in the northwest Himalayas, Sikkim, Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Tripura of India, Bhutan, and Bangladesh. The blooming cycle of this species has been reported to last 30-40 years (Arya et al., 2012). Many bamboo species are commercialized based on its productive potential and utility (Divya et al., 2023, Twinkle et al., 2023). *D. hamiltonii* is also one of the species under domestication and is utilized for construction, fencing, baskets, containers, and shoots eaten fresh or pickled. Tremendous socio-economic pressures besides jhum cultivation in the natural niche have often compelled the utilization of raw bamboo resources to a critical level. *In vitro* regeneration technique offers an effective policy for the expeditious propagation and mass propagation of edible bamboo species, considering their sustainable development and utilization (Devi and Sharma 2009). It can propagate through seed, culm, or rhizome cuttings using conventional propagation methods. Even though method of mass propagation has been emergence through the use of single node culm cuttings in Maggar bamboo, but due to limitations like time consumption, labor intensive conventional technique of macro propagation and less availability of explants creates hurdles in mass propagation. Another problem with bamboo propagation through seeds is the unfamiliar age of the mother plant, which shows mass flowering and the death of flowered clumps. Bamboo seeds are available only for specific period of time and their viability is very short. These conventional methods do not satisfy the demand for bamboo in the market; as a result, micropropagation secured a continuous supply of bamboo materials to the market in a very short period of time. Pest and disease also play significant roles in the success or failure of authentication of nurseries and plantations of bamboo stands (Singh et al., 2013). Micropropagation techniques can solve many of the problems associated with conventional methods and provide proficiency in developing large progenies from elite genotypes (Mustafa et al., 2021). Trial and error experiments are carried out to identify specific conditions for individual species, genotypes and the donor plant development stages when designing protocols for *in vitro* plant propagation in most of the cases. The purpose of designing a bamboo tissue culture protocol is to accomplish large-scale production of plants for operation, to conceive disease-free and genetically uniform planting material, and to furnish material for breeding programs and germplasm conservation (Sandhu et al., 2018). Hence, present study was undertaken to standardize protocol for tissue culture in *D. hamiltonii.*

MATERIAL AND METHODS

Explants source: Healthy segment (2.0- 2.5 cm length) of *D. hamiltonii,* were collected from Bambusetum established in the premises of Bamboo Resource Center, CoF, NAU, Navsari. Explants were collected from the secondary branch of the new culm of *D. hamiltonii* during December to March 2022. Bamboo internodes are highly loaded with bacteria, fungi, and endophytes; therefore, internodes with young buds covered with culm leaves were selected as explants for micropropagation.

Explants preparation, establishment and contamination control: *D. hamiltonii* explants were washed with tap water 2-3 times, consequently laboratory detergent Tween 20 (0.05%) added to distilled water and stir the solution with explants for 10 min and washed with sterile water 2-3 times, afterwards treated the explants with fungicide carbendazim (0.5%) and streptomycin (0.01%) for 30 min. The explants were washed 2-3 times with sterile distilled water at room temperature. Furthermore, plants were subjected to different contamination control treatments-C_1 -C_6 (Table 2) and observation on number of shoots, number of days taken for sprouting, length of longest shoot, per cent establishment and per cent contamination were recorded. Explants were washed 2-3 times with sterile distilled water to remove traces of mercuric chloride and inoculated on MS basal media (0.8%) gel at pH 5.8 and were incubated for 21 days at 24°C under 16 h light and 8 h dark cycles.

Shoot multiplication: Sprouted shoots were transferred onto MS medium containing cytokinines (BAP) at different concentrations (Table 3). The data were recorded for shoot multiplication. The excised shoots were incubated for 21 days at 24°C under 16 h light and 8 h dark cycles.

Rooting of shoots: Multiplied shoots were dissected into 3-4 shoots and inoculated on different rooting media. There were 33 combinations of rooting hormones (R_1 to R_{33}) were applied to *in vitro* regenerated shoots for root induction and root development (Table 1). Rooting treatments selected as per review in which generally three auxins (IAA, NAA and IBA) are widely utilized for inducing roots *in vitro*. Data were recorded after 40-45 days of incubation period at 24°C for 16 hours light and 8 hours dark cycles.

Acclimatization/ hardening: Rooted plants were transferred to seedling trays for 30-35 days. Trays were used, which contained different media for hardening (A_1 to A_4). The different types of media used in the study (Table 4). Trays were kept in a mist chamber for better survival of the tissue-cultured raised plants. *In vitro* regenerated plants were transferred to the field for better survival and vigorous growth.

Statistical analysis: Data was analyzed with the OPSTAT online.

RESULTS AND DISCUSSION

Establishment and contamination control: Significantly maximum number of shoots (2.21), length of longest shoot (5.35 cm), highest establishment (92.22 %), and lowest contamination (7.78 %) were in 70% ethyl alcohol for 30s followed by mercuric chloride (0.1%) for 5 min (Table 2). The 70% ethyl alcohol for 30s, followed by mercuric chloride (0.1%) for 3 min resulted in minimum number of days to sprout shoots (5.21 d). Culture establishment of *D. hamiltonii* in MS basal media without hormone is shown in Plate 1a.

Jha et al. (2013) reported similar results for the contamination control treatment of *D. hamiltonii* nodal explants. Mercuric chloride with 70 % ethanol is found satisfactory result in sterilized bamboo explants (Hu et al.,

Table 1. Rooting media treatments for *D. hamiltonii* multiplied shoots

Treatments code	Treatment details
R_1	MS + 1 mg/l IBA + 0.25 % Activated charcoal
R_2	MS + 0.1% IBA+ 2% Sucrose
R_3	MS + 1 mg/l IBA + 1 mg/l NAA + 2% Sucrose
R_4	MS + 1 mg/l IBA + 1 mg/l NAA
R_5	MS + 1.5 mg/l NAA + 3 mg/l IBA + 2% Sucrose
R_6	MS + 1 mg/l NAA + 0.3% Activated charcoal
R_7	MS + 20 mg/l IBA
R_8	MS + 0.5mg/l IBA
R_9	MS + 1 mg/l BAP + 3 mg/l NAA
R_{10}	MS + 1.8 mg/l NAA + 10 mg/l Coumarin
R_{11}	MS + 1.8 mg/l NAA
R_{12}	MS + 1 mg/l BAP + 1 mg/l NAA + 3% Activated charcoal
R_{13}	MS + 3 mg/l NAA+ 2% Sucrose
R_{14}	MS + 0.2 mg/l NAA + 0.2 mg/l IBA + 10 mg/l Coumarin
R_{15}	½ MS + 1.8 mg/l NAA + 2 mg/l IBA + 10 mg/l Coumarin + 2 % Sucrose
R_{16}	½ MS + 0.5 mg/l IBA + 0.5 mg/l NAA + 2 % Sucrose
R_{17}	½ MS + 1 mg/l IBA + 0.5 mg/l NAA + 2 % Sucrose
R_{18}	½ MS + 0.5 mg/l NAA
R_{19}	½ MS + 1 mg/l NAA
R_{20}	½ MS + 1 mg/l IBA
R_{21}	½ MS + 2 mg/l IBA
R_{22}	MS + 3 mg/l IBA
R_{23}	MS + 4 mg/l IBA
R_{24}	MS + 5 mg/l IBA
R_{25}	½ MS + 3 mg/l IBA
R_{26}	½ MS + 3 mg/l IBA + 3 mg/l IAA + 2% Sucrose
R_{27}	½ MS + 3 mg/l IBA + 10 mg/l Coumarin
R_{28}	½ MS + 0.2 mg/l IBA
R_{29}	½ MS + 1 mg/l IBA + 1 mg/l IAA
R_{30}	½ MS + 2 mg/l IBA + 1 mg/l IAA
R_{31}	½ MS + 5 mg/l IBA + 1 mg/l IAA
R_{32}	MS + 8μM BAP + 1μM NAA + 100 μM IBA
R_{33}	½ MS + 8μM BAP + 1μM NAA + 100 μM IBA

2011, Arshad et al., 2005). These entire chemicals successfully eliminated the surface contaminations; however, there is a hindrance in controlling the endophytic contamination. In such situation, antibiotics can be used during surface sterilization (Syandan and Md Nasim 2016).

Shoot multiplication: Significantly greater number of multiplied shoots was recorded in MS supplemented with 4.0 mg/l BAP in pooled over two years data (10.44), which was followed by treatment MS + 3.0mg/l BAP *i.e.,*7.66 shoots (Table 3). The treatment MS + 0.5 mg/l BAP resulted in poor shoot multiplication 3.80 (Table 3). Successful shoot multiplication of *D. hamiltonii* on MS media supplemented with 4 mg/L BAP is shown in Plate1b. As discussed by Jha and Das (2021) showed near to similar trend for number of shoot multiplication of *D. hamiltonii.* In contrast, Sayanika et al (2014) observed that the combined concentration of 2mg/L of kinetin and 3mg/L of BAP produced more shoots per explant in *B. tulda* and *M. baccifera.* Moreover, increase in the BAP concentration may escalate the multiplication but leaves of plants became tiny and more condense (Mudoi and Borkhadur 2009). This could be due to surplus of hormone which may leads to toxic for the plant, hence, smaller and abnormal leaves develop.

Root initiation and establishment: A total of 33 rooting treatment combinations ($R_1$to T_{33}) were applied for the rooting in *D. hamiltonii* (Table 1). Among them, only R_{26}*i.e.,* treatment combination of ½ MS + 3 mg/l IBA + 3 mg/l IAA + 2% sucrose responded to rooting and rest of treatments did not produce roots. T_{26} resulted in 86.68 per cent rooting with 12.84 roots per plant in the pooled data (Plate 1c). Length of longest root measured was 5.69 cm in the pooled data. Since all the treatments, except $T_{26,}$ showed zero result; hence, data is not provided in the table.

Murlidhran and Pandalai (2017) suggested that the best concentration of auxin for rooting is ½ MS + 3 mg/l IBA for *B. tulda*.Such combination also holds good for rooting of *D. giganteus* shoots (Ramanyake and Yakandawala 1997).Raju and Roy (2016) achieved optimum rooting efficiency in bamboo shoots within 15-22 days when 2.5 mg/l IBA was added in conjunction with 2.5 mg/l NAA. Effect of growth regulators on rooting also vary from species to species and also depend upon age of explants (Ramanayake et al., 2008).

Acclimatization / Hardening: The data on acclimatization of *D. hamiltonii* using *in vitro* regeneration techniques from nodal explants is given in Table 4. Result shows that the highest survival per centwas recorded in acclimatization

Table 3. Effect of shoot multiplication treatments on number of multiplied shoots of *D. hamiltonii*

Treatments	Pooled
T_1 - MS + 0.5 mg/l BAP	3.80
T_2 - MS + 1.0 mg/l BAP	4.67
T_3 - MS + 2.0 mg/l BAP	5.44
T_4 - MS + 3.0mg/l BAP	7.66
T_5 - MS + 4.0 mg/l BAP	10.44
CD (p=0.05)	0.46
CV %	5.95

Table 4. Effect of acclimatization treatments on survival per cent of *Dendrocalamus hamiltonii*

Treatments	Survival per cent pooled
A_1 - FYM + Soil + Sand (1:1:1)	63.33
A_2 - FYM+ Soil (1:1)	42.22
A_3 - FYM + Soil + Coco Coir (1:1:1)	55.00
A_4 - Soil + Sand + Vermicompost (1:1:1)	80.00
CD (p=0.05)	5.07
CV %	6.88

Table 2. Effect of contamination control treatments on shoot establishment parameters in *D. hamiltonii (*Pooled data for 2022 and 2023)

Treatments	No. of shoots	No. of days for sprouting	Length of longest shoot (cm)	Establishment (%)	Contamination (%)
C_1 - Mercuric Chloride (0.1%) for 3 Min	1.53	5.33	4.33	60.00	40.00
C_2 - Mercuric Chloride (0.1%) for 4 Min	1.64	5.34	4.06	66.11	33.89
C_3 - Mercuric Chloride (0.1%) for 5 Min	1.76	5.51	4.77	71.67	28.33
C_4 – Ethyl alcohol (70%) for 30 Sec + Mercuric Chloride (0.1%) for 3 Min	1.77	5.21	3.90	58.89	41.11
C_5 - Ethyl alcohol (70%) for 30 Sec + Mercuric Chloride (0.1%) for 4 Min	1.83	5.54	4.55	79.44	20.56
C_6 - Ethyl alcohol (70%) for 30 Sec + Mercuric Chloride (0.1%) for 5 Min	2.21	5.35	5.36	92.22	7.78
CD (p=0.05)	0.09	NS	0.24	3.31	3.31
CV %	4.02	3.87	4.54	3.89	9.71

(a) (b) (c)

Plate 1. a) Culture establishment in basal MS medium, b) Shoot multiplication in MS supplemented with 4mg/ l BAP, and c) Rooting of multiplied shoot in ½ MS media supplemented with 3 IBA+ 3 IAA with 2% sucrose

(a) (b) (c)

Plate 2. Acclimatized plantlets of *D. hamiltonii* through *in vitro* regeneration a) Primary hardening, b) Secondary hardening and c) Well-established plantlets

treatment A_4 [Soil + Sand + Vermicompost (1:1:1)] in individual years (78.89 % and 81.11 %, respectively) and it was 80 % in pooled data. In fact, A_1 treatment composed of FYM + Soil + Sand (1:1:1) also resulted in 64.44 and 62.22 per cent survival in the first and second year respectively with pooled value of 63.33 per cent survival (Table 4). In contrast, treatment-A_2 [FYM+ Soil (1:1)] resulted in lowest survival per cent of 42.22 % (in pooled data), 43.33 % in the first year and 41.11 % in second year. Acclimatized micro-propagated plantlet of D. hamiltonii is shown in Plate 2.

Jha and Das (2021) also suggested Soil: Sand: Vermicompost (1:1:1) as hardening media for obtaining optimum growth of *D. hamiltonii*. Arya et al. (2012) mentioned that plants, which are transferred into polybags containing Sand: Soil: FYM in a 1:1:1 ratio, resulted in elongated shoots as well as greener and expanded leaves.

CONCLUSION

Culture establishment of *D. hamiltonii* in MS basal media in the contamination control treatment containing 70% ethyl alcohol for 30 second + mercuric chloride @ 0.1% for 5 minutes performed well and produced a greater number of shoots, length of longest shoot with more culture establishment and less contamination. The least number of days taken for sprouting was recorded in C_4. Shoot multiplication was highest in the treatment containing MS media + 4.0 mg/l BAP (T_5). Roots were developed only in treatment combination of ½ MS media supplemented with 3mg/l IBA + 3mg/l IAA + 2% sucrose (R_{26}). Maximum *in vitro* plant survival was acclimatization treatment composed in soil: sand: vermicompost (1:1:1) (A_4). Study concludes that protocol developed in this study can be used for successful *in vitro* regeneration of *D. hamiltonii*.

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AUTHORS' CONTRIBUTION

Jayesh Pathak provided the study conception and design, Nidhi Patel helped in data collection, development of methodology, D.H. Prajapati and J.R. Chavda helped in statistical analysis and data interpretation, V. B. Patel supervised the experiment and helped in preparation of draft.

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Patterns and Perceptions of Human–*Rhesus macaque* Conflict in Humid Subtropical Climate of Jabalpur Forest Division

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Abstract: The human–rhesus macaque conflict has become a significant conservation and management issue in India, particularly in human-dominated landscapes where macaques increasingly depend on anthropogenic resources. The present study was undertaken to assess the impact of troop dynamics and spatio-temporal behaviour of rhesus macaques on human-wildlife conflict in Territorial Forest Division of Jabalpur, Madhya Pradesh. Jabalpur city is situated in the humid subtropical climatic zone. For present study the primary data were collected through direct observations, semi-structured questionnaire surveys of 350 respondents, and interviews with local communities, forest staff, and NGOs, supplemented with secondary literature. Results revealed that rhesus macaques were perceived as the most problematic species (40.86%), surpassing snakes, langurs, wild boars, leopards, crocodiles, and other animals. Conflicts were concentrated in residential colonies, religious sites, marketplaces, and croplands, where macaques found easy access to food, water and shelter. Medium-sized troops (11–30 individuals), primarily composed of adult females and juveniles, were most frequently encountered, with peak activity during morning and evening hours. The conflict was associated with economic losses, property damage, injuries, and psychological stress among local residents. The findings underscore the urgent need for integrated mitigation strategies, including waste management, controlled feeding practices, habitat restoration, and community-based awareness programs, supported by coordinated action from government agencies and non-governmental organizations.

Keywords: Human–rhesus macaque conflict, *Rhesus macaque*, Troop dynamics, Spatio-temporal behaviour

India is recognized as one of the most primate-rich regions globally, both in terms of species diversity and population density. Among these, the rhesus macaque (*Macaca mulatta*) is one of the most widely distributed non-human primate species, inhabiting a broad range of environments from dense forests to heavily human-modified landscapes (Kumara et al., 2010). It is estimated that 80–90% of India's *Rhesus macaques* reside in close proximity to human settlements (Pathak 2023), where they have developed a high dependency on anthropogenic food sources (Pragatheesh 2011). Devi and Saikia (2008) reported that primates across India have exhibited remarkable adaptability to human-dominated areas, facilitating their successful co-existence. However, this increased proximity has also led to intensified competition for space and resources, often resulting in direct human–macaque conflict (Patari and Dasgupta 2021). Across many regions, *Rhesus macaques* are considered a public nuisance damaging property, raiding crops, and causing frequent disturbances to daily human activity (Karayathil et al., 2023). The reverence of macaques as sacred being, combined with the absence of natural predators in urban environments and a national ban on primate export, has allowed macaque populations to grow unchecked (Govindrajan 2015). Consequently, human–macaque conflicts have escalated in both urban and rural settings. As natural habitats are increasingly fragmented by human expansion, macaques are compelled to infiltrate residential areas in search of food, water, and shelter. Their interactions often involve damage to household items, vehicles, and gardens, alongside aggressive behaviours such as food-snatching, intimidation, and biting. Efforts to deter them, such as the use of stones, loud noises, dogs, or firecrackers, are largely ineffective and sometimes provoke even more aggressive responses. Considering the above facts, the present study was undertaken to assess the impact of troop dynamics and spatio-temporal behaviour of rhesus macaques on human-wildlife conflict in Territorial Forest Division Jabalpur, Madhya Pradesh.

MATERIAL AND METHODS

Study area: The present study was conducted in the urban and semi-urban landscapes of Territorial Forest Division Jabalpur, Madhya Pradesh. Jabalpur is situated in central region of Madhya Pradesh, India, between 22°49′ to 23°07′ N latitude and 79°21′ to 80°35′ E longitude. It spans 5,211 km², with a mean elevation of 425.7 m and an average slope of 7.56%.

Data collection: Both primary and secondary data were collected with the methodology outlined by Singh and Sharma (2011). The information pertaining to the status of human–rhesus macaque conflict and the factors influencing its occurrence was collected through direct observations at identified conflict sites, interactions and interviews with local residents, forest frontline staff, non-governmental organizations, and volunteers as well as through a semi-structured questionnaire surveys in the affected areas. Secondary data refers to the analysis and use of information that has already been collected for purposes other than the present study. Its sources include published literature, earlier research studies, government reports, official records, scholarly journals, technical documents, books, computerized databases, and newspaper articles.

Semi-structured questionnaire survey: Field visits were conducted in areas affected by human–rhesus macaque conflict to design a semi-structured questionnaire. The questionnaire included a mix of open-ended and fixed-response questions. A pilot test was carried out in select conflict zones to validate the questionnaire before its final dissemination. The total of 350 respondents were surveyed, representing a diverse cross-section of stakeholders, including local residents, forest frontline staff, wildlife rescuers, volunteers, shopkeepers, farmers, students, housewives, saints, and devotees. Demographic information such as age, gender, and area of residence was recorded for each respondent. In addition to the survey, in-depth interviews and group discussions were conducted with forest staff, NGO personnel, wildlife rescuers, volunteers, and local inhabitants to gather insights into their experiences and perceptions regarding the conflict. Regular site visits were made to high-conflict zones, such as residential areas, marketplaces, temples, government offices, old buildings, military compounds, and agricultural lands frequently visited by rhesus macaques.

RESULTS AND DISCUSSION

Conflicting animal species in the region: The survey findings revealed that among the 350 respondents, the majority identified *Rhesus macaques* as the primary cause of human–wildlife conflict (40.86%) followed by snakes (18.57%), Hanuman langurs , wild boars , leopards , crocodiles (Table 1).

Rhesus macaques, Hanuman langurs, and wild boars were consistently reported as the most damaging species to agricultural crops, with rats also noted as crop pests in certain regions. Additionally, frequent leopard sightings and occasional crocodile encounters were reported, creating a significant psychological impact and fear among local residents. Areas with recurrent leopard sightings included Ranjhi, Khamariya, Thakurtaal, Nayagaon, Udana village, GCF Estate and New Colony, Andhuwa, Bahdan, Temar Bhita, Bhatauli, Dumna Road, Chulha Gulai, Bargi Hills, IIITDM, Kundam, and Indrana Beet. Crocodile sightings were mainly reported from Rithori, Pariyat, Ranjhi, Ghana, Khamariya, Nanak Nagar, Manegaon, Umariya, and Sonpur.

Snakes, while commonly encountered, were largely considered non-threatening, as the majority were non-venomous and did not cause direct harm. Even in instances involving venomous snakes, prompt action by trained snake rescuers and forest department rescue squads helped mitigate risk, reducing the perceived threat from these species.

The intensity and frequency of *R. macaque* attacks were reported to be particularly distressing for residents, many of whom expressed a sense of helplessness in dealing with the problem. A notable concern among respondents was the perceived lack of support from government and non-government organizations, which may have contributed to rhesus macaques being perceived as the most problematic wildlife species in the area.

These results partially align with the findings of Saraswat et al. (2015), who reported that the majority of respondents (87.11%) identified Nilgai as the most problematic species in terms of human–wildlife conflict, followed by jackals (58.11%), *R.macaques* (57.11%), wild boars (48.00%), snakes (42.89%), and leopards (15.67%). While the species involved and their rankings differ somewhat, the commonality lies in the multi-species nature of human–wildlife conflict and the strong influence of local context on perceptions of severity.

Troop size: The majority of respondents (24.86%) reported encountering troops of *R. macaques* consisting of 11–20 individuals followed by 18.85% in larger groups comprising 21–30 macaques (Table 2). These observations reflect the dynamic social structure of rhesus macaque populations, which tend to form multi-male, multi-female troops with variable group sizes depending on resource availability, habitat type, and anthropogenic influences (Hasan et al., 2013). The predominance of medium-sized troops (11–30

Table 1. People perception on the most conflicting animal species in Territorial Forest Division, Jabalpur

Species	Percentage
Rhesus macaque (*Macaca mulatta*)	40.86
Hanuman langur (*Semnopithecus entellus*)	16.00
Leopard (*Panthera pardus*)	06.28
Snake (*Serpentes*)	18.57
Crocodile (*Crocodylus*)	04.57
Wild boar (*Sus scrofa*)	08.29
Others (birds, jackal, rats)	05.43
Total	100

individuals) may be linked to urban-edge habitats, where food availability from human sources can support moderately large groups without leading to intra-group resource competition or conflict.

Troop composition: The adult females identified by the presence of red skin on the rump and estimated to be over three years of age constituted the largest proportion of individuals within the observed *R. macaque* troops, accounting for 35.43% (*n* = 124) followed by juveniles (aged between 1–3 years, typically seen in close proximity to their mothers), comprising 21.43% (Fig. 1).

Habitat use pattern: Most respondents reported that *R. macaques* mainly inhabited residential colonies and buildings (21.71%), followed by religious sites (20.29%) with easy access to food, water, and shelter. Croplands (17.72%) and marketplaces (14.86%) were also common habitats due to food availability, while 13.71% noted their presence in nearby forests and 11.71% in abandoned buildings or old monuments offering undisturbed shelter.

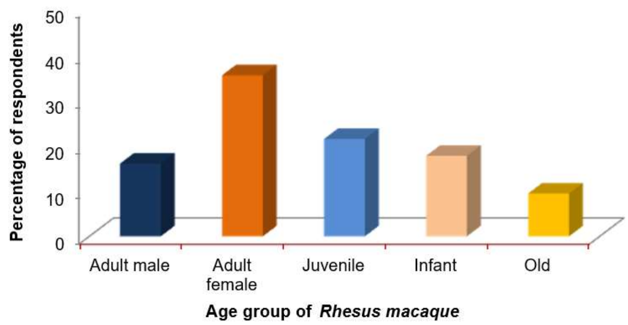

Fig. 1. Troop composition

Table 2. Troop size of rhesus macaques in the study area

Troop size	Percentage
1	07.43
2-5	12.00
6-10	16.86
11-20	24.86
21-30	18.85
31-40	09.14
41-50	06.00
51 and above	04.86
Total	100

Table 3. Time of maximum activity of rhesus macaques

Time of activity	Percentage
Morning	30.57
Afternoon	17.71
Evening	25.43
Night	03.14
All day	23.15
Total	100

Time of maximum activity: Majority of respondents reported increased sightings and activities of problematic *R. macaques* during the morning hours (30.57%). A notable proportion also indicated evening hours (25.43%) as peak activity periods. Interestingly, 23.15% of the respondents observed that the macaques exhibited activity throughout the day, indicating potential site-specific variations in behavioural patterns (Table 3). Das and Mandal (2015) also documented that *Rhesus macaques* frequently visit residential areas during morning hours (07:00–10:00) and again in the afternoon to early evening (14:00–18:00). Such bimodal patterns of activity are likely influenced by factors such as human movement, food availability, and environmental conditions at different sites.

CONCLUSION

This study underscores the growing severity of human–rhesus macaque conflict within the Territorial Forest Division of Jabalpur, driven largely by increasing anthropogenic pressures and shifting ecological dynamics. As macaques become more reliant on human-dominated environments, their interactions with people have intensified—often surpassing those with traditionally more feared species such as leopards and snakes. Conflict hotspots consistently include areas that offer easy access to food and shelter, such as residential zones, religious sites, markets, and agricultural lands. Medium-sized macaque troops, particularly those with a high proportion of adult females and juveniles, are most active especially during morning and evening hours. This pattern of behaviour reflects their adaptive strategies but also highlights the absence of effective deterrents or management frameworks. The resultant impact on local communities includes psychological distress, economic strain, and deteriorating tolerance towards wildlife. Effectively addressing the growing human–rhesus macaque conflict requires a comprehensive and coordinated strategy. Key measures include better waste management, strict regulation of feeding practices, habitat restoration, and targeted community awareness programs. Successful implementation will depend on collaboration among local communities, forest departments, and non-governmental organizations. Ongoing research and monitoring are also vital to guide these efforts and promote sustainable human–wildlife coexistence amid increasing urbanization.

AUTHORS CONTRIBUTION

Somesh Singh contributed to the conceptualization, data

curation, methodology, provision of resources, and review and editing of the manuscript. Sumit Kumar Patel was responsible for the investigation and preparation of the original draft. Meenakshi Dawar contributed to the investigation and review and editing of the manuscript. Babita Singh Kushwah performed the formal analysis. All authors read and approved the final manuscript.

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Tigers of Riverbanks: Ecological Patterns of Riparian Tiger Beetles along the Habitat Gradient in a Tropical Alluvial Plain

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Abstract: Although insects in general and tiger beetles in particular make up a large part of the river's biodiversity, their diversity and ecology are poorly understood from the Ramganga River. Diversity and habitat preferences of riparian tiger beetles in the alluvial plains of the Ramganga River was surveyed. Total 17 tiger beetle species from eight genera in the area. Among these two species originating exclusively from muddy habitat, seven species originating exclusively from sandy habitat, and eight species originating from multiple habitats such as grasslands, gravel and rocks, and shrubs. Similarity test (Anosim) and permutational multivariate analysis of variance (Permanova), indicated significant differences between tiger beetle communities in different habitats. By analysing the indicator value index , two species can be used as an indicator for gravel and rock habitats, three species as an indicator for muddy habitat, ten species as an indicator for sandy habitat, and one species as an indicator for shrub habitat. Market basket analysis using the apriori algorithm, showed two species were positively associated with muddy habitat and seven species were positively associated with sandy habitat.

Keywords: Caraboidea, Cicindelidae, Coleoptera, Conservation, Ramganga River

With more than 385,000 described species, beetles (Order- Coleoptera) account for about 40% of all described arthropod species and it is estimated that there are about 1.5 million beetle species worldwide (Stork et al., 2015, Bouchard et al., 2017). Beetles' great diversity is believed to be due to their extreme adaptive radiation, as they are found in all types of terrestrial and aquatic habitats (McKenna et al., 2019). Among the Coleoptera, tiger beetles are well-studied, brightly coloured predatory insects that are often used as flagship taxa for insect conservation (Knisley and Gwiazdowski 2020). Because of their ecological niche specificity and sensitivity to environmental degradation, they are considered bioindicators of habitat quality (Smith et al., 2021). So far, 2897 tiger beetle species have been reported worldwide, of which 247 species are found in India, including 127 endemic species (Pearson and Wiesner 2022).

Various aspects of tiger beetle ecology and behaviour have been studied in different regions of India over the past three decades. This research includes habitat preference in the river ecosystem, the impact of differential feeding on reproduction, larval tower building behaviour, reproductive behaviour, altitudinal distribution in the Himalayas, foraging and feeding ecology, and habitat association and use as a bioindicator (Ganeshaiah and Belavadi 1986, Shivashankar and Veeresh 1987, Shivashankar et al., 1988, Shivashankar and Pearson 1994, Uniyal and Mathur 2000, Sinu et al., 2006, Bhargav et al., 2008).

The Ramganga River is an important tributary of the Ganga River, which has its source in the Himalayan mountains and flows through the alluvial region of the Ganges. It serves as a habitat for many animals in this riverway, including conservation-dependent animals such as golden mahseer, gharial, lesser flamingo and otters (Ali et al., 2018, Gupta et al., 2020, Gangaiamaran et al., 2021, Vashistha 2022). Several species of benthic macroinvertebrates and insects have also been reported from this river (Nautiyal and Mishra 2022), but the diversity and ecology of tiger beetles have not been previously reported. Because of their importance in the food web, their abundance in different habitats, and their sensitivity to habitat structure and microclimate, ground beetles play an important role in habitat-insect relationship studies (Lange et al., 2023). As an important bioindicator and ecosystem service provider of the riverine landscape, it is important to understand the diversity of tiger beetles in the Ramganga River. Therefore, study aimed to determine tiger beetle diversity, species composition and their association between species and their respective habitat along the Ramganga River alluvial plain.

MATERIAL AND METHODS

Study was conducted in Ramganga River, a major tributary of the Ganga River. Geographically, this 642 km long river having catchment area of about 23,758 km^2 flows through two separate regions namely Himalayan mountainous terrain which is covered by forest in the state of Uttarakhand and Gangetic alluvial plain which is mainly covered by agricultural lands in the state of Uttar Pradesh (Bhattacharjee et al., 2022, Khan et al., 2022). Eleven study sites was selected with an interval of ~ 50 km along the Ramganga River from Biharipur Ahatmali, Uttar Pradesh to Khamdoopur, Uttar Pradesh (at the confluence of Ganga and Ramganga River) (Table 1, Fig. 1). Fieldwork was conducted in winter (November-December 2022) and summer (May-June 2023). At each site, depending on accessibility, a 50 m by 100 m plot was selected alongside either on the left or right bank of the river, for tiger beetle collection. The tiger beetles were collected between 10:00 –15:00 from five type of habitats namely grassland, gravel and rocks, mud, sand and shrub using a standard insect net (Dangalle et al., 2012). The samples were preserved in 96% ethanol and identified with the help of literature (Pearson et al., 2020). The final data set was composed of 55 sampling units, 11 for each habitat for specimen collection.

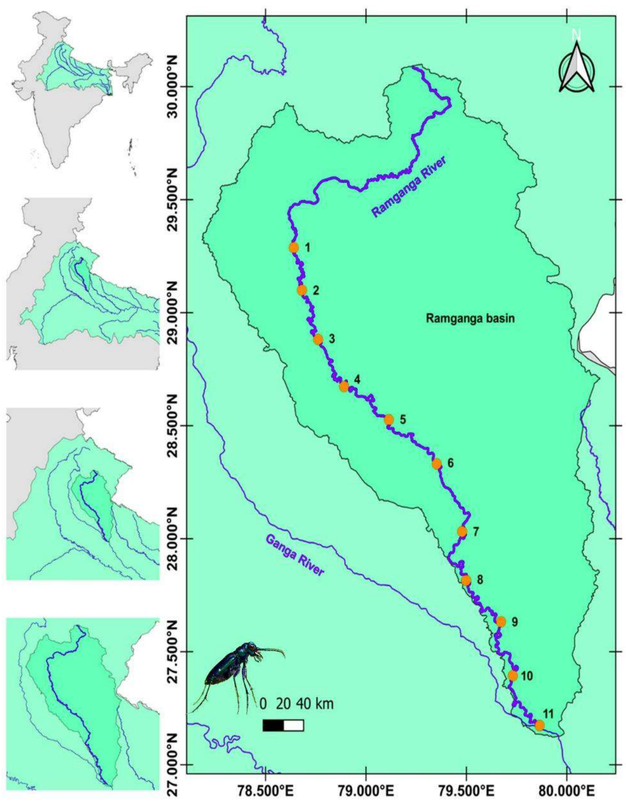

Fig. 1. Location of 11 study sites in the Ramganga River

Statistical analyses: For the analysis, summed species data (i.e., pooled over all seasons for all year) were used for each sampling site. For all statistical analyses, tiger beetle presence-absence data were used as they provide a natural basis for understanding relationships between multiple indicators of biodiversity at large geographic scales, lend themselves to the study of mobile species communities, and are worthy of describing ecological patterns (Arita et al., 2008, Dorazio et al., 2011, Dai et al., 2018, De et al., 2023).

Sample-based rarefaction curves (Gotelli & Colwell, 2001) were calculated to assess whether the sampling effort was sufficient to be representative of the tiger beetle diversity of the study area. To do this, the second-order Chao estimator (Chao2) (Colwell and Coddington 1994) and the first-order Jackknife estimator (Jackknife1) (Burnham and Overton 1978) were calculated using the 'BAT' package (Cardoso et al., 2014). For this Chao2 and Jackknife1 estimators were used because they are non-parametric, can use rare species frequencies for calculation, provide lower bound estimates for small sample fractions, can reduce bias, are more accurate and less sensitive to sample coverage, non-uniformity in species distributions and variability in capture probability, and are therefore reliable for studying invertebrate species richness (Smith and Pontius 2006, Hortal et al., 2006, Chao et al., 2009, Brito et al., 2021, Chiu 2023).

To find out whether the species composition of different habitats is similar or not, the nonparametric analysis of similarity (Anosim) test (Clarke 1993) and the nonparametric

Table 1. Study sites along the Ramganga river

Latitude	Longitude	Area
29°22'35.54"N	78°38'15.72"E	Mahpur, Afzalgarh, Uttar Pradesh
29° 9'32.60"N	78°40'35.95"E	Daryapur, Uttar Pradesh
28°56'6.59"N	78°44'42.54"E	Mohabaatpur, Moradabad, Uttar Pradesh
28°41'20.60"N	78°53'4.80"E	Tajpur Lakhan, Moradabad, Uttar Pradesh
28°31'8.40"N	79° 7'3.65"E	Bhopatpur, Uttar Pradesh
28°17'54.10"N	79°21'50.32"E	Fatehpur Thakuran, Bareilly, Uttar Pradesh
28° 2'51.24"N	79°29'6.43"E	Dandi, Dataganj, Badaun, Uttar Pradesh
27°49'0.38"N	79°29'32.57"E	Parour, Shahjahanpur, Uttar Pradesh
27°37'59.54"N	79°39'21.63"E	Manihaar, Uttar Pradesh
27°22'56.96"N	79°44'19.44"E	Sildaspur, Farrukhabad, Uttar Pradesh
27°10'31.45"N	79°51'45.35"E	Tera ghat, Farrukhabad, Uttar Pradesh

permutational multivariate analysis of variance (Permanova) (Anderson 2001) were performed in the 'vegan' package (Oksanen et al., 2019). To facilitate interpretation of the results from Anosim And Permanova, nonmetric multidimensional scaling (NMDS) was performed based on tiger beetles' composition. For bioindicator tiger beetles of specific habitats indicator value index (IndVal) (Dufrêne and Legendre 1997) were performed using 'labdsv' package (Roberts 2023). This index can assess a species' predictive value as an indicator of specific habitat which is beneficial for ecosystem conservation and management (Legendre 2024).

The Market basket analysis (MBA) is a data mining technique used to identify relationships between product groups, items, or categories (Aguinis et al., 2012). This analysis was performed with apriori algorithm (Agrawal et al., 1993) in the 'rule' package (Hahsler et al., 2005, 2011, 2023) to find out whether there is a connection between species and their respective habitats in the study area. The 'apriori algorithm' was used because it offers a good performance gain in data mining (Chee et al., 2018, Xie et al., 2019). The minimum support value was set at 0.038 and the confidence level at 0.95 to generate significant associations. The lift value was used as a measure of the association between species and habitat. If the lift value is greater than 1.0, the association is considered positive, and if the lift value is less than 1.0, the association is considered negative (Leote et al., 2020). All statistical analysis were performed in the R language and environment for statistical computing (R Core Team 2022).

RESULTS AND DISCUSSION

A total of 17 species of tiger beetles under eight genera were recorded from the study area. Among these species we found two species exclusively from muddy habitat, seven species exclusively from sandy habitat and eight species from multiple habitats like grassland, gravel and rocks and shrub (Fig. 2). The sample-based rarefaction curve of observed species richness based on the sampling data and the implemented non-parametric species richness estimators (Chao2 and Jackknife1) reached the asymptote for a species richness of 17, thus suggesting that the recorded species richness is likely representative of the tiger beetle diversity occurring in the study area (Fig. 3). The non-parametric Anosim test indicated significant differences exist between tiger beetle communities in different habitats (Anosim statistic R = 0.667) and the non-parametric Permanova test showed significant differences between tiger beetle communities in different habitats (Adonis, F = 19.087, R^2 = 0.604,). The Nmds plot (Fig. 4) illustrated that the habitats that had the similar species composition were clustered together. Two species (*Calomera chloris* and *Cylindera bigemina*) were identified as indicator of gravel and rocks habitat, three species (*Cylindera minuta*, *Lophyra striolata striolata* and *L. parvimaculata*) can be used as indicator of muddy habitat, ten species (*Calomera angulata*, *Calomera plumigera*, *Chaetodera vigintiguttata*, *Cicindela albopunctata*, *Cosmodela juxtata*, *Cylindera anelia*, *Cylindera cognata*, *Cylindera grammophora*, *Cylindera venosa* and *Myriochila dubia*) can be used as indicator of sandy habitat and one species (*Cicindela aurulenta*) can be used as indicator of shrub (Table 2, Fig. 5). No species were identified as indicator of grassland habitat. Two species (*Lophyra parvimaculata* and *Lophyra striolata striolata*) were positively associated (as lift value was 6.5 for both) with muddy habitat and seven species (*Calomera angulata*, *Calomera plumigera*, *Chaetodera vigintiguttata*, *Cosmodela*

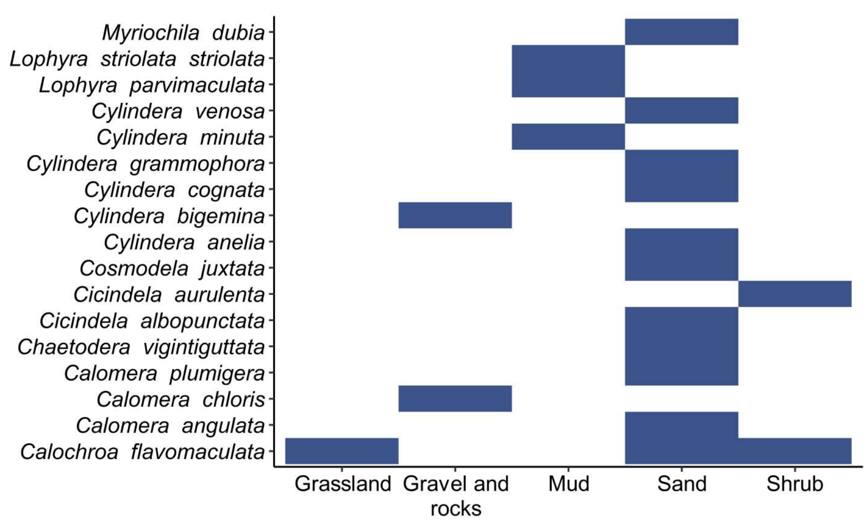

Fig. 2. Distribution of 17 species of tiger beetles in 5 habitat types

juxtata, *Cylindera anelia*, *Cylindera cognata* and *Cylindera venosa*) were positively associated (as lift value was 2.0 for all) with sandy habitat (Table 3).

The tiger beetles live in the transition area between terrestrial and aquatic ecosystems and can use both dry and wet habitats (Bobrek 2023). Thus, these species are among the few organisms that can be used for conservation monitoring in both terrestrial and aquatic ecosystems. The occurrence of 17 species (6.88% of all Indian species) of tiger beetles in the study area highlighted the important role of the Ramganga River in maintaining rich biodiversity. The eight tiger beetle species from multiple habitats which suggested the species-specific habitat utilizations of the tiger beetles where it is evident that within the same habitat, the tiger beetle population can coexist and escape competition for resources through niche partitioning (Brosius and Higley 2013, Vacher et al., 2016, Jaskuła and Płóciennik 2020). Furthermore, study found seven tiger beetle species from sandy habitats only and two tiger beetle species from muddy habitats only. This suggests that sandy substrates may provide more favorable ecological conditions such as suitable microclimate, easier burrowing for larval development, and greater prey availability compared to muddy habitats, which appear to support fewer specialized species (Pearson et al., 2020). Such differences highlight the importance of habitat type in shaping tiger beetle distribution and diversity patterns.

Among the six species of tiger beetles identified by Bhargav et al. (2008) as indicators of riverine or river-associated habitats in the Shivalik landscape of north-west India, the present study re-identified five species (*Calomera angulata*, *Calomera chloris*, *Chaetodera vigintiguttata*, *Cylindera bigemina* and *Cylindera venosa*) as indicators of specific habitats within the broader riverine riparian ecosystem. Their consistent reappearance as key habitat indicators highlighted the ecological significance of tiger beetles in monitoring habitat quality and heterogeneity within river systems. Notably, *Calochroa flavomaculata* did not emerge as an indicator of any particular habitat type in the present study, a pattern consistent with the earlier observations of Acciavatti and Pearson (1989) and Jaskuła (2011). However, evidence from previous research also suggested that this species may be shifting its distribution

Table 3. Species positively associated with sandy and muddy habitats

Species	Associated habitat	Lift
Lophyra striolata	Mud	6.5
Lophyra parvimaculata	Mud	6.5
Cylindera venosa	Sand	2.0
Calomera angulata	Sand	2.0
Chaetodera vigintiguttata	Sand	2.0
Cosmodela juxtata	Sand	2.0
Cylindera anelia	Sand	2.0
Cylindera cognata	Sand	2.0
Calomera plumigera	Sand	2.0

Table 2. Indicator species of tiger beetles for different habitats

Species	Habitat	Indicator value index (IndVal)	p value
Calomera chloris	Gravel and rocks	0.416	0.002
Cylindera bigemina	Gravel and rocks	0.298	0.02
Cylindera minuta	Mud	0.669	0.001
Lophyra striolata	Mud	0.636	0.001
Lophyra parvimaculata	Mud	0.273	0.036
Cylindera venosa	Sand	0.636	0.001
Calomera angulata	Sand	0.545	0.001
Chaetodera vigintiguttata	Sand	0.545	0.001
Cosmodela juxtata	Sand	0.545	0.001
Cylindera anelia	Sand	0.545	0.001
Cylindera cognata	Sand	0.545	0.001
Calomera plumigera	Sand	0.364	0.003
Myriochila dubia	Sand	0.364	0.003
Cicindela albopunctata	Sand	0.343	0.007
Cylindera grammophora	Sand	0.343	0.004
Cicindela aurulenta	Shrub	0.388	0.005

toward higher elevations, where it can potentially serve as a key climatic indicator (Singh et al., 2019). This absence from study area may be attributed to the species' broader ecological tolerance or less specialized habitat requirements compared to other tiger beetles, making it less suitable as a habitat-specific indicator. In the Ramganga River, human activities over the past few decades have substantially altered bank land-use patterns, and the river is increasingly threatened by pollution (Khan et al., 2019; Sarah et al., 2019; Bhattacharjee et al., 2022). With rapid urban development, sand extraction from rivers and coastal areas has intensified worldwide resulting in severe riverbed alteration, bank erosion, and the consequent loss of vital ecosystem services (Pandey et al., 2022; Rangel-Buitrago et al., 2023). Ramganga River is no longer an exception, as sand quarrying continues at various sites along its course (Daityari and Khan 2017; Alexander Speed et al., 2019; Nautiyal and Mishra 2022). These anthropogenic pressures threaten not

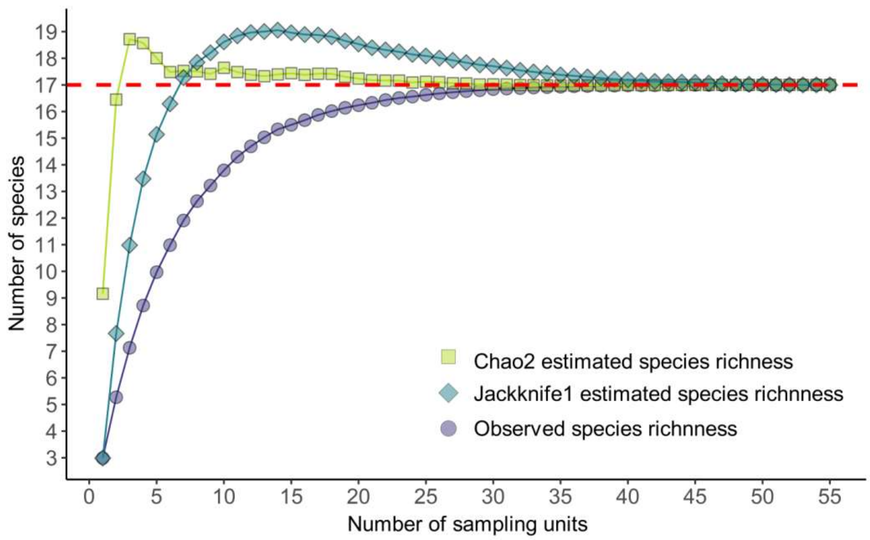

Fig. 3. Species accumulation curves for tiger beetles in Gangetic riparian zone at increasing sample size. The sampling effort yielded all the estimated species (17 species)

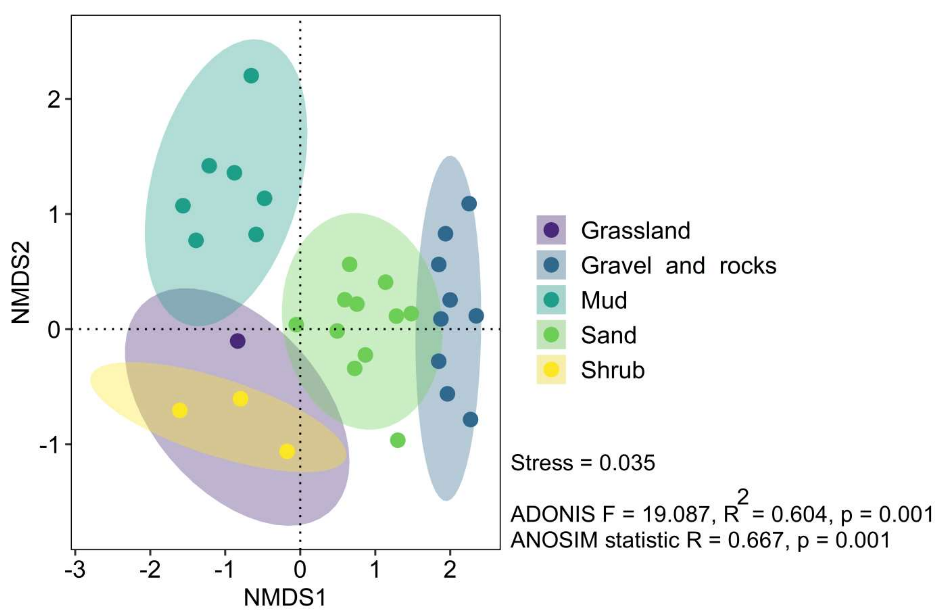

Fig. 4. Non-metric multidimensional scaling (NMDS) ordination plot illustrating differences among groups of habitats plotted in species space

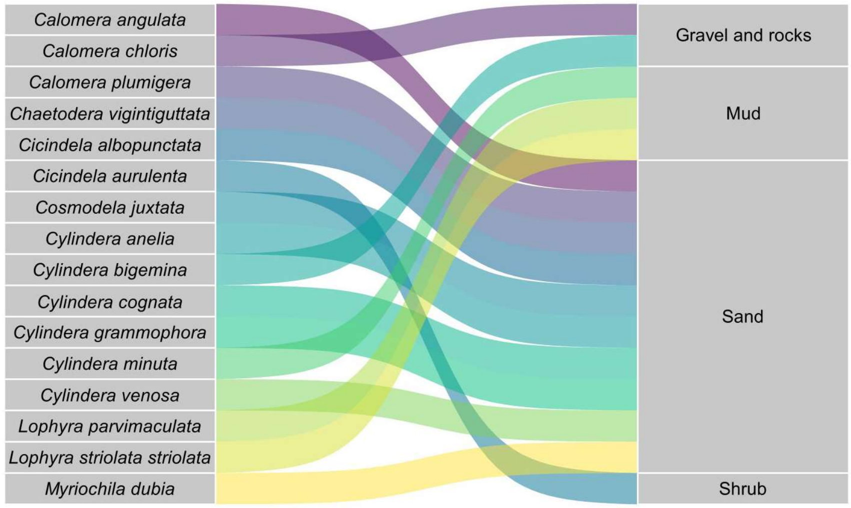

Fig. 5. Alluvial plot showing of 16 species of tiger beetles which are bioindicator of four habitat types. *Calochroa flavomaculata* is not shown was not significantly associated with any habitat and the grassland habitat is not shown was not significantly associated with any species

only riverine biodiversity but also the ecological functions that sustain local communities and biodiversity.

CONCLUSIONS

Present study provided the baseline information about the diversity of tiger beetles and species-specific habitat preferences in the Ramganga River. Moreover, study also concluded that tiger beetles are strongly associated with sandy habitats and riparian vegetation in riverbeds and riverbanks. Given the factors affecting their habitat, tiger beetles are highly habitat-specific and sensitive to environmental changes, the degradation of riparian zones directly risks their survival in the near future. Therefore, protecting riverbanks from uncontrolled sand mining, restoring natural substrates, and maintaining water quality are critical not only for the health of the river but also for conserving tiger beetle assemblages that serve as reliable bioindicators of ecosystem integrity. Integrating tiger beetle monitoring into river management plans can provide an early-warning system for ecological disturbances and help guide conservation actions. Ensuring the persistence of tiger beetle populations in the Ramganga River will thus play a vital role in conserving both biodiversity and the long-term resilience of riparian ecosystems.

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Rust Outbreak vs. Heat Stress: Resolving the Weather-Disease Nexus in Wheat

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Abstract: The present study was planned to evaluate the association between meteorological parameters and yellow rust severity in wheat during three contrasting seasons: 2012-13 (rust epidemic year), 2021-22 (heat stress year), and 2022-23 (recent rust year). Rust severity was recorded as 100, 56.75 and 58.42% in 2012-13, 2021-22, and 2022-23, respectively. Correlation analysis revealed that maximum temperature (Tmax) was positively associated with rust severity. Minimum temperature also showed a strong positive correlation Morning relative humidity (RHm) and evening relative humidity (RHe) exhibited negative correlations, with RHm r = -0.65 to -0.72 and RHe r = -0.73 to -0.79, strongest in 2021-22. Rainfall had a weak positive correlation in 2012-13 and 2022-23, but a significant negative one in 2021-22 (r = -0.64). Sunshine hours were positively correlated with rust severity (r = 0.88 in 2021-22). During the disease phases, maximum and minimum temperatures were lowest in 2012–13 and highest in 2021-22. Relative humidity and rainfall were highest in 2012-13, followed by 2022-23, and minimum in 2021-22. Sunshine hours were least in 2012-13 and highest in 2022-23. Thus, 2012-13 was characterized by cooler temperatures, higher humidity, and greater rainfall conditions highly favourable for rust development. In contrast, 2021-22 experienced higher temperatures, lower humidity, and reduced rainfall, contributing to heat stress conditions. The year 2022-23 exhibited intermediate weather conditions, favouring moderate rust development. These findings highlight the importance of integrated weather-based forecasting for effective yellow rust management in wheat.

Keywords: Heat stress, Meteorological parameters, Thumb rules, Weather disease window, Yellow rust

The Earth's average temperature has been steadily rising, stimulating more frequent and intense heat waves worldwide. In India, March and April 2022 were the hottest on record, with extreme temperatures surpassing normal levels by +8 to +10.8°C and rainfall decreasing by 60% to 99% in 10 out of 36 meteorological subdivisions. This period stands as a truthful example of how elevated temperatures and reduced rainfall collectively impacted agricultural production, particularly in northern and central India. The heat wave struck at a critical stage in wheat development i.e. grain filling leading to yellowing of grains, shrivelling and premature maturity, ultimately reducing yields by 15-25% (Bal et al., 2022). In addition to weather aberrations, wheat is attacked by different pathogens in northern India and stripe rust is a devastating fungal disease occurring as major wheat disease in north India. Stripe rust/yellow rust thrives well under cool and humid conditions. It is a major threat to wheat productivity globally, largely due to favourable weather conditions and the evolution of new virulent pathotypes (Hovmoller et al., 2008, Ali et al., 2017). The coexistence of these two contrasting climatic stressors—one triggered by elevated temperatures and the other thriving in cool-humid environments poses a challenge in predicting and managing wheat diseases under changing climatic conditions.

The correlation between weather parameters and the development of stripe rust has been well documented. Temperature, humidity, leaf wetness, sunshine hours and rainfall are critical in modulating the onset and progression of the disease (Brown et al., 2001, Chen 2005). Infection is favoured by daytime temperatures between 10-15°C, relative humidity above 85% and wet leaf surfaces for at least three hours (Line, 2002). In India yellow rust does not survive the summer temperatures of the plains but survives in cooler hill areas and returns with wind-dispersed uredospores during winter (Wang et al., 2010). In contrast, rising temperatures above 25°C which are common under climate change scenarios tend to suppress the disease, though newer stripe rust strains show adaptation to warmer climates (Wellings, 2007, Hubbard et al., 2015). The timing of weather anomalies, such as an unusually warm January or wet February, can trigger or suppress rust outbreaks. Studies have shown that February, with optimum conditions for stripe rust viz., maximum temperatures in range of 15–25°C and relative humidity around 86–98% frequently corresponds with high disease severity years (Sandhu et al., 2018, Kashyap et al., 2018). Moreover, rain splashes and wind currents facilitate spore dispersal and inoculum buildup (Geagea et al., 2000, Isard & Russo, 2011). Conversely, sunshine duration, which limits leaf wetness, has a negative correlation with disease development (Gill et al., 2012). Several models have been developed to forecast stripe rust incidence using meteorological data. ARIMA models, regression analyses, and temperature-humidity indices have all shown high predictive power in determining disease outbreaks (Poudyal et al., 2013, Sandhu et al., 2021, Khushboo et al. 2023). Yellow rust has historically caused up to 70% yield losses in epidemic years (Singh et al., 2004), while heat stress particularly during terminal growth stages can cause yield penalties of 20–40%, particularly in heat-sensitive cultivars. Heat stress, precisely during the reproductive and grain-filling stages, compromises yield and quality by accelerating senescence and reducing grain size (Milus et al., 2009). The convergence of these threats, particularly in wheat-growing belts such as the northwestern plains of India, calls for robust, localized and dynamic disease-forecasting systems.

This study investigates the validation of meteorological thumb rules for yellow rust severity using historical rust outbreak years (2012–13) and heat stress years (2021–22) and recent rust year (2022–23). The key weather variables viz., temperature, relative humidity, sunshine hours and rainfall across critical crop growth months were analysed. The main objective behind this analysis was to identify conditions conducive or suppressive for stripe rust development. This will not only enhance our understanding of the environment-pathogen interaction but will also contribute to adaptive disease management strategies under changing climatic conditions.

MATERIAL AND METHODS

Study area: The research experiments were conducted at Punjab Agricultural University, Ludhiana during different years under study viz., 2012-13, 2021-22 and 2022-23. Ludhiana is located in the trans-Gangetic agroclimatic zone of Punjab at 30°54' N latitude and 75°48' E longitude, lies at an altitude of 247 meters above mean sea level. The region has a subtropical, semi-arid climate. December and January are the coldest months, occasionally experiencing frost, while May and June are the hottest, with temperatures sometimes exceeding 45°C. The annual average maximum and minimum temperatures are 29.8°C and 16.7°C, respectively. Rainfall averages 760 mm annually, with 75-80% occurring during the monsoon season from June to September.

Meteorological data: The meteorological data w.r.t maximum and minimum temperature, morning and evening relative humidity, rainfall and sunshine hours for the study years was collected from the Department of Climate Change and Agricultural Meteorology at PAU, Ludhiana.

Disease incidence: The disease severity involves determination of the plant tissue proportion that was infected by disease. Different scales, such as the modified Mannar's (1960) scale for yellow rust of wheat was employed to estimate the severity in per cent on the leaves (Table 1). Disease severity was calculated from collected data by using following formula:

$$\text{Disease severity (\%)} = \frac{\text{Number of leaves infected} \times \text{Scale}}{\text{Total area of leaves infected} \times \text{Maximum grade}} \times 100$$

Correlation coefficient analysis: Correlation coefficient analysis was carried out between different meteorological parameters and yellow rust severity using R software. This analysis was carried out to study the strength and direction of linear relationship between two variables.

Validation of thumb rules: To anticipate disease outbreaks, “thumb rule” a simplified empirical guideline (Sandhu et al., 2021) based on key weather variables was validated. This section evaluates the validity of these rules across three contrasting years: a high rust severity year (2012-13), a heat stress year with low rust occurrence (2021-22), and a recent rust year (2022-23).

RESULTS AND DISCUSSION

Meteorological parameters during different years: Rust severity was 100, 56.75, and 58.42% in in 2012-13, 2021-22, and 2022-23 (Fig. 1). During disease initiation, the maximum temperature averaged 16.6°C, 15.3°C 2021-22, and 16.4°C in 2012-13, 2021-22, and 2022-23 (Fig. 2a). Minimum temperatures remained relatively cool, with means of 6.4°C,

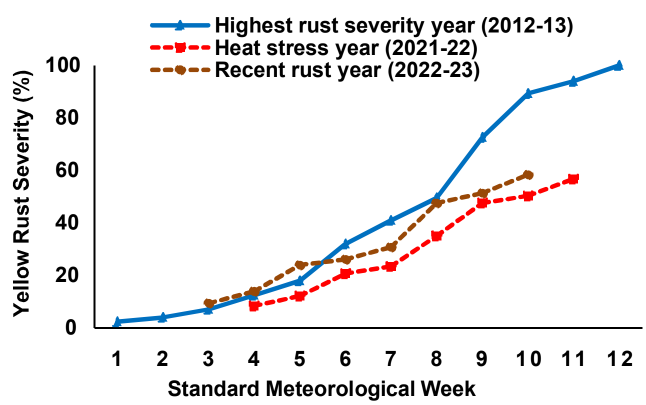

Fig. 1. Yellow rust severity during different years under study

Table 1. Rating scale for yellow rust of wheat

Code	Scale for severity of infection
5	Up to 5 per cent leaf area infected
10	Up to 10 per cent leaf area infected
20	Up to 20 per cent leaf area infected
50	Up to 50 per cent leaf area infected
75	Up to 75 per cent leaf area infected
100	Up to 100 per cent leaf area infected

8.5°C, and 5.8°C, respectively, and ranged between 4.3°C and 8.1°C (Fig. 2b). Morning relative humidity was consistently high across years, averaging 94.0, 93.8 and 91.8% in 2012-13, 21-22, and 22-23 (Fig. 3a). Evening humidity ranged from 49% to 75% in 2012-13 , higher in 2021–22 (74.4%, 68-83%) but relatively lower in 2022-23 (57.5%, 37-77%) (Fig. 3b). Total rainfall during the initiation phase was 8.2 mm in 2012-13, while it was negligible in 2021-22 and 2022-23 (Fig. 4a). Sunshine hours averaged 5.9 hours/day (0.2-8.4 h) in 2012-13, 4.9 hours/day (0.1-9 h) in 2021-22, and 5.2 hours/day (1.4-6.9 h) in 2022-23 (Fig. 4b). The combination of cooler temperatures, high humidity, low sunshine, and occasional rainfall during 2012–13 provided highly favourable conditions for early rust initiation and infection establishment. During disease progression, maximum temperatures increased to averages of 20.3°C (19.8-23.9°C) in 2012-13, 21.1°C (17.5-23.4°C) in 2021-22, and 23.9°C (20.3-27.5°C) in 2022-23. Minimum temperatures averaged 8.7°C, 8.2°C, and 10.7°C, respectively. Morning relative humidity was highest in 2012–13 (97.0%, 97-99%), compared to 91.0% in 2021–22 and 89.5% in 2022–23. Evening humidity averaged 63.0 % in 2012–13 declining to 50.5% in 2021–22 and 47.75% in 2022–23 . Total rainfall during the progression phase was 22.6 mm in 2012–13, whereas it was much lower (3-4 mm) in 2021-22 and 2022-23. Sunshine hours during this phase were lower in 2012-13 (5.8 hours/dayh) compared to 7.3 hours/day and 7.7 hours/day in 2021-22 and 2022-23, respectively. The cool, humid, and moderately rainy conditions in 2012-13 favoured rapid disease multiplication and widespread epidemic spread during this critical phase. In the end phase, maximum temperatures increased to 27.7°C in 2012-13, 29.6° in 2021-22, and 27.1°C in 2022-23. Minimum temperatures followed a similar rising trend, averaging 12.6°C, 15.3°C, and 14.7°C, respectively. Morning relative humidity declined slightly, averaging 95.0% in 2012-

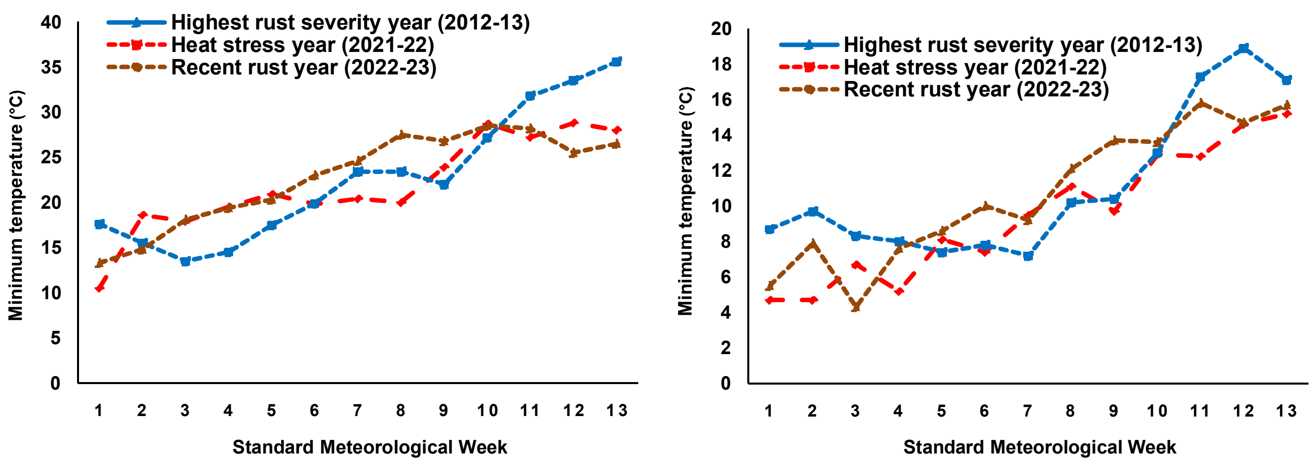

Fig. 2. Comparison of weekly maximum and minimum temperatures during yellow rust occurrence during different years under study

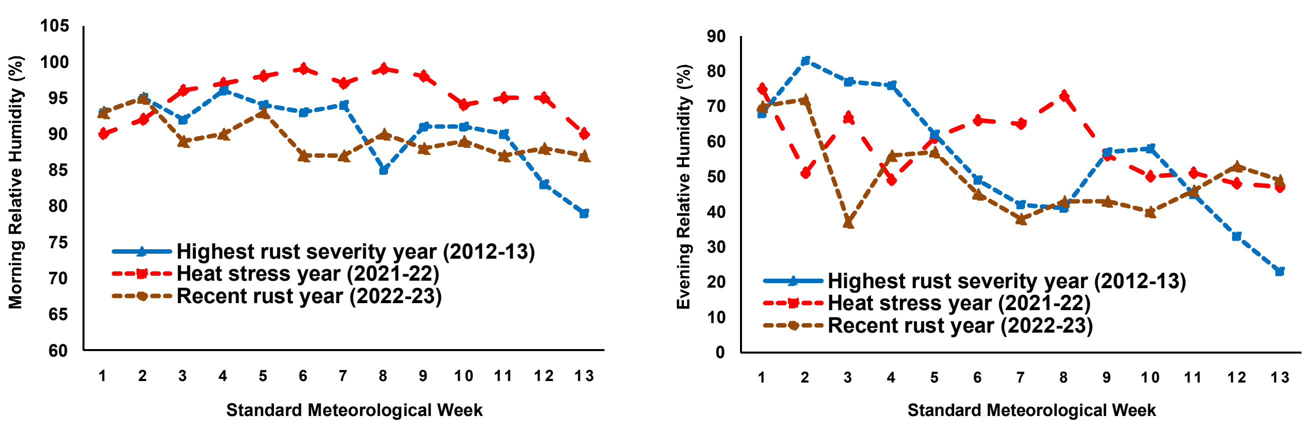

Fig. 3. Comparison of morning and evening relative humidities during yellow rust occurrence during different years under study

13, 87.0% in 2021-22, and 88.2% in 2022-23. Evening humidity was 55.0% in 2012–13, compared to 43.0% in 2021–22 and 46.0% in 2022–23. Total rainfall during the end phase was about 35 mm in 2012–13, almost negligible in 2021-22, and limited (~10 mm) during week 13 in 2022-23. Sunshine hours peaked during 2021-22 (9.4 hours/day) and remained relatively high in 2022-23 (8.2 hours/day) compared to 2012-13 (8.7 hours/. The higher temperatures, reduced humidity, and greater sunshine during the later stages of 2021-22 and 2022-23 led to early crop maturity, limiting late-stage disease development.

Weather-disease window: During the disease initial phase, maximum and minimum temperatures were lowest in 2012–13, intermediate in 2022-23, and highest in 2021-22 (Fig. 5). Morning and evening relative humidity were maximum in 2012-13, followed by 2022-23, and minimum in 2021-22. Rainfall was highest in 2021-22, intermediate in 2022–23, and lowest in 2012-13. Sunshine hours were least in 2012-13 and highest in 2021-22. In the disease progression phase, maximum and minimum temperatures were highest in 2022-23 and lowest in 2012–13. Relative humidity (both morning and evening) and rainfall were maximum in 2012–13, intermediate in 2021–22, and minimum in 2022–23. Sunshine hours were highest in 2022-23 and lowest in 2012-13. During the disease end phase, maximum and minimum temperatures were highest in 2022-23 and lowest in 2012-13. Morning and evening relative humidity were highest in 2012-13, intermediate in 2022-23,

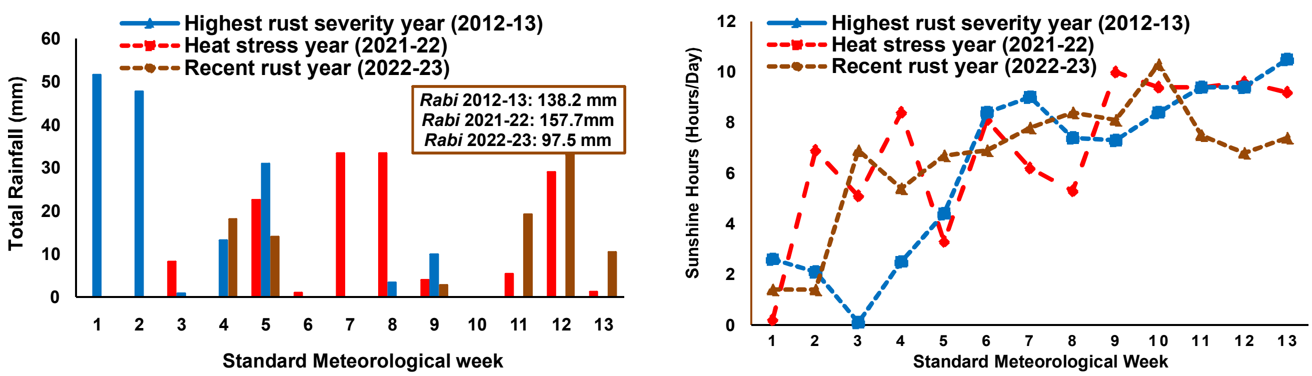

Fig. 4. Comparison of total rainfall and sunshine hours/day during yellow rust occurrence during different years under study

		Disease initial phase (SMW 1st – 4th)	Disease progression phase (SMW 5th – 8th)	Disease end phase SMW (9th – 13th)
Highest rust severity year (2012-13)	Tmax (°C)	10.5–19.5 (16.6)	20.9–20.4 (20.7)	20–28 (25.6)
	Tmin (°C)	4.7–6.7 (5.3)	8.1–11.1 (9.3)	9.7–15.2 (12.04)
	RHm (%)	90–97 (93.8)	98–99 (98.8)	98–90 (94.2)
	RHe (%)	49–75 (60.3)	61–73 (65.3)	56–47 (51.4)
	Total Rainfall (mm)	8.2	33.4	29
	Ssh (hours)	0.2–8.4 (5.1)	3.3–10 (5.7)	5.3–9.6 (8.7)
Heat-stress year (2021-22)	Tmax (°C)	13.5–17.6 (15.3)	17.5–23.4 (20.9)	22–35.6 (29.9)
	Tmin (°C)	7.4–8.7 (8.1)	7.2–10.2 (8.2)	10.4–17.1 (14.5)
	RHm (%)	92–96 (94)	94–85 (91.5)	91–79 (87)
	RHe (%)	68–83 (76)	62–41 (53)	57–23 (44.2)
	Total Rainfall (mm)	51.6	3.4	9.9
	Ssh (hours)	0.1–9 (3.5)	4.4–9 (7.1)	7.3–10.5 (8.7)
Recent rust year (2022-23)	Tmax (°C)	13.3–19.4 (16.4)	20.3–24.6 (22.9)	25.5–28.5 (27.1)
	Tmin (°C)	4.3–7.9 (6.1)	8.6–12.1 (10.5)	13.7–15.7 (14.7)
	RHm (%)	89–93 (91.5)	93–87 (89.3)	88–87 (88.2)
	RHe (%)	56–70 (62.3)	57–43 (47.8)	43–49 (46.2)
	Total Rainfall (mm)	18.1	0	33
	Ssh (hours)	1.4–6.9 (4.8)	6.7–8.4 (7.8)	6.8–8.4 (7.6)
Maximum among study years				
Intermediate				
Minimum among study years				

Fig. 5. Weather disease window for yellow rust during different phases of disease occurrence

and lowest in 2021-22. Rainfall was maximum in 2022-23, followed by 2012-13, and least in 2021-22. Sunshine hours remained highest in 2022-23 and intermediate in 2021-22. Thus, it is evident that 2012-13 was characterized by cooler temperatures, higher humidity, and higher rainfall conditions highly favourable for rust development. In contrast, 2021-22 experienced higher temperatures, lower humidity, and reduced rainfall, contributing to heat stress conditions. The year 2022-23 exhibited intermediate weather conditions, favouring moderate rust development.

Correlation coefficients: The correlation analysis between yellow rust severity and meteorological parameters during study revealed that both consistent and year-specific trends. Across all three years, maximum temperature and minimum temperature showed a consistently strong positive correlation with yellow rust severity. The maximum and minimum temperatures were significantly correlated with rust severity during three years and maintained significant positive relationships, confirming the critical role of temperatures in promoting yellow rust infection across years. Sunshine hours also showed a positive and significant correlation with rust severity throughout all years, being highest in 2022-23 followed by 2012-13 and 2021-22. This indicates that more sunshine days favoured rust development consistently, with slightly stronger effects observed in 2022-23. In contrast, relative humidity parameters showed negative correlations with rust severity, although their interpretation requires careful understanding of disease progression dynamics. Morning relative humidity (RHm) was significantly negatively correlated with yellow rust severity in 2012-13 and 2022-23 but was non-significant in 2021-22 .). Similarly, evening relative humidity (RHe) exhibited negative correlations across all three years,. Although higher humidity is essential for the initial establishment of yellow rust, as the season progressed, relative humidity declined, yet the disease continued to proliferate under conducive temperatures and sunshine conditions. Consequently, the overall season-long correlation between relative humidity and rust severity appeared negative, reflecting the later-season weather-disease dynamics rather than initial infection conditions. Rainfall exhibited a weak and inconsistent relationship with rust severity across years. In 2012-13 and 2021-22, rainfall was weakly and positively correlated with rust severity, though not significant. However, in 2022-23, rainfall showed a significant negative correlation.

The regression plots further reinforced these relationships by visually depicting the trends between meteorological variables and yellow rust severity. In the scatter plots for maximum and minimum temperatures across all three years, a strong positive slope was observed, indicating that as temperatures increased, yellow rust severity also increased. This positive trendline was steeper during 2012–13 and 2022–23, corresponding to the higher correlation coefficients during these years. In contrast, the regression lines for morning and evening relative humidity showed a negative slope, particularly sharp in 2022–23, further supporting the negative correlation pattern. However, this negative trend represents the later-season decline in humidity rather than the initial favourable conditions for rust initiation. Notably, the sunshine hours regression lines consistently showed a steep positive slope, with tighter clustering of data points around the trendline in 2022–23, highlighting the stronger dependence of yellow rust on sunshine during this year. Rainfall plots showed no consistent trend in 2012–13 and 2021–22, while a mild negative slope was visible in 2022–23, aligning with the observed negative correlation. The spread of data points (as reflected in the scatter) and the fitted regression lines, along with shaded confidence intervals, confirmed that temperature and sunshine were the most consistent and significant predictors of yellow rust severity, while humidity and rainfall played more variable roles depending on the year.

Validation of thumb rules: To anticipate disease outbreaks, several “thumb rules” i.e. simplified empirical guidelines based on key weather variables have been developed by different researchers. In this study the thumb rule developed for yellow rust forecasting by Sandhu et al. (2021) was validated (Table 2). This section evaluated the validity of these rules across three contrasting years: a high rust

Table 2. Validation of thumb rules for forewarning of yellow rust in Punjab

Month	Thumb rule*	Highest rust severity year (2012-13)	Heat stress year (2021-22)	Rust year (2022-23)
January	Tmax : 20-24°C	No	No	No
	Tmin : 7-13°C	No	Yes	No
	RHm : 86-98%	Yes	Yes	Yes
	Ssh : >8 hrs	No	No	No
	Rainfall : > 20 mm	No	Yes	No
February	Tmax : 15-25°C	Yes	Yes	Yes
	Tmin : 7-13°C	Yes	Yes	Yes
	RHm : 86-98%	Yes	No	Yes
	Ssh : 5-10 hrs	Yes	No	Yes
	Rainfall : > 20 mm	Yes	Yes	No
March	RHm : > 80%	Yes	Yes	Yes
	Frequent rainfall	Yes	No	Yes

severity year (2012-13), a heat stress year with low rust occurrence (2021-22), and a recent rust year (2022-23). The thumb rules for January suggests that yellow rust risk increases when maximum temperatures range between 20-24°C, minimum temperatures between 7-13°C, relative humidity (RH) is high (86-98%), sunshine hours exceeds 8 h, and rainfall is above 20 mm. Upon comparison with historical data, only relative humidity consistently matched across all three years, including both rust-prone and rust-free seasons. Notably, in 2012-13 and 2022-23 both rust years most of the other parameters were not aligned with the thumb rules. Conversely, in 2021-22, despite the occurrence of some conducive factors (e.g., high humidity and rainfall), no

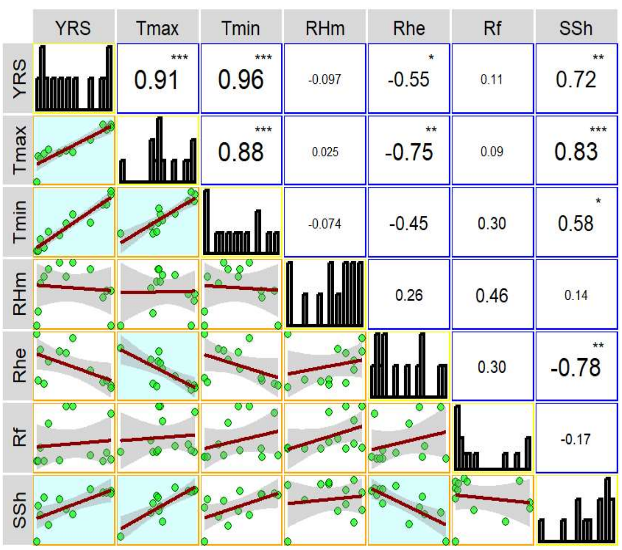

Fig. 6. Correlation coefficients between meteorological parameters and yellow rust severity during 2012-13 year

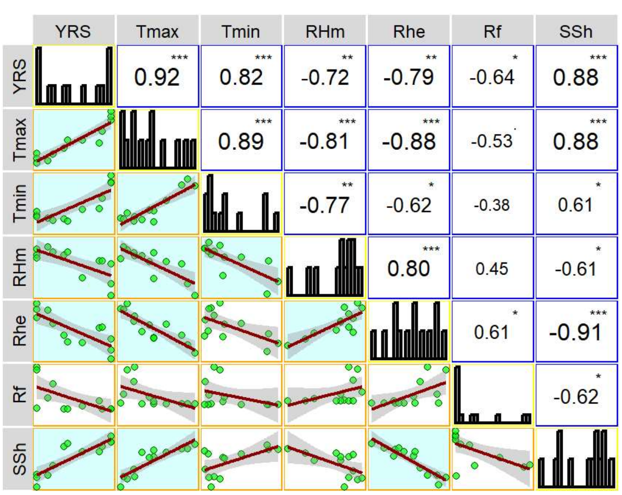

Fig. 7. Correlation coefficients between meteorological parameters and yellow rust severity during 2021-22 year

significant yellow rust outbreak was reported. Thumb rules for January exhibited limited predictive value when considered in isolation. Relative humidity appears to be the most consistently associated factor, but other parameters, such as temperature and rainfall, are not individually sufficient for early rust prediction. In February, the thumb rules hypothesize more rust risk when maximum temperatures fall between 15-25°C, minimum temperatures between 7-13°C, RH remains within 86-98%, sunshine ranges from 5-10 hours, and rainfall exceeds 20 mm. These criteria were fully met in 2012-13, which recorded the highest yellow rust severity, and largely satisfied in 2022-23, a more recent rust year. In contrast, 2021-22 met several thermal and rainfall criteria but did not experience a significant outbreak, potentially due to lower relative humidity and sunshine hour discrepancies. February exhibits the strongest validation of thumb rules. When multiple environmental factors (particularly temperature, RH, and rainfall) converge, the risk of yellow rust escalates considerably. This month serves as a critical window for disease surveillance and management interventions. For March, the thumb rules emphasize RH levels above 80% and the presence of frequent rainfall. All three years fulfilled these criteria. However, yellow rust was maximum during 2012-13. While March conditions can support disease progression, they are not independently predictive of outbreak initiation. Rather, they may contribute to sustained or secondary infection phases if early-season conditions were conducive. The comparative analysis confirms that the thumb rules are most predictive when evaluated cumulatively, particularly in February. Relative humidity is a consistently relevant factor across all months. However, relying on any single parameter may lead to false positives or negatives. Therefore, an integrated approach that considers multiple concurrent weather conditions - especially during February - is essential for accurate forecasting and timely management of yellow rust in wheat.

The comparative analysis of three contrasting wheat seasons: 2012–13 (epidemic year), 2021-22 (heat-stress year), and 2022-23 (recent rust year) provides valuable insights into the complex relationship between meteorological variables and the development of yellow rust. The results underscore that temperature, humidity, sunshine duration and rainfall interact in nuanced ways to influence the onset, progression, and suppression of the disease. Temperature emerged as a key driver of disease onset and severity. During both the 2012-13 and 2022-23 seasons, yellow rust outbreaks coincided with moderately low maximum temperatures (15-17°C) and cool minimum temperatures (5.8-6.4°C) during the initiation phase. These thermal conditions fall within the optimal range for *Puccinia striiformis* development, consistent with findings by Chen (2005) and Line (2002) where favourable growth and infection at day temperatures between 10-15°C and night temperatures between 4-8°C. Minimum temperature showed the strongest positive correlation with rust severity in 2012-13 and 2022-23), highlighting the role of night temperatures in prolonging leaf wetness duration and enhancing nocturnal fungal activity (Sandhu et al., 2021). Conversely, the 2021-22 season, marked by daytime and nighttime temperatures up to 35.6°C and 15.3°C, respectively, recorded minimal disease severity Wellings (2007) and Milus et al. (2009) also observed that higher temperatures negatively affect urediniospore viability, germination, and infection efficiency. Although newer pathotypes of *Pst* have shown some thermotolerance (Ali et al., 2017), the 2021-22 thermal profile likely exceeded even these adapted thresholds, supporting reduced epidemic potential under heat-stress scenarios. Relative humidity also played a pivotal role in disease modulation. Across all three years, morning RH levels were consistently high (91-94%) during the initiation phase, with maximum levels (97%) observed in 2012-13. However, evening humidity levels, which were substantially lower in 2021-22 (43%) and 2022-23 (46%), correlated negatively with rust severity, especially in the former year. The incomplete overlap of high relative humidity across morning and evening in 2021–22 could explain the limited disease spread despite the presence of other partially favourable conditions. Rainfall contributed to disease spread but was not a primary driver. Moderate rainfall during the progression phase in 2012-13 (22.6 mm) likely facilitated spore dispersal and canopy wetness, which is consistent with the disease-promoting role of rain splashes and wind-assisted dissemination described by Isard and Russo (2011) and Geagea et al., (2000). However, the overall weak correlation between rainfall and disease (r = 0.11 to 0.38) suggests that rainfall acts as a secondary or complementary factor, enhancing the conducive environment established by temperature and humidity. Sunshine duration displayed a somewhat paradoxical pattern. While longer sunshine hours are generally thought to reduce leaf wetness and inhibit disease, a positive correlation was observed in all years, particularly in 2021-22 (r = 0.88). Anand et al. (2023) demonstrated that optimal urediniospore germination of *Puccinia striiformis* occurred at 15°C, whereas *Puccinia triticina* peaked at 20°C, with a neutral pH (7) and intense light (1250 lux) further promoting germination. This anomaly may stem from the role of sunlight in regulating host physiology, such as photosynthesis and stomatal conductance, which can indirectly affect disease susceptibility (Brown et al., 2001). In 2021-22, the intense solar radiation and elevated temperatures may have offset any potential disease benefits of increased sunlight by hastening crop maturity and thereby shortening the rust's infection window. The research of relationship of illumination and temperature was instructive, since under natural conditions illumination would always be changing and may affect spore germination. Since, the initial studies by Dillon Weston (1931) and Stock (1931), light has been recognised to alter urediniospore germination of numerous species of rust fungi differently. The type of affect and the degree to which light affects germination, however, differ depending on the light source and the species of rust. There hasn't been a direct comparison of how light affects the urediniospores of different species of rust fungi. At specific temperatures, *P. striiformis* germination was accelerated by fluorescent light (Tollenaar and Houston 1966).

The validation of thumb rules proposed by Sandhu et al. (2021) highlighted February as the most critical month for reliable disease forecasting. In both 2012-13 and 2022-23, all major indicators like favourable temperature ranges (15-25°C max, 7-13°C min), high RH (86-98%), rainfall above 20 mm, and moderate sunshine were met, and yellow rust incidence was prominent. In contrast, the 2021-22 season, though satisfying some thermal and rainfall conditions, failed to meet crucial humidity and canopy wetness thresholds, explaining the absence of a significant outbreak. Poudyal et al. (2013) also emphasized the cumulative effect of multiple parameters rather than single-variable thresholds in predicting disease emergence. The January thumb rules showed limited alignment with actual disease patterns, especially in 2021–22, and March parameters were too general to independently predict disease initiation, the

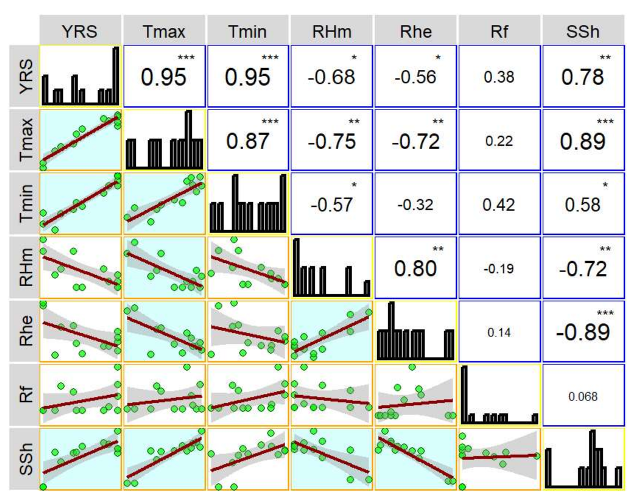

Fig. 8. Correlation coefficients between meteorological parameters and yellow rust severity during 2022-23 year

results strongly advocate for an integrated approach that considers concurrent interactions of temperature, humidity, and rainfall during February. This approach supports the development of robust, dynamic early warning systems that can optimize fungicide application timing and reduce unnecessary chemical use (Kashyap et al., 2018). Anand and Sandhu (2024) highlighted that the early sowing window, marked by ideal weather for rust proliferation, recorded the highest yellow rust severity compared to later sowing periods. Together, these insights underscore the intricate interplay of abiotic factors driving stripe rust epidemics and highlight the potential of predictive modelling in disease management. Overall, the study confirms that yellow rust outbreaks are not driven by single weather variables but by the synergistic effect of multiple favourable conditions, particularly during the disease initiation and progression windows. The reduced rust pressure under high-temperature regimes, as observed in 2021–22, also emphasizes the emerging trade-off between heat stress and rust outbreaks under climate change scenarios. As newer *Pst* pathotypes continue to evolve, continuous surveillance and adaptive forecast models integrating real-time weather data will be indispensable.

CONCLUSIONS

The findings of this study underline that yellow rust development is strongly driven by a combination of cooler temperatures, high humidity and adequate moisture availability. Years marked by such conditions lead to severe disease outbreaks. In contrast, elevated temperatures, lower humidity, and longer sunshine hours restricted disease progression, suggesting that rising temperatures due to climate change could potentially shift disease dynamics in future wheat-growing seasons. These insights point to the need for region-specific disease forecasting systems that integrate real-time weather data. Proactive surveillance and adaptive management strategies will be crucial to safeguarding wheat production under increasingly unpredictable weather patterns.

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Monitoring Biodiversity and Temperature-Dependent Patterns of Insects in Wet and Moist Sal Forest of Western Himalayas

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***Abstract*:** Sal forest ecosystems are home to a diverse array of insect communities. The Western Himalayan range, particularly the Shivaliks, is characterized by its sal forests, which exhibit significant ecoclimatic habitat variability. Sal forests of the Himalayas support a rich diversity of insect fauna. The study investigated, investigated the turnover of insect communities, species diversity, seasonal fluctuations, and their correlation with temperature across two distinct sal forests with different rainfall regimes. Through random sampling, we monitored the insect fauna in both wet and sal forest. The two forest types have significantly similar species composition and diversity. Order Lepidoptera predominates in both forests, and most insect communities exhibit higher species diversity during the warmer summer months. In total, 7,787 individuals representing 92 species and 13 orders were recorded, with 64 species shared between sites and relatively low turnover (31% dissimilarity). Principal component analysis revealed a strong temperature dependence, with insect communities thriving between 24–34°C, highlighting a distinct thermal niche across both forests. Further studies that emphasize the need for conservation and long-term monitoring of insect fauna in this region is recommended.

***Keywords*:** Biogeography, Diversity, Insects, Sal, *Shorea robusta,* Western Himalayas

The Western Himalayan region, with its complex terrain and diverse microclimates, supports some of the most ecologically significant forest ecosystems in South Asia. Among these, the wet and moist Sal forests, dominated by *Shorea robusta* Gaertn. f., are recognized for their high ecological and economic value. *S.robusta* exhibits a gregarious growth pattern and is distributed widely across the Himalayan foothills in India, Nepal, and Bangladesh (Baral et al., 2022, Majumdar et al., 2014), with further extensions into Bhutan and southern China (Nepal 2023). These forests thrive at altitudes ranging from near sea level up to 1500 meters, and they harbor a wide variety of insect taxa critical for pollination, nutrient cycling, and as indicators of ecosystem health.

Insects are key components of forest ecosystems, yet recent studies have raised concerns over widespread declines in insect populations globally, attributed to anthropogenic pressures, habitat loss, and climate change (Forister et al., 2011; Sánchez-Bayo and Wyckhuys 2021). These declines are particularly alarming in ecologically sensitive regions like the Himalayas, where insects play indispensable roles in sustaining biodiversity. Temperature, a dominant abiotic factor, influences insect physiology, phenology, and distribution patterns, making it a crucial variable in understanding insect responses to environmental change. Monitoring shifts in insect diversity and abundance along temperature gradients is therefore vital for anticipating the impacts of climate change on forest ecosystems (Montgomery et al., 2021).

Despite the ecological importance of sal forests, there remains a significant knowledge gap in the documentation and monitoring of their insect communities, particularly in different rainfall regimes of sal forests in the Western Himalaya. Some records highlight the presence of insect pests such as *Hoplocerambyx spinicornis*, a major defoliator of *S. robusta* in the region (Singh 2021), but there is limited data on the broader insect assemblages and their ecological relationships. Vudem et al. (2010) mentioned that the lack of extensive entomological surveys within Sal forest ecosystems, and no studies have yet examined how insect biodiversity in these forests correlates with local temperature regimes. Additionally, the species turnover of insects between the Sal forests of two distinct rainfall regimes is not documented. This research aims to address these gaps by conducting a systematic survey of insect diversity and assessing temperature-dependent patterns in the Western Himalaya's wet and moist Sal forests. Using established arthropod monitoring protocols,

MATERIAL AND METHODS

Description of sites: The sal forest of Ranibagh (hereafter referred to as RSF) is 600 meters above sea level. The area under survey is located between latitude 29.28882° and longitude 79.55140° and is flanked by the Gaula River. This area is marked by its excellent richness of flora and fauna, and is a moist Sal forest. The transects studied in the Sal forest of Kaladhungi (hereafter referred to as KSF) are located at an elevation of 400 meters and between

29.30476°N, 79.33974°E to 29.30828°N, 79.34312°E, and are flanked by the Boar River and are a wet sal forest. The elevation of transects was carefully selected to minimize the effect of altitudinal habitat heterogeneity, ensuring more uniform ecological and environmental conditions for the study.

Sampling: The study was conducted between May 2022 and June 2023. The forests were visited during the daytime and nighttime on consecutive days to record diurnal and nocturnal insects. Temperature was recorded throughout the day using a digital thermometer. Additionally, various trapping methods and instruments were used, such as light traps and sweep nets, and collecting jars, to collect specimens. 100m.sq. transects were randomly selected, and the line-transect method was followed at each transect to observe the insects.

Collection of insects: Insects were preserved using cotton-soaked fumigants (chloroform or 10% ethyl acetate) at the lab by stretching and pinning the insects following Upton and Mantle (2010). Furthermore, insect diversity and abundance were studied through observational approaches. Identification of insects was done after bringing the collected insects to the lab. The insects were examined for their physical characteristics and compared to the reference collections in the Insect Biodiversity Laboratory at the Department of Zoology, D.S.B. Campus, Kumaun University, Nainital, based on key descriptions. Those that could not be identified at the species level were identified at the genus level. Surveys were performed during the night to observe the nocturnal insects using insect Night traps following Jonason et al. (2014).

Statistical analysis: The data was analyzed statistical software Paleontological Statistics (PAST 4.03), Brodgar v2.7.5, and Microsoft Excel for developing models, graphs, diversity indices, evenness, accumulation, distribution, and visualization. α-Diversity indices were estimated using the PAST v4.03 statistical software. Population parameters, such as evenness and richness, include the Shannon and Gini-Simpson Index, and the Margalef and Menhinick Index, respectively. Whittaker and Wilson-Shmida β diversity index was estimated using PAST software. Indices such as Jaccard's Dissimilarity and Bray-Curtis Dissimilarity index were used to analyze the similarities of insect community distribution between the two sal forests.

Seasonal index and fluctuations: The total period of the survey was divided into 4 seasons: Summer=S, Rainy=R, Winter=W, and Autumn=A (Farooq et al., 2021). March, April, and May are considered Summer; June, July, and August as Rainy; September, October, and November as Autumn; and finally, December, January, and February as winter. Seasonal diversity was checked using the species frequencies in the PAST v4.03 Statistical software. Seasonal fluctuations and seasonal index were calculated using slight modifications to the method Mathew and Anto (2007) used. Seasonal Index = (Monthly mean/Overall mean) x 100 (1)

Status of species: The status of insect species was based on the number of sightings, following Farooq et al. (2021). The insects were assigned different status with slight modification to Farooq et al. (2021). Very Common (VC) >=70 sightings, Common (C) = 30-69, Occasional = (O) = 10-29, Rare = (R) =<=9

Evaluating impacts of temperature: PCA with variant-covariant was employed for analyzing non-parametric multi-dimensional arrays. The correlation was analyzed between the abundance of orders and their relationship to annual temperature using the Bray-Curtis correlation matrix for each site.

RESULTS AND DISCUSSION

General findings: Total of 7787 individuals of insects belonging to 92 species and 13 orders were recorded across the two aal forest areas, with 80 species of insects from RSF and 77 species from KSF were recorded. Of these, 64 species are common to both Sal forests, whereas 16 and 13 are unique to RSF and KSF, respectively (Table 1). Twelve orders were recorded at KSF and 13 orders at RSF. 3384 individuals were recorded from the Ranibagh Sal forest, belonging to 79 species of insects from 13 different Orders. These findings may be the consequences of high vegetative diversity within the two Sal forests, which was previously reported by Pandey et al. (2023). Such high biodiversity is also supported by slightly disturbed ecosystems aided by their geographic isolation (Hussain et al., 2023) since both forests stand away from anthropogenic disturbances. Order Lepidoptera was the most abundant order (40%) with 11 Families (45.5%) and 36 species, with family Pieridae (seven genera and eight species) having the highest number of individuals (10.12%) among all the orders, followed by order Diptera (13.7%), and Hymenoptera (13.17%). 4403 individuals were recorded from the KSF, belonging to 77 species of insects from 12 different Orders. Annual evenness shows congruence (9.7) in both forests. Out of these, Order Lepidoptera was the most abundant order (32%) with nine families (45.5%) and 31 species, with family Pieridae having the highest number of individuals among all the orders (13.6%). Bashar and Chowdhury (2021) form deciduous sal forests of Bhawal and Madhupur in central Bangladesh observed that dominant was Hymenoptera, accounting for 31% of the identified species. In present study Lepidoptera was the dominant order, accounting for 40% species at the moist Sal forest (RSF) and

Table 1. Insect fauna associated with Sal forests and their habitat preferences

Family	Taxa	RSF	KSF	Habitat
Order Lepidoptera				
Papilionideae	*Papilio polytes* (Linnaeus)	+	+	Wet & Moist
Papilionideae	*Papilio clytia dissimilis* (Linnaeus)	+	+	Wet & Moist
Papilionideae	*Papilio demoleus* (Linnaeus)	+	+	Wet & Moist
Papilionideae	*Papiliopta aristolochiae* (Fabricius)	+	-	Moist
Pieridae	*Zemeros flegyas* (Cramer)	+	+	Wet & Moist
Pieridae	*Eurema hecabe* (Linnaeus)	+	+	Wet & Moist
Pieridae	*Leptosia nina* (Fabricius)	+	+	Wet & Moist
Pieridae	*Catopsilia pomona*(Fabricius)	+	+	Wet & Moist
Pieridae	*Catopsilia pyranthe* (Linnaeus)	+	+	Wet & Moist
Pieridae	*Belenois aurota* (Fabricius)	+	+	Wet & Moist
Pieridae	*Pieris brassicae* (Linnaeus)	+	+	Wet & Moist
Pieridae	*Cepora nerissa* (Fabricius)	+	+	Wet & Moist
Nymphaladeae	*Tirumala limniaceae* (Cramer)	+	+	Wet & Moist
Nymphaladeae	*Junonia lemonias* (Linnaeus)	+	+	Wet & Moist
Nymphaladeae	*Phalanta phalantha* (Drury)	+	-	Moist
Nymphaladeae	*Symbrenthia lilaea* (Hewitson)	+	-	Moist
Nymphaladeae	*Ypthima baldus* (Fabricius)	+	+	Wet & Moist
Nymphaladeae	*Euthalia aconthea (Cramer)*	-	+	Wet
Hesperidae	*Telicota bambusae* (Moore)	+	-	Moist
Riodinidae	*Abisara bifasciata* (Moore)	+	-	Moist
Lychenidae	*Zizeeria karsandra* (Moore)	+	+	Wet & Moist
Lychenidae	*Lampidus boeticus*(Linnaeus)	+	+	Wet & Moist
Arctiidae	*Cyana adita* (Moore)	+	-	Moist
Carambidae	*Luma sericea* (Butler)	-	+	Wet
Carambidae	*Endocrossis flavibasalis* (Moore)	+	+	Wet & Moist
Carambidae	*Conogethis punctiferalis* (Guenée)	+	+	Wet & Moist
Geometridae	*Ornithospila avicularia* (Warren)	+	+	Wet & Moist
Geometridae	*Campaea perlata* (Guenée)	-	+	Wet
Geometridae	*Pelagodes spp.* (Holloway)	+	+	Wet & Moist
Geometridae	*Hypomecis cineracea* (Moore)	+	-	Moist
Geometridae	*Timandra correspondens* (Hampson)	+	+	Wet & Moist
Geometridae	*Pingasa ruginaria* (Guenée)	+	-	Moist
Erebidae	*Perina nuda* (Fabricius)	+	+	Wet & Moist
Erebidae	*Oraesia emarginata* (Fabricius)	+	+	Wet & Moist
Erebidae	*Mocis undata* (Fabricius)	+	+	Wet & Moist
Noctuidae	*Helicoverpa armigera* (Hubner)	+	+	Wet & Moist
Noctuidae	*Athetis transversa* (Walker)	+	+	Wet & Moist
Noctuidae	*Mythimna separata* (Walker)	+	-	Moist
Noctuidae	*Spodoptera litura* (Fabricius)	+	-	Moist
Eupterotini	*Eupterote undata* (Blanchard)	-	+	Wet
Eupterotini	*Eupterote bifasciata* (Kishida)	-	+	Wet

Cont...

Table 1. Insect fauna associated with Sal forests and their habitat preferences

Family	Taxa	RSF	KSF	Habitat
Order Orthoptera				
Tettigoniidae	*Tettigoniidae spp.* (Continua)	+	+	Wet & Moist
Tettigoniidae	*Sathrophyllia rugosa* (Stål)	-	+	Wet
Acrididae	*Arphia conspersa* (Cudder)	+	-	Moist
Acrididae	*Acrida exaltata* (Walker)	-	+	Wet
Gryllidae	*Gryllus confirmatus* (Walker)	+	+	Wet & Moist
Gryllidae	*Teleogryllus testaceus* (Walker)	-	+	Wet
Order Coleoptera				
Scarabaeidae	*Mimela fulgidivittata* (Blanchard)	+	-	Moist
Scarabaeidae	*Mimela splendens* (Gyllenhal)	-	+	Wet
Scarabaeidae	*Holotricia serrata* (Fabricius)	+	+	Wet & Moist
Scarabaeidae	*Onthophagus orientalis* (Harold)	+	+	Wet & Moist
Scarabaeidae	*Agamopus unguicularis* (Harold)	+	+	Wet & Moist
Scarabaeidae	*Lepidiota albistigma* (Burmeister)	+	+	Wet & Moist
Scarabaeidae	*Maladera castanea* (Arrow)	+	+	Wet & Moist
Coccinellidae	*Coccinella septempunctata* (Linnaeus)	+	+	Wet & Moist
Carabidae	*Bradycellus spp.* (Erichson)	+	+	Wet & Moist
Elateridae	*Adelocera spp.* (Latreille)	+	+	Wet & Moist
Cerambycidae	*Dorysthenus huegelii* (Redtenbacher)	+	+	Wet & Moist
Meloidae	*Zonitoschema pallidissima* (Reitter)	+	+	Wet & Moist
Brentidae	*Caenorhychodes diagramma* (Boisduval)	-	+	Wet
Order Odonata				
Libellulidae	*Orthetrum taeniolatum* (Schneider)	+	+	Wet & Moist
Libellulidae	*Crocothermis serivilia* (Drury)	+	+	Wet & Moist
Order Blattodea				
Blattidae	*Blatta orientalis* (Linnaeus)	+	+	Wet & Moist
Blattidae	*Periplaneta americana* (Linnaeus)	-	+	Wet & Moist
Order Mantodeae				
Hymenopodidae	*Creobroter gemmatus* (Saussure)	+	+	Wet & Moist
Mantidae	*Mantis religiosa* (Linnaeus)	+	+	Wet & Moist
Gonypetidae	*Gonypetyllis spp.* (Wood-Mason)	+	+	Wet & Moist
Order Neuroptera				
Chrysopidae	*Chrysoperla carnea* (Stephens)	+	-	Moist
Chrysopidae	*Leucochrysa insularis mina* (Walker)	+	+	Wet & Moist
Order Trichoptera				
Rhyacophilidae	*Rhyacophila spp.* (Pictet)	+	-	Moist
	Order Hemiptera			
Largidae	*Physopelta gutta* (Burmeister)	+	+	Wet & Moist
Pyrrhocoriidae	*Dindymus versicolor* (Herrich-Schaeffer)	+	+	Wet & Moist
Pyrrhocoriidae	*Dysdercus sidae* (Montrouzier)	+	+	Wet & Moist
Pentatomidae	*Nezara viridula* (Linnaeus)	+	+	Wet & Moist
Rhyparochromidae	*Diecuches notatus* (Dallas)	+	+	Wet & Moist
Miridae	*Closterotomus spp.* (Fieber)	+	+	Wet & Moist

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Table 1. Insect fauna associated with Sal forests and their habitat preferences

Family	Taxa	RSF	KSF	Habitat
Order Diptera				
Stratiomyidae	*Hermetia illucens* (Linnaeus)	+	+	Wet & Moist
Tabanidae	*Tabanus rubidus* (Wiedemann)	+	-	Moist
Sarcophagidae	*Sarcophaga argyrostoma* (Robineau-Desvoidy)	+	+	Wet & Moist
Muscidae	*Musca domestica* (Linnaeus)	+	+	Wet & Moist
Culicidae	*Anopheles fluviatilis* (James)	+	+	Wet & Moist
Culicidae	*Culex pipiens* (Linnaeus)	+	+	Wet & Moist
Culicidae	*Aedes aegypti* (Linnaeus)	-	+	Wet
Order Hymenoptera				
Vespidae	*Vespa tropica* (Linnaeus)	+	+	Wet & Moist
Vespidae	*Polistes olivaceus* (DeGeer)	+	+	Wet & Moist
Apidae	*Apis cerana* (Fabricius)	+	+	Wet & Moist
Apidae	*Apis dorsata* (Fabricius)	+	+	Wet & Moist
Formicidae	*Oecophylla smaragdina* (Fabricius)	+	+	Wet & Moist
Ichneuonidae	*Enicospilus* sp. (Stephens)	+	+	Wet & Moist
Order Isoptera				
Termitidae	*Microtermes obesi* (Holmgren)	+	+	Wet & Moist
Order Ephimeroptera				
Ephemeridae	*Ephemera* sp1 (Linnaeus)	-	+	Wet
Ephemeridae	*Ephemera* sp2 (Linnaeus)	+	-	Moist
Baetidae	*Labiobaetis spp.* (Novikova and Kluge)	+	+	Wet & Moist
Total individuals		3378	4403	7781

32% at the Wet Sal forest (KSF). The mean annual temperature of RSF is reportedly higher than KSF. Higher mean temperatures at RSF can increase the abundance and diversity of Lepidopterans, especially moths in dry and moist habitats, which aligns with the findings of Choi (2008), Goswami et al. (2023) and Mishra et al. (2016).

Apis cerana (6.32%) was the most sighted species in RSF. A total of eight species were rare at RSF and KSF, *Physopelta gutta* (5.8%) was the most sighted species, followed by *Apis cerana* and *Apis dorsata* (Table 1) Only three species were rare visitors at KSF, which include *Gonypetyllis sp*. (0.18%) and *Labiobaetis sp*. (0.09%). Overall, *Apis cerana* (4.5%) was the most sighted species, followed by *Physopelta gutta* (5.9%) and *Apis dorsata* (5.1%). Five rare species existed overall, which are *Rhyacophila sp*. (0.6%), *Labiobaetis sp*. (0.6%), *Sathrophyllia rugosa* (0.01%), *Arphia conspersa* (0.01%), and *Ephemera sp*. (0.01%).

Seasonal community dynamics: The Shannon index indicates the highest evenness during the summer season at RSF and KSF (3.95 and 3.90, respectively), while the least during the autumn at KSF (3.05) and during winters at RSF (3.46) (Table 2). The Margalef index was highest for the year (9.78) at RSF. Highest species richness was recorded during the autumn season at RSF (10.47) and during the summer season at KSF (9.33). The least richness was during the months of autumn (KSF=5.40) and winter (RSF=6.90). Such seasonal trends align with previous studies of macro-invertebrate communities (Sun et al., 2024). Similarly, the highest abundance of insects at KSF was seen during the summers, aligning with Müller et al. (2024).

The seasonal fluctuation of insects at RSF and KSF has been demonstrated in Figure 1. The fluctuation of insects was the highest for May (rainy season), followed by April. On the other hand, the slightest fluctuation of insects can be seen in October or the winter season. At KSF, the abundance of insects was the highest in July, followed by June. The slightest fluctuation of insects can be seen in October or winter. The high abundance of insects before and during the rainy season may be due to favorable temperature and humidity, as reported in previous studies (Zhao et al., 2023).

Relationship of insect community dynamics with temperature: Principal components for KS explain a maximum variance of 94.1% by temperature in PC1 and PC2, and 93.3% variance for RSF. The biplots demonstrated high collinearity in principal components 1 and 2 (Fig. 2).

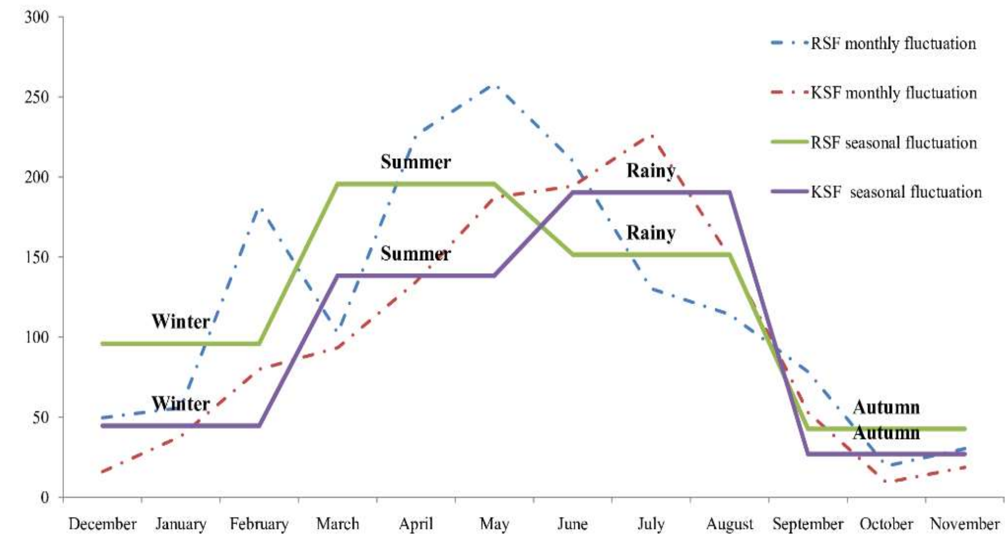

Fig. 1. Seasonal and monthly fluctuations derived from the seasonal index of insects in both forests

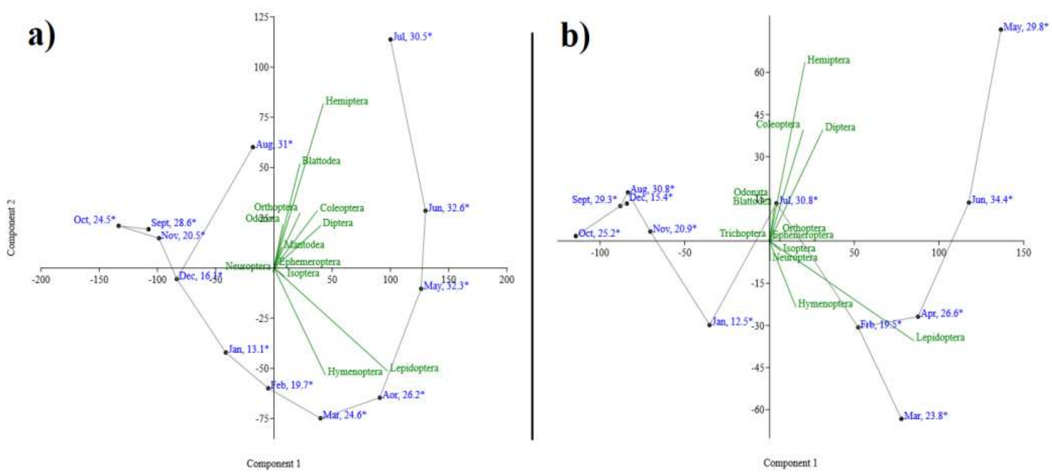

Fig. 2. PCA analysis of annual insect fluctuation showing the relationship with temperature. a) Scatter plot of PC-1 & PC-2 for KSF b) Scatter plot of PC-1 &PC-2 for RSF

Table 2. Descriptive seasonal insights of insect communities at RSF and KSF

Index	Site	Winter	Summer	Rainy	Autumn
Taxa S	RSF	46	71	67	60
	KSF	37	70	67	32
Simpson (1–D)	RSF	0.96	0.975	0.976	0.948
	KSF	0.952	0.971	0.965	0.928
Shannon (H')	RSF	3.467	3.959	3.917	3.513
	KSF	3.267	3.905	3.774	3.054
Evenness (e^H/S)	RSF	0.696	0.738	0.750	0.559
	KSF	0.709	0.709	0.650	0.662
Margalef	RSF	6.909	9.618	9.580	10.470
	KSF	5.574	9.334	8.784	5.407

Table 3. Turnover in the regional biogeography of the two sites

Diversity Index	Value	Interpretation
Jackard's Similarity Index	0.68	68% similarity
Jackard Distance of Dissimilarity	31.18%	31% dissimilarity
Wilson and Shmida, β	0.184	Low turnover
Whittaker, β	0.184	Low turnover
Bray-Curtis dissimilarity	0.294	29.4% dissimilarity

The most communities of insects at KSF thrive at temperatures between 24 to 30°C, and 23.8 to 34.4°C at RSF. Most insect communities fall within the first and the fourth quadrants in both wet and moist forests. The PCA highlights that most insect communities of insects show less diversity at temperatures corresponding to the months from September to January, and thrive at temperatures between 24 and 34°C. Such findings on insect thermal niche have also been reported in previous studies (Dingha, 2009; Wu et al., 2017; Brewer et al., 2021; Qiu et al., 2012). Various other biotic and abiotic factors, such as temperature, altitude, humidity, understory vegetation, and solar intensity, contribute to the potential growth of insects within these two distinct forests, which requires further investigation.

Species turnover: Statistically significant ($p<0.05$) Pearson's correlation coefficient of 0.82 exists between the species diversities of both sites (Table 3). The two sites present 68% similarity in species but also show 31% dissimilarity in the diversity of communities, suggesting relatively low species turnover between wet and moist forests.

This study limits the environmental parameters to temperature only. Further research is required on these al ecosystems, considering other parameters along with temporal dynamics. The study will serve as a foundation and baseline for future research on insect ecology and conservation in forest ecosystems with similar rainfall regimes.

CONCLUSION

This study shows that the wet and moist Sal forests of the western Himalaya support a high and largely overlapping insect fauna (7,787 individuals, 92 species; 64 species shared between sites) with relatively low species turnover (29-31% dissimilarity). Lepidoptera were dominant in both forest types, and insect diversity and abundance peaked in the warmer months. Multivariate analysis reveals that most insect communities are concentrated between 24-34°C, indicating a strong temperature dependence of community dynamics. These results provide a practical baseline for detecting climate-driven changes in insect assemblages in Sal forests. The standardized, long-term insect monitoring program in the region with continuous temperature loggers and repeat surveys (pre-monsoon/monsoon focus) should be initiated and need to protect habitat heterogeneity.

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Morphological and Molecular Characterization of Native Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium rileyi* from North Coastal Andhra Pradesh

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Abstract: The investigation was undertaken on isolation and assessment of native entomopathogenic fungi from the North coastal districts of Andhra Pradesh at the Department of Entomology, Regional Agricultural Research Station, ANGRAU, Anakapalle, Andhra Pradesh during 2024-25. The soil samples were collected from both natural and agricultural habitats across Srikakulam, Vizianagaram, Visakhapatnam and Anakapalle districts. Two entomopathogenic fungal species were successfully isolated: *Beauveria bassiana* (AKP Bb-cd) from *Neolamarckia cadamba* soil and *Metarhizium rileyi* (AKP Mr-2m) from an insect cadaver collected from maize field. Morphological examination revealed white mycelial colonies in both isolates with *M. rileyi* transitioning to olive green upon sporulation. Molecular characterization using ITS1 and ITS4 primers produced 600 bp amplicons for both the isolates and sequences were deposited in NCBI GenBank. Phylogenetic analysis using Neighbour Joining method in MEGA 12 confirmed their genetic identity.

Keywords: Entomopathogenic fungi, Isolation, *B. bassiana*, *M. rileyi*, Biocontrol

Biological control offers an environmentally sustainable approach for managing insect pests. Among the key agents employed are entomopathogenic fungi, which play a vital role in reducing pest populations and minimizing crop damage. The success of these fungi as biocontrol agents depends largely on the insect's susceptibility and the fungus virulence. Unlike other biological control organisms, entomopathogenic fungi do not require ingestion to infect, they penetrate the host directly through the cuticle (Inglis et al., 2000). Entomopathogenic fungi have been widely explored as promising biological control agents due to their natural occurrence, mode of action through cuticular penetration and environmental safety. Different entomopathogens such as *Metarhizium anisopliae*, *M. rileyi*, *Beauveria bassiana*, *Isaria* and *Lecanicillium* are the most extensively studied EPFs for pest control applications (Chandel et al., 2018, Timmi and Joshi 2024). Several studies have emphasized their potential as alternatives to chemical insecticides, especially under integrated pest management strategies (Fang et al., 2014, Niu et al., 2019). The genus *Metarhizium* is known for its ecological diversity and infect a wide range of insect hosts (Wang et al., 2016, Moonjely and Bidochka 2019). Likewise, *Beauveria* is another extensively researched entomopathogen with proven efficacy in insect pest control. Furthermore, indigenous fungal isolates, adapted to local environmental conditions often outperform commercial strains in terms of persistence and efficacy (Abott 1925, Abid et al., 2022). Molecular identification using ITS regions has further enabled accurate characterization and differentiation of native strains (Rehner and Buckley 2005). Species identification can be achieved through the analysis of total genomic DNA digested with restriction enzymes and separated electrophoretically using restriction fragment length polymorphisms (RFLPs) for strain differentiation. Additionally, Random Amplified Polymorphic DNA (RAPD) analysis has also been used for characterizing fungal isolates as demonstrated by Kosir et al. (1991) and Visalakshi et al. (2020).

MATERIAL AND METHODS

Sampling and insect cadaver collection: A total of 32 soil samples were collected from natural and agricultural ecosystems and two entomofungal isolates were isolated, one from *Neolamarckia cadamba* tree soil collected from Devipuram, Anakapalle district located at 17.977°N, 83.329°E coordinates and another from an insect cadaver collected from Maize crop, Padmanabham, Visakhapatnam district located at 17.698°N, 83.003°E coordinates in Andhra Pradesh, India. The soil samples were collected from the rhizosphere zone between 5 to 10 cm from the soil surface and approximately 100 g of soil sample were collected at each location from different soil habitats in separate sterile polythene bags and labelled properly with name of the crop, date and place of collection and stored in a darker place until further testing. Insect cadaver collected from maize crop was surface sterilized with 4% solution of sodium hypochlorite for one minute followed by rinsing in sterile distilled water to remove the external contaminants.

Isolation of entomopathogenic fungi: Entomopathogenic fungi were isolated from soil samples by using serial dilution method, with dilutions ranging from 10^{-4} to 10^{-8}. The procedure was replicated three times and plated on SDAY media, leading to the successful recovery of fungal isolates. In addition, fungal inoculum scraped from the insect cadaver was also plated on SDAY media to initiate the growth of the fungus.

Morphological identification of entomopathogenic fungi: The morphological characteristics such as spore size, shape, colour, colony morphology, shape of the conidia, length and width ratio of the spore of fungal isolates were examined. Microscopic observations were conducted at 10x and 40x magnifications and digital images were captured at 40x magnification using V-image 2013 software.

Preparation of SDB (Sabouraud's dextrose broth): Sabouraud's Dextrose Broth (SDB) was prepared using glucose, peptone and yeast extract, excluding agar. The medium was sterilized in an autoclave at 121°C for 15 minutes under 15 psi pressure. Five mm disc of mycelial mat from fungal cultures grown on SDAY medium was aseptically transferred into conical flasks containing the sterilized SDB. These flasks were incubated at 25°C for two weeks. After incubation, the mycelial mat was separated by filtering the broth through filter paper, washed with distilled water and dried on tissue paper. The dried mat was wrapped in aluminum foil and stored at -20°C. After 24 hours, the preserved fungal mat was used for DNA extraction.

Molecular Identification of Entomopathogenic Fungi

Fungal DNA extraction: The DNA was extracted using Sambrook and Russell protocol (2001). Fungal mats of both the isolates were homogenized **in** liquid Nitrogen **using** a pre-sterilized mortar and pestle. The macerated fungus was transferred to sterile 1.5 ml eppendorf tubes followed by the addition of pre-heated (65°C) 2% CTAB buffer. These samples were incubated in a dry bath at 65°C for one hour. After incubation, an equal volume of Chloroform and Phenol (1:1 ratio) was added and the samples were centrifuged in a refrigerated centrifuge (Centrifuge 5418 R) at 10,000 rpm for 10 min at 24°C. The supernatant was transferred to 1.5 ml fresh eppendorf tubes. Centrifugation was repeated and supernatant again collected into fresh tubes. Equal volume of Chloroform and Isoamyl alcohol mixture was added and centrifuged at 10,000 rpm for 10 min. The supernatant was collected in a separate eppendorf tube and 0.6 volume of ice-cold isopropanol, 0.1volume of sodium acetate buffer (0.3M, pH 5.0) were added and then incubated for 24 hours at -20°C. After incubation, the tubes were taken out and centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was discarded and pellet was washed with 70 per cent ethanol and again centrifuged at 10,000 rpm for 10 min at 4°C. After discarding the supernatant, the pellets were air dried and dissolved in 100µl of molecular grade water. The DNA samples of fungal isolates were stored at -20°C.

PCR amplification of ITS region: The identification of the purified isolates based on morphological characteristics was complemented with the sequencing of the Internal transcribed region (ITS) sequences. The amplification of ITS region was carried out using Universal primers ITS 1 and ITS 4 primers. Thermocycling conditions included initial denaturation at 95°C for 5 min, followed by 35 amplification cycles of 94°C for 1 min, 55°C for 1min, 72°C for 1 min, followed by final extension at 72°C for 10 min.

Running of gel electrophoresis: Agarose gel electrophoresis of PCR amplified DNA was carried out using a 1% (w/v) agarose gel prepared by dissolving 1.0 g of MB-grade agarose in 100 ml of 1x TAE buffer. The solution was heated until fully melted, then cooled to 50-55°C before adding 4 µl of ethidium bromide (10 mg/ml). The gel was poured into a casting tray fitted with 0.5 mm combs, ensuring bubble free pouring. Once solidified, the comb was gently removed and the gel was placed in a tank containing 1xTAE buffer. DNA samples and a 1 kb ladder were loaded and electrophoresis was performed at 100 V for 45-60 minutes. DNA band migration was visualized and documented using a Vilber E-Box gel documentation system in auto exposure mode. The amplified PCR products were excised and sent to a sequencing service provider for partial sequencing. The Internal Transcribed Spacer (ITS) region sequences obtained were compared with reference sequences using the BLAST tool available in the NCBI GenBank database to identify similarity with known sequences. Phylogenetic trees for *Beauveria* and *Metarhizium* constructed using MEGA software (version 12.0) to analyze their evolutionary relationships.

RESULTS AND DISCUSSION

The soil sample and insect cadaver collected from natural and agricultural ecosystems from north coastal districts of Andhra Pradesh yielded two fungal isolates that showed typical characteristics of entomopathogenic fungi when observed under the microscope at 40x magnification.

Morphological identification of EPF: In this study, *Beauveria bassiana* (AKP Bb-cd) was isolated from rhizosphere of *Neolamarckia cadamba* tree while *Metarhizium rileyi* (AKP Mr-2m) was isolated from an insect cadaver collected from maize crop. The preliminary characterization showed that the isolate *B. bassiana* initially produced white cottony colony growth which later turned into powdery growth. In contrast, the isolate *M. rileyi* initially showed white colony growth and later turned into olive green colour with velvety spores upon sporulation (Table 1, 2).

Cokola et al., (2023) examined the morphological characteristics of fungal structures, including shape and size of the conidia, conidiophores and identified the isolates as belonging to the genus *Beauveria*. The genus *Metarhizium* is characterized by cylindrical to ellipsoid conidia in mature colonies, which were dark green in colour, formed chains of equal length in the clusters obtained. The conidia were typically oblong, elliptical in shape varying 9.0 µm in length and 4.3 µm in width (Mathulwe et al., 2021).

Molecular characterization of EPF: The molecular identification was done for both the isolates by using ITS Universal primers. The primers ITS 1 and ITS 4 were used for PCR amplification of DNA. Along with primers, DNA sample, Dream Taq (2x) master mix and nuclease free water were added in a PCR tube and PCR amplification was done and the ladder DNA of 1 kb was taken as reference for the gel run. A single discrete PCR amplicon band of 600 bp was observed for both the isolates when resolved under gel documentation system (Vilber E-box). Molecular analysis confirmed the identification of *Beauveria* and *Metarhizium* through amplication and sequencing of the ITS1-5.8S-ITS2 region of recombinant DNA followed by sequence comparison using databases such as NCBI and fungal barcoding resources demonstrated by Kearse et al. (2012).

DNA sequencing: PCR products were eluted and sequenced with an automated DNA sequencing facility. The obtained Sequences were identified by homology using Basic Local Alignment Search Tool (BLAST). The nucleotide sequences of *B. bassiana* and *M. rileyi* in the present study were compared with other *B. bassiana* and *M. rileyi* sequences available in the National center for Biotechnology Information (NCBI) GenBank database (http://www.ncbi.nlm.nih.org). Phylogenetic tree was constructed for both the isolates using the Neighbour joining method and 1000 bootstrap replicates using MEGA 12.0 version software (Kumar et al., 2024).

Phylogenetic tree analysis of EPF using MEGA software: The phylogenetic tree was constructed to elucidate the evolutionary relationships among various *B. bassiana* and *M. rileyi* isolates from different geographic regions using sequence data retrieved from NCBI database for comparison. The phylogenetic tree was constructed ~~by~~ using Neighbour joining method with bootstrap analysis to assess the robustness of each clade. Bootstrap values shown at the nodes indicate the percentage of 1000 bootstrap replicates in which the associated group of taxa clustered together. The image (Fig. 3) represents a phylogenetic tree constructed using sequence data from various isolates of *Beauveria bassiana* along with a single outgroup *Metarhizium rileyi*. The phylogenetic analysis of *Beauveria bassiana* isolates revealed distinct clustering patterns reflecting their geographic origins. Isolates from Kerala, Raichur, Gujarat and Puducherry formed a well-supported clade, whereas isolates from southern India, including Bangalore, Chennai, Tirupati and Devipuram grouped together with strong bootstrap support. The Devipuram isolate, AKP Bb-cd

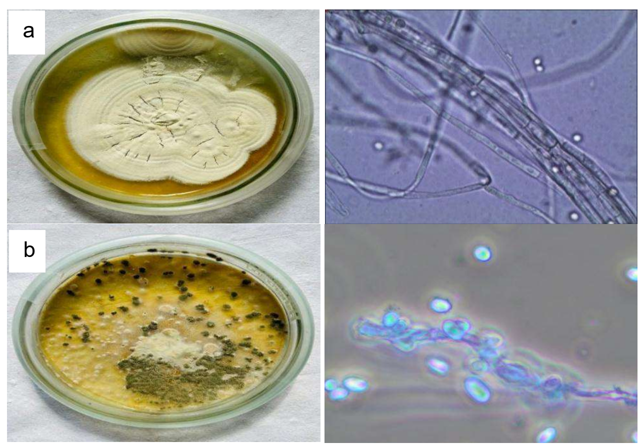

Fig. 1. a. Morphological identification of *B. bassiana* AKP Bb-cd; b. Morphological identification of *M. rileyi* AKP Mr-2m

Table 1. Morphological characteristics of entomopathogenic fungal isolates

Fungal isolate	Colony colour	Colony shape	Colony elevation	Radial growth	Shape of the conidia	Colony diameter (cm)
B. bassiana AKP Bb-cd	White	Round	Initially raised and then flat	Fast	Globose	6 cm
M. rileyi AKP Mr-2m	Olive green	Round	Flat	Slow	Round to oval	4 cm

Table 2. Growth characteristics of *B. bassiana* (AKP Bb-cd) and *M. rileyi* (AKP Mr-2m)

Isolates	Colony colour	Colony elevation	Shape of the conidia	Spore size (µm) (40X)		L/W ratio
				Length	Width	
AKP Bb-cd isolate	White	Initially raised and then flat	Globose	7.26	3.63	2.0
AKP Mr-2m isolate	Olive green	Flat	Round to oval conidia	6.83	2.91	2.34

L/W – Length to width ratio

(PV791015.1) clustered closely with the Araku Valley isolate (OR345217.1) with high confidence (bootstrap value of 99) indicating a close evolutionary relationship. The use of *Metarhizium rileyi* as an outgroup confirmed the divergence of *B. bassiana* isolates, highlighting the genetic diversity within the species.

The tree represents the evolutionary relationships among *Metarhizium rileyi* isolates across different regions in India and other countries with one *Beauveria bassiana* isolate used as an outgroup (Fig. 4). The isolates formed multiple

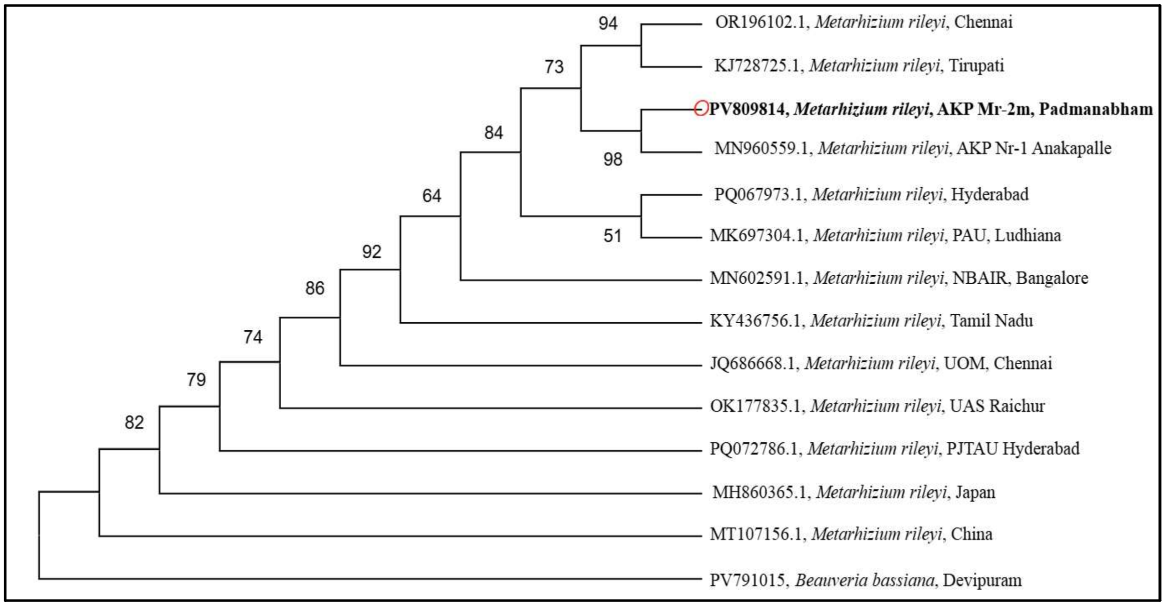

Fig. 3. Phylogenetic tree based on the nucleotide sequences of ITS 1-5.8S-ITS 4 r DNA region of isolated *B. bassiana* AKP Bb-cd along with closely related reference sequence retrieved from the NCBI database

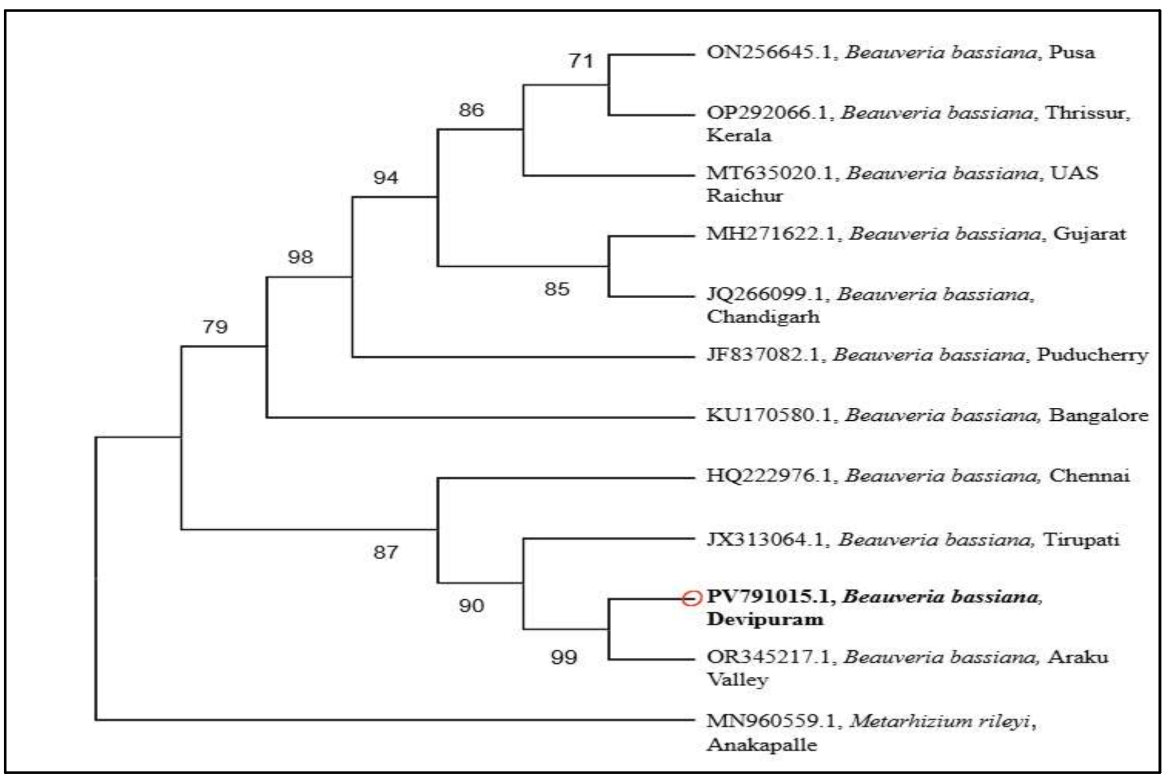

Fig. 4. Phylogenetic tree based on the nucleotide sequences of ITS 1-5.8S-ITS 4 r DNA region of isolated *M. rileyi* AKP Mr-2m along with closely related reference sequences retrieved from the NCBI database

clades, each supported by bootstrap values that indicated the reliability of the branching. The clade containing isolates from Chennai (OR196102.1), Tirupati (KJ728725.1) and Padmanabham (PV809814) showed strong bootstrap support (94-98). The native isolate AKP Mr-2m Padmanabham isolate (PV809814) clustered with the Anakapalle isolate (MN960559.1) and other closely related isolates from Hyderabad and Ludhiana, suggesting genetic relatedness across geographically distinct locations. The strong bootstrap value of 98% for this cluster confirmed a robust evolutionary relationship. Isolates from southern India (Chennai, Tirupati, Anakapalle, Hyderabad, Bangalore, Tamil Nadu and Raichur) were distributed across different clades, showing moderate to strong bootstrap support. Interestingly, isolates from Japan (MH860365.1) and China (MT107156.1) also clustered within the Indian lineages suggesting a broader evolutionary conservation of *M. rileyi*.

CONCLUSION

Two entomopathogenic fungal isolates were obtained in this study: *Beauveria bassiana* AKP Bb-cd, isolated from natural ecosystem using the soil serial dilution method and *Metarhizium rileyi* AKP Mr-2m, isolated from an agricultural ecosystem as an insect cadaver. Morphological characteristics played a crucial role in distinguishing between the two isolates. Notable differences were observed in colony morphology as well as in the size and shape of conidia, thereby facilitating the identification and comparison of the entomopathogenic fungi. Molecular techniques provided additional insights into the genetic identity and evolutionary relationships of the isolates.

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Notes on Genus Chondromorpha Silvestri, 1897 (Diplopoda: Polydesmida: Paradoxosomatidae) with New Distributional Records

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Abstract: This paper reports three species of the genus *Chondromorpha* Silvestri, 1897 namely, *C. mammifera* Attems, 1936, *C. kelaarti* (Humbert 1865) and *C. xanthotricha* (Attems 1898) from the eastern and north-eastern regions of India. Among these, *C. mammifera* is recorded for the first time from Assam and West Bengal, and *C. kelaarti* is newly documented from Nagaland, India.

Keywords: *Chondromorpha*, Gonopod, India, Millipede, New state record

The class Diplopoda is the largest of the four myriapod classes (Blower 1985). This group of terrestrial arthropods comprising approximately 11,000 described species distributed across more than 2,000 genera, over 140 families, and 16 extant orders, occurring on all continents except Antarctica (Golovatch 2025). Millipedes fauna in India currently includes 287 recognized species, classified into 95 genera, 26 families, and 12 orders (Subramanian and Senraj 2024).

The millipede genus *Chondromorpha* Silvestri, 1897 is South Asian in origin (Sankaran and Sebastian 2017) and belongs to the family Paradoxosomatidae, which is one of the largest and most diverse families in the class Diplopoda, dominating the millipede fauna of the Indo-Australian region (Likhitrakarn et al., 2015). The genus *Chondromorpha* comprises eight described species, six of which are found in India. The type species *C. severini* Silvestri, 1897 was originally described from Tamil Nadu (Sankaran and Sebastian 2017). *C. kelaarti* (Humbert, 1865) has been recorded from Gujarat, Kerala, Tamil Nadu, West Bengal, and other parts of southern India and Sri Lanka (Bhakat 1989, Sankaran and Sebastian 2017, Dave and Sindhav 2025). *C. mammifera* Attems, 1936 occurs in Gujarat, Jharkhand, Maharashtra, Odisha, Karnataka and Kerala (Sankaran and Sebastian 2017). *C. kaimura* Turk, 1947 is known to occur from the Kaimur Plateau in Bihar (Turk 1947). The recently described *C. lakroda* Dave and Sindhav, 2025 contributes to the genus diversity of Gujarat (Dave and Sindhav 2025). *C. xanthotricha* Attems, 1898 is considered a pantropical species native to South India and Sri Lanka, but it is also found in Central and North America, the Caribbean and northern South America, and Southeast Asia (Almeida et al., 2022). Although Golovatch and Wesener (2016) considered the records of *C. xanthotricha* in India to be dubious, a recent report from Gujarat by Dave and Sindhav (2025) confirmed its occurrence in India. In addition to India, *C. greke* Golovatch, 2023 has been reported in Nepal (Golovatch 2023), and *C. stadelmanni* (Verhoeff, 1930), which is endemic to Sri Lanka, is considered doubtful because it is known only from a female holotype (Dave and Sindhav 2025). This study is the first to report, the presence of the millipede species *C. mammifera* in Assam and West Bengal, and *C. kelaarti* in Nagaland.

MATERIAL AND METHODS

The millipede specimens were collected from Assam, Nagaland, Odisha, and West Bengal (Fig. 1) using the hand-picking method (Blower 1985) and preserved in 70% ethanol, and deposited at the Museum and Taxidermy section, Zoological Survey of India, Kolkata, and assigned registration numbers as well. The specimens were subsequently studied in the laboratory using a Leica EZ4 educational stereo microscope. Photographs of the preserved specimens were captured under a Leica M205A stereomicroscope using a Leica DMC-4500 camera. The photographs were processed using LAS V4.12 software. The photographs were edited using Adobe Photoshop 7.0 software, and the map was mapped using QGIS 3.40 Bratislava (QGIS Development Team 2025). Identification was carried out based on published literature, including works by Almeida et al., 2022, Attems 1936, Bano and Murthy 1997, Dave and Sindhav 2025, Deker and Tertilt 2012, Golovatch 2023, Likhitrakarn et al., 2017, Sankaran and Sebastian 2017, Sankaran 2023 and Turk 1947.

RESULTS AND DISCUSSION

TAXONOMY

Class -Diplopoda de Blainville in Gervais, 1844

Order -Polydesmida Pocock, 1887

Family- Paradoxosomatidae Daday, 1889

Genus -*Chondromorpha* Silvestri, 1897

Type species- *Chondromorpha severini* Silvestri, 1897, type by monotype.

Diagnosis: The genus is distinguished from other genera by a unique combination of characteristics. It has 20 body segments; well-developed paranota, often identifiable by their shape, which may or may not bear an anterolateral tooth, a feature used to differentiate between species. The metazona is typically granulated with setae (small hairs) and the pleural keels are indistinct. Male legs lack tarsal brushes and sternal lamellae are present between the fourth pair of male coxae. In addition, the gonopodal femorite is notably short. A comparative table (Table 1) was prepared to display the distinguishing characteristics of the three species in the genus *Chondromorpha*.

***Chondromorpha mammifera* Attems, 1936** (Fig. 2)

Material examined: 1 ♂ (ZSIK-TM112), Nischintapur, Purulia, West Bengal, India; 23°13'11" N, 86°00'14" E, Coll. U. K. Chaudhary, 4 Aug. 2024; 1 ♂ (ZSIK-TM113), Bhursu, Purulia, West Bengal, India 23°12'59" N, 85°59'06" E, Coll. U. K. Chaudhary, 5 Aug. 2024; 2 ♂ (ZSIK-TM114 and ZSIK-TM115), Tarafeni Dam, Jhargram, West Bengal, India, 22°40'44" N, 86°47'15" E, Coll. U. K. Chaudhary, 7 Aug. 2024; 9 ♂ (ZSIK-TM116, ZSIK-TM117, ZSIK-TM118, ZSIK-TM119, ZSIK-TM120, ZSIK-TM121, ZSIK-TM122, ZSIK-TM123 and ZSIK-TM124), Kuldiha, Jhargram, West Bengal, India, 22°39'13" N, 86°44'37" E, Coll. U. K. Chaudhary, 9 Aug. 2024; 1 ♂ (ZSIK-TM125), forest opposite of Jhargram Zoological Park, Jhargram, West Bengal, India, 22°26'56" N, 87°01'13" E, Coll. U. K. Chaudhary, 10 Aug. 2024; 1 ♂ (ZSIK-TM126), Kangsabati ghat, West Midnapore, West Bengal, India, 22°24'23" N, 87°18'13" E, Coll. U. K. Chaudhary ,11 Aug. 2024; 1 ♂ (ZSIK-TM127), Gopalpur, West Midnapore, West Bengal, India, 22°24'49" N, 87°17'42" E, Coll. U. K. Chaudhary, 12 Aug. 2024; 1 ♂ (ZSIK-TM128), Chaupahari Jangal, Burdwan, West Bengal, India, 23°37'55"N, 87°34'27"E, Coll. D. Mondal, 26 Sept. 2023; 1 ♂ (ZSIK-TM129), Barbil, Keonjhar, Odisha, India, 22°06'40" N, 85°23'09" E, Coll. S. Mitra, 31 Aug. 2023 and 1 ♂ (ZSIK-TM130), Tukura, Goalpara, Assam, India, 26°10'34" N, 90°37'30" E, Coll. S. Mukherjee, 2 Oct. 2024.

Diagnosis: *C. mammifera* can be identified by presence of sternal lamella between male coxae 4 as paired, globular prominences (Fig. 2H). It shares a close relationship with *C. kaimura* Turk, 1947, which is characterized by paired, rectangular prominences. Prefemur long and dense setose. The femorite was short, sub-cylindrical, and showed no evidence of torsion (Fig. 2D-F). The solenophore is moderately long and mesally curved. The solenomere was simple and long, with a broad base, gradually tapering to a filiform shape, and smoothly curved distolaterally. Length mostly 26-35 mm (♂) and width of mid body 3.5-4.0 mm (♂). Colouration greyish black. Angular part of paranota yellowish orange. The anterior angle of the lateral keels is notably rounded, whereas the posterior angle is acute and extends beyond the border.

Distribution: India**:** Gujarat, Jharkhand, Karnataka, Kerala, Maharashtra and Odisha (Sankaran and Sebastian 2017), Assam, and West Bengal.

Remarks: *C. mammifera* is recorded for the first time in Assam and West Bengal. This species is abundant in West Bengal.

***Chondromorpha kelaarti* (Humbert 1865)** (Fig. 3)

Material examined: 1 ♂ (ZSIK-TM109), Triple Falls Seithekema, Dimapur, Nagaland, India, 25°48'23"N, 93°48'59"E, Coll. P. G. S. Sethy & Party, 5 Dec. 2024; 1 ♂ (ZSIK-TM046), Beside Damodar River, Tirat, Paschim Burdwan, West Bengal, India, 23°37'03" N, 87°02'28" E, Coll. U. K. Chaudhary, 3 Aug. 2024; 2 ♂ (ZSIK-TM047 and ZSIK-TM048), Pogrodi Aambagan near Tunturi, Purulia, West Bengal, India, 23°12'26" N, 85°55'07" E, Coll. U. K. Chaudhary, 5 Aug. 2024; 3 ♂ (ZSIK-TM049, ZSIK-TM050 and ZSIK-TM051), Bhunighra, Purulia, West Bengal, India, 23°13'41" N, 86°16'19" E, Coll. U. K. Chaudhary, 6 Aug. 2024; 1 ♂ (ZSIK-TM110), 46 Rajni Mukherjee Road, New Alipore, Kolkata, West Bengal, India, 22°30'26" N, 88°19'54" E, Coll. U. K. Chaudhary, 7 June 2024; and 1 ♂ (ZSIK-TM111), FPS Building, Indian Museum Campus, Kolkata, West Bengal, India, 22°33'24" N, 88°21'06" E, Coll. S. Mukherjee, 21 Mar. 2025.

Diagnosis: *C. kelaarti* can be identified by the presence of a

Table 1. Distinguishing characters of the species of *Chondromorpha* Silvestri, 1897

Species	Sternal lamella between male coxae 4	Gonopods
C. mammifera (Attems 1936)	Paired, juxtaposed globular prominences (Fig. 2H)	Simple with short femorite. (Fig. 2D-F)
C. kelaarti (Humbert 1865)	Trapezoid having two short anterior processes (Fig. 3H)	Simple with elongated femorite. (Fig. 3D-F)
C. xanthotricha (Attems 1898)	Paired, conical in shape (Fig. 4H)	Simple with stout femorite. (Fig. 4D-F)

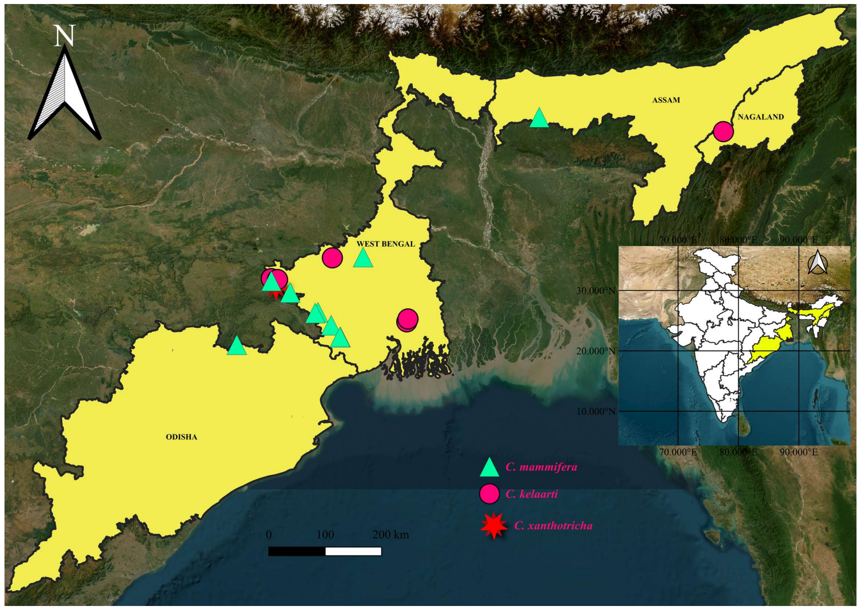

Fig. 1. Distribution of species of the genus *Chondromorpha* Silvestri, 1897 across Assam, Nagaland, Odisha, and West Bengal, India

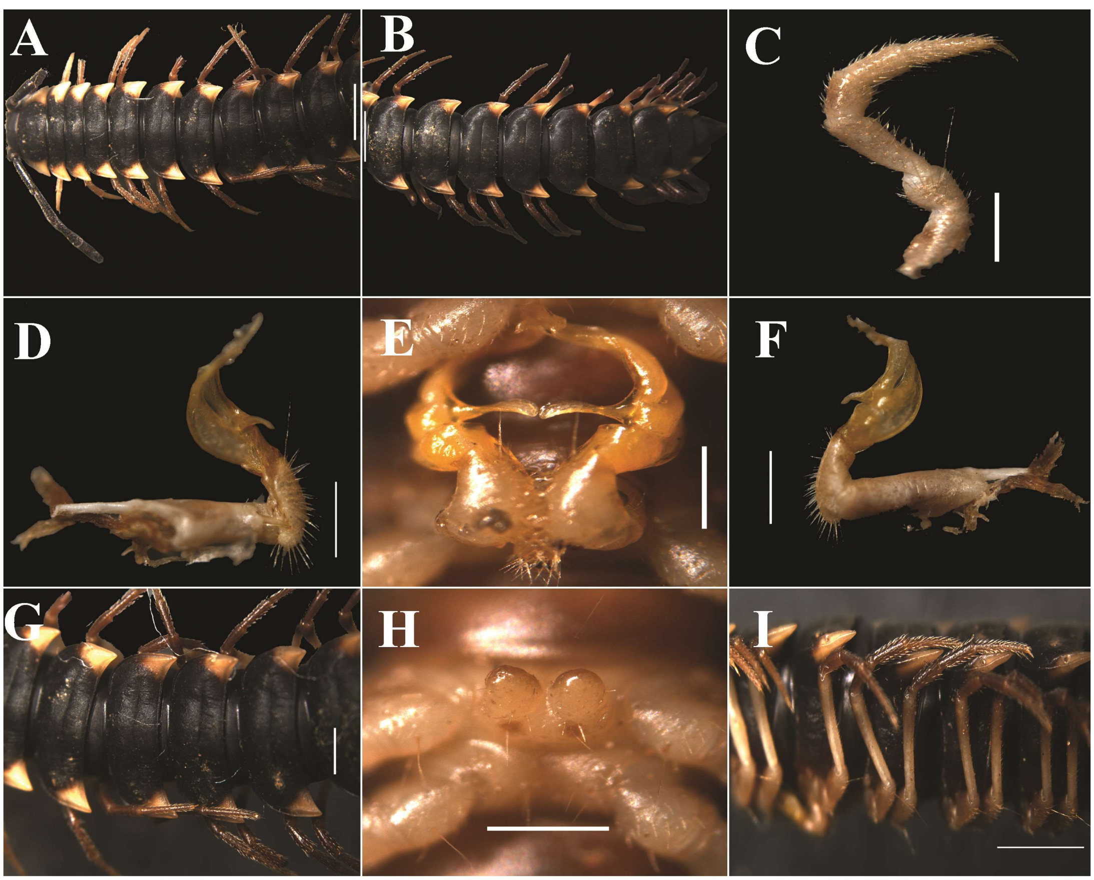

Fig. 2. *Chondromorpha mammifera*, male **A**) Anterior part of body, dorsal view; **B**) Posterior part of body, dorsal view; **C**) Anterior left leg I, mesal view; **D**) Left gonopod, mesal view; **E**) Gonopods, ventral view; **F**) Left gonopod, prolateral view; **G**) Body segments 8-10, dorsal view; **H**) Sternal lamella between male coxae 4, ventral view; **I**) Body segments 8-10, lateral view. Scale bars: A - B, I = 2 mm; G = 1 mm; C - F, H = 0.5 mm

sternal lamella between the male coxae 4, which is trapezoidal in shape, with two short, paired anterior processes (Fig. 3H). It shares a close relationship with *C. severini* Silvestri, 1897, which is characterized by globular prominence without processing. The prefemur was elongated and densely setose with long, stiff setae. The femorite was short and subcylindrical, with a median fold, and the seminal groove ran along the medial side of the femorite (Fig. 3D-F). Solenophore long and mesally curved, with distal lappet having triangular mesal process. Solenomere elongated. Length mostly 22-25 mm (♂) and width of mid body 2.5-3.2 mm (♂). Colour brownish-black. The angular part of the paranota is yellowish. The anterior angle of the lateral keels is nearly rounded, while the posterior angle forms broad triangles in the anterior body segments and acute triangles in the posterior segments.

Distribution: India: Gujarat, Kerala, Tamil Nadu, and West Bengal (Bhakat 1989, Sankaran and Sebastian 2017, Dave and Sindhav 2025), Nagaland.

Remarks: *C. kelaarti* is recorded for the first time in Nagaland. This species is abundant in the west Bengal.

***Chondromorpha xanthotricha* (Attems 1898)** (Fig. 4)

Material examined: 2 ♂ (ZSIK-TM132 and ZSIK-TM133), Andhra Alias Hathinada, Purulia, West Bengal, India, 23°13'24'' N, 86°06'02'' E, Coll. U. K. Chaudhary, 5 Aug. 2024.

Diagnosis: *C. xanthotricha* can be identified by the presence of sternal lamella between male coxae 4 as paired, conical in shape (Fig. 4H). It resembles C. *kelaarti* (Humbert 1865), which is characterized by trapezoidal prominence with two short paired anterior processes (Fig. 3H). Prefemur densely setose. The femorite was stout with a distolateral sulcus that demarcated it from the postfemorite (Fig. 4D-F). The solenophore comprises a conspicuous trifid lamina that supports a long flagelliform solenomere. Length mostly 20-27 mm (♂) and width of mid body 2.3-3.0 mm (♂). Coloration blackish. Metaterga granular with setae. Sterna sparsely setose. Paranota yellowish in colour.

Distribution: India: Gujarat, West Bengal (Chakraborty 2018, Dave and Sindhav 2025).

In the present study, millipede species belonging to the genus *Chondromorpha* were recorded in the Indian states of Assam, Nagaland, Odisha, and West Bengal. Three species

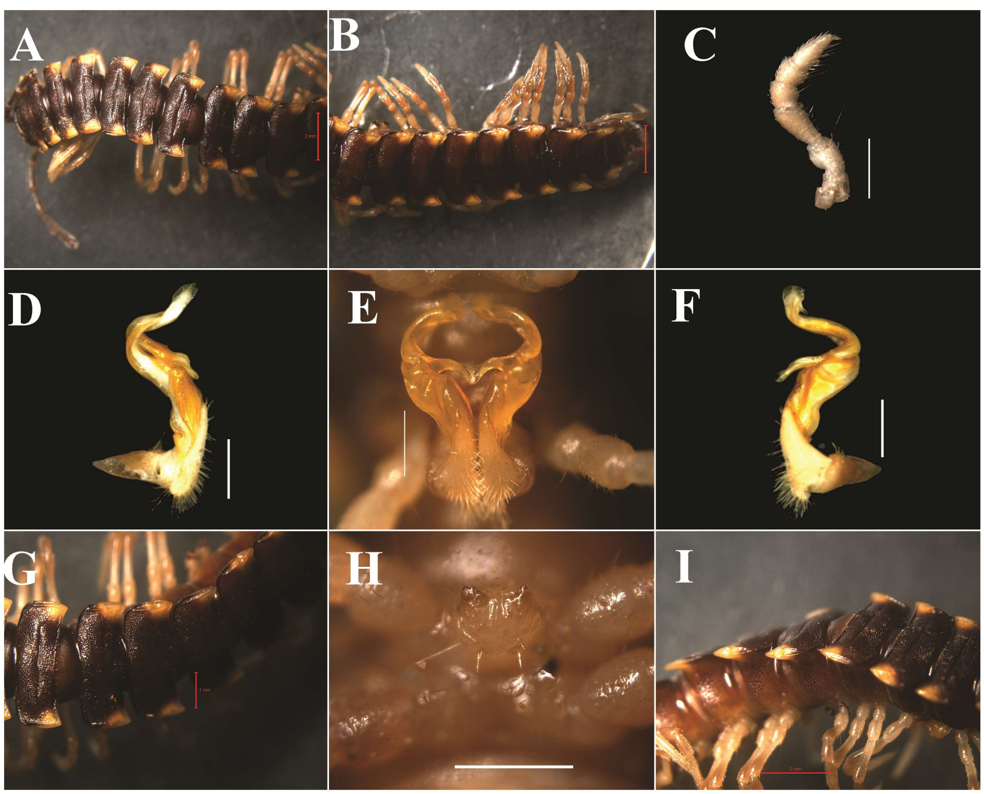

Fig. 3. *Chondromorpha kelaarti*, male **A**) Anterior part of body, dorsal view; **B**) Posterior part of body, dorsal view; **C**) Anterior left leg I, mesal view; **D**) Left gonopod, mesal view; **E**) Gonopods, ventral view; **F**) Left gonopod, prolateral view; **G**) Body segments 8-10, dorsal view; **H**) Sternal lamella between male coxae 4, ventral view; **I**) Body segments 8-10, lateral view. A - B, I = 2 mm; G =1 mm; C - F, H = 0.5 mm

have been identified like, *C. mammifera*, *C. kelaarti*, and *C. xanthotricha*. *C. mammifera*. Attems (1936) has been observed at altitudes ranging from as low as 12 m near Mumbai, Maharashtra to approximately 919 m in the vicinity of Bangalore, Karnataka. In addition to these regions, the species is distributed across Kerala, Odisha, and Jharkhand (Sankaran and Sebastian 2017). In the present study, *C. mammifera* was recorded in the Keonjhar district of Odisha. This species was reported for the first time in Goalpara, Assam, and has also been reported in Burdwan, Jhargram, Purulia, and West Midnapore districts in West Bengal. These records indicate that *C. mammifera* is extending its distribution range from the southern India to northeast India.

C. kelaarti (Humbert, 1865) was originally described as *Polydesmus kelaarti* (Humbert 1865) from Sri Lanka. Subsequently, Sankaran and Sebastian (2017) synonimized several species and subspecies of *C. kelaarti*, including *C. atopus* (Chamberlin, 1920), *C. indus* (Chamberlin, 1920), *C. kelaarti kelaarti* (Humbert, 1865), *C. kelaarti longipes* (Verhoeff 1936) and *C. kelaarti valparaiensis* Carl, 1932. This species is widely distributed across India, with confirmed records from Gujarat, as recently reported by Dave and Sindhav (2025), Kerala, Tamil Nadu (Sankaran and Sebastian 2017), and West Bengal (Bhakat 1989). Additionally, *C. kelaarti* has been recorded in Great Britain as an introduced species (Pocock 1906). Although Bhakat (1989) reported the presence of *C. kelaarti* in the Birbhum district of West Bengal, it was based solely on ecological characteristics. In the present study, *C. kelaarti* was recorded in the Kolkata, Paschim Burdwan, and Purulia districts of West Bengal. This study also documents the first confirmed record of this species from Dimapur, Nagaland. These findings suggest that in addition to *C. mammifera*, *C. kelaarti* expands its distributional range to northeast India.

C. xanthotricha (Attems, 1898) was originally described as *Prionopeltis xanthotrichus* Attems, 1898. According to Golovatch and Wesener (2016), the occurrence of the pantropical species, *C. xanthotricha* in India was previously considered doubtful. However, a recent study by Dave and Sindhav (2025) described the species from Gujarat, thereby confirming its presence in India. Previously, Chakraborty (2018) reported *C. xanthotricha* from West Midnapore (Paschim Medinipur), West Bengal; however, no taxonomic description of the species was provided at the time.

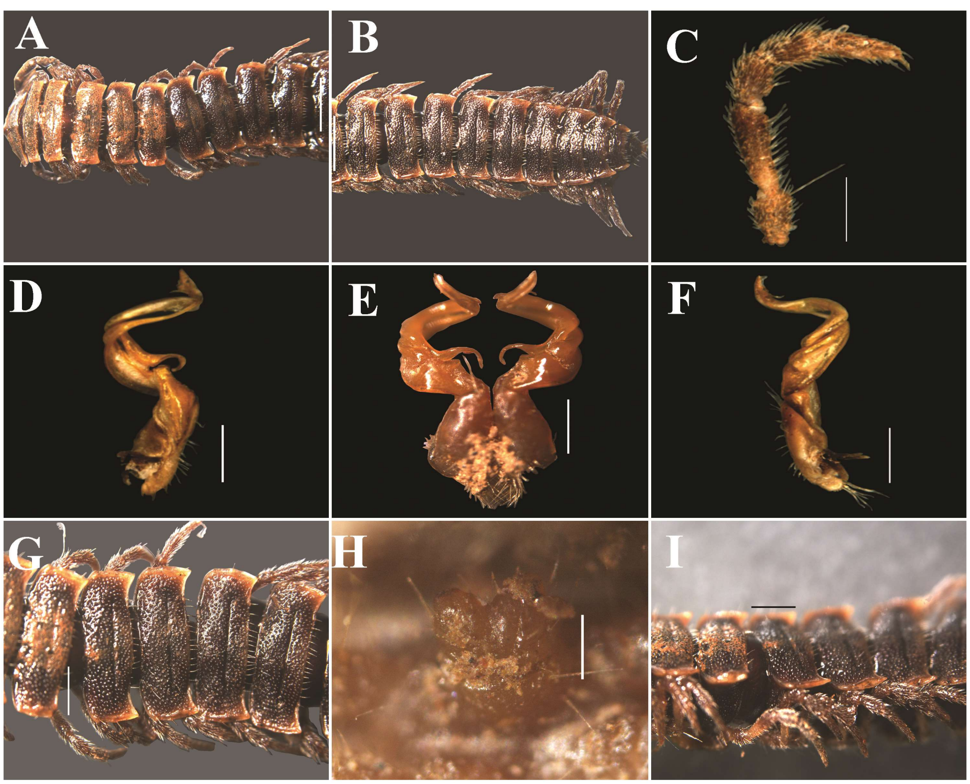

Fig. 4. *Chondromorpha xanthotricha*, male **A**) Anterior part of body, dorsal view; **B**) Posterior part of body, dorsal view; **C**) Anterior left leg I, mesal view; **D**) Left gonopod, mesal view; **E**) Gonopods, ventral view; **F**) Left gonopod, prolateral view; **G**) Body segments 8-10, dorsal view; **H**) Sternal lamella between male coxae 4, ventral view; **I**) Body segments 8-10, lateral view. A - B = 2 mm; G, I = 1 mm; C - F = 0.5 mm; H = 0.2 mm

Millipedes, particularly *Chondromorpha* species remain insufficiently documented across India, especially in the northeastern states, resulting in significant knowledge gaps. This also underscores the need for further exploration of millipede diversity in these relatively underexplored areas of Northeast India..

CONCLUSIONS

The species of the genus *Chondromorpha* was recorded in states of Assam, Nagaland, Odisha, and West Bengal, India. Notably, *Chondromorpha mammifera* is reported for the first time from Assam and West Bengal, while *C. kelaarti* is recorded for the first time from Nagaland. These findings indicate a range extension of the genus into the northeastern regions of India beyond its previously known distribution in the southern and western parts of the country. This highlights the need for further exploration in northeastern India, which may lead to the discovery of additional *Chondromorpha* species as well as other millipede taxa.

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AUTHOR'S CONTRIBUTION

UKC collected the specimens, performed identification, photographed the specimens and drafted the manuscript. SD confirmed the identification and edited the manuscript. MDA helped with the literature. PGSS collected specimens and edited the manuscript. AP assisted in photography of specimens and edited the manuscript.

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Butterflies (Insecta: Lepidoptera: Papilionoidea) Fauna of Papum Pare district of Arunachal Pradesh, India

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Abstract: The Papum Pare district of Arunachal Pradesh was surveyed from April 2019 to March, 2022 to record the butterfly diversity. Total 209 species referable to 127 genera under six families *i.e.*, Nymphalidae, Pieridae, Papilionidae, Lycaenidae, Riodinidae and Hesperiidae were recorded from Papum Pare district. The family Nymphalidae was with maximum number of species i.e., 85 species under 42 genera (40%), followed by Lycaenidae, 49 species under 38 genera (23%), Hesperiidae, 36 species under 27 genera (17%), Pieridae, 20 species under 12 genera (10%), Papilionidae, 18 species under 7 genera) (9%), and Riodinidae, 1 species under 1 genus (1%). The diversity revealed that of these 209 species, 60 species (29%), were common, 84 species (40%) uncommon and remaining 65 species (31%) less common. Out of 209 species, 37 species are in the Wildlife (Protection) Amendmant Act, 2022.

Keywords: Butterfly, Diversity, Papum Pare, Wildlife (Protection) Amendmant act, Arunachal Pradesh

Arunachal Pradesh is one of the 28 states of India, situated in the country's Northeast. The state is 83,743 square kilometers in size. The state is bordered by Assam and Nagaland in the south and shares international borders with Bhutan in the West, Myanmar in the East and the People's Republic of China in the North. Most of Arunachal Pradesh is mountainous and terrain consists of lofty, haphazardly aligned ridges that separate deep valleys and rise to the peaks of the Great Himalayas. Geographically speaking, Arunachal Pradesh is essentially a hilly region tucked away in the Himalayan foothills. Four major climate categories and five major forest types—plus a sixth category of secondary forests—can be used to categorize the vegetation of Arunachal Pradesh. These are tropical forests, sub tropical forests, pine forests, temperate forests and alpine forests. Arunachal Pradesh is the largest in area among North – Eastern states and is the second largest forest covered state next to Madhya Pradesh in the country.

Butterflies, the flying flowers, form a crucial component in the food web-chain and are at the primary trophic level and important pollinators (anthophilous). Their habitat requirement are very diverse in terms vegetation, water and moisture and also depend on sun and warmth, shadow, etc. (Warren 1985). Butterflies are a valuable tool for monitoring the health of the environment and can provide early warning signs of environmental stress or pollution. Abundance of butterflies usually indicates a healthier ecosystem. The 1.87% of all insects worldwide are butterflies, with 18768 species currently recognized (van Nieukerken et al., 2011). From the Indian Himalaya, a total of 1013 species from six families are known.

Doubleday (1845) was the first person to study butterflies of North –Eastern India from Lower Dibang Valley. Earlier British Lepidopterists had explored and published reports from Abor and Mishimi hills (Moore 1857, Evans 1912, South 1913). The other important contributions on the butterfly fauna of Arunachal Pradesh were made by Betts (1950), Varshney and Chandra (1971), Arora amd Mondal (1981), Mondal (1985), Gupta and Shukla (1988), Radhakrishnan (1988), Athreya (2006), Borang *et al*. (2008), Gogoi (2012), Roy (2013), Sondhi and Roy (2013), Sondhi and Kunte (2014), Sondhi and Kunte (2016), Singh (2017), Gayen et al. (2019), Mazumder et al. (2019), Sharma and Prathana (2020, 2021, 2021a). The main objective of the study is to provide the inventory of butterflies from Papum Pare district of Arunachal Pradesh and this is the first study on the diversity of butterflies from Papum Pare district.

MATERIAL AND METHODS

The collections and observations were made at Papum Pare district of Arunachal Pradesh from April 2019 to March, 2022. Papum Pare District is situated in the Northeastern part of Arunachal Pradesh. It is extended between 26°55′ and 28°40′ North latitude and 92°40′ and 94°21′ East longitudes. The state of Assam borders the district on the South, Lower Subansiri district on the East, East Kameng district on the West, and Kra Daadi district on the North. The district's name is taken from the names of its two principal rivers, Papum and Pare. The district covers an area of 3462 sq. kms. approximately. The land is mostly mountainous with Himalayan ranges. The hill ranges approximately varies from 45 to 1200 mtrs. above sea level. The natural vegetation of

Papum Pare district comprises chiefly humid semi-evergreen and sub-tropical evergreen forests. The vegetation is largely made up of ferns and rhododendrons at higher elevations, and tall trees, shrubs, bamboo, wild bananas, cane, and various other types of creepers found at lower elevations.

The specimens were collected with the help of a specified butterfly net. Being soft, the species need a light pressure at thorax while killing. After killing, the specimens were transferred to the insect folder labelled with name of locality, date, latitude, longitude and altitude. Later in the laboratory, the specimens were relaxed in desiccator and stretched. After drying in the drying chamber for 3 days, the adults were shifted to fumigated insect storage boxes. All the well stretched specimens labelled with the information such as name of collector, place of collection, date of collection, latitude, longitude, and altitude. The identified specimens have been deposited in the National Zoological Collections (NZC) at, Arunachal Pradesh Regional Centre, Itanagar. The butterflies were determined as per the available literature (Evans, 1932, Talbot, 1939, 1947, Wynter-Blyth, 1957, Kehimkar, 2008, Sidhu and Kumar, 2016). The geographic coordinates and altitude were obtained, by Orgon 550 Garmin GPS.

RESULTS AND DISCUSSION

Total 209 species referable to 127 genera under six families i.e., Papilionidae, Pieridae, Nymphalidae, Riodinidae, Lycaenidae, and Hesperiidae were recorded from the Papum Pare district of Arunachal Pradesh. The family Nymphalidae was with maximum number of species i.e., 85 species under 42 genera (40%), followed by Lycaenidae, 49 species under 38 genera (23%), Hesperiidae, 36 species under 27 genera (17%), Pieridae, 20 species under 12 genera (10%), Papilionidae, 18 species under 7 genera) (9%), and Riodinidae, 1 species under 1 genus (1%) (Table 1, Fig. 1). Out of 209 species, 60 species (29%), were found to be common, 84 species (40%) uncommon and remaining 65 species (31%) less common (Table 1, Fig. 2).

In all the six butterfly families, Nymphalidae is the only family having the dry and wet-season forms in some genera. Seasonal morphological variations were noted in the fifteen

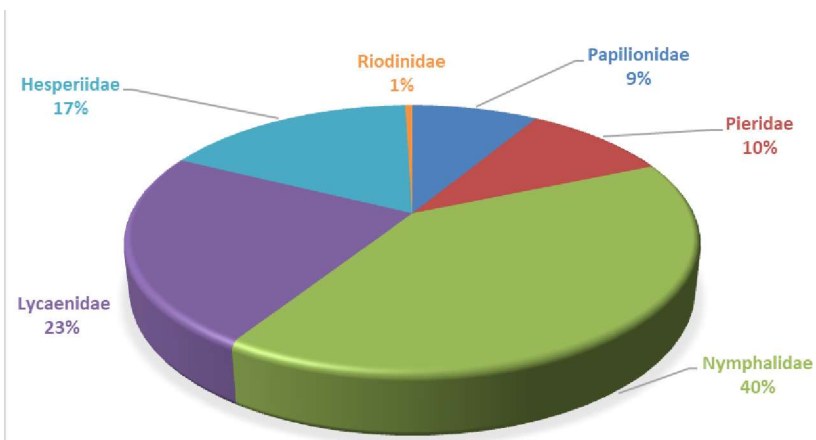

Fig. 1. Family-wise number of species of butterflies

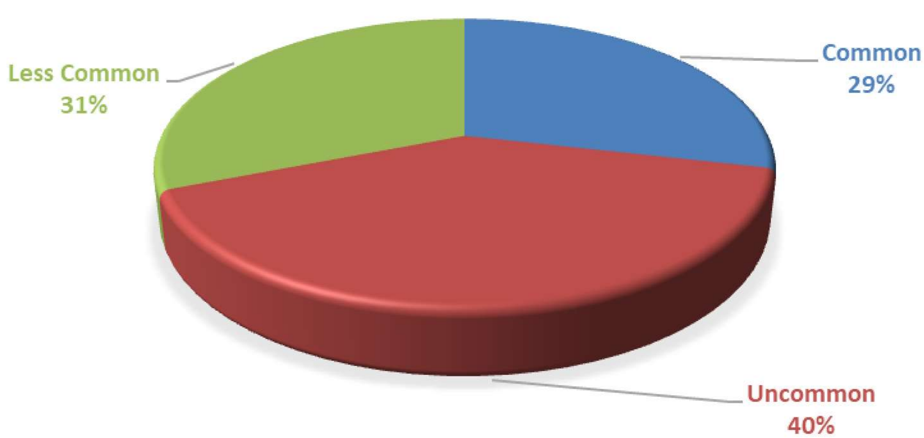

Fig. 2. Relative abundnance of butterflies

Table 1. Inventory of Butterflies (Rhopalocera) recorded from Papum Pare District, Arunachal Pradesh

Scientific name	Common name	Status	Wild Life (Protection) Amendment Act, 2022
Family: PAPILIONIDAE Subfamily PAPILIONINAE			
Pachliopta aristolochiae (Fabricius 1775)	Common Rose	Uncommon	
Troides aeacus (C. & R. Felder 1860)	Golden Birdwing	Common	Schedule II, part H
Troides helena (Linnaeus 1758)	Common Birdwing	Uncommon	Schedule IV, Appendix II
Byasa dasarada (Moore 1858)	Great Windmil	Uncommon	
Papilio bianor Cramer [1777]	Common peacock	Uncommon	
Papilio clytia Linnaeus 1758	Common Mime	Uncommon	Schedule II, Part H
Papilio demoleus Linnaeus 1758	Lime Butterfly	Common	
Papilio helenus Linnaeus 1758	Red Helen	Common	
Papilio memnon Linnaeus 1758	Great Mormon	Common	
Papilio polytes Linnaeus 1758	Common cormon	Common	
Papilio paris Linnaeus 1758	Paris Peacock	Less Common	
Papilio alcmenor C. & R. Felder [1864]	Redbreast	Uncommon	
Papilio protenor Cramer [1775]	Spangle	Uncommon	
Graphium agamemnon (Linnaeus 1758)	Tailed Jay	Less Common	
Graphium sarpedon (Linnaeus 1758)	Common Bluebottle	Common	Schedule II, Part H
Graphium xenocles (Doubleday 1842)	Great Zebra	Uncommon	
Lamproptera curius (Fabricius 1787)	White Dragontail	Less Common	
Meandrusa payeni (Boisduval 1836)	Yellow Gorgon	Uncommon	
Family HESPERIIDAE Subfamily COELIADINAE			
Hasora chromus (Cramer [1780])	Common Banded Awl	Less Common	
Subfamily PYRGINAE			
Tagiades japetus (Stoll [1781])	Suffused Snow Flat	Less Common	
Tagiades litigiosa Moeschler 1878	Water Snow Flat	Less Common	
Celaenorrhinus leucocera (Kollar [1844])	Common Spotted Flat	Uncommon	
Pseudocoladenia dan (Fabricius 1787)	Fulvous Pied Flat	Common	
Spialia galba (Fabricius 1793)	Indian Skipper	Less Common	
Subfamily HESPERIINAE			
Ochus subvittatus (Moore 1878)	Tiger Hopper	Uncommon	
Ampittia dioscorides (Fabricius 1793)	Bush Hopper	Uncommon	
Aeromachus jhora (de Niceville 1885)	Grey Scrub Hopper	Uncommon	
Halpe zola Evans 1937	Long-banded Ace	Uncommon	
Pithauria stramineipennis Wood-Mason & de Niceville [1887]	Light Straw Ace	Uncommon	
Astictopterus jama C. & R. Felder 1860	Forest Hopper	Uncommon	
Iambrix salsala (Moore [1866])	Chestnut Bob	Common	
Koruthaialos butleri (de Niceville [1884])	Dark Velvet Bob	Uncommon	
Ancistroides nigrita (Latreille [1824])	Chocolate Demon	Uncommon	
Notocrypta curvifascia (C. & R. Felder 1862)	Restricted Demon	Common	
Notocrypta paralysos (Wood-Mason & de Niceville 1881)	Common Banded Demon	Uncommon	
Gangara thyrsis (Fabricius 1775)	Giant Redeye	Uncommon	
Udaspes folus (Cramer [1775])	Grass Demon	Common	

Cont...

Table 1. Inventory of Butterflies (Rhopalocera) recorded from Papum Pare District, Arunachal Pradesh

Scientific name	Common name	Status	Wild Life (Protection) Amendment Act, 2022
Scobura isota Swinhoe 1893	Swinhoe's Forest Bob	Uncommon	
Erionota torus Evans 1941	Banana Skipper	Less Common	
Parnara guttatus (Bremer & Grey [1852])	Straight Swift	Uncommon	
Borbo cinnara (Wallace 1866)	Rice Swift	Less Common	
Pelopidas assamensis (de Niceville 1882)	Great Swift	Uncommon	
Pelopidas mathias (Fabricius 1798)	Variable Swift	Uncommon	
Pelopidas conjuncta (Herrich-Schaeffer 1869)	Conjoined Swift	Uncommon	
Pelopidas sinensis (Mabille 1877)	Large Branded Swift	Uncommon	
Polytremis eltola (Hewitson1869)	Yellow Spot Swift	Uncommon	
Polytremis lubricans (Herrich-Schaeffer 1869)	Contiguous Swift	Less Common	
Baoris farri (Moore 1878)	Paintbrush Swift	Common	
Baoris pagana (de Niceville 1887)	Figure of Eight Swift	Uncommon	
Caltoris philippina (Herrich-Schaeffer 1869)	Philippine Swift	Uncommon	
Caltoris tulsi (de Niceville [1884])	Purple Swift	Uncommon	
Potanthus pallidus (Evans 1932)	Pale Dart	Less Common	
Potanthus mara (Evans 1932)	Sikkim Dart	Less Common	
Telicota bambusae (Moore 1878)	Dark Palm Dart	Uncommon	
Family PIERIDAE Subfamily COLIADINAE			
Catopsilia pomona (Fabricius 1775)	Common Emigrant	Common	
Catopsilia pyranthe (Linnaeus 1758)	Mottled Emigrant	Common	
Gandaca harina (Horsfield [1829])	Tree Yellow	Less Common	
Eurema blanda (Boisduval 1836)	Three-spot Grass Yellow	Less Common	
Eurema hecabe (Linnaeus 1758)	Common Grass Yellow	Common	
Subfamily PIERINAE			
Leptosia nina (Fabricius 1793)	Psyche	Common	
Pieris brassicae (Linnaeus 1758)	Large Cabbage White	Common	
Pieris canidia (Linnaeus 1768)	Indian Cabbage White	Common	
Ixias pyrene (Linnaeus 1764)	Yellow Orange Tip	Less Common	
Appias indra (Moore 1857)	Plain Puffin	Uncommon	Schfedule II, Part H
Appias libythea (Fabricius 1775)	Striped Albatross	Common	
Appias lyncida (Cramer [1777])	Chocolate Albatross	Common	Schedule II, Part H
Cepora nadina (Lucas 1852)	Lesser Gull	Uncommon	Schedule II, Part H
Appias galba (Wallace 1867)	Indian Orange Albatross	Uncommon	
Cepora nerissa (Fabricius 1775)	Common Gull	Less Common	
Delias acalis (Godart 1819)	Redbreast Jezebel	Uncommon	
Delias descombesi (Boisduval 1836)	Red-spot Jezebel	Common	
Delias pasithoe (Linnaeus 1767)	Red-base Jezebel	Uncommon	
Pareronia avatar (Moore [1858])	Pale Wanderer	Less Common	Schedule II, Part H
Hebomoia glaucippe (Linnaeus 1758)	Great Orange-tip	Common	
Family RIODINIDAE Subfamily RIODININAE			
Zemeros flegyas (Cramer [1780])	Punchinello	Common	
Family LYCAENIDAE Subfamily PORITINAE			
Poritia hewitsoni Moore [1866]	Common Gem	Uncommon	Schedule II, Part H

Cont...

Table 1. Inventory of Butterflies (Rhopalocera) recorded from Papum Pare District, Arunachal Pradesh

Scientific name	Common name	Status	Wild Life (Protection) Amendment Act, 2022
Subfamily MILETINAE			
Taraka hamada (Druce 1875)	Forest Pierrot	Uncommon	
Spalgis epius (Westwood [1851])	Common Apefly	Less Common	
Subfamily LYCAENINAE			
Heliophorus epicles (Godart [1824])	Purple Sapphire	Common	
Heliophorus tamu (Kollar [1844])	Powdery Green Sapphire	Less Common	
Subfamily APHNAEINAE			
Spindasis lohita (Horsfield [1829])	Long-banded Silverline	Uncommon	Schedule II, Part H
Subfamily THECLINAE			
Arhopala centaurus (Fabricius 1775)	Centaur Oakblue	Less Common	
Surendra quercetorum (Moore [1858])	Common Acacia Blue	Less Common	
Loxura atymnus (Stoll 1780)	Yamfly	Less Common	
Yasoda tripunctata (Hewitson 1863])	Branded Yamfly	Less Common	Schedule II, Part H
Horaga onyx (Moore 1858)	Common Onyx	Uncommon	
Cheritra freja (Fabricius 1793)	Common Imperial	Common	
Suasa lisides (Hewitson [1863])	Red Imperial	Uncommon	Schedule II, Part H
Hypolycaena erylus (Godart [1824])	Common Tit	Common	
Zeltus amasa (Hewitson [1865])	Fluffy Tit	Less Common	
Deudorix epijarbas (Moore 1857)	Cornelian	Uncommon	
Rapala nissa (Kollar [1844])	Common Flash	Uncommon	
Rapala pheretima (Hewitson 1863)	Copper Flash	Less Common	
Subfamily POLYOMMATINAE			
Anthene emolus (Godart [1824])	Common Ciliate Blue	Common	
Una usta (Distant 1886)	Singleton	Uncommon	Schedule II, Part H
Petrelaea dana (de Nicéville [1884])	Dingy Lineblue	Uncommon	
Nacaduba beroe (C. & R. Felder [1865])	Opaque Six-Lineblue	Common	
Nacaduba kurava (Moore [1858])	Transparent Six-Lineblue	Uncommon	
Prosotas aluta (Druce 1873)	Banded Lineblue	Uncommon	Schedule II, Part H
Prosotas bhutea (de Nicéville [1884])	Bhutia Lineblue	Less Common	Schedule II, Part H
Prosotas nora (C. Felder 1860)	Common Lineblue	Less Common	
Prosotas dubiosa (Semper [1879])	Tailless Lineblue	Less Common	Schedule II, Part H
Ionolyce helicon (C. Felder 1860)	Pointed Lineblue	Uncommon	Schedule II, Part H
Caleta elna (Hewitson 1876)	Elbowed Pierrot	Uncommon	
Jamides alecto (C. Felder 1860)	Metallic Cerulean	Common	Schedule II, Part H
Jamides bochus (Stoll [1782])	Dark Cerulean	Less Common	
Jamides elpis (Godart [1824])	Glistening Cerulean	Less Common	
Catochrysops panormus (C. Felder 1860)	Silver Forget-me-not	Uncommon	
Catochrysops strabo (Fabricius 1793)	Forget-me-not	Less Common	
Lampides boeticus (Linnaeus 1767)	Pea Blue	Common	
Leptotes plinius (Fabricius 1793)	Zebra Blue	Less Common	
Castalius rosimon (Fabricius 1775)	Common Pierrot	Common	
Tarucus ananda (de Nicéville [1884])	Dark Pierrot	Uncomon	

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Table 1. Inventory of Butterflies (Rhopalocera) recorded from Papum Pare District, Arunachal Pradesh

Scientific name	Common name	Status	Wild Life (Protection) Amendment Act, 2022
Zizeeria karsandra (Moore 1865)	Dark Grass Blue	Common	
Pseudozizeeria maha (Kollar [1844])	Pale Grass Blue	Common	
Zizina otis (Fabricius 1787)	Lesser Grass Blue	Common	
Zizula hylax (Fabricius 1775)	Tiny Grass Blue	Less Common	
Talicada nyseus (Guérin-Méneville 1843)	Red Pierrot	Uncommon	
Megisba malaya (Horsfield [1828])	Malayan	Uncommon	Schedule II, Part H
Celastrina lavendularis (Moore 1877)	Plain Hedge Blue	Uncommon	
Celastrina hueglii (Moore 1882)	Large Hedge Blue	Uncommon	
Celastrina argiolus (Linnaeus 1758)	Hill Hedge Blue	Uncommon	
Acytolepis puspa (Horsfield [1828])	Common Hedge Blue	Uncommon	
Chilades lajus (Stoll [1780])	Lime Blue	Less Common	
Family NYMPHALIDAE Subfamily DANAINAE			
Danaus chrysippus (Linnaeus 1758)	Plain Tiger	Common	
Danaus genutia (Cramer [1779])	Common Tiger	Common	
Danaus melanippus (Cramer [1777])	White Tiger	Uncommon	
Parantica aglea (Stoll [1782])	Glassy Tiger	Common	
Tirumala septentrionis (Butler 1874)	Dark Blue Tiger	Less Common	
Euploea mulciber (Cramer [1777])	Striped Blue Crow	Common	
Euploea midamus (Linnaeus 1758)	Spotted Blue Crow	Less Common	Schedule II, Part H
Subfamily CHARAXINAE			
Polyura bharata (C & R Felder [1867])	Common Nawab	Less Common	
Charaxes bernardus (Fabricius 1793)	Tawny Rajah	Less Common	Schedule II, Part H
Charaxes marmax Westwood 1847	Yellow Rajah	Less Common	Schedule II, Part H
Subfamily MORPHINAE			
Discophora sondaica Boisduval 1836	Common Duffer	Less Common	Schedule I, Part I
Discophora timora Westwood [1850]	Great Duffer	Uncommon	
Subfamily SATYRINAE			
Elymnias hypermnestra (Linnaeus 1763)	Common Palmfly	Common	
Elymnias malelas (Hewitson 1863)	Spotted Palmfly	Uncommon	Schedule II, Part H
Elymnias nesaea (Linnaeus 1764)	Tiger Palmfly	Uncommon	
Penthema lisarda (Doubleday 1845)	Yellow Kaiser	Uncommon	Schedule II, Part H
Ethope himachala (Moore 1857)	Dusky Diadem	Less Common	
Melanitis leda (Linnaeus 1758)	Common Evening Brown	Common	
Melanitis zitenius (Herbst 1796)	Great Evening Brown	Uncommon	Schedule II, Part H
Lethe chandica (Moore [1858])	Angled Red Forester	Common	
Lethe confusa Aurivillius [1898]	Banded Treebrown	Less Common	
Lethe distans Butler 1870	Scarce Red Forester	Uncommon	Schedule I, Part I
Lethe europa (Fabricius 1775)	Bamboo Treebrown	Uncommon	Schedule I, Part I
Lethe kansa (Moore [1858])	Bamboo Forester	Uncommon	
Lethe mekara (Moore [1858])	Common Red Forester	Uncommon	
Mycalesis anaxias Hewitson 1862	White-bar Bushbrown	Uncommon	Schedule II, Part H
Mycalesis francisca (Stoll [1780])	Lilacine Bushbrown	Uncommon	
Mycalesis gotama Moore 1857	Chinese Bushbrown	Uncommon	Schedule II, Part H

Cont...

Table 1. Inventory of Butterflies (Rhopalocera) recorded from Papum Pare District, Arunachal Pradesh

Scientific name	Common name	Status	Wild Life (Protection) Amendment Act, 2022
Mycalesis intermedia (Moore [1892])	Intermediate Bushbrown	Uncommon	
Mycalesis mineus (Linnaeus 1758)	Dark-branded Bushbrown	Common	
Mycalesis perseus (Fabricius 1775)	Common Bushbrown	Common	
Mycalesis visala Moore [1858]	Long-branded Bushbrown	Less Common	
Orsotriaena medus (Fabricius 1775)	Nigger	Common	
Ypthima baldus (Fabricius 1775)	Common Five-ring	Common	
Ypthima huebneri Kirby 1871	Common Four-ring	Common	
Ypthima nareda (Kollar [1844])	Large Threering	Less Common	
Subfamily LIMENITIDINAE			
Neptis cartica Moore 1872	Plain Sailer	Less Common	
Neptis clinia Moore 1872	Sullied Sailer	Less Common	Schedule II, Part H
Neptis hylas (Linnaeus 1758)	Common Sailer	Common	
Neptis magadha C. & R. Felder [1867]	Spotted Sailer	Less Common	
Neptis miah Moore [1858]	Small Yellow Sailer	Uncommon	
Neptis sappho (Pallas 1771)	Rusty Sailer	Less Common	
Neptis soma Moore 1858	Creamy Sailer	Less Common	Schedule II, Part H
Pantoporia hordonia (Stoll [1790])	Common Lascar	Less Common	
Pantoporia sandaka (Butler 1892)	Extra Lascar	Uncommon	
Athyma asura Moore [1858]	Studded Sergeant	Uncommon	
Athyma nefte (Cramer [1780])	Colour Sergeant	Common	
Athyma perius (Linnaeus 1758)	Common Sergeant	Less Common	
Athyma ranga Moore [1858]	Blackvein Sergeant	Uncommon	Schedule II, Part H
Athyma selenophora (Kollar [1844])	Staff Sergeant	Less Common	
Athyma zeroca Moore 1872	Small Staff Sergeant	Less Common	
Moduza procris (Cramer [1777])	Commander	Common	
Sumalia daraxa (Doubleday [1848])	Green Commodore	Uncommon	
Tanaecia lepidea (Butler 1868)	Grey Count	Common	
Tanaecia jahnu (Moore [1858])	Plain Earl	Less Common	
Euthalia aconthea (Cramer [1777])	Common Baron	Less Common	Schedule II, Part H
Subfamily HELICONIINAE			
Argynnis hyperbius (Linnaeus 1763)	Tropical Fritillary	Less Common	
Phalanta phalantha (Drury [1773])	Common Leopard	Less Common	
Vagrans egista (Cramer [1780])	Vagrant	Less Common	
Vindula erota (Fabricius 1793)	Cruiser	Less Common	
Cirrochroa aoris Doubleday [1847]	Large Yeoman	Common	
Cirrochroa tyche C. & R. Felder 1861	Common Yeoman	Less Common	
Subfamily BIBLIDINAE			
Ariadne merione (Cramer [1777])	Common Castor	Common	
Subfamily APATURINAE			
Mimathyma ambica (Kollar [1844])	Indian Purple Emperor	Uncommon	
Hestinalis nama (Doubleday 1844)	Circe	Uncommon	
Subfamily CYRESTINAE			
Cyrestis thyodamas Boisduval 1846	Common Map	Uncommon	

Cont...

Table 1. Inventory of Butterflies (Rhopalocera) recorded from Papum Pare District, Arunachal Pradesh

Scientific name	Common name	Status	Wild Life (Protection) Amendment Act, 2022
Pseudergolis wedah (Kollar [1844])	Tabby	Uncommon	
Subfamily NYMPHALINAE			
Symbrenthia hypselis (Godart [1824])	Spotted Jester	Less Common	
Symbrenthia lilaea (Hewitson 1864)	Northern Common Jester	Common	
Aglais caschmirensis (Kollar [1844])	Indian Tortoiseshell	Uncommon	
Junonia almana (Linnaeus 1758)	Peacock Pansy	Common	
Junonia atlites (Linnaeus 1763)	Grey Pansy	Common	
Junonia iphita (Cramer [1779])	Chocolate Pansy	Common	
Junonia lemonias (Linnaeus 1758)	Lemon Pansy	Common	
Junonia hierta (Fabricius 1798)	Yellow Pansy	Less Common	
Junonia orithya (Linnaeus 1758)	Blue Pansy	Less Common	
Hypolimnas bolina (Linnaeus 1758)	Great Eggfly	Common	
Hypolimnas misippus (Linnaeus 1764)	Danaid Eggfly	Uncommon	Schedule II, Part H
Kallima inachus (Boisduval 1846)	Orange Oakleaf	Less Common	
Doleschallia bisaltide (Cramer [1777])	Autumn Leaf	Uncommon	
Rhinopalpa polynice (Cramer [1779])	Wizard	Uncommon	
Subfamily ACRAEINAE			
Acraea issoria (Huebner [1819])	Yellow Coster	Common	
Cethosia biblis (Drury [1773])	Red Lacewing	Less Common	Schedule II, Part H
Cethosia cyane (Drury [1773])	Leopard Lacewing	Common	
Subfamily LIBYTHEINAE			
Libythea lepita Moore [1858]	Common Beak	Uncommon	Schedule II, Part H

species of the Nymphalidae family during the course of the current studies. These species shows dry-season forms (DSF) and wet-season forms (WSF*) i.e., Junonia orithya* (Linnaeus), *J. hierta* (Fabricius), *J. almana* Linnaeus, *Ypthima baldus* Fabricius, *Y. huebneri* Kirby *Melanitis leda* Linn., *M. zitenus* (Herbst), *Mycalesis anaxias* Hewitson, *M. perseus* (Fabricius), *M. francisca* (Stoll), *M. mineus* (Linn.), *M. gotama* Moore*, M. visala* Moore, *M. intermedia* (Moore), and *Orsotriaena medus* (Fabricius). The seasonal variations in butterfly forms are entirely caused by environmental factors, specifically temperature and humidity, which have an impact on the internal chemical physiology of the immature butterflies. These differences are not genetic in nature. We refer to these non-genetic differences as polyphenism. As a result, DSF butterflies have reduced marginal ocelli, more angulate wings, and cryptic patterns on their undersides than WSF butterflies in monsoon regions.

Flight duration also noted during study period, although most butterflies prefer to fly during sun-hours but members of subfamily Satyrinae (Family Nymphalidae) i.e., *Mycalesis anaxias* Hewitson, *M. perseus* (Fabricius), *M. francisca* (Stoll), *M. mineus* (Linn.), *M. gotama* Moore*, M. visala* Moore, *M. intermedia, Lethe confusa* Aurivillius*, Lethe chandica* Moore*, Lethe distans* Butler*, Lethe kansa* (Moore), *Melanitis leda* Linn., *Melantis zitenus* (Herbst), *Ypthima huebneri* Kirby and *Ypthima baldus* (Fabricius) were also active during dusk and dawn hours or cloudy days. The species *Kallima inachus* (Boisduval)*,* and *Doleschallia bisaltide* (Cramer) shows camouflage mimicry and resemble to dry oak-leaf. The species, *Discophora sondaica* Boisduval and *Discophora timora* Westwood were observed sitting near the light source.

Out of 209 species, 37 species are included in the Wildlife (Protection) Amendment Act, 2022. Three butterfly species are included in Schedule I, Part I, 33 species, are are included in the Schedule II, Part H and one species is included in the Schedule IV, Appendix II of the Act (Table 1). During the course of present studies, four species i.e., *Troides aeacus* (C. & R. Felder), *Graphium sarpedon* (Linnaeus), *Appias lyncida* (Cramer) and *Jamides alecto* (C. Felder) were found common and should be removed from the Act.

The four species *i.e., Ypthima nareda* (Kollar), *Talicada nyseus* (Guérin-Méneville), *Pantoporia sandaka* (Butler*)* and *Caltoris philippina* (Herrich-Schaeffer) were recorded only from Papum Pare district of Arunachal Pradesh. The species *Suasa lisides* (Hewitson) is a very rare species and recording of this species from Papum Pare district (Ganga Lake, Itanagar) is the second sighting in Arunachal Pradesh.

CONCLUSION

Out of these 209 species, 60 species (29%) were common, 84 species (40%) were uncommon, and the remaining 65 species (31%) were less common. 37 species out of 209 are covered by the Wildlife (Protection) Amendment Act of 2022. Four species—*Caltoris philippina* (Herrich-Schaeffer), *Pantoporia sandaka* (Butler), *Talicada nyseus* (Guérin-Méneville), and *Ypthima nareda* (Kollar)—were first documented from Arunachal Pradesh and were only found in the Papum Pare district. The second sighting of the extremely rare species *Suasa lisides* (Hewitson) in Arunachal Pradesh was recorded from the Papum Pare district (Ganga Lake, Itanagar).

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Ecological Distribution and Infestation Dynamics of Rice Panicle Mite, *Steneotarsonemus spinki* Smiley

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***Abstract*:** Field surveys conducted across major rice-growing villages of the North Coastal districts of Andhra Pradesh between 2017 and 2021 confirmed *Steneotarsonemus spinki* Smiley as the principal factor associated with spikelet sterility and grain discolouration. Mite colonies were predominantly located within the intercellular spaces of the upper leaf sheath and basal midrib regions, producing characteristic necrotic streaks. Infestations began on leaf sheaths during booting and later extended to developing grains. Incidence ranged from 1–15% in most surveyed locations and reached 20–30% in Amadalavalasa. Cultivars such as RNR-15048, BPT-5204 and BPT-3219 recorded higher susceptibility. Mites persisted in stubbles and ratoon rice and survived on associated weeds (*Cyperus rotundus*, *Echinochloa crusgalli, Cynodon dactylon*), confirming their role as off-season hosts. Peak populations occurred during the booting and soft dough stages. The study highlights the increasing economic importance of *S. spinki* in Andhra Pradesh and emphasizes the need for integrated management strategies targeting both the mite and its associated pathogens.

Keywords: *Steneotarsonemus spinki*, Rice, Host range, Distribution

Among non-insect pests of rice, mites are considered significant due to their impact on both field production and storage. In India, as many as 61 species of mites have been reported to be associated with rice cultivation and storage systems (Rao et al., 1999). Of these, the rice panicle mite, *Steneotarsonemus spinki* Smiley (Acarina: Tarsonemidae) is important, as it infests the flag leaf sheath of rice, leading to brown discolouration. Infestation on panicles causes chaffy grains and discoloration of filled or partially filled grains (Srinivasa et al., 2004), while feeding on floral reproductive structures can result in grain sterility (Rao et al., 2000).

Steneotarsonemus spinki has emerged as a major constraint to rice production across Asia and other global rice-growing regions since the 1970s (Tseng 1978). The earliest indirect reference in India dates to Ramaiah (1931), describing a “tiny moving arthropod” infesting rice. The species was formally described by Smiley (1967) from specimens in Louisiana, USA. First documented as a rice pest in China in 1968 (Ou et al., 1977), the mite was detected in India in 1975 (Rao and Das 1977). It has since spread widely, causing severe outbreaks in several countries, including 30–90% yield losses in Cuba (Almaguel et al., 2000). In India, *S. spinki* was reported in 1992 from paddy fields in the south, later becoming a major pest responsible for significant reductions in both the quality and quantity of rice production in Orissa (Rao and Prakash 1992), Gujarat (Rai et al., 1998) and West Bengal (Karmakar 2008; Karmakar and Debnath 2016).

In Andhra Pradesh, widespread spikelet sterility and grain discolouration were observed in 1999 across major rice varieties, including MTU1001 and BPT5204, in the West and East Godavari districts, affecting up to 50% of the fields in certain villages. Subsequent investigations confirmed the involvement of *S. spinki* as the primary causal agent, often in association with fungi and nematodes (Rao et al., 2000). Typical symptoms included black sheath lesions, malformed grains, and mite infestation on seed-raised seedlings, indicating possible seedborne transmission. Over the years, the pest has spread to other parts of Andhra Pradesh, causing grain discolouration and chaffy grains in several widely cultivated rice varieties. In recent years, grain discolouration has become more pronounced in *kharif* (wet season) rice cultivated in the North Coastal districts of Andhra Pradesh, leading to considerable qualitative and quantitative losses. Owing to its frequent association with sheath rot (*Sarocladium oryzae*) and uncertainties surrounding its off-season survival, the present study was undertaken to assess the prevalence and morphological characteristics of *S. spinki* in North Coastal Andhra Pradesh.

MATERIAL AND METHODS

Field surveys were conducted across 24 villages in the districts of Srikakulam, Vizianagaram, and Visakhapatnam during 2017–2021. The specific objectives were (i) to assess the prevalence of *S. spinki* on major rice varieties and (ii) to evaluate the role of ratoon rice and weeds in paddy fields as possible inoculum sources contributing to infestations in subsequent cropping seasons. Rice fields were monitored every two weeks starting from four weeks after planting until harvest covering green ring, early boot, late boot, milk, soft dough and hard-dough stages.

During the surveys, discoloured rice panicles were collected in polythene bags from different rice varieties. To determine possible infestation sources, collected four types of substrates from field sites: (1) rice-associated weeds, (2) soil, (3) rice grains of different ages (pre- and post-harvest), and (4) rice stubbles. Samples were brought to the laboratory, placed on a glass slide and examined under stereo binocular microscope for diagnostic symptoms and mite presence. Morphological identification was carried out using stereo binocular microscopy. Life stages were measured to determine morphometrics. Images of the mycosis were captured using Capture Pro-Camera control software.

RESULTS AND DISCUSSION

Survey on grain discolouration and spikelet sterility: Surveys from October to December (2017–2021) confirmed widespread spikelet sterility and grain discolouration in all 24 surveyed villages (Fig. 1). The most affected rice varieties included MTU1001, RNR 15048, BPT5204, BPT3219, NDLR-7, DRR-DHAN45 (Fig. 2 a-f). The infestation exhibited a patchy distribution pattern across 24 villages and the infestation ranged from 1-15% in most villages, with the highest incidence (20-30%) recorded in Amadalavalasa. Varietal-specific symptoms were also documented: leaf sheath discolouration was observed on Srikurma at Ragolu and MTU1156 at Amadalavalasa. Grain discolouration occurred in MTU1001 (5-10%), Sambhamahsuri (5-10%), and Sonamahsuri (5-10%) at Kusuma Polavalasa (Polaki mandal). In Amadalavalasa (Srikakulam district), discolouration was observed on MTU1239 (5-10%), MTU1156 (5-10%), RNR15048 (20-30%), and NDLR-7 (15-20%). In Anakapalle district, MTU1001, BPT 5204 and RNR 15048 exhibited 20-25% grain discolouration in Chodavaram and Koyyuru mandals during early November, while discolouration of the leaf midrib was observed on RGL 2537 at Anakapalle during November-December. Rao et al. (2000) reported that the cultivars MTU-1001, MTU-2067, MTU-2077, MTU-7029, BPT-5204 and PLA-1100 were most susceptible to rice panicle mite. The variation in incidence among varieties and locations may be attributed to differences in varietal susceptibility, local microclimatic conditions, and cropping intensity. Higher humidity and moderate temperatures prevailing during the *kharif* season likely favoured the population build-up of *S. spinki* which is consistent with previous reports from India and Southeast Asia (Rao et al., 2000).

The panicle mite was observed from the booting to harvest stages, with higher incidences recorded during the *kharif* season compared to the late *kharif* period. The population density peaked at the booting and soft dough grain maturity stages, reaching 78-110 mites per panicle. Thereafter, mite numbers declined progressively from the medium to hard dough stages. Following harvest, *S. spinki* migrated to alternative sources of moisture and sustenance, including rice stubble, ratoon crops, seedlings from fallen grains, and other Poaceae hosts, where it could continue to survive and reproduce. The present findings are consistent with those reported by Begam et al. (2019) who reported that peak incidence of mite occurred at the ripening stage but significantly higher number of mite population and damage symptoms in all plants were observed at the panicle emerging to ripening stage.

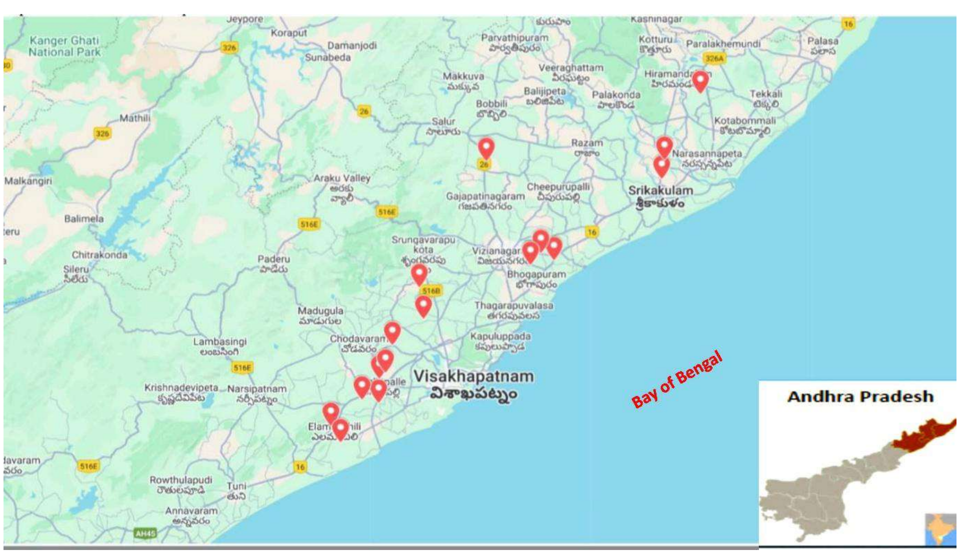

Fig. 1. Map of Coastal Andhra Pradesh showing the mandals of Srikakulam, Vizianagaram and Visakhapatnam districts surveyed

Morphological identification: The panicle rice mite is not visible to the naked eye and requires a minimum 20× hand lens for observation inside the leaf sheath. Stereomicroscopic examination of infested rice samples confirmed *S. spinki* as the causal organism associated with grain discolouration. Mites were observed in colonies within the intercellular spaces of the upper leaf sheath, necrotic lesions, and the basal part of the midrib of the leaf blade, causing necrotic streaks in the interveinal epidermis (Fig. 3). Affected plants exhibited poorly exerted panicles and necrotic leaf sheaths. Mites were primarily located between the stem and leaf sheath, and the affected glumes showed brown to black discolouration of the lemma and palea with shrivelled ovaries.

Fig. 2. Grain discolouration in different varieties (a-e) : (a) Basmati variety; (b) NDLR -7; (c) RNR 15048; (d) DRR Dhan-45; (e) MTU1001; (f) BPT 3219

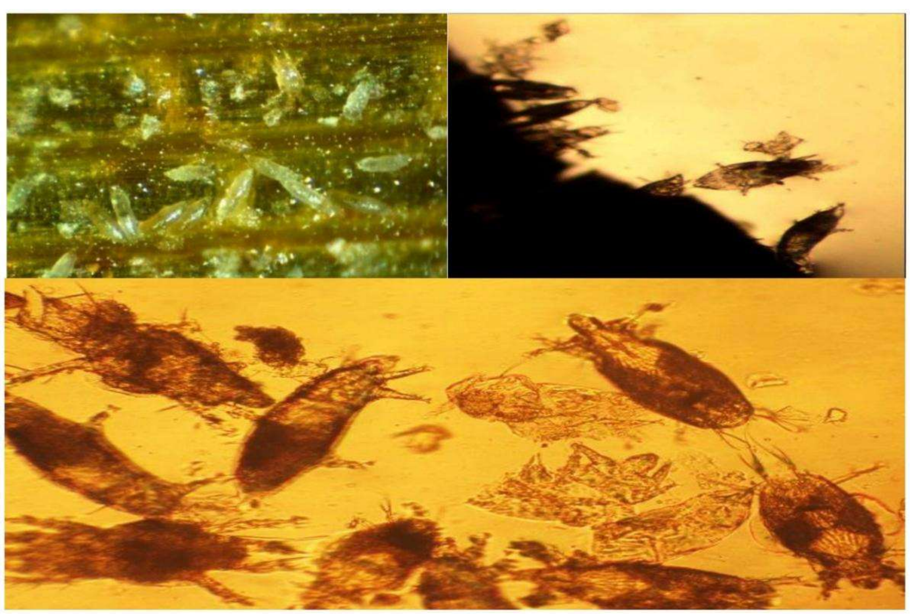

Fig. 3. Colonies of *S. spinki* in the inter cellular space of the leaf sheaths

Life stages of *S. spinki:* These mites are clear to straw-coloured and measure approximately 250 µm in length. Males have elongated rear legs with a pair of prominent spines, which they carry above their bodies. They are highly active and can be seen moving on the leaf surface. Females are ovoid in shape. The larval stages and eggs are about half the size of adults.

Egg: Morphologically, the eggs were cloudy, creamy white to yellowish white, and elongated in shape (Fig. 4a). As embryonic development progressed, the eggs turned more whitish. The eggs measured an average of 0.12 ± 0.006 mm in length and 0.068 ± 0.007 mm in breadth. Eggs laid by adult females were found deposited within the intercellular spaces of rice leaf sheaths, either singly or in small clusters containing 2–5 eggs. These findings are consistent with the observations of Patel and Purohit (2009).

Nymph: Male and female nymphs were distinguished primarily by size (Fig. 4b). Male nymphs were smaller and exhibited a transparent white colouration (Fig. 4d). The average body length and width of male nymphs were 0.148 ± 0.017 mm and 0.069 ± 0.006 mm, respectively, while female nymphs measured 0.174 ± 0.018 mm in length and 0.070 ± 0.006 mm in width. The present findings show slight variation from those reported earlier by Patel and Purohit (2009).

Quiescent stage: The mature larva entered a quiescent stage and feeding of the larva was restricted. Quiescent stage measured 0.213 ± 0.012 mm in length and 0.073 ± 0.014 mm in width.

Adult: Adult males and females were nearly transparent. Males were broader, with characteristic dagger-shaped setae (Fig 4c), whereas females were narrower but slightly longer (Fig. 4d). Morphometric analysis revealed that adult males measured 0.234 ± 0.011 mm in length and 0.106 ± 0.010 mm in width. Adult females measured 0.286 ± 0.016 mm in length and 0.080 ± 0.011 mm in width which are in conformity with Patel and Purohit (2009).

Life cycle: Rice Panicle mite, *S. spinki* has fast and efficient reproduction with females producing 50 to 70 eggs in their lifetimes. Reproduction is arrhenotokous parthenogenetic, whereby virgin female produce male off springs. The sex ratio of female: male *S. spinki* to be 22:1, 32:1 and 8:1at 32^{0}C, 28^{0}C under field condition respectively. Increasing temperature significantly shortened generation time from 11. 3 days at 20^{0}C to 7.8 days at 23.9^{0}C and 4.9 days at 33.9^{0}C and *S. spinki* can produce 48 -55 generations per year under ideal climatic conditions. Thus, a large population of S. spinki can develop very quickly in a rice crop during a single growing season. These findings align with Chaudhari et al. (2019).

Damage symptoms: Microscopic examination of several affected plant samples confirmed *S. spinki* as the predominant causal organism in all cases. Four distinct combinations of visual symptoms were identified on infested

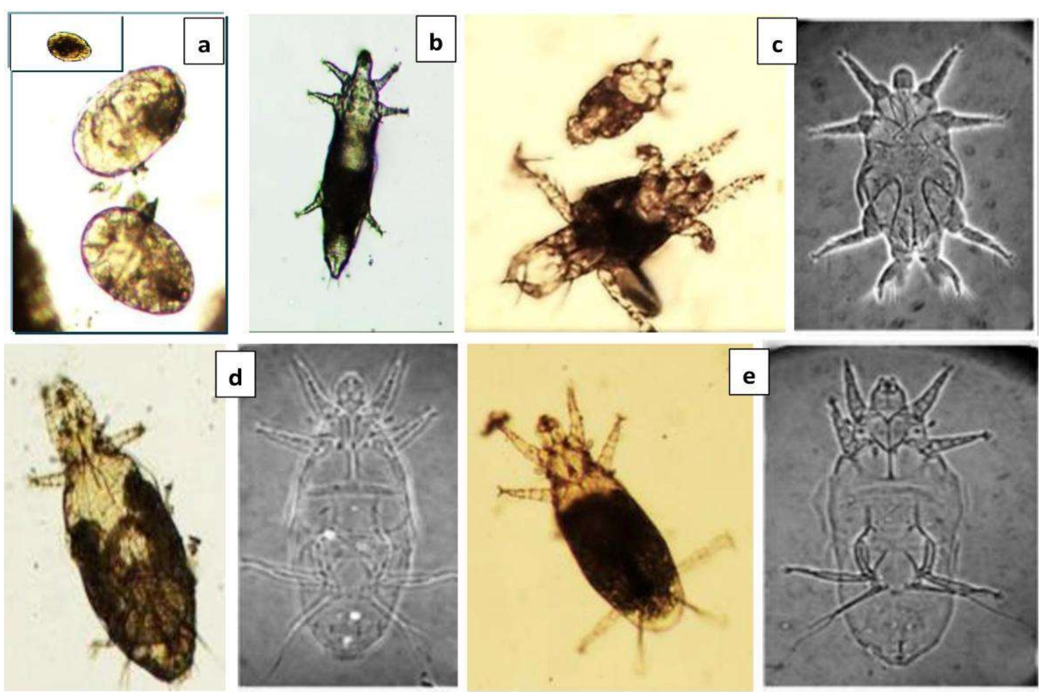

Fig. 4. Life stages of *Steneotarsonemus spinki* (a) Egg (b) Nymph (c) Female adult- Dorsal side & Ventral side (d) Male adult -Dorsal side &Ventral side

plants: (i) mite damage alone; (ii) mite + saprophytic fungus; (iii) mite + saprophytic fungus + sheath rot fungus; and (iv) mite + white-tip nematode (*Aphelenchoides besseyi)* + other saprophytic fungal damage. The present findings corroborate the observations of Rao et al. (1993). Characteristic field symptoms included black lesions on the leaf midrib (Fig. 5a) and leaf sheath (Fig. 5b), discoloured grains (Fig. 5c), partially or completely chaffy grains (Fig. 5d) and other grain deformities (Fig. 5f). These observations are consistent with earlier reports of Karmakar (2008). Distinct mite-associated symptoms were also observed on the leaves of seedlings raised from infested seed material, suggesting the possibility of seed-to-plant transmission of the tarsonemid mite. These observations agree with the findings of Rao et al. (2000). The frequent association of *S. spinki* with fungal pathogen such as *Sarocladium oryzae* observed in the present study suggests a possible synergistic interaction that may exacerbate the severity of grain discolouration and spikelet sterility. Another plausible explanation for this co-occurrence is that feeding damage caused by *S. spinki* predisposes the panicle and sheath tissues to secondary infections, thereby facilitating the entry of pathogens responsible for panicle blight and sheath rot. Further investigations are warranted to elucidate the precise nature of these interactions and to develop integrated management strategies that simultaneously target the mite and its associated pathogens. Rao and Prakash (2003) also found *S. spinki* on plants from which no pathogens were isolated, they concluded that the grain discolouration could be caused by a chemical reaction to toxic saliva of *S. spinki* whereas Chen et al. (1979) found that *S. spinki* carried spores of *Acrocylindrium oryzae* Sawada (*S. oryzae*) on their body and attributed the plant symptoms to a combination of *S. spinki* damage and disease.

The panicle mite causes both direct and indirect damage to rice. Direct injury results from feeding on leaf tissues within the sheath and developing grains during the milk stage, while indirect damage occurs from transmission of fungal and bacterial pathogens. The mite punctures epidermal cells with its stylets, producing brown necrotic lesions on the upper leaf sheath and grain hulls. Feeding weakens tissues and facilitates fungal infection, leading to panicle sterility, grain malformation, and discolouration. Feeding occurs mainly beneath the leaf sheath, where chocolate-brown streaks develop. As new leaves emerge, females migrate to younger sheaths for oviposition, and damage becomes visible only after removing outer sheaths. The mites progress to the innermost sheath and eventually colonize the developing panicle. Infestations during booting to milk stages cause grain sterility, chaffy and deformed grains with a "parrot-beak" shape, and sheath discolouration (Hummel et al., 2009). The mites also carry fungal spores on their body, aiding pathogen spread. These symptoms often resemble sheath rot caused by *Sarocladium oryzae*. Mite populations peaked at the booting stage and declined as the crop matured, consistent with earlier observations (Chandrasena et al., 2018). Infestations were associated with necrotic sheaths, poorly exerted panicles, and discoloured or malformed grains. Similar damage including "unfilled grain syndrome," has been documented in other Asian countries (Srinivasa et al., 2004, Mutthuraju et al., 2014).

Influence of weather parameters : Weather factors such as temperature, relative humidity, rainfall, and sunshine hours play a crucial role in regulating the population dynamics of *S. spinki*. Periods of reduced rainfall and increased sunshine were conducive to the proliferation of *S. spinki*. High temperatures coupled with low rainfall during August–October favoured rapid population buildup. Optimal development occurred at around 28°C and relative humidity above 80%, with maximum growth observed at 90–95%. Rainfall and relative humidity showed a negative correlation with *S. spinki* populations, whereas temperature and sunshine hours exhibited a positive correlation. Continuous rice cultivation and the movement of infested stubbles or contaminated equipment between fields further facilitated the spread and persistence of *S. spinki* populations. Intermittent heavy showers negatively affected mite populations, whereas prolonged dry periods promoted their growth. These observations agree with the findings of Chandrasena et al. (2018).

Sources of infestation: The incidence of *S. spinki* was higher in the *kharif* season than in *rabi*. Ratoon rice plants present during the fallow period (December–June) served as survival habitats, facilitating the persistence of the panicle rice mite between two consecutive kharif seasons. No mites were found in soil samples, but infestations in grains and rice stubbles confirmed these as major inoculum sources (Prabhakara 2002). Three grassy weeds (1) *Cyperus rotundus*, (2) *Cynodon dactylon*, and (3) *Echinochloa crusgalli* were served as alternate hosts, facilitating mite survival and carryover. (Fig. 6 a-c). Khimji (2005) found that *S. spinki* survived on graminaceous weeds such as *Cyperus difformis* and *Cynodon dactylon*, as well as on post-harvest rice residues, acting as a source of infestation for the subsequent *kharif* crop. Srinivasa and Prabhakara (2007) also detected eggs and active stages on *C. dactylon* and *Echinochloa colonum* near rice fields in Karnataka. In Sri Lanka, Chandrasena et al. (2016) identified *Sacciolepis interrupta* as a major alternate host, while *E. crusgalli* and *Leptochloa chinensis* played minor roles. Similarly, Tran et al. (2023) reported *E. crusgalli* and

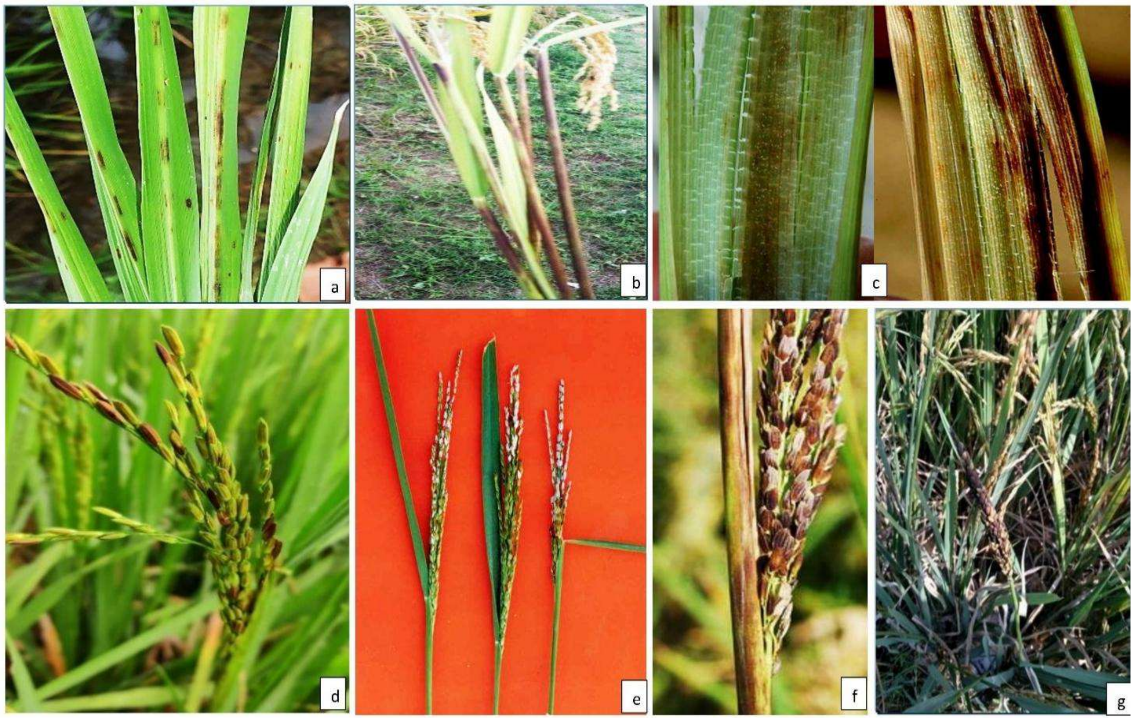

Fig. 5. Damage symptoms of *S. spinki* in rice (a-f): (a) Midrib discolouration; (b) Leaf sheath discolouration (c) Necrotic lesions inside the leaf sheath; (d) Grain discolouration; (e) Chaffy grains; (f) Poor exertion and (f) Malformed panicle

Fig. 6. Alternate hosts of *S. spinki:* Grassy weeds (a) *Cynodon dactylon;* (b) *Cyperus rotundus;* (c) *Echinochloa crusgalli*

Fimbristylis spp. as alternative hosts and important sources of infestation. No tarsonemid mites were detected in soil samples from infested fields, indicating that stubbles serve as the primary site for off-season survival. The rice panicle mite was found on grains of different ages and in stubbles, confirming these as key inoculum sources between cropping seasons. The mite can spread through infested rice seeds since larvae can briefly survive under dry conditions and by passive dispersal via wind or insect vectors such as planthoppers. Even a small number of surviving eggs or immatures can rapidly multiply under favourable conditions due to parthenogenetic reproduction. Thus, both rice grains and stubbles act as important sources of *S. spinki* infestation in successive crops. These findings emphasize the importance of eliminating ratoons and weed hosts during crop-free periods as an effective cultural strategy for managing *S. spinki* under integrated pest management programs in North Coastal Andhra Pradesh and are consistent with the observations of Kayal et al. (2021) and Tran et al. (2023).

Impact on grain quality and yield: In the North Coastal districts of Andhra Pradesh, the highest economic impact of *S. spinki* was recorded in Srikakulam district, where yield losses of 10–20% occurred in medium-duration rice varieties such as MTU 1001, BPT 5204, BPT 3219, and RNR 15048. (Rao et al., 2000) also reported severe grain deterioration characterized by ill-filled, chaffy, and discoloured grains in medium-irrigated rice varieties. Similar results have been documented in India and elsewhere, with *S. spinki* infestations causing yield losses ranging from 5% to 90%. In India, losses have been estimated at 4.9–23.7% (Rao and Prakash 1996) and 10–15% (Rao et al., 2000), while global estimates indicate damage ranging from 5% to 95% across several countries (Navia et al., 2010, Pushpakumari et al., 2010).

CONCLUSIONS

The study confirms that infestation by the panicle mite, *S. spinki* is the primary cause of grain discolouration and chaffy grains in *kharif* rice across the North Coastal districts of Andhra Pradesh. Mite populations peak during the booting stage and persist through ratoons and weeds, leading to significant yield and quality losses. The association of mites with fungal spores suggests a potential role in transmitting secondary pathogens. Therefore, an integrated management strategy is recommended, involving early scouting during the booting stage, removal of ratoon and weed hosts, cultivation of less susceptible varieties, and timely application of acaricides with fungicides to effectively suppress mite populations and reduce grain discolouration and chaffiness.

AUTHOR's CONTRIBUTION

B. Bhavani was responsible for the conceptualization of the study, planning and execution of experiments, supervision of data collection, data analysis, interpretation of results, and preparation of the original manuscript draft. P. Kishore Varma supported the surveys and microscopic examination of affected samples and contributed to data validation. M. Visalakshi assisted in experimentation and data recording, reviewed and edited the manuscript.

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Diversity and Abundance of Insect Pollinators in Sesame, *Sesamum indicum* Linnaeus

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Abstract: Field study was conducted during the *Rabi* 2023–24 at the Agricultural College, ANGRAU, Bapatla, to investigate the diversity and abundance of insect pollinators in sesame (*Sesamum indicum* L.). Thirty insect species belonging to four orders were recorded visiting sesame flowers. Among these, *Apis cerana indica* (Indian honey bee) was the most dominant pollinator. The family Megachilidae was dominating among the pollinator guild. Pollinator activity varied throughout the day, gradually increasing from 09:00 h and peaking between 09:30 and 11:05 h, before declining after 14:00 h. However, species *Megachile laticeps*, *Megachile lanata*, *Megachile cephalotes*, *Ceratina binghami*, and *Ceratina* sp. exhibited peak activity between 12:30 and 14:05 h. The Shannon–Wiener index (H) was lowest during the early morning hours but increased throughout the day, showing two peaks corresponding to morning and noon activity. Both the Berger-Parker dominance index (d) and Shannon's diversity index showed a positive correlation with floral abundance. These findings highlight the diversity, diurnal activity, and ecological significance of pollinators in sesame, emphasizing the need for their conservation and sustainable management.

Keywords: Sesame, Diversity, Pollinators, *Sesamum indicum*

Pollination is one of the most critical ecosystem services, directly and indirectly benefiting human societies through enhanced agricultural productivity and biodiversity conservation. Among various pollination mechanisms, insect-mediated pollination plays a pivotal role by facilitating the transfer of pollen within and between flowers (Fisher et al., 2009). Globally, about 35% of crop production depends on pollinators, particularly insects, which contribute to yield improvement in nearly 75% of major crop species (Klein et al., 2007). Among the insects, bees are considered the most efficient and dominant pollinators in both natural and agricultural ecosystems, providing sustainable pollination services.

Sesame (*Sesamum indicum* L.), a member of the family Pedaliaceae, is a valuable oilseed crop and ranks third globally after soybean and mustard. Sesame seeds contain approximately 50% oil and are widely used in the food, pharmaceutical, and chemical industries (Blal 2013, Elleuch et al., 2007, Namiki 2007). Although sesame is commonly regarded as a self-pollinating crop, its floral morphology favours cross-pollination. The rate of cross-pollination varies significantly from 0.5 to 65%, depending on insect activity, surrounding flora, and environmental conditions (Free 1993, Sharma and 2010). Despite the importance of pollinators in sesame, comprehensive studies on pollinator diversity and foraging behaviour in different agro-climatic regions, particularly in Andhra Pradesh, remain limited. Given the increasing interest in sustainable agriculture and pollinator-friendly practices, understanding the diversity and behaviour of native pollinators in sesame ecosystems is crucial. This study was therefore undertaken to document the diversity, abundance, and diurnal activity of insect pollinators visiting sesame flowers during *rabi* 2023–24. The findings aim to support conservation strategies and promote pollinator-friendly agricultural practices to enhance sesame productivity.

MATERIAL AND METHODS

Experimental site and crop details: The study was conducted during *Rabi* 2023–24 at the Agricultural College, Bapatla, ANGRAU. The experimental site is located at 15.900° N latitude, 80.460° E longitude, and an altitude of 8 meters above mean sea level, with an average annual rainfall of 914 mm. Sesame (*Sesamum indicum* L.) variety 'Sarada' (YLM-66) was sown under irrigated conditions in a 1400 m² field, with 1000 m² designated for observations. Land preparation involved two ploughings and levelling. Seeds were treated with carbendazim @ 3 g/kg and mixed with sand in a 1:1 ratio before sowing at a seed rate of 2.5 kg/ha. Manual sowing was done at a spacing of 30 × 10 cm. Thinning was carried out at 20 and 40 days after sowing to maintain proper plant spacing. Standard agronomic practices, except for plant protection measures, were followed throughout the crop season.

Flower visitors and pollinators: Insect visitation to sesame flowers was observed visually during the flowering period. Five random spots of 1 m² each were selected within the observation plot. Visual counts of flower-visiting insects were recorded for five minutes at each spot using a stopwatch. Observations were made at four time intervals: 06:30, 09:30, 12:30, and 15:30 h, at 25, 50, 75, and >90% flowering stages. Insects were collected using fine mesh sweep nets, preserved, and later identified by taxonomic experts at ICAR–National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru.

Pollinator diversity: Pollinator diversity and dominance were evaluated using standard ecological indices:

- Shannon-Wiener Diversity Index (H) (Shannon and Wiener, 1963):
- Simpson's Diversity Index: Measure of species richness and evenness.
- Berger-Parker Dominance Index (d) (Berger and Parker, 1970):
- Shannon Evenness Index

Abundance and diurnal activity of pollinators: To analyze the diurnal activity of pollinators, observations were made at 3-hour intervals between 06:30 h and 15:30 h during peak flowering. In each 1 m² spot, insect visitors were counted for five minutes per interval. The mean number of pollinators per square meter per five minutes was calculated and later standardized to hourly data for assessing relative abundance. This method was adapted with modifications from Revanasidda and Belavadi (2019), previously applied in studies on coriander and muskmelon

RESULTS AND DISCUSSION

Thirty species of insect pollinators were recorded of which seventeen belonged to Hymenoptera, nine to Diptera, three to Lepidoptera and one to Coleoptera (Table 1). Among these, the most effective and abundant pollinator was the Indian honey bee, *Apis cerana indica* L . Hymenoptera emerged as the most dominant group followed by Diptera. These findings are consistent with previous studies reporting Hymenoptera as the dominant order in sesame ecosystems (Pasthe and Shylesha 2013, Rao et al., 2022, Kamel et al., 2013).

Abundance of insect pollinators across flowering stages: At 25% flowering, *A. cerana indica* was most abundant (1.00–1.67 individuals/m²/5 min), followed by *Ceratina* sp., *M. lanata*, *M. laticeps*, and *C. binghami* (each with 0.00–1.00 individuals/m²). At 50% flowering, *A. cerana indica* (1.67–2.00), *C. binghami*, and *M. lanata* showed increased abundance, along with *Nomia* sp. and *Xylocopa* sp. At 75% flowering, a similar trend was observed, with *A. cerana indica*, *M. lanata*, *Sarcophaga* sp., and *M. cephalotes* being dominant. At 90% flowering, *A. cerana indica* peaked (up to 4.67 individuals/m²), with notable increases in *M. laticeps*, *Xylocopa* sp., *Eristalinus* sp., and *Ceratina* sp. These results confirm a positive correlation between floral density and pollinator abundance, consistent with previous

Table 1. Diversity of insect pollinator species of sesame at Agricultural College, Bapatla

Species	Family
Hymenoptera	
Apis cerana indica	Apidae
Xylocopa sp. (1)	
Xylocopa sp. (2)	
Ceratina binghami	
Ceratina sp.	
Amegilla confusa	
Amegilla cingulata	
Thyreus sp.	
Megachile laticeps	Megachilidae
Megachile lanata	
Megachile cephalotes	
Megachile sp.	
Coelioxys sp. (1)	
Coelioxys sp. (2)	
Halictus sp.	Halictidae
Nomia sp.	
Bembix priesneri	Crabronidae
Diptera	
Episyrphus sp.	
Eristalinus sp.	Syrphidae
Eristalinus obscuritarsis	
Sarcophaga sp.	Sarcophagidae
Musca sp.	Muscidae
Stomorhina sp.	Rhiniidae
Bombylius sp.	Bombyliidae
Physiphora sp.	Ulidiidae
Chrysomya sp.	Calliphoridae
Lepidoptera	
Catopsilia sp.	Pieridae
Papilio demoleus	Papilionidae
Danaus chyrsippus	Nymphalidae
Coleoptera	
Mylabris sp.	Meloidae

Table 2. Simpson's diversity and evenness index of insect visitors at different floral densities of sesame

Stage of the flowering	Simpson's diversity index	Evenness index
25% flowering	0.85	0.89
50% flowering	0.94	0.71
75% flowering	0.95	0.67
>90 % flowering	0.97	0.61

findings in muskmelon and other crops (Revanasidda and Belavadi 2019).

Diurnal activity of pollinators: Pollinator activity varied significantly throughout the day**.** Overall activity increased from 06:30 h, peaked between 09:30 and 11:05 h, and declined after 14:00 h. *A. cerana indica*, *Xylocopa* sp., *Nomia* sp., *Thyreus* sp., and *Halictus* sp. were most active during the mid-morning hours. *M. lanata*, *M. cephalotes*, *C. binghami*, and *Ceratina* sp. reached peak activity between 12:30 and 14:05 h. Sajjanar and Eswarappa (2015) and Mohapatra &

Table 3. Shannon-Weiner Diversity Index (H) of insect visitors at different floral densities of sesame during *Rabi*, 2023-24

Hour of the day	Floral density (%)			
	25	50	75	>90
6.30-08:05	1.37	1.97	2.03	2.15
09.30-11.05	2.54	2.56	2.77	2.86
12:30-14:05	2.36	2.48	2.55	2.85
15:30-17:05	0.00	0.95	1.03	1.07

Table 4. Dominance of insect species at different floral densities of sesame during *Rabi,* 2023-24

Species of visitor	Flowering density							
	25%		50%		75%		90%	
	Dominance Index							
	d	1/d*	d	1/d*	d	1/d*	d	1/d*
Apis cerana indica	0.12	8.63	0.14	7.06	0.12	8.19	0.12	8.09
Megachile laticeps	0.04	23.00	0.03	37.67	0.02	43.67	0.04	22.25
Megachile lanata	0.07	13.80	0.06	16.14	0.06	16.38	0.04	25.43
Megachile cephalotes	0.03	34.50	0.05	18.83	0.06	16.38	0.05	19.78
Megachile sp.	0.01	69.00	0.04	28.25	0.02	43.67	0.02	59.34
Ceratina binghami	0.04	23.00	0.09	11.30	0.05	21.84	0.03	29.67
Ceratina sp.	0.07	13.80	0.04	22.60	0.05	21.84	0.04	22.25
Xylocopa sp.(1)	0.04	23.00	0.04	28.25	0.03	32.76	0.04	22.25
Xylocopa sp.(2)	0.06	17.25	0.04	28.25	0.03	32.76	0.03	29.62
Amegilla confusa	0.01	69.00	0.02	56.50	0.05	21.84	0.04	22.25
Amegilla cingulata	0.01	69.00	0.03	37.67	0.02	43.67	0.04	25.39
Coelioxys sp.(1)	0.03	34.50	0.04	28.25	0.02	65.51	0.01	178.01
Coelioxys sp.(2)	0.01	69.00	0.02	56.50	0.01	131.02	0.01	89.01
Halictus sp.	0.03	34.50	0.04	22.60	0.04	26.20	0.03	29.67
Nomia sp.	0.01	69.00	0.04	22.60	0.02	43.67	0.03	35.67
Thyreus sp.	0.01	69.00	0.01	113.00	0.02	43.67	0.02	59.34
Bembix priesneri	0.04	23.00	0.03	37.67	0.02	43.67	0.03	35.60
Episyrphus sp.	0.03	34.50	0.03	37.67	0.05	21.84	0.04	25.47
Eristalinus sp.	0.04	23.00	0.03	37.67	0.05	21.84	0.04	25.43
Eristalinus obscuritarsis	0.00	0.00	0.03	37.67	0.03	32.59	0.04	25.47
Musca sp.	0.04	23.00	0.03	37.67	0.02	43.67	0.02	44.50
Stomorhina sp.	0.00	0.00	0.00	0.00	0.02	65.18	0.01	88.56
Sarcophaga sp.	0.04	23.00	0.02	56.50	0.04	26.20	0.03	29.67
Bombylius sp.	0.01	69.00	0.00	0.00	0.02	65.51	0.02	44.50
Physiphora sp.	0.00	0.00	0.01	114.14	0.02	65.51	0.03	35.53
Chrysomya sp.	0.03	34.50	0.02	56.22	0.02	43.67	0.02	59.34
Catopsilia sp.	0.03	34.50	0.03	37.67	0.03	32.76	0.03	35.60
Papilio demoleus	0.03	34.50	0.00	0.00	0.02	43.67	0.03	35.60
Danaus chyrsippus	0.04	23.00	0.05	18.83	0.03	32.76	0.02	44.50
Myalabris sp.	0.03	34.50	0.03	37.67	0.02	43.67	0.03	35.60

Sontakke (2012) also observed peak foraging activity of pollinators in sesame between 10:00 and 13:00 h.

Diversity Indices

Shannon-Wiener diversity index (H): Pollinator diversity was lowest in early morning and late afternoon and increased significantly during peak hours. The Shannon-Wiener Index values were 00–1.37 at 25% and increased to 1.07–2.15 at 90%flowering (Table 2). Two activity peaks were observed one in the morning (09:30–11:05 h) and in the afternoon (12:30–14:05 h). This suggests a bimodal foraging pattern linked to floral resource availability and favourable microclimatic conditions.

Simpson's diversity index (D): Simpson's Index also indicated increasing diversity with flowering rising from 0.85 at 25% flowering to 0.97 at 90% flowering respectively. This confirms that both species richness and evenness improved during peak bloom periods. Similar patterns were also reported by Prakash & Bijoy (2021) in ash gourd (Table 3).

Shannon evenness index: Evenness decreased with increasing flowering, 0.89, 0.71, 0.67, and 0.61 across the four flowering stages. This decline indicates dominance by a few species primarily *A. cerana indica*, *Megachile* spp., and *Ceratina* sp.in later stages of flowering. Comparable results were also reported in bitter gourd by Yogapriya et al. (2019) (Table 2).

Dominance patterns: The Berger-Parker Dominance Index revealed that *A. cerana indica* was consistently dominant (d = 0.12–0.14), followed by *Ceratina* sp., *M. lanata*, *M. cephalotes*, and *C. binghami*. The dominance index decreased with increasing floral abundance, indicating greater pollinator diversity at later flowering stages. The inverse of dominance (1/d) ranged from 8.63 to 178.01, indicating a richer and more even distribution of pollinators during peak flowering (Table 4).

Pollination behaviour and floral traits: The significant plant-pollinator interaction was observed. The sesame flower's bell-shaped corolla and pinkish-white colour likely enhanced pollinator attraction through visual cues. Rich nectar and pollen resources further supported frequent visitation. While foraging, most insects contacted the pollen-laden anthers, resulting in sternotribic pollination (pollen transfer via the ventral body). This efficient pollination mechanism has also been reported in previous studies (Shankar and Mukhtar, 2022). Pasthe and Shylesha (2013) documented that honey bees formed 77.67% of sesame flower visitors. Rao et al. (2022) also observed Hymenoptera as the dominant order (62%) in sesame. Blal et al. (2013) and Gebremedhn and Tadesse (2014) reported peak foraging during mid-day due to high nectar availability which is in consistent with the present study. Selvakumari et al. (2022) found highest diversity index between 10:00–12:00 h, aligning with the current findings.

CONCLUSION

The study documented diverse assemblage of 30 insect pollinator species visiting sesame flowers with Hymenoptera, particularly *Apis cerana indica* and Megachilidae bees, being dominant. Pollinator abundance and diversity were positively correlated with floral density, showing two activity peaks in mid-morning and early afternoon. Diversity indices confirmed increased richness and dominance of key species during peak flowering. Sesame's floral traits, including bell-shaped corolla and abundant nectar, effectively attracted foragers, facilitating efficient sternotribic pollination. These findings highlight the ecological importance of native pollinators and emphasize the need for their conservation through pollinator-friendly practices, reduced pesticide use, and enhancement of local floral diversity.

AUTHOR'S CONTRIBUTION

G. Alekhya carried out the implementation, writing of original draft, review, editing and data curation. S.R. Koteswara Rao was responsible for conceptualization, investigation, project administration, and validation, T. Madhumathi provided supervision and validation and contributed to the manuscript through review and editing. D. Ramesh performed the formal analysis.

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New Record of Predator and Parasitoid on Carpenter Bee *Xylocopa fenestrata* F.

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Abstract: *Xylocopa fenestrata* F. is a significant buzz pollinator, playing a vital role in the pollination of various field crops and contributing to the stability and productivity of agroecosystems. In the present study, conducted at the Agricultural Research Station, Vijayarai, Andhra Pradesh, during 2023-2025, detailed observations were made on the nesting biology, nesting preferences, and foraging activity of *X. fenestrata*. The artificial nesting substrates made from bamboo nodes were deployed to monitor and document the nesting behavior of this species. The study also identified two natural enemies associated with *X. fenestrata* nests: a predator belonging to the genus *Horia* (Coleoptera: Meloidae), and a parasitoid, *Coelopencyrtus krishnamurtii* (Hymenoptera: Encyrtidae). Out of the 400 nests installed, 60 (15%) were infested with predator/parasitoid. These findings highlight previously ecological interactions and potential threats faced by this important pollinator and have significant implications for its conservation and management.

Keywords: *Xylocopa fenestrata*, Pre-pupae, Grub, *Horia* sp., *Coelopencyrtus krishnamurtii*

Pollination is the transfer of pollen from anther to stigma, enabling fertilization and seed or fruit development. It occurs via wind, water, birds, or insects. Insects such as bees form mutualistic relationships with plants, gaining nectar or pollen while transferring pollen between flowers. Some bees perform buzz pollination vibrating their flight muscles to release pollen from tubular or poricidal anthers using higher-frequency vibrations that enhance pollen discharge (Boucher et al., 2025). Floral traits such as shape, tube length, color, and nectar properties determine which pollinators can access rewards, acting as selective filters. Studies show that different pollinators exert distinct selective pressures, shaping floral morphology as in *Stellera chamaejasme* (Zhang et al., 2022) and *Rhododendron* species influenced by both pollinators and climate (Basnett et al., 2024). The floral syndromes describe trait sets linked to specific pollinators, they often exist along a continuum, especially when multiple pollinators or shifts in pollination modes occur (Li et al., 2022).

Although some carpenter bee species, such as *Xylocopa nasalis*, exhibit social behavior where one female builds and forages while others guard the nest, *Xylocopa fenestrata* is generally regarded as solitary. Foraging behavior in *X. fenestrata* is influenced by environmental factors like temperature, humidity, and light, and has been shown to increase significantly with the addition of brood hormones (Pankiw 1998). The genus *Xylocopa* includes large-bodied bees that excavate nests in hardwood. For over 200 years, the circumtropical genus *Horia* (Coleoptera: Meloidae) has been associated with *Xylocopa* spp. and is known to parasitize their nests (Guilding 1824, Cros 1924, 1938). This indicates an antagonistic relationship. The tribe Horiini, primarily tropical, includes three species across two genera in the Palaearctic: *Horia* Fabricius, with two species, and *Synhoria* Kolbe, with one. None have been previously recorded in Israel (Bologna and Pinto 2002, Bologna and Turko 2007, Ricciieri et al., 2023). However, *Horia fabriciana* is known from parts of the Afrotropical region and the southern Palaearctic, including Egypt, Saudi Arabia, the UAE, and Yemen (Löbl and Smetana 2008).

The large parasitoid wasp family Encyrtidae includes approximately 455 genera and 3,710 species. Most are primary parasitoids, particularly of Hemiptera, but some target larvae, eggs, or even ticks. They are globally distributed and play a significant role in biological control. However, their impact can be ecologically significant, as seen with the endangered butterfly *Papilio homerus*, whose egg mortality rate from parasitism reaches up to 77% (Segoli et al., 2010). Among Encyrtidae, *Coelopencyrtus* species are recognized by their relatively narrow female antennal segments, a thorax that is elevated, and a non-exserted ovipositor. In contrast, *Nesencyrtus* has broader antennal segments, an enlarged mouth opening to accommodate large mandibles, a depressed thorax, and a slightly exserted ovipositor. Males differ in antennal structure and head morphology (Burks 1958). Despite extensive research on *Xylocopa* pollination ecology, there are no documented natural enemies of *X. fenestrata* from India or elsewhere. The present study reports, for the first time, a predator (*Horia* sp.) and a parasitoid (*Coelopencyrtus krishnamurtii*) associated with its brood.

MATERIAL AND METHODS

The present study was conducted at the Agricultural Research Station, Vijayarai, Andhra Pradesh, India, during the period 2023–2025. The research site is located at latitude 16.81210° N and longitude 81.03270° E. To study the nesting biology and behavior of *X. fenestrata*, bamboo nodes with diameters ranging from 1.0 to 1.8 cm and lengths of 3 to 4 feet were used as artificial nesting substrates. These bamboo nests were installed within a pollinator shed established at three different locations within the research station premises to encourage nesting activity under semi-natural conditions. Regular observations were made to document the nesting preferences, nest construction, and other biological behaviors of *X. fenestrata*. Detailed records were maintained on nest establishment, structure, occupancy, and developmental stages. During the course of the study, two natural enemies a predator and a parasitoid were also observed interacting with the nests of *X. fenestrata*. The total number of nests installed and observed was 400, with 60 nests infested by predators/parasites (15%). Adults were preserved in 70% ethanol and sent to ZSI, Kolkata for expert identification.

RESULTS AND DISCUSSION

X. fenestrata preferred nest of diameter of 1.5 cm and 1.6 cm in 2023-24 and 2024-25 respectively. The females of *X. fenestrata* collected pollen and nectar from various crops such as Sunhemp (*Crotolaria juncea*), Diancha (*Sesbania bispinosa*), Redgram (*Cajanaus cajan*), Sesame (*Sesamum indicum*) and Niger (*Guizotia abyssinica*).

The female *X. fenestrata* excavated the bamboo node wood with its mandibles, creating circular discs of wood shavings and laid eggs in a mass provision of pollen and nectar placed between two such excavated wooden discs. The female mass provisioned pollen and nectar within the space. The egg, larval and pupal period was for 3, 16 and 26 days respectively. The bamboo nodes were cut longitudinally to observe various stages of grubs of *X. fenestrata*. (Fig. 1. A

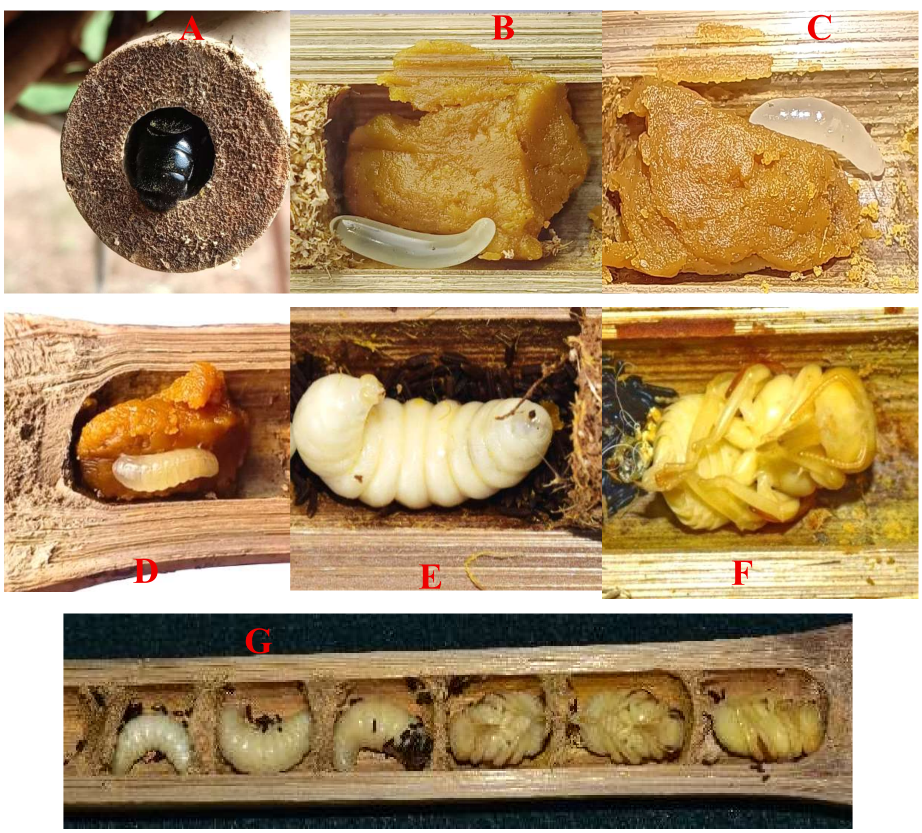

Fig 1. Developmental stages of *X. fenestrata* in bamboo node at, ARS, Vijayarai at 2023-24. (A to H) A: *Xylocopa fenestrata nesting* in 1.5 cm bamboo node. B: Egg laid in provisioned food (pollen+ nectar), C: Hatched egg, D: Grub, E: Pre-pupa F: Pupa, G: Different grub stages of *X. fenestrata*

– G) *X. fenestrata* preferred bamboo as nesting material. The nests were built in an angled position in the shadow which might be due to convenience and protection from rain. Despite a range of bamboo node diameters (1.0−2.70 cm) being provided, *X. fenestrata* showed a preference for nodes with diameters of 1.5, 1.6, and 1.7 cm for nest construction. The highest mean acceptance rate was observed in 1.5 cm nodes (41.75%), followed by 1.6cm (35.36%), with the lowest acceptance in 1.7cm nodes (22.87%)

During our observation, the grub of *Horia* sp. was found predating upon the grubs of *X. fenestrata*. The grub of *Horia* sp. closely resembled the *X. fenestrata* grub, likely facilitating concealment within brood cells. But upon close observation, the larvae of *Horia* sp. could be clearly identified by three pairs of prolegs on the thorax often called as triungulin larvae, which is the first instar larvae of the family Meloidae, whereas in the larval stages of *X. fenestrata,* the grubs were immobile and did not have either prolegs or abdominal legs with reduced head and poorly developed sense organs. It was observed that the campodeiform larvae of *Horia* sp. is characterized by well developed head, strong mandibles, clear segmentation of the body and was actively mobile and predating upon the grubs of *X. fenestrata*. The adult beetle laid its egg in the nectar and pollen provisioning in the second cell from the nodal septum. The grub of *Horia* sp. consumed both pollen-nectar mass provision and the grubs of *X. fenestrata*. It had strong mandibles by which it bored through the wooden circular discs/septum and predated upon the grub of *X. fenestrata*. The grub of the predator voraciously predated upon each grub within one day. The time of laying of egg by adult predator was not traceable. The grub changed its colour from white to pale yellow in colour in pupal stage. The adult was collected and sent to Zoological Survey of India, Kolkata for authentic identification. The adult was identified as *Horia* sp. (Fig. 2. A – G).

Another parasitoid belonging to the family Encyrtidae named *Coelopencyrtus krishnamurtii* (Mahdihassan) was recorded in the late grub stage of *X. fenestrata* in one of the trap nests having a diameter of 1.5 cm (Bamboo node) (Fig. 2). The grub which was located in the end of bamboo node near to nodal septum and was the only grub that is infested by *C. krishnamurtii*. The adult wasp of parasitoid laid thousands of eggs in the pre-pupal body of *X. fenestrata*. The larvae fed upon the entire body contents of *X. fenestrata* and the pre-pupa of *X. fenestrata* appeared sac-like and the entire larval body of *X. fenestrata* was occupied with pre-pupae of *C. krishnamurtii*. The infested grub was kept in a glass vial enclosed with cotton plug to record the emergence of adult wasp parasitoids (Fig. 2). The pre-pupae/ late stage grub of *X. fenestrata* did not emerge into adult. The pre-pupae of *X. fenestrata* became transparent white when the grub was entirely occupied with pre-pupae of *C. krishnamurtii*. All the

Fig. 2. A) Second grub from the left- Triungulin larva of *Horia sp*. in Bamboo node; B) Predatory activity of grub of *Horia sp*. on the grub of *X. fenestrata;* C) Late-stage grub of *Horia* sp; D) Prepupa of *Horia* sp.; E) Pupa of *Horia* sp.; F) Emerged adult of *Horia* sp.; G) Adult *Horia* sp

pre-pupae of *C. krishnamurtii* became clearly visible from the outside body of pre-pupae of *X. fenestrata* grub. The colour of pre-pupae of *X. fenestrata* was changed from pale white initially to transparent white and then to light brown in colour. Then all the adults of *C. krishnamurtii* completely emerged from the pre-pupae of *X. fenestrata* in the glass vial (Fig. 2). The adults were collected and sent to Zoological Survey of India, Kolkata for authentic identification of parasitoids. The parasitoid was identified as *C. krishnamurtii*.

Triungulin larva of *Horia sp* active, campodeiform (elongated and flattened), with well-developed legs, three claws, having predatory activity on the grub of *X. fenestrata* in Bamboo node. The name triungulin means three claws on each foot and is distinctive when compared to later larval instars. The later instars are apodous, creamy white in colour without having well developed head capsule. Later it transforms into pre pupa within the bamboo node. Exarate pupa having appendages clearly visible and not glued to the integument. Reddish adult with black markings on the elytra having long serrate antennae. Adult beetle abdominal segments exposed beyond the elytra (pygidium). (Fig. 2. A – G). *Coelopencyrtus krishnamurtii* is an endoparasite of *X. fenestrata* prepupal or pupal stage. Female lays an egg inside the prepupa of *X. fenestrata*. The parasitoid larva consumes the host internally. Pupation occurs within the host cell. Emergence occurs through a small circular hole bored through the cocoon or cell wall. (Fig. 2. H – J). Guilding (1824) reported that some species in the genus *Horia* live in the brood colonies of *Xylocopa* bees. *Horia maculata* was introduced to Hawaii to control *Xylocopa* but the beetles did not establish a stable population. (Clausen 1940).

Horia fabriciana was identified as a parasitoid of *Xylocopa pubescens* in Egypt (incorrectly listed under *Xylocopa aestuans* (Linnaeus 1758)) in the Palaearctic region (Blair 1924, Cros 1924, 1927, 1929, 1938, Bologna and Laurenzi 1994), as well as of an unidentified species of *Xylocopa* in the United Arab Emirates on the same mulberry tree trunk, inhabited by *X. pubescens*. These results support current findings.Bologna et al. (2013) observed the taxonomic characters of *Horia* sp. Male metafemora inflated, noticeably broader than mesofemora. Males with mandibles short, their length less than half that of head and with head widest at eyes Pronotum length usually greater than head length to apex of mandibles. Fourteen specimens were collected in Papua New Guinea (Port Moresby) that may be assigned to a new species closely related to *Horia blairi*. The first instar larvae of three African and Oriental species were described, which were phoretic and parasitoids of anthophorine bees (Apidae) (Bologna et al., 2013). The scutellum of *Coelopencyrtus krishnamurtii* is large bluish with some bronzy shine medially (Hayata et al., 2014). Segoli et al. (2010) reported that *C. krishnamurthii* had the cerci forwards on the metasoma (and the resulting distortion of the tergites), and a substantially expanded mesopleuron with

Fig. 2. H) Pre-pupae of *X. fenestrata* infested by *C. krishnamurtii* (first one from the top near nodal septum); I) The pre-pupae of *X. fenestrata* occupied with pre-pupae of *C. krishnamurtii;* J) Adult parasitoids emerged from larval case of *X. fenestrata;* K) Adult parasitoid *Coelopencyrtus krishnamurtii* (Mahdihassan)

anteriorly positioned mesocoxae make it relatively straightforward to distinguish wasps of this family from other Chalcidoidea.

CONCLUSION

The present study documents for the first time, both globally and from India, the occurrence of *Horia* sp. (Meloidae) and *Coelopencyrtus krishnamurtii* (Encyrtidae) as natural enemies of the carpenter bee *Xylocopa fenestrata*. This finding emphasizes the complexity of pollinator–natural enemy interactions and highlights the need for further studies on the biology, seasonal occurrence and impact of these species on bee populations.

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Impact of Inundative Releases of Parasitoids (*Bracon hebetor* and *Goniozus nephantidis*) against Coconut Black Headed Caterpillar under Severe Infestations

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Abstract: Caterpillar of coconut black headed caterpillar (*Opisina arenosella*) attacks palms of any age from nursery to grown up plants causing severe damage to the foliage and resulting in yield losses up to 50 percent. During 2023-24, a high infestation of black headed caterpillar was observed in the Dr.B.R. Ambedkar Konaseema, West Godavari and Eluru districts of Andhra Pradesh with the incidence ranging from 21.29 to 68.55% from January 2023 to June 2024, A total of 15 lakhs numbers of larval parasitoids *Bracon hebetor* and *Goniozus nephantidis* were released in pest affected coconut plantations. To study the impact of the parasitoid releases, 10 leaflets/ palm were randomly collected from three sample gardens in three villages (Allipalli, Appanacheruvu and Sannavilli) and the pest population recorded., Larval population of *O. arenosella* in the affected gardens decreased by 19.59 -30.64 percent after three months and up to 72.79-85.16 percent after six months of release of parasitoids and no leaf damage and pest population was recorded in the newly emerged leaves. In the random leaflet samples collected the percentage of parasitized larval population recovered was 2.18-3.52 per cent after three months and increased to 12.69-34.59 per cent after six months of release of parasitoids. After six months of release the impact of inundative release of bioagents in the suppression of the pest was visible as pest population could be brought down to very negligible level by the timely release of stage specific parasitoids.

Keywords: Coconut black headed caterpillar, Biological control, Inundative releases, Parasitoids, *Opisina arenosella, Goniozus nephantidis*, *Bracon hebetor*

Coconut (*Cocos nucifera* L.) is the most valuable palm which occupies a unique position among plantation crops worldwide. In India, coconut cultivation is primarily confined to the four southern states, accounting 90% of the area under coconut, among which Andhra Pradesh shares about an area of 1.07 lakh hectares producing 17.07 lakh nuts with a productivity of 15,899 nuts per hectare (CDB, 2023-24). In Andhra Pradesh, Dr. B. R. Ambedkar Konaseema, East Godavari, West Godavari, Eluru, Srikakulam and Visakhapatnam are important coconut growing coastal districts. One of the major factors that contribute to the loss of production and productivity in coconut is the damage due to pests. More than 900 species of vertebrate and invertebrate pests are reported to be associated with cultivated and wild coconut palm (Patel et al., 2023). Of these coconut black headed caterpillar, *Opisina arenosella* Walker is a serious pest of coconut palm causing significant yield loss in all the coconut growing tracts of India (Kumara et al., 2015). The first report of this pest is from Sri Lanka in the year 1900 and the earliest record of the pest on coconut palm in India was in 1909 from Bapatla, Andhra Pradesh (Sathiamma 1993, Subaharan and Ravindran 2009). During severe outbreaks, the pest multiplies rapidly and devastates the leaf lamina and also feeds on the green surfaces of the petioles, spathes and nuts. In case of nurseries or newly planted young palms may lead to the death of seedlings and button drop in the gardens resulting in reduced yields (Muthiah 2007).

The occurrence of periodic out breaks is common in coastal coconut plantations of east coast region. Andhra Pradesh has favorable climatic conditions and ample water resources, besides a long coastline of 974 km for aquaculture. Intensive and successive foliar applications of broad spectrum chemical insecticides for controlling larval stages leads to environmental pollution and disturbance in the natural balance in addition to insecticide resistance development. Pest incidence can be checked by the bio-control methods utilizing indigenous parasitoids. Bio-intensive pest management strategy has been developed for the management of this pest when it is in an epidemic form (Nair et al.,1997).Some indigenous and introduced species of parasites have been quite amenable to laboratory rearing and utilization in the field as biological control agents In the light of these facts, the Horticultural Research Station, Ambajipeta has intensified research and besides identifying the natural bioagents, has standardized methods for mass multiplying them under laboratory conditions and supply to the farmers to release in infested gardens. Present study was carried out in the black headed caterpillar affected gardens in Allipalli, Sannavilli and Appanacheruvu villages of Andhra Pradesh to evaluate the impact of the biocontrol based pest management and contain the spread of pest to neighboring coconut growing mandals.

MATERIAL AND METHODS

For recording pest and parasitoid population of coconut black headed caterpillar, *O. arenosella* 10 leaflets/ palm were collected from 10 sample palms in the villages Allipalli (17° 11′ 8.86″ N, 80° 59′ 51.07″ E), Appanacheruvu (16° 31' 2.8344" N, 81° 43' 31.2312" E) and Sannavilli (16.5720° N, 82.0993° E) before release at three and six months interval and larval population is expressed as mean larval and pupal population /10 leaflets along with parasitized larval population. Sample leaflets were collected from lower / middle whorl of leaves through destructive sampling (41-60% leaflets of 20% leaves from the lower or middle whorl). In each of the sample palms, population of larvae and pupae of the pest and the associated parasitoids present were recorded from the leaflets. Before releasing the parasitoids in the field were fed with honey and newly emerged parasitoids are released in the field after three days of emergence. The newly emerged parasitoids are exposed to the host odours for 72 hours (smell of the volatiles of the frass from galleries obtained from infested leaflets) as this improves the host searching efficiency of *Goniozus nephantidis* and *Bracon hebetor.* The releases of parasitoids were carried out at fortnightly intervals depending on the stage of the pest population to affect an inundative release in each location

From April 2023 to June 2024, about 16, 69,642 numbers of these parasitoids were produced in bio-control lab at Dr. YSRHU-HRS, Ambajipeta for distribution and released in pest affected gardens in the Allipalli village. Around 115 ha. of Allipalli village was covered and parasitoids were released. In case of Appanachervu village 500 palms and for Sannavilli 500 palms were selected area. Removal and burning of heavily infested 2-3 outer fronds and inundative release of stage specific parasitoids (*G. nephantidis* and *B. hebetor*) were done for the management of black headed caterpillar in three experimental locations.

Usually the parasitoids *G. nephantidis* are to be released at the rate of 20 parasitoid/palm and *B. hebetor* at the rate of 30 parasitoid /palm in at least ten percent of the infested palms in each village at fortnightly intervals in case of low (few damaged leaflets here and there) to medium (2–3 damaged fronds with clear drying) intensity damage. But as there is high intensity (all the lower whorls of leaves or entire crown damaged) inundative release of parasitoids was taken up in the affected gardens of Allipalli, Appanacheruvu and Sannavilli villages.

Statistical analysis: Data was analyzed by using Paired t test in the OPSTAT Software.

RESULTS AND DISCUSSION

The severe incidence of coconut black headed caterpillar was observed in coconut plantations of Alliaplli in area of115 ha. in November 2023 and 500 palms of Appanacheruvu in December 2023 and 1000 palms in Sannavilli in January 2024. The cropping system and date of initiation of release of parasitoid in the infested villages is given in Table 1.

The mean prerelease larval population in the Allipalli, Appanachervu and Sannavilli was 91.38, 79.05 and 76.75 larvae/ 10 leaflets in November 2023, December 2023 and January 2024 respectively. Removal and burning of heavily infested 2-3 outer fronds was done before the inundative release of parasitoids at 15 days intervals. After three months of the inundative release of the parasitoids in the three villages has resulted in the decrease of larval population of black headed caterpillar to 65.64, 63.56 and 53.23 larvae for 10 leaflets at Allipalli, Appanacheruvu and Sannavilli villages respectively. After six months of release, low larval population/ 10 leaflets was further reduced at Allipalli (13.56), Appanacheruvu (19.31) and Sannavilli (20.88) villages (Table 2). The total number of fresh pupae of *O. arenosella* and paralised larvae with cocoons of parasitoids present in the collected samples were also recorded. The number of CBHC pupae present before the release of parasitoids was high in all the experimental villages (21.81,19.76 and 22.52).The decrease in the number of pupae recorded after three months of release of parasitoids differ being lowest in Allipalli (14.79) followed by

Table 1. Number of parasitoids released in the affected villages for the management of coconut black headed caterpillar

Name of the village & District	Date of initiation	Area infested & cropping system	Name of the parasitoid	No. of parasitoids released (lakhs)
Allipalli, Eluru	03.11.2023	115 ha. (Sole coconut gardens and intercropped with cocoa)	*B. hebetor* *G. nephantidis*	8.08 5.41
Appanacheruvu, West Godavari	20.12.2023	500 palms (Fish pond bunds & Sole gardens)	*B. hebetor* *G. nephantidis*	0.6 0.5
Sannavilli, Dr.B.R.Ambedkar Konaseema	08.01.2024	1000 palms (Fish pond bunds & Sole gardens)	*B. hebetor* *G. nephantidis*	0.6 0.5

Table 2. Larval and pupal population of coconut black headed caterpillar, *O. arenosella* before and after release of parasitoids (Mean + SE)

Village	Larval population per 10 leaflets			T value	T value	Pupal population per 10 leaflets			T value	T value
	Pre release	Post-release		Pre- 3 months	Pre- 6 months	Pre release	Post- release		Pre- 3 months	Pre- 6 months
		After three months	After six months				After three months	After six months		
Allipalli	91.38 + 6.19	65.64 + 3.74	13.56 + 2.93	3.68 (0.008)	11.12 (<0.05)	21.81+ 1.38	14.79 + 1.77	3.05 + 0.50	2.47 (0.04)	13.41 (<0.05)
Appanacheruvu	79.05 + 4.67	63.56 + 1.86	19.31 + 3.79	3.46 (0.01)	13.70 (<0.05)	19.76 + 2.16	14.18 + 2.41	2.87 + 0.67	1.82 (NS)	8.08 (<0.05)
Sannavilli	76.75+ 6.34	53.23 + 8.30	20.88 + 2.53	1.67 (NS)	7.65 (<0.05)	22.52 + 2.39	15.56 + 2.25	4.36 + 0.76	2.10 (NS)	5.95 (<0.05)

Table 3. Parasitized larval population of *O. arenosella* before and after release of parasitoids (Mean + SE)

Village	Parasitized larval per 10 leaflets			T value	T value
	Pre release	Post release		Pre- 3 months	Pre- 6 months
		After three months	After six months		
Allipalli	0.74+0.28	1.85+ 0.38	4.69+ 0.53	2.89 (0.02)	7.16 (<0.05)
Appanacheruvu	0.94+ 0.22	2.24+0.28	3.13+0.65	3.18 (0.015)	2.88 (0.023)
Sannavilli	0.83 +0.23	1.69+ 0.39	2.65+ 0.55	2.00 (NS)	2.97 (0.021)

Appanacheruvu (14.18) and Sannavilli village (15.56). But after six months of release of parasitoids, the decrease in the number of pupae significantly differed in all the experimental gardens being 3.05, 2.87 and 4.36 in Allipalli, Appanacheruvu and Sannavilli. The decrease in the number of pupae represents the reduced pest population of the target pest in the parasitoid released gardens (Table 2).

The naturally parasitized CBHC larvae recorded very low before the release of parasitoids and significantly increased after three months of release of parasitoids except in Sannavilli village. After six months of parasitoids release, there is an increase in the parasitized larval population from 0.74 to 4.69 per 10 leaflets in collected samples of Allipalli which demonstrates the impact of biological control. This indicate that parasitized larval population increased significantly after six months when compared to prerelease showing the impact of inundative release of parasitoids in the CDB-LoDP programme. In case of Appanachervu and Sannavilli parasitized larval population varied 0.94 to 3.13 and 0.83 to 2.65 per 10 leaflets respectively after six months of parasitoids release (Table 3).

In the LODP villages the larval and pupal population of *O.arenosella* in the affected gardens decreased after six months of release of parasitoids and no leaf damage and pest population was recorded in the newly emerged leaves. In the random leaflet samples collected the percentage of parasitized larval population increased after six months of release of parasitoids. The adoption on large area can effectively manage coconut leaf black headed caterpillar and pest population could be brought down to very negligible level by the timely release of stage specific parasitoids. Mohan and Nair (2002) also reported 52.6 and 94.7% reduction in pest population after one and two years, respectively of parasitoid release in heavily infested homestead coconut in Neendakara (Kerala). Chalapathi Rao et al. (2018) also observed similar findings in the area of 675 ha in Allavaram. For large area field management of the coconut black headed caterpillar in an epidemic out-break, inundative releases of parasitoids in the initial periods in coconut plantations of at fortnight intervals and further monitoring and augmentative releases during probable periods of pest build up is required. The self-perpetuating parasitoids prevented the spread of the outbreak to surrounding plantations and regulated

CONCLUSION

The present studies conclude that after six months of inundative releases of parasitoids, *Bracon hebetor* and *Goniozus nephantidis* resulted in the suppression of the coconut black headed caterpillar. The success of biological control programmes needs to be promoted so as to educate the farmers, and replicate them in all the places where it is needed.

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Population Dynamics of Pod Feeders of Green Gram under Red and Lateritic Zone of West Bengal

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Abstract: Pod feeders such as pod bug (*Clavigralla gibbosa* Spinola), flea beetle (*Madurasia obscurella* Jacoby), pod borer (*Helicoverpa armigera* Hubner), and blue butterfly (*Lampides boeticus* Fabricius) cause substantial damage to green gram crops in eastern India. This study quantified their population dynamics across two consecutive pre-kharif seasons (2023 and 2024) within the red and lateritic soils of West Bengal, alongside evaluation of key abiotic factors. Peak populations were observed between 15 and 16 weeks , with pod bug 3.37 and 3.40 individuals per plant, flea beetle 1.84 and 1.90, pod borer larvae 1.28 and 1.20, and blue butterfly adults 3.43 and 3.20 in 2023 and 2024, respectively. Pearson's correlation analysis revealed a strong positive relationship between minimum temperature and pest populations (r = 0.53 to 0.74), whereas rainfall and relative humidity had weak or non-significant effects. Linear regression analyses underscored minimum temperature as the dominant explanatory factor, especially for pod borer populations ($R^2 = 0.72$ in 2023). These findings provide vital insights for climate-informed integrated pest management strategies in West Bengal's green gram agroecosystem.

Keywords: Abiotic factors, Green gram, Pod feeders, Population dynamics, West Bengal

Green gram (*Vigna radiata* (L.) Wilczek) is a vital pulse crop valued for its nutritional quality and nitrogen-fixing capabilities that contribute to sustainable agriculture. Despite its economic importance, green gram productivity is frequently compromised by an array of insect pests, among which pod feeders pose a significant threat by damaging pods, leading to yield loss and reduced seed quality (Mahesh et al., 2021). The pod feeders of primary concern include pod bug (*Clavigralla gibbosa* Spinola), flea beetle (*Madurasia obscurella* Jacoby), pod borer (*Helicoverpa armigera* Hubner), and blue butterfly (*Lampides boeticus* Fabricius), all of which infest the crop during critical reproductive stages (Dutta and Ghosh 2019, Mehendra et al., 2019). Abiotic factors such as temperature, rainfall, and relative humidity profoundly influence the population dynamics of these pests. Temperature, especially minimum temperature, notably affects development rates and survival while rainfall and humidity can either suppress or facilitate pest outbreaks depending on intensity and timing (Sharma et al., 2019, Singh et al., 2019). Although several studies have focused on pod feeders in varying agroclimatic conditions, specific data for West Bengal's red and lateritic soils remain limited (Meena et al., 2021, Kundu et al., 2021). These soils possess distinctive properties affecting microclimate and pest ecology, warranting localized studies to enable precise pest forecasting and management planning (Umbarkar et al., 2010, Chaudhuri et al., 2020). The aim was to understand environmentally driven pest dynamics and support development of efficient and sustainable integrated pest management interventions appropriate for the regional pulse production system.

MATERIAL AND METHODS

Field trials were conducted at the Agricultural Farm of Seacom Skills University, Kendradangal, Birbhum district, West Bengal (latitude 23.70°N, longitude 87.64°E). The experimental site is characterized by sandy loam, acidic red lateritic soils and experiences a dry sub-humid subtropical climate with an annual average rainfall of approximately 1000 mm predominantly received during monsoon months. Green gram (cv. *Samrat*) was sown during the last week of February in 2023 and 2024 in a randomized complete block design with three replicates with spacing 30cm X 10cm in a plot size of 4m X 3m. Standard cultivation practices, including recommended fertilization and weed management, were implemented (Samant 2014). Weekly pest sampling occurred from 9th to 19th standard meteorological week (SMW) during both years. Five randomly selected tagged plants per plot within each replication were surveyed. Pod feeders, pod bug (*Clavigralla gibbosa* Spinola), flea beetle (*Madurasia obscurella* Jacoby), pod borer (*Helicoverpa armigera* Hubner) larvae, and blue butterfly (*Lampides boeticus* Fabricius) adults, were counted manually on three pods per plant across canopy layers during early morning hours. Daily meteorological parameters including minimum and maximum temperatures (°C), rainfall (mm), and relative humidity (RH) (%) were recorded onsite from a campus weather station to capture accurate microclimatic conditions concurrent with pest sampling.

Statistical analysis: Mean weekly pest populations were computed by averaging counts across replications. Pearson's correlation coefficients quantified relationships between pest populations and individual weather parameters for both years separately. Linear regression analyses were performed to model pod feeder population responses to minimum temperature. Statistical significance was determined at $p < 0.05$ with SPSS version 26.

RESULTS AND DISCUSSION

Climatic trends showed increasing minimum temperatures from early March (SMW 9) with maxima of 24.5 °C around mid-April (SMW 16), followed by gradual decrease toward May (SMW 19) (Table 1). Rainfall was generally low in early weeks and increased modestly following pest population peaks. Population trends for all major pod feeders during both 2023 and 2024 are indicating clear seasonal synchrony of pest peaks across years, typically between SMW 15 and 16 (Fig. 1).

Pod Borer: Pod borer populations began appeared in early reproductive weeks, rapidly rising to maxima of 1.28 larvae per plant in 2023 and 1.20 larvae per plant in 2024, before subsiding as crop maturity approached (Fig. 1). Correlation analysis revealed a strong positive relationship between minimum temperature and pod borer density (r = 0.71 in 2023, r = 0.70 in 2024), with maximum temperature also showing moderate, but weaker positive correlation (Table 2). Rainfall showed a marginal effect, and relative humidity fluctuations were not significantly associated with population changes. The resultant multiple regression equations indicate that minimum temperature exerts the strongest positive influence on larval abundance, in line with findings from Mahesh, Kumar, and Reddy (2021), and similar to reports in related cropping systems (Bera et al., 2023). The high R^2 demonstrates robust explanatory power for temperature variables, suggesting thermal cues are key for forecasting pod borer outbreaks in this zone (Table 3).

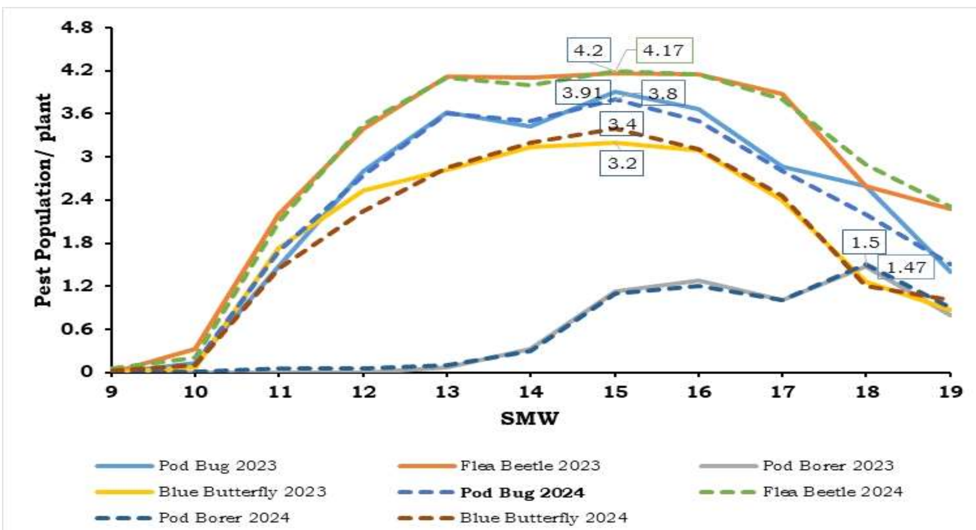

Fig. 1. Population dynamics of pod feeders of green gram in 2023 and 2024

Table 1. Weekly meteorological data for pre-*kharif* (2023 and 2024)

SMW	Min temp (°C) 2023	Max temp (°C) 2023	Rainfall (mm) 2023	RH (%) 2023	Min temp (°C) 2024	Max temp (°C) 2024	Rainfall (mm) 2024	RH (%) 2024
9	16.5	29.8	3.4	65.2	16.2	30.0	4.1	64.5
10	17.2	30.1	2.7	63.7	17.5	30.3	5.0	63.2
11	18.8	31.2	1.5	59.8	18.4	31.5	0.9	60.6
12	20.1	32.0	0.0	56.1	19.3	32.2	0.0	56.0
13	21.5	33.0	0.0	49.8	21.0	33.5	0.0	48.0
14	22.5	34.0	0.0	38.5	22.3	34.2	0.0	36.5
15	24.0	35.0	0.0	35.0	23.5	35.5	0.0	34.5
16	24.5	35.8	5.0	40.5	24.0	36.1	3.4	40.2
17	23.5	34.2	7.0	45.0	23.8	35.7	6.0	46.0
18	22.0	33.8	12.0	51.2	21.8	33.5	9.0	52.0
19	20.5	32.5	10.5	55.5	20.7	32.0	11.5	56.0

Table 2. Pearson correlation coefficients (r) between pod feeder populations and meteorological parameters (2023 and 2024)

Pest	Year	Min temp (°C)	Max temp (°C)	Rainfall (mm)	RH (%)
Pod bug	2023	0.65*	0.51**	0.15 **	0.17**
	2024	0.63*	0.49 **	0.12 **	0.15**
Flea beetle	2023	0.59*	0.41 **	0.12 **	0.13 **
	2024	0.58*	0.39 **	0.14 **	0.12**
Pod borer	2023	0.74*	0.61*	0.18 **	0.20**
	2024	0.72*	0.59*	0.19 **	0.20**
Blue butterfly	2023	0.62*	0.48 **	0.11 **	0.16**
	2024	0.63*	0.46 **	0.13 **	0.15**

*Significant at $p < 0.05$; ** = non-significant

Table 3. Multiple linear regression equations for pod feeders of green gram (2023 and 2024)

Insect & Year	Regression equation	R^2
Pod Borer (*H. armigera* Hubner), 2023	$Y = -3.40 + 0.07X_1 + 0.11X_2 + 0.01X_3 + 0.02X_4$	0.72
Pod Borer (*H. armigera* Hubner), 2024	$Y = -3.10 + 0.06X_1 + 0.10X_2 + 0.01X_3 + 0.02X_4$	0.71
Pod Bug (*C. gibbosa* Spinola), 2023	$Y = -2.10 + 0.06X_1 + 0.13X_2 + 0.01X_3 + 0.01X_4$	0.43
Pod Bug (*C. gibbosa* Spinola), 2024	$Y = -1.90 + 0.06X_1 + 0.12X_2 + 0.01X_3 + 0.01X_4$	0.42
Flea Beetle (*M. obscurella* Jacoby), 2023	$Y = -0.99 + 0.03X_1 + 0.08X_2 + 0.01X_3 + 0.01X_4$	0.34
Flea Beetle (*M. obscurella* Jacoby), 2024	$Y = -0.93 + 0.03X_1 + 0.07X_2 + 0.01X_3 + 0.01X_4$	0.33
Blue Butterfly (*L. boeticus* Fabricius), 2023	$Y = -1.50 + 0.04X_1 + 0.11X_2 + 0.01X_3 + 0.01X_4$	0.37
Blue Butterfly (*L. boeticus* Fabricius), 2024	$Y = -1.36 + 0.04X_1 + 0.09X_2 + 0.01X_3 + 0.01X_4$	0.36

Y: pest population per plant; X□: max temp (°C); X□: min temp (°C); X□: rainfall (mm); X□: relative humidity (%)

Pod Bug: Pod bug populations peaked at 3.37 per plant in 2023 and 3.40 in 2024, closely following the trends of temperature rise (Fig. 1). Correlation coefficients with minimum temperature were significant in both years while those for maximum temperature, rainfall, and relative humidity were positive but far less significant (Table 2). The moderate R^2 value suggests temperature is the primary, but not exclusive, driver, possibly acting in concert with plant phenology (Sarkar et al., 2019) (Table 3). Mahesh, Kumar, and Reddy (2021), observed that rise in minimum temperature enhances adult and nymph development, leading to high pest loads during pod set and filling stages.

Flea beetle: Flea beetle infestations increased steadily from SMW 10, peaking with 1.84 insects per plant in 2023 and 1.90 in 2024 (Fig. 1). There was significant positive correlation with minimum temperature with negligible impacts from rainfall and humidity (Table 2).

Blue butterfly: Blue butterfly adults appeared late vegetative through flowering stage, peaking at 3.43 (2023) and 3.20 (2024) adults per plant (Fig. 1). Correlation with minimum temperature remained statistically significant whereas other abiotic factors showed no clear trends (Table 2). The study demonstrate that rises in minimum temperature provide the main ecological cue for population expansion of all major pod feeders in green gram, aligning with Mahesh et al. (2021) and Bera et al. (2023). Rainfall and relative humidity, within the experienced typical ranges, present limited limiting force. This confirms the critical importance of climate-driven forecasting for timely, sustainable management against these pests in Bengal's pulse systems.

This detailed two-year investigation demonstrates that pod feeders viz. pod bug, flea beetle, pod borer, and blue butterfly exhibit repeatable seasonal population dynamics in green gram under the red and lateritic zone of West Bengal. Their abundance peaks mid-season in close synchrony with rising minimum temperatures, which strongly predict population surges. Rainfall and humidity provide minor influences. The derived regression models enable timely prediction and management. Integrating these climate-based insights into regional pest management strategies can substantially reduce yield losses, minimize pesticide overuse, and promote sustainable green gram farming in eastern India.

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Implication Pesticide Residues on Abundance and Diversity of Anurans in Transplanted Rice Crop

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***Abstract*:** During present study, six anuran species which belonged to order anura and families bufonidae and dicroglossidae from transplanted rice crop fields during 2021 and 2022. The species included *Duttaphrynus melanostictus, D. stomaticus, Euphlyctis cyanophlyctis, E. adolfi, Hoplobatrachus tigerinus* and *Fejervarya limnocharis* among which *E. adolfi* had patchy occurrence, whereas *E. cyanophlyctis* was most abundant species Analysis of monthly differences of anuran abundance at all study sites showed July and August as high peak activity periods in all rice fields, about 13% increase in anuran abundance was during 2022 as compared to previous year. However, 36% increase in anuran population was recorded in unsprayed as compared to sprayed transplanted rice crop fields. Overall increase in body weights of male and female anuran species was 14.21 and 11.17%, respectively in unsprayed as compared to sprayed fields. Positive correlation was observed between amphibian population with atmospheric temperature, relative humidity and rainfall. Pesticide residues were below the limit of quantification (0.01 mg kg^{-1}) in soil, excretory contents and water samples without any amphibian morphological abnormality. The study suggests rice agroecosystems, in partnership with neighboring natural aquatic and terrestrial habitats without use of pesticides can serve as a sanctuary for anuran biodiversity.

***Keywords*:** Abundance, Anuran, Diversity, Frog, Pesticide residue, Rice crop

Expansion and intensification of agricultural land use are the most significant anthropogenic changes in the last century (Pascual and Perrings 2007). All long-term land use changes resulted in the conversion of natural ecosystems to seminatural or artificial systems which have negatively affected biodiversity composition and ecological processes (Kanianska 2016). Land use changes often likely resulted in decreased species diversity as changes in forest cover, vegetation type and composition, soil characteristics and topography will provide different microhabitats and resources for wildlife than existed prior to destruction. In Punjab, paddy accounts for 87% of total kharif crop area which during 2021-22 occupied 31.45 lakh hectares with total paddy production of 203.71 lakh tonnes. In addition to providing a necessary food source, irrigated rice cropping systems also serves as artificial wetland for a variety of species including amphibians (Natuhara 2013). Rice agro-ecosystem creates a landscape mosaic of hydroperiods, which improves the richness of regional species. The usage of rice fields as a substitute for natural wetlands may be contingent on management practises such as the use of agrochemicals or organic agriculture, irrigation water source and offseason crop area use (Donald 2004). Amphibian species forage and breed in rice fields and adjacent irrigation ditches (Frost et al., 2008). In terms of diversity, amphibians play a key role in many natural ecosystems and greatly contributes to the global biomass. Among vertebrates, amphibians (41%) is the most threatened group with extinction as compared to mammals (27%) and birds (13%) (IUCN 2021) which breathe through lungs (in adult stage) and gills (in tadpole stage) thus, hold a key position in the ecosystem as 'ecological indicators' (Simon et al., 2011). In rice agro-ecosystem, amphibians play important role in pest control and in enhancing soil fertility and productivity. Many factors are responsible for their decline, such as climate change, habitat destruction, pollution, deforestation, infectious diseases, heavy metals, pesticide contamination and UV radiation exposure (Blaustein et al., 2003). At global level, habitat modification is a key factor which contributes to amphibian declines with an estimated 63% of all amphibian species affected and approximately 87% of the threatened amphibian species affected (Chanson et al., 2008). Overuse of pesticides, field water contamination with heavy metals agricultural fields especially rice fields are not suitable habitat for amphibian species (Bruhl et al., 2013),as these possess the potential to interfere with physiology, growth, behaviour and regulation of reproductive, developmental processes and alterations in the neurological and immune system of the organism (Andreia et al., 2019). The effect has been seen on clutch size, larval survival rate hence, directly affecting the population trajectories of amphibians. Loss of habitat and increased pesticide inputs are two significant factors that have a synergistic effect in the contribution of reduction in amphibian population (Stuart et al., 2004, Kiesecker 2010).

In India, due to the patchy and fragmented amphibian species distribution very little information is available at the population, abundance and diversity levels. They have neither been adequately recorded nor monitored over time in relation to extinction risks, population vulnerability and human-induced changes (Aravind et al., 2004). During the last decade alone, there has been an increase in the discovery of amphibians across India which has led to the reports of higher amphibian species diversity and description of as many as 100 amphibian species (Hebbar et al., 2019). The present study was conducted for two seasons to analyse the abundance, diversity and effect of any pesticide residue on amphibian species in rice crop fields.

MATERIAL AND METHODS

Study area: The present study was carried out in rice crop fields of six selected villages of district Ludhiana during 2021 and 2022. Ludhiana is the largest district located in the central region of Punjab (India), having tropical steppe, hot and semi-arid climate with very hot summers and chilly winters. The south-west monsoon, which arrives during final week of June and departs towards the end of September, accounts for around 78% of annual rainfall. Rice crop fields having two different management practices were selected: (1) with use of pesticides to control insect pests and diseases and use of chemical fertilizers to fulfil nutritional requirements as per recommended doses (2) unsprayed rice crop fields, where application of any pesticide was prohibited and nutritional requirements were fulfilled by the use of farm yard manure.

Sprayed rice crop fields include application of herbicides such as pretilachlor (500 grams per acre acre) before and after transplanting rice crop for the control of grasses and weeds and application of insecticides like cartap hydrochloride , emamectin benzoate and carbendazium for the control of insect-pests whereas fungicides like tebucoazole and veldamycin were sprayed to control diseases. In unsprayed rice crop fields, no pesticide and chemical fertilizers were applied on rice crop from the last 10 years. The climatic factors like atmospheric temperature (^{0}C), rainfall (mm) and relative humidity (%) and the edaphic factors like soil pH, electrical conductivity and organic carbon content were also recorded and correlated with the amphibian population.

Data collection: Visual encounter survey method (VESM) was applied for estimating the anuran population. For estimation of individuals from rice crop fields, each plot consists of 0.4 ha area with three replications. The count of individuals from each plot was recorded from four corners and centre as five belt transects with size of each belt transect as 50×4 m. All observations were recorded at fortnight intervals (pooled at month level) from May to October months during 2021 and 2022, mostly during early morning 06:00 am to 08:00 am. Capturing of anurans from water was done using scoop net and each specimen was checked for any morphological abnormalities and was released back in their natural habitat. Anurans were identified by using respective identification keys Daniel and Seakar (1989), Daniel (2005) and from ZSI (Zoological Survey of India). Different indices like Simpson's index, Shannon-Weiner index, species evenness and species richness were calculated. Each anuran was measured for its body weight and morphometric measurements (mm) using a digital vernier calliper (Themisto® TH-M61). Spearman's correlation was employed to analyse the anuran community (richness and abundance) versus ambient temperature, rainfall and relative humidity.

Sampling of soil and excretory contents: From the selected sprayed transplanted rice crop fields, the soil samples were collected after spray of different pesticides on rice crop at 7-day interval. Uniform slices of soil samples were taken out separately and packed in cloth bags and related information like date, location, time, crop, depth, sample number was labelled with the help of marker. Urine and faecal matter samples were taken using Krogh (1973) and Bennett (1999) methods, respectively from anurans of sprayed and unsprayed transplanted rice crop fields at monthly intervals. Urine and faecal samples were collected in plastic vials and plastic packets, respectively and deep freeze at -4°C.

Extraction and clean-up procedure: The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method for estimating pesticide residues was used to prepare the soil and faecal samples. Liquid-liquid phase extraction (LLE) method was used to prepare water and urine samples.

Estimation by GLC: Different soil and excretory content samples were estimated for any pesticide residues using Gas Liquid Chromatography (Shimadzu model GC-2010) equipped with ECD/FTD supplied by M/S Shimadzu (Japan) in Toxicology laboratory, Department of Entomology, Punjab Agricultural University, Ludhiana.

Estimation by HPLC: Soil samples were estimated for pesticide residues using High Performance Liquid Chromatograph (HPLC): Shimadzu (Model DGU-2045) equipped with reversed-phase C18 column and PDA detector supplied by M/SShimadzu (Japan) in the Toxicology laboratory, Department of Entomology, Punjab Agricultural University, Ludhiana. The HPLC analysis was carried out at column temperature 25°C under isocratic condition acetonitrile/water (80/20, v/v) with pump flow at 0.4 mL min^{-1}.

Recovery studies: Recovery experiments were implemented to evaluate the competence of the analytical method used. Samples of soil and excretory contents were spiked with pesticides at 0.05, 0.0005 and 0.01 mg kg^{-1} levels, respectively. The reagent blank was also processed in the same way so as to find out the interference, if any due to reagents. The following formula was used for quantification of pesticide residues:

$$\text{Residue(ppm)} = \frac{\text{ng of standard injected}}{\text{Area of standard}} \times \frac{\text{Area of sample}}{\mu\text{L of sample injected}} \times \frac{\text{Final Volume of sample (mL)}}{\text{Weight of sample (g)}}$$

RESULTS AND DISCUSSION

Diversity and abundance of anurans in rice crop fields: Total of 3945 individuals of six anuran species were recorded from the transplanted rice crop fields (sprayed and unsprayed) which belonged to order anura; and families bufonidae and dicroglossidae during 2021 and 2022 (Table 1). Recorded species included *Duttaphrynus melanostictus* (Schneider 1799)*, D. stomaticus* (Lütken 1864)*, Euphlyctis cyanophlyctis* (Schneider 1799)*, E. adolfi* (Gunther 1860)*, Hoplobatrachus tigerinus* (Daudin 1802) and *Fejervarya limnocharis* (Gravenhorst 1829) among which *E. adolfi* had patchy occurrence and were present at Sites A, D and F only. *E. cyanophlyctis* was the most abundant species in all the selected sites as it remains active during whole year, whereas other species were active during summer (May, June) or monsoon (July, August) season. The incidence of all the six anurans took place during May to October. Analysis of monthly differences of anuran abundance at all study sites showed July and August as high peak activity periods (Fig. 1) as these months received maximum precipitation due to which there is enough water in crop fields and other water bodies which along with suitable temperature leads to congenial environmental conditions for the survival and reproduction of anurans. Site D was most diverse whereas Site B least diverse as indicated by Shannon-Wiener index (H') and Simpson diversity index (D) (Table 2). Higher species evenness recorded at Site C indicated that anuran species were more evenly distributed as compared to other sites.

In sprayed rice crop fields, mean anuran populations in 2022 increased by 3.85% at Site A, 13.92% at Site B, and 5.34% at Site C compared to 2021. *E. cyanophlyctis* was the most abundant species across all sites. The least abundant species varied by site: *E. adolfi* at A, *D. melanostictus* at B, and *F. limnocharis* at C, all showing statistically significant

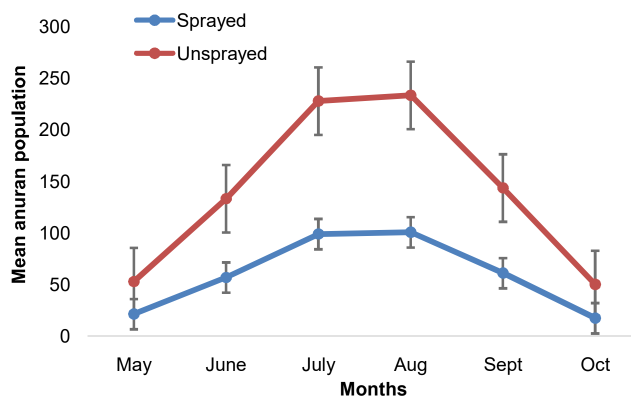

Fig. 1. Relative incidence of anurans in sprayed and unsprayed transplanted (2021 and 2022) rice crop fields

Table 1. Inventory of anuran species recorded in transplanted rice crop fields

Common name	Scientific name	Family	Order	IUCN status
Common Asian toad	*Duttaphrynus melanostictus* (Schneider 1799)	Bufonidae	Anura	Least concern
Indian marble toad	*Duttaphrynus stomaticus* (Lütken 1864)	Bufonidae	Anura	Least concern
Indian skittering frog	*Euphlyctis cyanophlyctis* (Schneider 1799)	Dicroglossidae	Anura	Least concern
Adolf's speckled frog	*Euphlyctis adolfi* (Gunther 1860)	Dicroglossidae	Anura	Least concern
Rice field frog	*Fejervarya limnocharis* (Gravenhorst 1829)	Dicroglossidae	Anura	Least concern
Indian bull frog	*Hoplobatrachus tigerinus* (Daudin 1802)	Dicroglossidae	Anura	Least concern

Table 2. Alpha diversity indices for anurans in different selected sites in rice crop fields

Biodiversity indices	Sprayed rice crop fields			Unsprayed rice crop fields		
	Site A	Site B	Site C	Site D	Site E	Site F
Shannon-Wiener index	1.738	1.598	1.606	1.771	1.601	1.762
Simpson diversity index	0.182	0.205	0.202	0.173	0.203	0.176
Index of similarity	0.818	0.795	0.798	0.827	0.797	0.824
Species evenness	0.970	0.993	0.998	0.988	0.995	0.983
Species richness	6.0	5.0	5.0	6.0	5.0	6.0

differences. In unsprayed rice crop fields, mean anuran populations in 2022 rose by 13.51% at Site D, 8.07% at Site E, and 10.44% at Site F compared to 2021. *E. cyanophlyctis* remained the most abundant species across all sites. The least abundant species were *E. adolfi* at Sites D and F, and *D. melanostictus* at Site E, all statistically distinct from others. Among sprayed rice fields, Site A (Noorpur Bet) was the most diverse, while Site B (Ladian Kalan) showed the highest population increase in 2022 due to nearby water bodies supporting breeding and food availability. In unsprayed fields, Site F (Burj Lambran) was most diverse, but Site D (PAU) had the highest population increase. Overall, anuran abundance was greater in unsprayed fields, with a 36.03% higher mean increase due to more breeding sites and insect food availability compared to pesticide-treated fields.

No significant difference in anuran species diversity was observed between sprayed and unsprayed rice fields, suggesting that landscape structure plays a more crucial role than farming practices. These rice crop fields are subjected to a variety of agricultural management practises, including the degree of vegetation conservation at field edges, crop rotation, agrochemicals and their mode of application, the management level, the availability of uncultivated regions in the field and the surrounding landscape environment (Attademo et al., 2005) which directly or indirectly affect anuran guilds. Overall, pesticide-free agriculture supports greater biodiversity by reducing environmental stressors, though landscape elements may exert a stronger influence than the type of crop management applied. Vegetation around the rice fields can have an impact on amphibian population sizes in natural environments (Weibull et al., 2000).

Environmental cues like temperature, moisture or the timing and amount of precipitation are the main components that affect anuran phenology directly. Inadequate rainfall, extreme drought and shortened hydro-periods have all been linked to a decrease in anuran calling activity, catastrophic reproductive failure in many pond-breeding amphibians, metamorphosis at smaller body sizes, the potential local elimination of paedomorphosis and local extinctions. Pounds et al. (2006) provided an illustration of the intricate linkages between anuran population losses and changes in the ecosystem at large scale.

Body weights and morphometric measurements: The observed body weights of anuran species from unsprayed transplanted rice crop fields were more as compared to sprayed transplanted rice crop fields which may be due to more food availability and reduced toxic stress. In unsprayed habitats, the abundance of insects and other invertebrates provides ample nutrition, which directly supports better growth, body condition, and reproductive fitness in frogs and toads. Conversely, pesticide application in sprayed fields reduces the diversity and density of prey organisms, thereby limiting food resources (Table 4). In case of *D. melanostictus, D. stomaticus, E. cyanophlyctis, F. limnocharis* and *H. tigerinus* the males exhibited increase in body mass ranging from 0.81-10.63% heavier, while females ranging from 0.21-15.90% during 2021 and 2022. The maximum difference was recorded in case of *E. adolfi* males and females during the study period which indicates this species may be particularly sensitive to pesticide exposure, showing a stronger negative impact on growth. The overall, increase (%) in SVL, HL: SVL, HL: HW, HL: HD, SL: HL, SL: SVL, EN: NS, EN: HL, ED: HL, ED: SL, ED: SVL and ED: EN ratios of both male and female anuran species sampled from unsprayed transplanted rice crop fields were 6.14, 16.74, 6.39, 3.97, 10.76, 32.05, 12.14, 29.96 15.98, 7.74, 34.89 and 2.65, respectively as compared

Table 3. Mean Anuran population (individuals/200m^2) recorded in selected sprayed and unsprayed transplanted rice crop fields of district Ludhiana

Villages	Mean anuran population (2021 & 2022)	Increase in population (%) (2021 to 2022)
Noorpur Bet	10.31	3.85
Ladian Kalan	9.37	13.92
Kothe Sherjang	9.99	5.34
Mean	9.89	-
Research area (School of Organic Farming), PAU	13.59	13.51
Sahibana	12.49	8.07
Burj Lambran	14.30	10.44
Mean	13.46	-

*Mean anuran population values represent the average number of individuals per sampling unit (individuals/200m^2) calculated across three replications during 2021 and 2022

Table 4. Percent difference (between mean body weights of anuran species recorded from sprayed and unsprayed transplanted rice crop fields of district Ludhiana

Anuran species	Mean (2022 over 2021)	
	Male	Female
Duttaphrynus melanostictus	1.72	2.70
D. stomaticus	4.23	1.38
Euphlyctis cyanophlyctis	13.26	12.50
E. adolfi	61.40	41.85
Fejervarya limnocharis	2.96	3.08
Hoplobatrachus tigerinus	1.70	5.55
Mean	14.21	11.17

Table 5. Percentage (%) difference between morphometric parameters (mm) of anuran species from sprayed and unsprayed transplanted rice crop fields of district Ludhiana (2022 over 2021)

Morphometric parameters	*Duttaphrynus melanostictus*		*Duttaphrynus stomaticus*		*Euphlyctis cyanophlyctis*		*Euphlyctis adolfi*		*Fejerverya limnocharis*		*Hoplobatrachus tigerinus*		Mean
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	
SVL	5.99	5.63	5.31	8.52	4.21	5.34	10.83	15.29	4.57	3.15	2.36	2.54	6.14
HL: SVL	21.42	9.02	5.49	12.77	8.45	11.51	21.05	75.00	6.34	14.09	9.13	6.63	16.74
HL: HW	3.53	7.96	3.30	3.98	7.35	12.87	12.38	10.71	2.97	3.48	3.03	5.13	6.39
HL: HD	4.48	4.71	1.62	2.15	6.15	5.12	2.65	10.43	4.25	2.35	1.32	2.44	3.97
SL: HL	6.49	16.00	3.57	8.11	9.92	18.27	16.92	8.82	7.90	16.66	7.64	8.87	10.76
SL: SVL	59.09	35.71	52.00	19.88	26.27	20.26	42.85	8.69	36.60	30.60	37.39	15.33	32.05
EN: NS	5.76	2.85	2.81	6.42	10.97	8.01	4.00	81.81	6.89	7.33	5.08	3.82	12.14
EN: HL	19.86	32.14	45.95	40.23	11.47	21.71	32.17	64.28	20.66	20.19	34.26	16.66	29.96
ED: HL	7.76	15.72	15.22	16.36	21.72	14.57	18.51	30.35	24.16	10.41	11.06	5.98	15.98
ED: SL	6.47	4.95	3.06	4.68	9.13	3.32	6.77	15.20	9.98	3.61	12.09	13.72	7.74
ED: SVL	8.13	18.04	36.00	37.24	54.36	44.27	17.14	100.0	29.41	26.08	34.56	13.55	34.89
ED: EN	2.18	2.88	2.20	3.58	1.21	2.46	3.18	3.70	0.71	3.04	1.88	4.86	2.65

* HL= Head length (from back of mandible to tip of snout) (mm), HW= Head width (left side back of mandible to right side back of mandible) (mm), HD= Head depth at angle of jaw (depth of head at angle of jaw) (mm), SL= Snout length (distance between anterior comer of eye to the tip of the snout) (mm), EN= Eye to nostril distance (distance between anterior point of eye and nostril) (mm), NS= Nostril to snout distance (distance between anterior point of nostril to tip of the snout) (mm) and ED= Eye diameter (mm)

to sprayed fields (Table 5). This indicates that pesticides may exert sub-lethal physiological effects on amphibians, such as metabolic stress, impaired nutrient assimilation, or disruption of endocrine functions, which can negatively influence growth parameters and body condition. The positive correlation was found between anuran population with atmospheric temperature (˚C), relative humidity (%) and rainfall (mm).

Pesticide residues in soil, water and excretory contents: Use of different pesticides by farmers to raise rice crop, the pesticide residues were below the limit of quantification (0.01 mg kg^{-1}) in soil, water and excretory contents (urine and feaces) samples of selected transplanted rice crop fields. The collected amphibians do not show any morphological abnormality due to toxicological effect of pesticide residues which was supported by the absence of any pesticide residues in excretory contents (urine and feaces). From the soil samples, a range of 88.36-108.50%, 76.27-98.61% and 76.15-105.58% were recovered in case of synthetic pyrethroid, organochlorines and organophosphates, respectively. Similarly from the water samples, 76.16-105.40%, 73.71-104.16% and 81.78-107.20%. From the urine samples, percent range of 78.46-101.30, 79.84- 103.71 and 76.11-102.46 were recovered in case of synthetic pyrethroids, organochlorines and organophosphates, respectively. From the faecal matter samples, a range of 79.54-101.27%, 86.43-107.13% and 73.35-107.38% were recovered in case of synthetic pyrethroids, organochlorines and organophosphates, respectively.

CONCLUSION

The study conclude that when rice agroecosystems are combined with nearby natural aquatic and terrestrial habitats, they help to preserve a dynamic, heterogeneous system that acts as a haven for biodiversity. Farmers can improve ecosystem quality to conserve anurans by enhancing judicious usage of pesticides and increasing uncultivated areas like ponds and ditches near the crop fields especially rice crop.

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Inefficacy of Cement and Cellulose-Based Baits as Rodenticides against Synanthropic Rodents in Subtropical Climatic Zone

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Abstract: Effective management of synanthropic rodent pests using natural, eco-friendly materials instead of chemical rodenticides represents a vital advancement in integrated pest management strategies. The present study evaluated the potential of cement- and cellulose-based baits as rodenticides under both controlled laboratory and field conditions. Various bait formulations were tested, including chapatti pieces coated with cement, wheat flour mixed with cement in varying proportions (4:1, 2:1, 1:1, and 1:2), only cement, and cereal-based baits containing 25% and 50% cellulose powder. These were offered to *Bandicota bengalensis* and *Rattus rattus* in no-choice and bi-choice feeding trials. Despite significant bait consumption, the rats exhibited no mortality, maintained normal activity levels, and showed no deviation in their water intake. Residues of both cement and cellulose were evident in their feces. Field trials further reinforced these findings. Placement of cement-coated chapatti pieces near active burrows of *B. bengalensis* in sugarcane fields failed to produce any reduction in burrow activity or signs of toxicity, despite evident bait consumption. This study provides conclusive evidence that cement- and cellulose-based baits are ineffective as rodenticides. These findings underscore the critical need for continued research to explore and develop effective, environmentally safe alternatives to chemical rodenticides for sustainable rodent pest management.

Keywords: Cellulose, Cement, Rodenticide, Rodents, Toxicity

Rodents represent the most diverse mammalian order, Rodentia (Burgin et al., 2018) and majority play essential ecological roles, a small but highly impactful proportion (7–10%) are notorious agricultural and infrastructural pests (Hernandez et al., 2021). These inflict substantial economic losses by ravaging field crops and stored grains (Legese and Bekele 2023). More alarmingly, rodents serve as primary reservoirs of zoonotic parasites, transmitting serious diseases directly or vector-mediated (Singla et al., 2016, Brar et al., 2021, Mandla et al., 2022, Brar et al., 2024). Effectively controlling rodent populations necessitates an integrated pest management strategy combining both lethal and non-lethal approaches. Key control measures include environmental sanitation, rodent-proofing, trapping, and the strategic use of rodenticides (Stuart et al., 2025). Zinc phosphide remains the most widely used acute rodenticide worldwide due to its high efficacy, affordability, and relatively favourable safety profile (Eason et al., 2013). However, it presents significant challenges, including bait aversion and the survival of sub-lethally poisoned rodents, leading to control failures (Horak et al., 2018). Chronic anticoagulant rodenticides such as warfarin, coumatetralyl, bromadiolone, and brodifacoum are effective but pose serious risks of resistance development and secondary toxicity (Hernandez-Moreno et al., 2013, Runge et al., 2013, Garg et al., 2017, Sharma et al., 2024). Given these limitations, there is an urgent need for alternative rodent control strategies that are cost-effective, environmentally sustainable, resistance-free, and safer for non-target species. Developing such alternatives is critical for ensuring long-term success in rodent pest management.

Cellulose, a fundamental component of plant cells, exhibits a mode of action distinct from conventional chemical rodenticides (Jokic et al., 2014). Research has demonstrated that cellulose-based corn cob baits possess potent rodenticidal properties (Issa et al., 2021). The groundbreaking ecologically friendly rodenticide derived from powdered maize cobs (40–45% cellulose) achieved an impressive 91.66% efficacy in controlling *Mus musculus* populations (Jokic et al., 2006). Similarly, the cellulose-based product *Natromouse* was proven highly effective, reducing rodent populations by 86–98% in alfalfa and wheat fields (Jokic et al., 2007). Cement, a fine gray or white powder primarily composed of cement kiln dust, is a critical component of concrete and mortar. Its composition includes calcium, silicon, aluminum, ferric, and magnesium oxides, with trace elements such as potassium sulfate, sodium sulfate, chromium, and nickel (Akinola et al., 2008). The content of hazardous metals in cement particles varies according to the raw material used. Heavy metals, including cadmium (Cd), lead (Pb), chromium (Cr), cobalt (Co), Nickel (Ni), Manganese (Mn), Iron (Fe) and mercury (Hg), present in high amounts in cement dust particles, are known to be harmful even in small doses (Ogunbileje et al., 2013). Many of these components exhibit mortality and significant occupational and long-term health hazards to humans and animals (Donato et al., 2016, Owonikoko et al., 2021, Paithankar et al., 2021). Chemicals present in cement also cause allergy and complications related to the respiratory system in workers exposed to cement (Rahmani et al., 2018). The lethal action of cement- and cellulose-based rodenticidal baits stems from their ability to induce rapid dehydration, severe blood volume reduction, tissue necrosis, cardiac arrest, and eventual death (Jokic et al., 2006). These compelling findings have fueled growing scientific interest in evaluating their effectiveness against rodents. This study assessed the potential of cement- and cellulose-based baits as rodenticides targeting synanthropic rodent species, *Bandicota bengalensis* and *Rattus rattus*, that have significant economic and health importance, being agricultural pests and reservoirs of pathogens.

MATERIAL AND METHODS

The lesser bandicoot rat, B. bengalensis, and house rat, *R. rattus,* were live-trapped from agricultural fields, fish markets, and poultry farms in Ludhiana, India, which has a subtropical climate. In the laboratory, the captured rats were housed individually in cages and acclimatized for 15 to 20 days before the commencement of experiments. During this period, food and water were provided *ad libitum*. The diet consisted of a standardized bait mixture prepared using cracked wheat, powdered sugar, and peanut oil (WSO bait) in the ratio of 96:2:2. Following acclimatization, healthy adult rats of both sexes were weighed and used for further studies. Cement and cellulose were evaluated for their potential as rodenticides by assessing the acceptance and toxicity of treated baits through no-choice and bi-choice laboratory feeding trials. Individually housed rats were randomly assigned to groups, with five rats per group. Depending on the experimental setup, each cage was equipped with either one or two feeders along with a water dish.

In the first experiment, a total of seven groups of rats were used. Two groups were offered cement-coated chapatti pieces, one under no-choice conditions and the other under bi-choice conditions. Four groups were provided with bait formulations consisting of a mixture of wheat flour and cement dust in varying ratios of 4:1, 2:1, 1:1, and 1:2 under no-choice conditions. The seventh group was exposed to cement dust alone, in a no-choice test. All groups were exposed to their respective bait formulations for five consecutive days. Fresh bait was supplied regularly, and the amount of bait consumed by each rat was recorded daily. Water was replenished daily, and water consumption was similarly monitored. Throughout the trial and post-treatment period, rats were closely observed for any clinical signs of toxicity, mortality, changes in fecal consistency and colour, and any behavioural abnormalities. The toxicity of each bait formulation was assessed based on bait consumption patterns, water intake, clinical observations, and mortality, if any.

In the second experiment, cement-coated chapatti pieces were placed near 20 active burrows of *B. bengalensis* located in sugarcane fields at Ludhiana for four consecutive days. The effectiveness of the bait was assessed by recording bait consumption and changes in burrow activity. Burrows that were closed one day in the evening and reopened the following day in the morning were considered active or live.

In the third experiment, *R. rattus* rats were divided into groups (n = 5 per group) and exposed to WSO bait formulations containing 25% and 50% cellulose powder in both no-choice and bi-choice feeding trials for eight consecutive days. Water was provided *ad libitum* throughout the trial. Bait consumption was measured daily, replenished as needed, and expressed as g/100g body weight (bwt). Rats were monitored for mortality during the treatment period and for up to one month post-treatment. Additionally, daily observations were made to record clinical symptoms, mortality, fecal consistency and colour, and any behavioural abnormalities.

Statistical analysis: The results were expressed as mean ± standard deviation (SD), and differences were considered statistically significant at $P<0.05$ employing SPSS Software version 30.0.

RESULTS AND DISCUSSION

The cement-coated chapatti pieces, baits containing different proportions of wheat flour and cement, and cement alone exhibited no toxic effects on rats. The highest cement consumption (g/100g bwt) was in bait containing wheat flour and cement in a 1:2 ratio (28.43), followed by cement-coated chapatti pieces in the no-choice tests. The lowest consumption was observed for cement-coated chapatti pieces (4.15 g/100g bwt) in the bi-choice test and for cement alone (4.55 g/100g bwt) in the no-choice test. In other bait formulations containing wheat flour and cement at ratios of 1:1, 2:1, and 4:1 in no-choice, the cement consumption was 6.73, 5.40, and 5.64 g/100g bwt, respectively (Table 1). No mortality occurred during or after the treatments, and all rats remained active and healthy without any signs of abnormal behaviour.

The highest average daily water consumption was in rats fed on wheat flour: cement (1:2) bait (17.98 ml), followed by those offered only cement in a no-choice. In contrast, the lowest water intake was observed in rats fed cement-coated chapatti pieces during the no-choice trial (8.66 ml). These results suggest that there was no consistent or direct relationship between cement content in the bait and water consumption by rats.

Field application of cement-coated chapatti pieces near the active burrows of *B. bengalensis* in sugarcane fields did not yield significant control effects. One week after treatment, 18 out of 20 burrows (90%) remained active (Table 2), indicating that cement-coated chapatti pieces were non-toxic and ineffective for rodent control under field conditions. No direct studies are available in the literature that specifically evaluated the role of cement as a rodenticide. Akinola et al. (2008) exposed albino rats to cement dust for six weeks, resulting in pronounced pathological and toxic effects. They reported heavy metal bioaccumulation in the lungs and structural damage to vital organs such as the liver, heart, and kidneys, thereby highlighting the occupational hazards faced by cement factory workers. Similarly, Owonikoko et al. (2021) exposed male rats to cement dust for 14 and 28 days (5 hours daily) and reported clear signs of clinical toxicity, elevated serum heavy metal levels, reduced gastrointestinal motility, altered hematological parameters, poor movement coordination, abnormal posture, cephalic fur loss, and even mortality of one rat after two weeks of exposure. Contrary to these findings, the present study found no evidence of toxic effects of cement when ingested by rats, either in terms of mortality or any observable signs of abnormal behaviour, fur loss, or physical distress. This striking difference may be attributed either to the inherently non-toxic nature of cement when consumed orally or to the relatively short duration of exposure provided in the present study.

In no-choice feeding trials, rats consumed a significantly higher amount of cellulose when offered bait containing 50% cellulose for 8 days (37.73 g/100g bwt) compared to bait with 25% cellulose (18.23 g/100g bwt). Similar trend was observed in bi-choice tests, where cellulose intake over 8 days was greater with 50% cellulose bait (16.31 g/100g bwt) than with 25% cellulose bait (10.08±1.50 g/100g bwt). Overall, cellulose ingestion was significantly higher in no-choice tests than in bi-choice tests at both bait concentrations (Table 3). No mortality was observed in rats fed bait containing either 25% or 50% cellulose under both feeding conditions. Additionally, there were no noticeable changes in behaviour or adverse clinical signs in the treated rats.

The scientific literature on the effectiveness of cellulose-based rodenticides remains highly debated. Cellulose-based rodenticides have also proven effective against common voles (*Microtus arvalis*) in wheat and alfalfa crops (Jokic et al., 2010). Issa et al., (2021) observed 100% mortality of *Rattus norvegicus* in corn-based bait. However, Schmolz (2010), observed no mortality and very low palatability of commercial cellulose-based baits in both house mice (*Mus musculus*) and Norway rats (*R. norvegicus*). Present results also showed *no* mortality in rats fed cellulose baits. Based on the results of both laboratory and field tests, Zhelev et al. (2013) concluded that the cellulose-based rodenticide *'Eradirat'* is highly unpalatable to black rats (*R. rattus*). Furthermore, a 90-day subchronic toxicity study by Ong et al. (2020) found *no* systemic toxicity associated with the consumption of fibrillated cellulose. The study determined that the no observed adverse effect level for fibrillated cellulose was 2194.2 mg/kg/day for male and 2666.6 mg/kg/day for female Sprague Dawley rats. Experiments with traditional cellulose have demonstrated that rats can tolerate even higher levels, with no negative effects observed from a 30% cellulose diet, even after an extended 72-week period. This strongly underscores the safety and non-toxicity of cellulose in rodenticide applications.

In the present study, after baiting with various cemented and cellulose baits, fecal samples collected from the experimental cages showed traces of both cement and cellulose. This strongly indicates that the cement and cellulose were metabolized and subsequently excreted from the rats' bodies. The rats remained fully active and

Table 1. Effects of cement-coated chapatti pieces, wheat flour mixed with cement in different ratios, and only cement on *B. bengalensis* rats

Exposure	Treatment	Body weight (g) (n = 5 each)	Mean daily consumption (g/100g bwt)	Total consumption of treated bait (g/100g bwt)	Total consumption of cement (g/100g bwt)	Mean daily consumption of water (ml)	Total consumption of water (ml)
No-choice	Cement-coated chapatti pieces	336.00±37.28	7.94±1.20	39.68±6.02	11.90±1.81	8.66±1.44	43.32±7.19
Bi-choice	Cement-coated chapatti pieces	294.00±58.57	2.76±0.85-T 5.79±0.71-P	13.82±4.27	4.15±1.28	-	-
No-choice	Wheat flour: cement (4:1)	277.60±34.70	5.63±1.43	28.14±7.17	5.64±1.45	12.60±2.62	63.0±13.12
No-choice	Wheat flour: cement (2:1)	245.00±47.70	8.10±1.58	16.20±3.16	5.40±1.05	17.98±5.24	35.97±10.49
No-choice	Wheat flour: cement (1:1)	278.00±29.46	6.77±0.93	13.53±1.86	6.73±0.91	15.07±1.11	30.13 ±2.22
No-choice	Wheat flour: cement (1:2)	261.50±39.80	6.56±1.49	37.23±10.56	28.43±7.04	15.43±3.08	86.42±23.41
No-choice	Cement	257.00±24.04	1.15±0.35	4.55±1.34	4.55±1.34	16.40±2.55	65.65±10.25

T-Treated bait, P-Plain bait, No mortality was achieved in any treatment; All values are Mean±SE

Table 2. Evaluation of cement-coated chapatti pieces against rodents in sugarcane fields

Burrow no. *	Consumption (g) of treated bait out of the total 20 g kept daily				Mean daily consumption (g)	Total consumption in 4 days (g)
	Day 1	Day 2	Day 3	Day 4		
1	5.0	13.0	20.0	18.0	14.0	56.0
2	20.0	20.0	20.0	20.0	20.0	80.0
3	20.0	20.0	20.0	20.0	20.0	80.0
4	20.0	20.0	20.0	20.0	20.0	80.0
5	20.0	15.0	14.0	20.0	17.2	79.0
6	20.0	20.0	20.0	15.0	18.7	75.0
7	20.0	20.0	20.0	20.0	20.0	80.0
8	20.0	20.0	20.0	20.0	20.0	80.0
9	20.0	20.0	20.0	20.0	20.0	80.0
10	20.0	20.0	18.0	16.0	18.5	74.0
11	20.0	20.0	17.0	20.0	19.2	77.0
12	20.0	14.0	20.0	20.0	18.5	74.0
13	20.0	20.0	20.0	20.0	20.0	80.0
14	20.0	20.0	20.0	20.0	20.0	80.0
15	20.0	20.0	20.0	12.0	18.0	72.0
16	20.0	20.0	20.0	20.0	20.0	80.0
17	20.0	16.0	20.0	11.0	16.7	67.0
18	20.0	20.0	15.0	20.0	18.7	75.0
19	20.0	20.0	20.0	20.0	20.0	80.0
20	20.0	20.0	20.0	13.0	18.2	73.0
Mean	19.2	18.9	19.2	18.2	18.9	76.0

*18 burrows were still live after one week of treatment termination

Table 3. Effect of bait containing different concentrations of cellulose powder in *R. rattus*

Exposure	Cellulose concentration (%)	Body weight (g) (n=5 each)	Mean daily consumption of treated bait (g/100g bwt)	Total consumption of treated bait (g/100g bwt)	Total cellulose consumed in 8 days (g/100g bwt)
No-choice	25	118.0	9.12[a]	72.92[a]	18.23[a]
Bi-choice	25	117.8	5.04[b]	40.30[b]	10.08[b]
No-choice	50	110.2	9.43[a]	75.46[a]	37.73[a]
Bi-choice	50	112.0	4.08[b]	32.62[b]	16.31[b]

Values with different superscripts (a-b) in a column differ significantly at P<0.05, No mortality was achieved in any treatment

responsive throughout the baiting process. No fur loss or signs of inactivity were observed in our study. In contrast, Issa et al., (2021) reported significant adverse effects, including fur loss, weight loss, sunken eyes, immobility, and insensitivity in rats treated with agricultural waste containing cellulose as the active ingredient.

CONCLUSIONS

The laboratory and field experiments, which examined the rodenticidal potential of cement and cellulose baits over 4-8 days, unequivocally revealed *no* toxic effects. This strongly suggests that cement and cellulose are not viable alternatives to chemical rodenticides. Extending the exposure period further is unlikely to yield positive results and would only increase labour and costs, making it an impractical solution. Therefore, further research must be conducted to identify truly effective and environmentally friendly alternatives for rodent pest management.

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Toxic and Antifertility Potential of Neem Seed and Andrographolide Based Bait against Male House Rat (*Rattus rattus* Linnaeus, 1758)

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Abstract: Anti-reproductive and toxic properties of neem seed alcoholic hexane and andrographolide extract-based bait were investigated in house rat*, Rattus rattus.* Using these extracts three treated baits were prepared, the doses of both extracts were similar in these baits; difference was only in the method of their preparation. In treated bait 1, polymer was used to increase the palatability and bioavailability of secondary metabolites. In treated bait 2, both polymer and bio stabilizer (for thermo-, photo-stability, and bioavailability of secondary metabolites) were added. In treated bait 3, nanoemulsions of both extracts were used. Percentage acceptance of treated bait 2 was also maximum. However, percentage mortality was maximum with treated bait 3 due to Tween 20 added to it. The weight of reproductive organs, and sperm parameters were adversely affected in rats fed on treated bait 2. Histological studies revealed maximum interstitial space, percent seminiferous tubules with disorganized germinal epithelium, and minimum number of Leydig, germ, and Sertoli cells, in rats fed on treated bait 2. Both toxic and antifertility effect of treated bait 2 might be due to the increase in the palatability, bioavailability, and stability of secondary metabolites which can be used for long-term reduction of rodent population.

Keywords: Andrographolide, Neem seed extract, Toxic agent, Antifertility agent, House rat

Rodents cause significant damage to a range of crops as well as under commensal situations (Brown et al 2013). They are extremely adaptable to any environment and have a very high reproductive potential, so they can keep their population at the maximum possible level (Pradhan and Talmale 2011). Rodenticide baits and trapping are commonly used management methods under field and commensal situations respectively. As chemical rodenticides are poisonous, they cause numerous ecological issues necessitating the use of plant-based products (anti-fertility agents and rodenticides) to manage the rodent population (Mandal and Dhaliwal 2007). Moreover, the reproductive potential of the residual population left after the application of these control methods increases so much that the population of rodents again reaches the same level in a short duration after the treatment. To check the sudden increase in the reproductive potential of rodents, antifertility agents based baits are required for the long-term management of rodents.

Plant-based anti-fertility agents and their products are used for ages to regulate the population by affecting spermatogenesis, oogenesis, and other reproductive processes. Flavonoids, Tannins, limonoids, and other compounds found in these plants are responsible for antifertility activities (Daniyal and Akram 2015, Samal 2016). These plant-based compounds are safe, eco-friendly, and have long-lasting effects (Shah et al 2016). Antifertility and toxic effects of *Azadirachta indica* and *Andrographis paniculata* are well known (Idu et al., 2023, Pandey and Rao 2018). Secondary metabolites in neem like azadirachtin, nimbin, nimbidin (Terpenoids), flavonoids, tannins, alkaloids, and phytosterols have toxic and antifertility effects, that could disrupt gonadotropin hormone and spermatogenesis (Kumar and Kumar 2014, Seriana et al., 2021). All the previous studies on antifertility effects were conducted using either pure compounds or by using extracts of polar or non-polar solvents as oral doses against rodents (Seriana et al., 2021). For the application of plant-based extracts as antifertility agents in fields against rodents, it was necessary to mix them in a bait.

Andrographolide and seeds of *A.indica* are well known for their bitter taste. Therefore, it was very difficult to make the rats feed on bait based on these extracts. Moreover, secondary metabolites in neem seed extract also degrade with time being photo-and thermo-labile (Madaki 2015). So, there was a need to formulate stable and palatable bait using these extracts against rodents to use both toxic and antifertility properties of andrographolide and NSAH (Neem seed alcoholic hexane) extracts under field conditions against rodents. Previous studies revealed that oral doses of NSAH extract and andrographolide have toxic and antifertility effects (Chawla 2018, Kaur 2019, Kavita 2021, Verma et al., 2023, Verma et al., 2024, 2025). However, when fed as baits to rats, their toxic and antifertility effects were not as pronounced as with oral doses, which might be due to less bioavailability of secondary metabolites in treated bait to rats because of the reduction in absorption of secondary metabolites by dietary fats, proteins, and fibers (Bushra et al., 2011, Chawla 2018, Kaur 2019, Kavita 2021). Tran and Hinds (2013) reported that treatment with a higher concentration of promising plant extracts in conjugation with one another can lead to permanent sterility in rodents. As both plants have a different mode of action for their antifertility properties, both can be used in synergism to produce more antifertility effects (Al-Batran et al., 2013). Therefore, during the present investigation, neem seed extract and andrographolide were mixed in bait to synergize their toxic and antifertility effects against rodent pests.

MATERIAL AND METHODS

Experimental details: The study was conducted at Punjab Agricultural University (PAU), Ludhiana located at an intersection of 30˚55' N parallel of latitude and 75˚54' E line of longitude. Approval of Institutional Animal Ethics Committee, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana was obtained for the usage of animals during the 58th Meeting of IAEC held on 26.3.2021 vide Memo No. GADVASU/2021/IAEC/58/05. The male house rats, *R. rattus* were trapped with the help of single and multi-catch rat traps from the storehouses, grocery shops, fish markets, and poultry farms around PAU, Ludhiana. Adult rats (body weight 150-200g) with fully descended testes were selected out of the trapped rats. These rats were acclimatized in laboratory cages (each of size 36 x 23 x 23cm) at 25°C temperature, 30-50% humidity and 14 hour dark/10 hour light cycle for 15-20 days before the commencement of the experiment with food and water provided *ad libtium*. After acclimatization, healthy rats were again weighed and grouped for experimentation (Table 1). The treatment period was 25 days for group 2, 15 days for group 3 and 8 days for group 4. Treatment period varied because of the variation in the toxicity of different treated baits. At the start of experiment, treatment period was planned for 30 days. As rats started dying because of the toxic effect of synergistic bait, rats were necropsied earlier to study the antifertility effect of each treated bait. The toxic effect of NSA bait was not anticipated before starting the experiment. Plastic trays were kept under each laboratory cage to collect and dispose of animal faeces and urine.

Procurement of plant material and chemicals used: Mature dried seeds of *Azadirachta indica* were collected from Punjab Agricultural University, Ludhiana. Andrographolide was procured from Shubhasya Biotech, Bangalore, India. The bio-stabilizer (UV protector, antioxidant, and polymerization inhibitor) and polymer (Inert, non-poisonous, deep pored with stable matrix and occupying large surface area) required for the preparation of treated baits were provided by Orion Organics Private Limited, Ludhiana, India.

Preparation of the neem seed alcoholic hexane (NSAH) extracts and andrographolide powder alcoholic extract (APE)

Three types of NSAH extracts (1, 2 & 3) and 2 types of APE (1 and 2) were prepared.

NSAH extract 1: NSAH extract 1 was prepared as per the method of Subramanian et al (2019) (Fig. 1).

NSAH extract 2: To prevent the degradation of secondary metabolites in neem seeds, 5.3 ml of bio-stabilizer was added to 133g of neem seeds at the time of preparation of its powder to prevent the degradation of active ingredients in the neem seeds (Fig. 1).

NSAH extract 3: Nano emulsion of this neem seed extract was prepared by homogenizing concentrated stock of NSAH extract and 27ml of Tween 20 for 10 minutes (Fig. 1). This solution was then probe sonicated (amplification 25% and 20 KHz) for 30 minutes by keeping the NSAH extract in an ice bath to get the nano emulsion of NSAH extract (Ghotbi et al., 2014).

Andrographolide powder extract (APE 1): Andrographolide (2.67g) was dissolved in 27ml of warm ethanol to get the andrographolide powder ethanol extract (Chen et al 2010).

Nano emulsion of andrographolide powder extract (APE 2): Nano emulsion of andrographolide powder extract was prepared by dissolving 2.67g of AP in tocopherol and ethanol

Table 1. Layout of experiment

Groups	Feed	Duration of treatment (Days)	Number of rats used
Group 1 (Control)	Plain maize sugar (96:4) bait	-	5
Group 2 (Treated group 1)	Treated bait 1 (APE 1(2.67 g AP) and NSAH 1 (133 g NSP) adsorbed on polymer and mixed in maize and sugar (4 gm) to prepare 100g bait)	25	5
Group 3 (Treated group 2)	Treated bait 2 (APE 1 and NSAH 2 adsorbed in polymer and mixed in maize and sugar (4 gm) to prepare 100 g bait).	15	5
Group 4 (Treated group 3)	Treated bait 3 (APE 2 and NSAH 3 adsorbed in polymer and mixed in maize and sugar (4gm) to prepare 100g bait).	8	5

AP: Andrographolide powder; NSP: Neem Seed Powder; APE 1: Andrographolide Powder Alcoholic Extract; APE 2: Nano emulsion of APE 1; NSAH 1: Neem Seed alcoholic hexane (NSAH) extract 1(133g NSP); NSAH 2: NSAH 1 containing bio stabilizer; NSAH 3: Nano emulsion of NSAH 1 in a 1:1 ratio (4.67g of tocopherol and 4.67g of ethanol) and 4.67g of Tween 20 was then added to this solution. This solution was then homogenized for 10 minutes and then probe sonicated for 30 minutes to get the nano emulsion of andrographolide (Yen et al 2018).

Qualitative analysis of the NSAH extract: Qualitative analyses of the NSAH 1, 2, and 3 were carried out . Benedict's Reagent Test for glycosides confirms the presence of glycosides (Biu et al., 2009) while a ferric chloride test was conducted to check the presence of tannins (Biu et al., 2009). To test the presence of saponins, a foam test was performed (Kosma et al., 2011). Salkoniski reaction was performed for the presence of phytosterols (Sasidharan et al., 2011).The appearance of a brownish to the red colour indicated the presence of reducing sugars after adding and boiling an equal quantity of both benedict reagent and NSAH extract (Biu et al., 2009).To test for the presence of terpenoids and alkaloids, the salkoniski test and Mayer's reagent test were performed respectively (Khanal 2021).

Preparation of treated baits using andrographolide and neem seed alcoholic hexane extracts: For the present investigation, three treated baits 1, 2, and 3 were prepared using APE and NSAH extracts.

Treated bait 1: For the preparation of 100g of this bait, stock NSAH extract 1 & APE 1 were adsorbed in a polymer to mask the bitter taste of extracts. Polymer with NSAH 1 and APE 1 was coated with 1 ml sugar syrup and then mixed with maize flour and sugar (4g) to prepare 100g bait.

Treated bait 2: For the preparation of 100gm of this bait, stock NSAH extract 2 & APE 1 were adsorbed in a polymer. This polymer with NSAH 2 and APE 1 was coated with 1 ml sugar syrup and then mixed with maize flour and sugar to prepare 100g bait.

Treated bait 3: For the preparation of 100g of this bait, stock NSAH extract 3 & APE 2 were adsorbed in a polymer. This polymer with NSAH 3 and APE 2 was coated with 1 ml sugar syrup and then mixed with maize flour and sugar to prepare 100g bait.

Toxicity and consumption of treated baits : Adult male rats were divided into four groups with five rats in each group (Table 1). Group 1 was fed on plain maize sugar-based bait mixed in 96:4 ratio. Groups 2, 3 & 4 were fed on treated baits 1, 2 & 3 for 25, 15 and 8 days respectively under bi-choice conditions. Consumption of baits was recorded after every 24 hours. Daily bait consumption (g/100 g body weight), consumption of active ingredients in treated baits, and percent acceptance of treated baits were calculated (Dhar

$$\text{Daily bait consumption (g/l00g bw)} = \frac{\text{Daily bait consumption by rat}}{\text{Weight of rat}} \times 100$$

$$\text{Active ingredient consumed} = \frac{\text{Quantity of NSP or Ap powder taken}}{\text{Quantity of treated bait given}} \times \text{Consumption of treated bait (g/100g BW)}$$

$$\text{Percent acceptance} = \frac{\text{Consumption of treated bait}}{\text{Consumption of treated bait + untreated bait}} \times 100$$

and Singla 2014).

Antifertility effect of treated baits: Immediately after the treatment periods (Table 1), male house rats of each group were necropsied to observe the effect of treated baits on the weight of their reproductive organ, sperm parameters, and histomorphology of testes and cauda epididymis.

Effect on sperm parameters: To examine the effect of treated baits on various sperm parameters, sperm motility, viability, abnormalities, and sperm concentration (million/ml) were recorded in the cauda epididymal fluid of untreated and treated rats. Cauda epididymis of three animals from each group was used for the study. Immediately after cutting, one drop of cauda epididymal fluid was kept on the microscopic slide and observed under a microscope to study sperm motility at 5 different sites of a microscopic field of each rat. For sperm concentration, the sperms were counted in twenty-five large squares of charged hemocytometer, and an average number of spermatozoa/square were calculated, which was then multiplied by 10^6 to obtain a million sperms per ml (MJF 2015). Hypo-Osmotic swelling test (HOS test) of caudal epididymal sperms was also performed to determine functionally viable sperms (Jeyendran et al., 1984). To study sperm abnormalities, one drop of cauda epididymal fluid was stained with trypan blue and, smear prepared. Hundred sperms/rat were recorded to calculate % sperm abnormalities, % viability and % sperms with coiled tail. Each parameter was studied at 400x magnification

Effect on histomorphology of reproductive organs: Testis and cauda epididymis were dissected out, free from fat, and fixed in neutral buffered formalin (NBF) for 72 hours followed by dehydration, clearing, and then embedding in paraffin wax (Humason 1979). Transverse sections of testis and cauda epididymis were cut at 5μm thickness and stained with Periodic acid Schiff (PAS) reagent and Haematoxylin respectively. Stained Slides were studied by using Magvision software. The diameter (μm) of seminiferous tubules, epididymis, and their lumen across the major and minor axis were recorded from three rats of each group. The seminiferous tubular diameter was taken for 3 tubules for each stage of seminiferous epithelial cycle (SEC). Several abnormalities of seminiferous tubules were identified and percent disorganized tubules were calculated. Different stages (13 stages) of the seminiferous epithelial cycle (SEC) were identified (Lagarrigue et al., 2011). The number of spermatogonia, spermatocytes (leptotene, zygotene, pachytene, and diplotene), round spermatids, elongating and elongated spermatids, and Sertoli cells was assessed from seminiferous tubules of different stages of SEC (n=3 for each stage from each group of rats (n=3). Frequency of different stages of SEC in each group was also calculated. Hundred tubules for each group were assessed. Fetal and adult Leydig cells in interstitial tissue were identified and counted from each group of rats (n=3). The true count of almost round cells was calculated (Abercrombie 1946). Each type of cell counted from different stages of seminiferous epithelial cycle were then aggregated.

Where correction factor =

$$\frac{Section\ \ thickness}{Section\ \ thickness + \sqrt{\left(average\ \frac{diameter}{2}\right)2 - \left(average\ \frac{diameter}{4}\right)2}}$$

Statistical analysis: Significance of difference was determined at a 5% level of significance using SAS version 9.4.

RESULTS AND DISCUSSIONS:

Phytochemical studies of NSAH extracts: Qualitative analysis of all three extracts of NSAH (NSAH 1, 2, and 3) revealed the presence of glycosides, tannins, phytosterols, phenolic compounds, saponins, reducing sugar, terpenoids, and alkaloids. The presence of maximum secondary metabolites was in NSAH 2 followed by NSAH 1 and NSAH 3 (Table 2). NSAH extracts were prepared using ethanol and hexane to extract all the secondary metabolites (polar and non-polar like azadirachtin, nimbin, nimbidine, nimbidol, picrin, sialin etc.) with antifertility and toxic properties from the neem seeds (Kumar and Kumar 2014, Fokunang et al., 2019, Braga et al., 2021). The presence of more active components in NSAH 2 could be attributed to the addition of a biostabilizer to neem seeds, which prevented the degradation of secondary metabolites in seeds. The NSAH 3 had the highest rate of degradation of secondary metabolites. NSAH 3 is a nano emulsion of NSAH 1. Secondary metabolites in neem being thermolabile might degrade during the preparation of nano emulsion due to the generation of heat during sonication.

Consumption and toxic effect of treated baits: For the first five days of the treatment period, consumption of all three baits was significantly lower than that of plain bait, but thereafter, there was a non-significant difference between the consumption of plain and treated baits, indicating good palatability. The average percent acceptance and daily consumption of active ingredients (NSP & AP) was significantly more with treated baits 2 & 3 as compared to the treated bait 1 (Table 3, Fig. 2). Increased palatability observed was likely due to the bitter extracts' adsorption on deep-pored, non-reactive stable polymers, which masked the bitter taste and increased the baits' palatability. This polymer also has a large surface area. NSAH extract and andrographolide adsorbed on polymer spread on a large surface area, resulting in increased absorption and so bioavailability of secondary metabolites. Feeding of rats on treated baits 1, 2 & 3 resulted in 20% mortality in 17-25 days, 40% in 12-15 days, and 20-100 % in 2-8 days respectively indicating maximum toxic effect of treated bait 3 (Table 3). Earlier and more mortality with treated bait 2 as compared to bait 1 might be due to more stability and bioavailability of secondary metabolites with toxic effects, due to the addition of bio-stabilizer while preparing neem seed powder. This bio-stabilizer is a polymerization inhibitor and UV protector. The neem seed extract is resinous, it agglomerates rapidly resulting in the least absorption. The maximum toxic effect of treated bait 3 was due to the addition of Tween 20 in NSAH 3 and APE 2 (Eskandani et al., 2013). Previous studies revealed that direct oral administration of andrographolide powder (20, 40, 80 & 500 mg/kg bw) and NSAH extract (6.67g NSP) can cause 40-100% mortality in 24 hours (Kavita 2021, Kaur 2022). Delayed mortality in the present study with treated baits might be due to the still less bioavailability of active ingredients in treated bait to rats because of the reduction in absorption of active ingredients by dietary fats, proteins, and fibers (Bushra et al., 2011).

Effect of treated baits on body weight and reproductive organs: There was a significant reduction in the final body weight of the treated groups as compared to the untreated group (Fig. 3). Weight of the male reproductive organs (testis) also reduced significantly in the treated rats as compared to the untreated rats but a non-significant

Table 2. Qualitative analysis of neem seed alcoholic hexane extracts (NSAH)

Secondary metabolites	NSAH 1	NSAH 2	NASH 3
Glycosides	++	++++	+++
Tannins	+	+++	-
Phytosterols	+++	++++	++
Phenolic compounds	+++	++++	+
Saponins	++++	++++	+++
Reducing sugar	+++	+++	++
Terpenoids	++	+++	+
Alkaloids	++++	++++	+++

NSAH 1: Neem Seed alcoholic hexane (NSAH) extract 1(133g NSP), NSAH 2: NSAH 1 containing bio stabilizer , NSAH 3: Nano emulsion of NSAH1 , ++++: Very high;+++: High; ++: Low; +: Very low

difference in weight was among treated groups, although the reduction in the weight of reproductive organs was more in rats fed on treated bait 2 as compared to the rats fed on other treated baits (Table 3). This indicated the maximum antifertility effect of treated bait 2, which might be due to the stabilization of secondary metabolites having both toxic and anti-fertility properties. Changes in the color (blue-black) of reproductive organs and other organs of the body in group 4 rats indicated the maximum toxic effect of treated bait 3 due to the addition of Tween 20 in this bait.

Effect on sperm parameters: Drastic and significant reduction in percent sperm motility, viability, HOS +ve sperms , and sperm concentration (millions/ml) was recorded in group 3 rats (3.00, 14.00, 22.0, 0.12 respectively) followed by group 2 rats fed on treated baits 2 and 1 respectively as compared to the untreated group (89.4, 86.80, 78.33, 2.69 respectively). Sperm abnormalities like head-tail separation, coiled tail, abnormal head, irregular shape, bent mid-piece, and double tail were also recorded. These sperm abnormalities (%) increased significantly in group 3 rats followed by group 2 rats (Table 4). The treated bait 2 was fed to rats for a shorter duration (15 days only) as compared to treated bait 1 (25 days), however, effects on sperm parameters were almost similar. Therefore, results for sperm parameters indicated the more antifertility effect of treated bait 2 in shorter duration as compared to treated bait 1. The effect on sperm parameters was more drastic in rats fed on treated bait 2 than that reported earlier with individual extracts (Kaur 2019). Most sperms were immotile and dead, with abnormalities reaching 83.33% after only 15 days, likely due to the polymer and bio stabilizer enhancing palatability, stability, and bioavailability, as well as the synergistic action of the compounds. In contrast, treated bait 3 showed limited impact, possibly due to thermo-degradation of antifertility metabolites during nano emulsion preparation. Previous studies also reported adverse effects: neem leaf meal (NLM) in rabbits improved sperm traits only at low inclusion (≤0.85%) but reduced quality at higher levels (Ogbuewu et al., 2022) and aqueous wood-ash extract of *A. indica* decreased sperm motility, viability, and concentration in mice (Auta and Hassan 2016).Neem leaf extract also reduced motility and increased abnormalities in rats (Mishra and Singh 2005). These parameters are clearly indicating the effect on sperm parameters (immotile, dead and abnormalities reaching up to 80%) was severe with treated bait 2 as compared to treated bait 1 and 3.

Effect on testicular histomorphology: To determine the effect of treated baits on testicular histomorphology, the total number of different germ and Sertoli cells in different stages (1-13) of the seminiferous epithelial cycle was determined (Table 5). Compared to the treated groups, the number of different germ and Sertoli cells in different stages of SEC was

Table 3. Comparison of consumption of plain and treated baits, weight of reproductive organs and toxicity among groups

Groups N=5 (Treatments)	Consumption (g/100gm BW)					Weight (g/100g bw)			Percent mortality (Time to death)
	Pre-treatment period (Plain bait)	Treatment period: Plain bait	Treatment period: Treated bait	AIC (AP)	AIC (NSP)	Testes	Corpus and caput epididymis	Cauda epididymis	
Control	4.99	4.9^{a1}	4.29^{a1}	-	-	0.77^{a}	0.16^{a}	0.26^{a}	-
Treated bait 1	4.53	4.31^{a1}	2.35^{b2}	0.063^{a}	3.13^{a}	0.64^{ab}	0.18^{a}	0.14^{a}	20 (17 to 25 days)
Treated bait 2	4.98	3.86^{a1}	2.95^{b2}	0.07^{b}	3.93^{b}	0.40^{b}	0.08^{a}	0.10^{a}	40 (12 to 15 days)
Treated bait 3	4.22	3.76^{a1}	2.87^{b2}	0.077^{ab}	3.84^{ab}	0.57^{ab}	0.28^{a}	0.18^{a}	100 (2-8 days)

All values are Mean±SE;; a, b indicate significance difference (p≤0.05) along the columns; 1,2 indicate significant difference (p≤0.05) between the consumption of plain and treated baits, NSAH 1: Neem Seed alcoholic hexane (NSAH) extract 1(133g NSP); NSAH 2: NSAH 1 containing bio stabilizer; NSAH 3: Nano emulsion of NSAH 1 Treated bait 1: APE 1 and NSAH 1 adsorbed in polymer and mixed in maize and sugar (4gm) to prepare 100g bait; Treated bait 2: APE 1 and NSAH 2 adsorbed in polymer and mixed in maize and sugar (4gm) to prepare 100g bait; Treated bait 3: APE 2 and NSAH 3 adsorbed in polymer and mixed in maize and sugar (4gm) to prepare 100g bait Plain bait: Maize: Sugar-96:4; AP: Andrographolide powder; NSP: Neem Seed Powder; APE 1: Andrographolide Powder Alcoholic Extract; APE 2: Nano emulsion of APE 1;

Table 4. Effect of baits on sperm parameters

Groups (Treatments)	Treatment period (days)	Motility (%)	Concentration (million/ml)	Viability (%)	HOS+VE (%)	Sperm abnormalities (%)
Control	-	89.4^{y}	2.69^{y}	86.80^{y}	78.33^{y}	12.66^{y}
Treated bait 1	25	4.25^{x}	0.35^{x}	19.75^{x}	21.66^{x}	76.00^{x}
Treated bait 2	15	3.00^{x}	0.12^{x}	14.00^{x}	22.0^{x}	83.33^{x}
Treated bait 3	8	88.5^{y}	1.10^{y}	82.50^{y}	75.0^{y}	23.00^{y}

See footnote of Table 3 for treatment details significantly higher in the control group. Overall, treated bait 2 has a substantial effect on the number of different types of spermatogonia (stem cells for spermatogenesis) and Sertoli cells (somatic cells and play a very important role in spermiogenesis). These findings indicated that bio stabilizer added in treated bait 2 increased the stability and thus Bio efficacy of extracts and can cause permanent sterility in rats if further stabilized since it had the maximum effect on germ and somatic stem cells. The total number of seminiferous tubular cells reduced in the treated groups as compared to the control group. Reduction in the total number of cells and spermatids was more in treated group 3 in later stages of SEC. Histomorphological studies also revealed that 16.64% of seminiferous tubules of untreated group 1 were disorganized (Fig. 4). However, the percentage of seminiferous tubules with disorganized epithelium increased in treated groups and was maximum in group 3 rats indicating that treated bait 2 has a maximum effect on the organization of germinal epithelium . The percent area of interstitial space was minimum in the control group as compared to the treated groups (Fig. 5, 7), being maximum in rats of Group 3 due to the maximum shrinkage of seminiferous tubules. The number of adult Leydig cells and fetal Leydig cells was significantly more in group 1 as compared to the treated groups. Among treated groups, the number of adult Leydig cells and fetal Leydig cells was also significantly less in group 3 followed by groups 2 and 4 (Fig. 6), indicating the maximum antiandrogenic effect of treated bait 2. Thasmi et al (2021) reported that when rats were treated with neem leaf extract @ 200mg/kg bw, the number of Leydig cells decreased resulting in a low level of testosterone in male rats. When neem leaf meal was given to rats (15%), the number of Leydig cells decreased. An increase in interstitial space and reduction in LH and FSH levels was also reported when rats were treated with neem oil @1.2 ml/animal (Shaikh et al., 2017).

In the treated groups, seminiferous tubular diameter and luminal diameter decreased markedly in all the stages of SEC, indicating shrinkage of seminiferous tubules. However, due to the disruption of the germinal epithelium, its thickness increased in treated groups 2, 3, and 4 (Fig. 7). Earlier studies also reported a reduction in the diameter of seminiferous tubules in male rats when fed orally on neem seed extract @159 mg/kg bw (Daniyal and Akram 2015). When male quails were fed on neem seed (40%) based bait, the diameter of seminiferous tubules of treated males decreased significantly (Gois et al 2019). In groups 2 & 4 rats, vacuolization of germinal epithelium was prominent. Pathological changes observed in group 3 rats include tubular atrophy, exfoliation and depletion of germ cells, shrinkage of seminiferous tubules, the occurrence of pyknotic cells, and vacuolization in seminiferous tubules indicating impaired spermatogenesis (Fig. 7). Previous studies supported our finding as an aqueous extract of neem leaf (200mg/kg bw/day for 30 days) caused vacuolization in seminiferous tubules (Santra and Manna 2009). In some of the seminiferous tubules, cellular associations were also disturbed due to the drastic reduction in the number of various germ cells and exfoliation of germinal epithelium. The treated bait 2 affected spermatogenesis and spermiogenesis, by targeting stem germ cells (spermatogonia and Sertoli cells), and Leydig cells due to synergistic effect of stabilized secondary metabolites of NSAH extract and andrographolide in treated bait 2 leading to permanent sterility in male house rats. Spermatogenesis occurs under the influence of FSH and Leydig cells produce testosterone under the influence of LH. Reduction in the number of different types of testicular cells during the present study clearly indicated hormonal disturbances in treated rats leading to severe effect on both spermatogenesis and spermiogenesis (Santi et al., 2020). Spermatogonia in the testis are the most primitive spermatogonial stem cells (SSCs), which play an important role in maintaining highly productive spermatogenesis by self-renewing and continuously generating daughter spermatogonia that differentiate into spermatozoa (Kubota and Brinster 2018). In the absence of Sertoli cells, haploid round spermatids are unable to form their tails and remain immotile, preventing them from going through the spermiogenesis process. Sertoli cells give metabolic assistance and sustenance to growing sperms. Because spermatogonia are precursors of spermatogenesis and

Table 5. Effect of baits on true count of cells of seminiferous tubules

Groups	Number of cells per seminiferous tubule									Total number of cells
	SG	PL	L	P	Z	D	RS	EL	SC	
Control	22.13[a]	20.93[a]	36.96[a]	40.87[a]	28.72[a]	30.28[a]	91.32[a]	62.27[a]	4.17[a]	338
Treated bait 1	15.47[ab]	14.30[b]	28.59[ab]	27.68[a]	21.19[a]	21.64[b]	62.97[b]	45.16[b]	3.44[b]	240
Treated bait 2	5.23[b]	3.20[c]	23.67[b]	31.00[a]	21.66[a]	21.74[b]	64.15[b]	44.49[b]	1.63[c]	217
Treated bait 3	12.67[ab]	11.70[b]	29.75[ab]	30.04[a]	26.59[a]	28.49[ab]	70.53[ab]	53.50[ab]	3.51[b]	267

SG: Spermatogonia, PL: Preleptotene, L: Leptotene, P: Pachytene, Z: Zygotene, D:Diplotene, RS: Round cells, EL: Elongating cells, SC: Sertoli cells

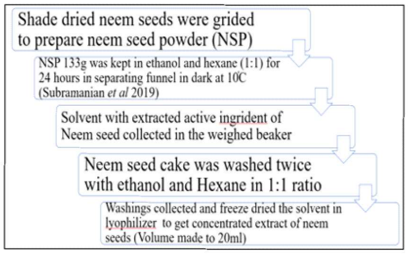

Fig. 1 Method of preparation of neem seed alcoholic hexane (NSAH) extract.

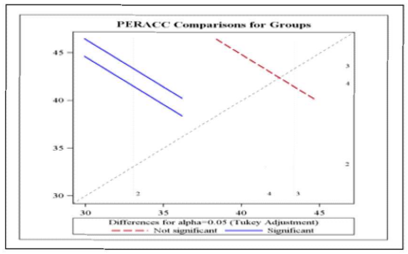

Fig. 2: Comparison of percent acceptance (PERACC) of treated baits among different groups of rats

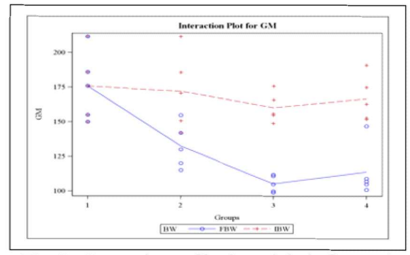

Fig. 3: Comparison of body weight before and after the treatment period among groups. IBW & FBM: Initial & Final body weight

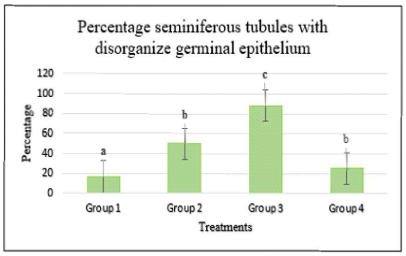

Fig. 4 Percent seminiferous tubules with disorganized germinal epithelium

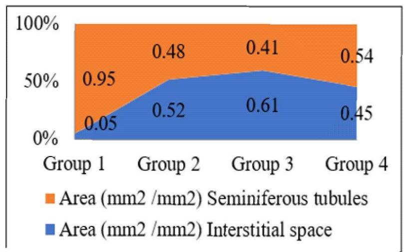

Fig. 5 Percentage area occupied by interstitial tissue and seminiferous tubules in different groups

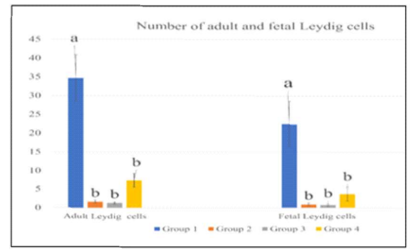

Fig. 6: Effect of treated baits on the number of adult and fetal Leydig cells

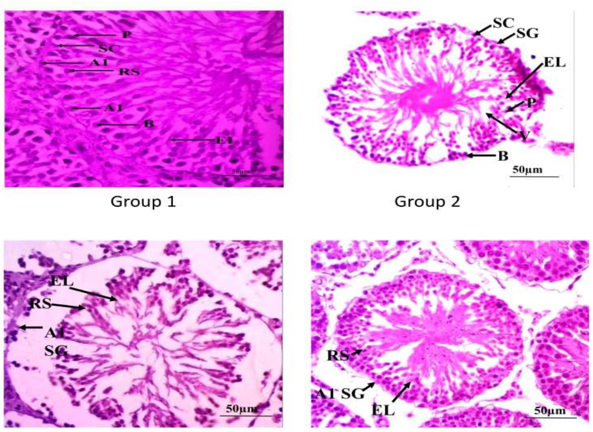

Fig. 7. Seminiferous tubules in different groups (PAS stained slides) showing vacuolization in groups 2 and 4 and exfoliation of germinal epithelium, pyknosis and reduction in the number of round spermatids in group 3, Magnification-400X

Sertoli cells supply nutrients and a favorable environment for growing spermatozoa, their absence or reduction in quantity in rats might result in lifelong infertility (Griswold 2018). Andrographolide affected spermatogenesis in male rats by preventing cytokinesis of the dividing spermatogenic cell lines. Sertoli cells were completely damaged and the spermatotoxic effect was also observed (Akbarsha and Murugaian 2000). Another study reported reduction in sperm density and the number of primary and secondary spermatocytes in rats treated with neem oil (Purohit et al., 2008). Ethanolic extract of all parts of *A. paniculata* given orally to adult male DDI mice @ 45mg/30 g bw impaired spermatogenesis and damaged Sertoli cells (Halim et al., 2005).

CONCLUSIONS

There was minimum antifertility effect of treated bait 3, which might be due to the thermo-degradation of secondary metabolites in the extracts at the time of preparation of their nano emulsion due to production of heat during probe sonication. Both the antifertility and toxic effect of treated bait 2, might be due to the synergistic effect of neem seed extract and andrographolide, addition of bio-stabilizer in this bait, which increased the thermo- and photostability and bioavailability of secondary metabolites in the bait. Adsorption of extracts in polymer masks the taste and also increased the bioavailability of secondary metabolites increasing its efficacy. As treated bait 2 is palatable and has both toxic and antifertility effects, can be successfully applied under field conditions for the long-term management of rodent pests. However, further studies are required to determine, the efficacy, the shelf life and stability of treated bait 2 under real field conditions.

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Agricultural Income Gains under Pradhan Mantri Krishi Sinchai Yojana (PMKSY): Evidence from North Eastern Karnataka, India

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Abstract: The Pradhan Mantri Krishi Sinchai Yojana (PMKSY), launched by the Government of India in 2015, aims to support small and marginal farmers through micro-irrigation facilities. The impact of PMKSY's on cropping patterns and income in the North Eastern Karnataka region was valuated. Comparative analysis shows that PMKSY beneficiaries have diversified their crops more significantly than non-beneficiaries, allocating greater area to high-value crops such as maize and groundnut during kharif, and Bengal gram and jowar during rabi, while non-beneficiaries predominantly grew traditional crops. Beneficiaries cultivated a gross cropped area of 132.44hectares and a net cropped area of 86.41hectares with a cropping intensity of 152.56 %, compared to cropping intensity of 135.72 % for non-beneficiaries. Income analysis reveals 23.11% increase in total income for beneficiaries, driven by a 28.87% rise in crop production income and 17.77% increase in income from other occupations. However, wage income for beneficiaries declined by 37.71 per cent reflecting reduced reliance on manual labour. The DID(Difference in difference) analysis reveals that the beneficiary farmers realised higher farm income per hectare compared to control farmers (₹73809/ha). These findings underscore the effectiveness of PMKSY in enhancing cropping efficiency and farmer income, highlighting its potential for future agricultural policy interventions.

Keywords: PMKSY, Cropping pattern, Income, difference in difference (DID)

Agriculture serves as the backbone of India's economy, employing a significant portion of the population and contributing substantially to the nation's GDP (Reddy et al., 2020, Suresh et al., 2019). However, the sector faces persistent challenges, including dependency on erratic rainfall, limited irrigation coverage, and inefficient water use. Recognizing these issues, the Government of India launched the Pradhan Mantri Krishi Sinchai Yojana (PMKSY) in 2015 (Anonymous 2015) aimed at achieving comprehensive irrigation coverage and improving water use efficiency under the themes of "Har Khet Ko Paani" and Per Drop More Crop (PMKSY 2025).

The North Eastern Karnataka region, characterized by semi-arid conditions and uneven rainfall patterns, represents an area where PMKSY has the potential to significantly transform agricultural practices and uplift socio-economic conditions. Small and marginal farmers, constituting the majority of the agricultural community in this region, often grapple with water scarcity, low crop yields, and financial instability. The implementation of PMKSY, particularly its micro-irrigation initiatives, offers a promising pathway to address these challenges by promoting sustainable water management and enhancing agricultural productivity. This study investigates the impact of PMKSY on the cropping pattern and income of respondent farmers in North Eastern Karnataka region by analysing changes in crop yields and water use efficiency. The findings will help gauge the effectiveness of the scheme in promoting sustainable agricultural practices and its role in improving the socio-economic conditions of farmers in water-scarce regions.

MATERIAL AND METHODS

Sampling design: The multistage random sampling procedure was used to select respondents for the study, conducted in two districts of the North Eastern Karnataka region, Koppal and Kalaburagi, based on their large area under micro-irrigation (Fig. 1). Two taluks from each district were selected, and a total of 120 respondents were chosen, including 60 beneficiaries and 60 non-beneficiaries of the Pradhan Mantri Krishi Sinchai Yojana (PMKSY). From each taluk, 3 villages were selected, and within each village, 5 beneficiaries and 5 non-beneficiaries were randomly chosen, resulting in 15 beneficiaries and 15 non-beneficiaries per taluk. Non-beneficiaries are farmers who do not receive PMKSY subsidies but may still use micro-irrigation systems.

Analytical Tools

Tabular presentation: Data on number of beneficiaries involved in the scheme, income and cropping pattern of beneficiary farmers under the PMKSY were analyzed by tabular analysis technique.

Difference in difference technique: Difference-in-Differences (DiD) is a quantitative method often used to estimate and compare change in outcome before and after scheme for beneficiary and non-beneficiary farmers. The advantage of using the double difference method is that it nets out the effects of additive factors that have fixed (time-invariant) impacts on income indicator, or that reflect common trends affecting beneficiary and non-beneficiary equally such as changes in income (Duflo et al., 2004, Verner et al., 2005, Sarma et al., 2015, Ravallion et al., 2005).

DD = (Y p1 – Y p0) – (Ynp1 – Y np0) -------(1)

Where,

DD=Income difference between the respondents

Y p1 = outcome (e.g., income) of beneficiaries after the PMKSY scheme;

Y p0 = outcome of beneficiaries before the PMKSY scheme;

Y np1 = outcome of non beneficiaries after the PMKSY scheme; and

Y np0 = outcome of non beneficiaries before the PMKSY scheme.

Paired 't' test: Paired 't' test was employed to assess the impact of PMKSY on income of beneficiary farmers in study area. The level of significance of difference was tested using pairedt-test.

$$X_1 = X_i - \bar{X}$$

$$Y_i = (Y_1 - Y_i)$$

Then t is defined as

$$\sqrt{\frac{n(n-1)}{\sum_{i=1}^{n}(\bar{x}_i - \bar{Y}_i)^2}}$$

Where,

X_1 and Y_1= two paired sample of beneficiary farmers and non-beneficiary farmers income respectively

n=sample size

n– 1 degree of freedom

The DID regression technique also provide us the same estimator along with the significance level (Gertler et al., 2010, Sinha and Laha 2019). The empirical specification of the regression can be written as follows:

$$Y = \alpha + \beta T + \gamma I + \theta(T.I) + €$$

Where *T* is a time dummy variable (*t* = 1 for after PMKSY started, *t* = 0 for before PMKSY started), and I is a treatment variable (*i* = 1 for beneficiary of PMKSY and *i* = 0 for non-beneficiary of PMKSY). The interaction effect (or the composite variable) *T.I* is a dummy variable (*t* = *i* =1 for PMKSY beneficiary's income after PMKSY scheme).

RESULTS AND DISCUSSION

Comparative cropping pattern of beneficiary and non-beneficiary farmers : During the *kharif* season, beneficiaries used 44.23 per cent of their gross cropped area, while non-beneficiaries used 53.46 per cent (Table 1). In the *rabi* season, beneficiaries allocated 32.62 per cent of their gross cropped area, compared to 26.35 per cent by non-beneficiaries. Beneficiaries also cultivated summer crops on 1.83 per cent of their gross cropped area, whereas non-beneficiaries did not grow any summer crops. Annual crops accounted for 21.31 per cent of the gross cropped area for beneficiaries and 20.19 per cent for non-beneficiaries.

Among beneficiaries, the major *kharif* crop was maize, covering 19.71 per cent of the gross cropped area (26.10ha), followed by bajra at 8.40 per cent and cotton at 7.56 per cent. In the *rabi* season, jowar was the leading crop at 13.83 per cent, with Bengal gram at 7.41 per cent and groundnut at 6.42

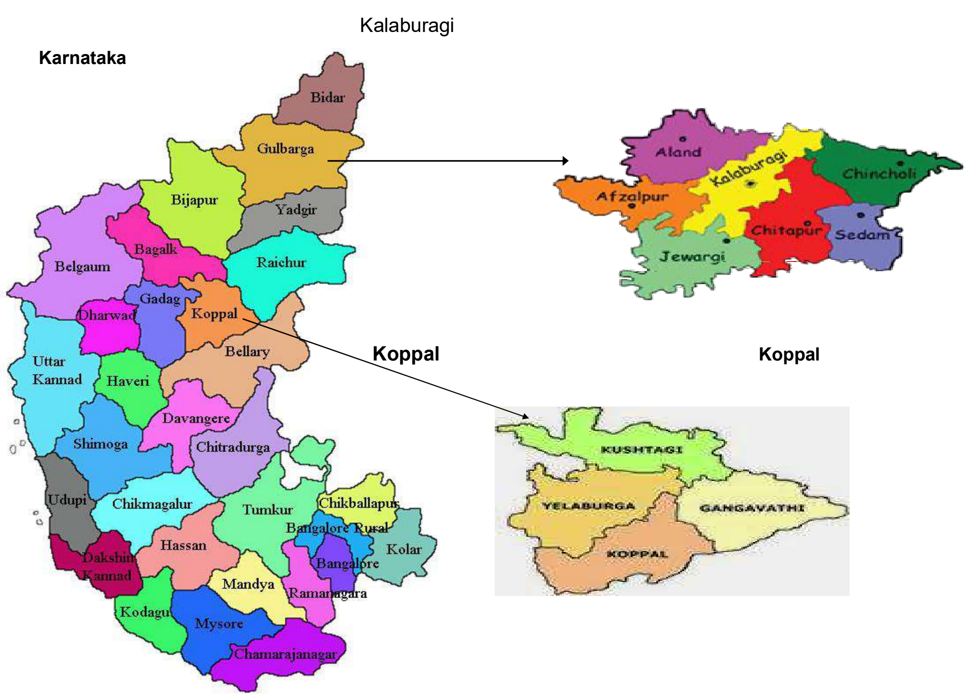

Fig. 1. Map showing the study area

per cent. Beneficiaries also allocated 1.83 per cent of their gross cropped area for summer vegetables, and their annual crops included were banana (11.69 per cent) and pomegranate (9.63 per cent). Non-beneficiaries primarily grew pigeonpea during the *kharif* season, accounting for 17.56 per cent of their gross cropped area (27.72ha), with maize covering 11.41 per cent and bajra at 8.46 per cent. In the *rabi* season, jowar was the primary crop at 7.18 per cent, followed by groundnut at 6.35 per cent and Bengal gram at 5.26 per cent. Non-beneficiaries did not grow summer crops, and their annual crops consisted of banana at 11.73 per cent and pomegranate at 8.46 per cent.

The gross cropped area of non-beneficiaries was 157.83 haand 132.44hectares for beneficiaries of PMKSY. The net cropped area was 86.81hectares for beneficiaries and for non-beneficiaries was116.25hectares. The PMKSY beneficiaries exhibited a higher cropping intensity of 152.56 per cent, compared to 135.77 per cent for non-beneficiaries indicating PMKSY beneficiaries exhibited a higher cropping intensity and adopted a more diverse cropping pattern compared to non-beneficiaries, especially in the *rabi* season. Beneficiaries allocated 32.62 per cent (43.20 ha) of their gross cropped area to *rabi* crops, while non-beneficiaries allocated 26.35 per cent (41.58ha). This indicated that more intensive land use by beneficiaries, supported by PMKSY, particularly in the cultivation of crops like jowar, Bengal gram, and groundnut. The adoption of sprinkler irrigation systems under PMKSY likely contributed to increased land utilization, allowing beneficiaries to manage water resources effectively for better crop productivity. Reddy et al. (2020), o studied the impact of PMKSY-Watersheds project in Srikakulam district of Andhra Pradesh and revealed that a series of farm ponds were constructed in farm fields and the beneficiaries started judicious use of farm pond water from different cultivation operations and more over farmers initiated the growing of horticultural crops like cashew, pomegranate, guava etc., on embankments in order to earn higher sustainable income.

Cropping pattern of beneficiary farmers of PMKSY: The cropping pattern of beneficiaries under PMKSY, revealed significant changes in crop allocation and intensity when compared the period before and after the implementation of the programme (Table 2). Before PMKSY, beneficiaries allocated 58.16 per cent of their gross cropped area to *kharif* crops, which decreased to 44.23 per cent after the program. Conversely, the area dedicated to *rabi* crops increased from 28.57 per cent to 32.62 per cent, indicating a more intensive use of land during

Table 1. Comparative cropping pattern of beneficiary and non-beneficiary farmers

Season	Crops	Beneficiaries		Non-beneficiaries	
		Area (ha)	%	Area (ha)	%
Kharif	Maize	26.10	19.71	18.01	11.41
	Bajra	11.13	8.40	13.35	8.46
	Cotton	10.02	7.56	10.93	6.92
	Groundnut	7.08	5.35	4.45	2.82
	Pigeonpea	4.25	3.21	27.72	17.56
	Paddy	0.00	0.00	9.92	6.28
	Sub total	58.58	44.23	84.38	53.46
Rabi	Jowar	18.31	13.83	11.33	7.18
	Bengalgram	9.81	7.41	8.30	5.26
	Groundnut	8.50	6.42	10.02	6.35
	Safflower	6.58	4.97	6.07	3.85
	Paddy	0.00	0.00	5.87	3.72
	Sub total	43.20	32.62	41.58	26.35
Summer	Vegetables	2.43	1.83	0.00	0.00
Biennial/ perennial	Banana	15.48	11.69	18.51	11.73
	Pomegranate	12.75	9.63	13.35	8.46
Sub total		30.66	23.15	31.87	20.19
Gross cropped area (ha)		132.44		157.83	
Net cropped area (ha)		86.41		116.25	
Cropping Intensity (%)		152.56		135.77	

this season. Beneficiaries also began cultivating summer vegetables, accounting for 1.83 per cent of the gross cropped area post-PMKSY, whereas no summer crops were grown pre-PMKSY. There was also a significant increase in annual crops, with pomegranate and banana expanding from 13.27 per cent to 21.31 per cent of the gross cropped area.

Specifically, maize remained the dominant *kharif* crop post-PMKSY, covering 19.71 per cent of the gross cropped area (58.58 ha), though its area decreased by 10.42 per cent compared to the pre-PMKSY period. Bajra and cotton became significant crops, contributing 8.40 per cent and 7.56 per cent respectively. During the *rabi* season, beneficiaries increased their focus on jowar, which covered 13.83 per cent of the gross cropped area marking to 54.70 per cent increase compared to pre-PMKSY. Bengalgram and groundnut also saw increased cultivation areas. The introduction of summer vegetables and the rise in annual crops like pomegranate (9.63%) and banana (11.69%) demonstrated the program's impact on diversifying cropping patterns.

The total gross cropped area increased slightly from 118.98 hectares pre-PMKSY to 132.44 hectares post-PMKSY, while the net cropped area grew marginally from 84.99 hectares to 86.81hectares. Cropping intensity improved from 140.00 per cent to 152.56 per cent. Although there was a reduction in the area allocated to *kharif* crops, the percentage of land dedicated to *rabi* and annual crops increased, showcasing how PMKSY helped beneficiaries diversify crop selection and utilize land more effectively. The adoption of micro irrigation systems, such as sprinkler and drip irrigation systems through PMKSY also, likely contributed to these improvements, leading to better land management, enhanced productivity, and increased cropping intensity. Suresh et al. (2019) in study on micro-irrigation development in India: an analysis of distributional pattern and potential correlates observed similar trend.

Comparative crop productivity among beneficiary farmers and non-beneficiary farmers The beneficiary farmers achieved a significant increase in crop productivity compared to non-beneficiaries, highlighting the impact of the PMKSY program and the use of micro-irrigation systems. Bajra productivity was 47.39 q/ha among beneficiaries, 33.94 per cent higher than the 35.38 q/ha produced by non-beneficiaries (Table 3). Bengalgram showed the largest difference with beneficiary farmers producing 20.95 q/ha, which was 60.61 per cent more than the 13.05q/ha harvested by non-beneficiaries. Similarly, in cotton, beneficiaries achieved a yield of 27.11 q/ha, 30.13 per cent higher than the 20.83 q/ha of non-beneficiaries. Groundnut yields among beneficiaries reached 23.5 q/ha, 24.48 per cent greater than the 18.88 q/ha from non-beneficiaries. For *rabi* jowar crop,

Table 2. Cropping pattern of beneficiary farmers of PMKSY

Season	Crops	Before PMKSY (2020)		After PMKSY (2023)		% Change
		Area (ha)	%	Area (ha)	%	
Kharif	Bajra	9.71	8.16	11.13	8.40	14.58
	Cotton	11.23	9.44	10.02	7.56	-10.81
	Groundnut	7.89	6.63	7.08	5.35	-10.26
	Maize	29.14	24.49	26.10	19.71	-10.42
	Pigeonpea	11.23	9.44	4.25	3.21	-62.16
	Sub total	69.20	58.16	58.58	44.23	-15.35
Rabi	Bengalgram	9.00	7.57	9.81	7.41	8.99
	Groundnut	8.09	6.80	8.50	6.42	5.00
	Jowar	11.84	9.95	18.31	13.83	54.70
	Safflower	5.06	4.25	6.58	4.97	30.00
	Sub total	33.99	28.57	43.20	32.62	27.08
Summer	Vegetables	0.00	0.00	2.43	1.83	-
Biennial/ perennial	Pomegranate	6.07	5.10	12.75	9.63	110.00
	Banana	9.71	8.16	15.48	11.69	59.38
Sub total		15.78	13.27	28.23	23.15	78.85
Gross cropped area		118.98		132.44		11.31
Net cropped area		84.99		86.81		2.14
Cropping Intensity (%)		140.00		152.56		8.97

beneficiaries produced 30.94 q/ha, 25.96 per cent more than the 24.56q/ha yielded by non-beneficiaries. In maize, beneficiaries saw a 31.07 per cent difference, producing 59.53 q/ha compared to 45.42 q/ha for non-beneficiaries. Pigeonpea productivity for beneficiaries was 18.01 q/ha, 33.03 per cent improvement over the 13.54 q/ha of non-beneficiaries, while safflower yields among beneficiaries stood at 11.19 q/ha, 39.38 per cent higher than the 8.03 q/ha recorded by non-beneficiaries. Among horticultural crops, banana and pomegranate also showed higher productivity. Beneficiaries produced 698.40 q/ha of banana, 14.71 per cent more than the 608.85 q/ha of non-beneficiaries, while pomegranate yields for beneficiaries were 155.28 q/ha, 5.83 per cent above the 146.73 q/ha for non-beneficiaries. These figures underscore the effectiveness of the PMKSY program in boosting crop yields through the use of micro-irrigation systems such as sprinkler and drip irrigation, leading to improved efficiency in water use for beneficiary farmers.

Comparative income level among beneficiary farmers and non-beneficiary farmers: The results in Table 4 demonstrate significant differences in income levels between beneficiary and non-beneficiary farmers. Beneficiary farmers reported higher income from crop production (₹5, 59, 050) compared to non-beneficiaries (₹4, 33, 800), showing a 28.87 per cent increase. Income from other occupations was also higher for beneficiaries (₹57,239) than non-beneficiaries (₹48,603), with a 17.77 per cent difference. Conversely, wage income was lower among beneficiaries (₹22,954) compared to non-beneficiaries (₹36,850), reflecting a 37.71 per cent lower income. Overall, the total income of beneficiary farmers (₹6, 39, 243) was higher than that of non-beneficiaries (₹5, 19, 253), indicating a 23.11 per cent increase. These findings highlight the positive impact of the intervention on beneficiaries' income levels, particularly through enhanced crop production. The results are in line with results reported by Kiran (2023) conducted study on socio-economic performance of Ganga Kalyana Yojana (GKY) in Ballari district of Kalyana Karnataka Region.

Table 3. Comparative crop productivity among beneficiary farmers and non-beneficiary farmers

Crops	Beneficiaries (n=60)	Non-beneficiaries (n=60)	% difference
Cereals			
Bajra	47.39	35.38	33.94
Rabi jowar	30.94	24.56	25.96
Maize	59.53	45.42	31.07
Pulses			
Bengalgram	20.95	13.05	60.61
Pigeonpea	18.01	13.54	33.03
Oil seed			
Groundnut	23.50	18.88	24.48
Safflower	11.19	8.03	39.38
Commercial crop			
Cotton	27.11	20.83	30.13
Horticultural crops			
Banana	698.40	608.85	14.71
Pomegranate	155.28	146.73	5.83

Impact of PMKSY on income of beneficiary farmers: Before the program, beneficiary farmers had a crop production income of ₹3, 27, 782, which increased significantly to ₹5,59,050 after PMKSY with a 70.56 per cent increase and statistically, demonstrating the program's strong positive effect on crop productivity (Table 5). Income from other sources, including livestock and poultry, increased from ₹46,520 to ₹57,239, marking a 23.04 per cent rise. This increase is also statistically significant indicating that PMKSY positively impacted additional income sources. Wage income for beneficiaries decreased by 20.03 per cent, from ₹28,705 to ₹22,954,indicated that reduction in reliance on wage labour, potentially due to higher income from other sources. Overall, beneficiaries' total income increased from ₹4,03,007 to ₹6,39,243, reflecting a 58.62 per cent rise. This increase, statistically significant, underscores the comprehensive positive impact of PMKSY on beneficiaries' financial well-being. Hence PMKSY led to significant improvements in crop production and other income sources for beneficiaries, despite a reduction in wage income. The program has effectively enhanced the overall financial stability and economic prospects of the farmers.

Double difference estimates of impact of PMKSY: The

Table 4. Comparative income level among beneficiary farmers and non-beneficiary farmers

Particulars	Total income (₹ per farm)			
	Non-beneficiary (n=60)	Beneficiary (n=60)	t-statistics	% difference
Crop production/year	4,33,800	5,59,050	5.05*	28.87
Other occupation	48,603	57,239	6.81*	17.77
Wage income	36,850	22,954	-8.43*	-37.71
Total income	5,19,253	6,39,243	4.72*	23.11

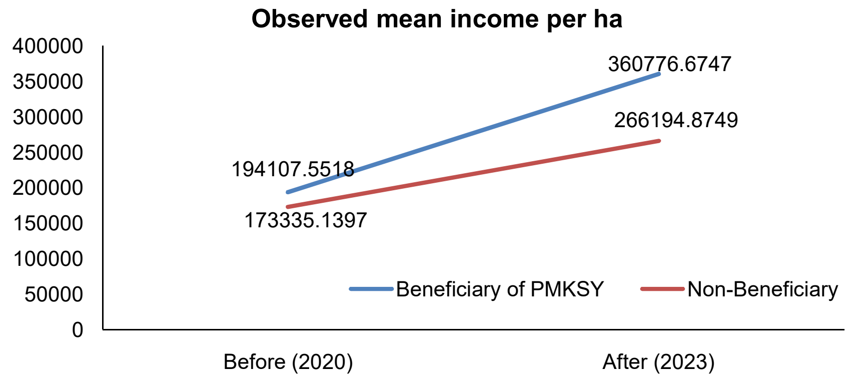

Fig. 2. Parallel trend in income per ha

Table 5. Impact of PMKSY on income of beneficiary farmers of PMKSY

Particulars	Total income (₹ per farm)			
	Before PMKSY	After PMKSY	t-statistics	% Change
Crop production/year	3,27,782	5,59,050	7.94*	70.56
Other occupation	46,520	57,239	19.36*	23.04
Wage income	28,705	22,954	-14.15*	-20.03
Total income	4,03,007	6,39,243	7.28*	58.62

Table 6. Double difference estimates of impact of PMKSY on income per ha

Particulars	Beneficiary of PMKSY	Non-Beneficiary	Difference
Before (2020)	194107.55	173335.14	20772.41
After (2023)	360776.67	266194.87	94581.80
Change	166669.12	92859.74	73809.39

Table 7. Difference-in-difference regression of income per hectare

Variables	Coefficients	Std. Error	t stat	Prob
Intercept	173335	31880	5.437	1.35e-07 ***
Treatment (I)	20772	45085	0.461	0.6454
Time (T)	92860	45085	2.060	0.0405**
DID (T*I)	73809	63760	1.158	0.2482
Adjusted R-squared: 0.0707				
F-statistic: 7.061*** p-value: 0.0001452				
No of observations :240				

mean income per hectare difference of the beneficiary and non-beneficiary farmers before and after the PMKSY was ₹ 20772 and ₹ 94581.80, respectively (Table 6). The positive mean double income difference of about ₹ 73809was realized between the beneficiary and non-beneficiary farmers. The PMKSY significantly benefited the beneficiary farmers (Fig. 2). The impact of PMKSY on farmers profit per hectare per year was ₹ 73809. The interaction between treatments (I) and time (T) variable shows a positive and significant impact of ₹73809/ha/season increase among PMKSY Beneficiary farmers after the participation in the PMKSY scheme (Table 7). Therefore, the DiD regression results are confirming the tabular results, which indicates that the PMKSY scheme has made an impact on beneficiary farmers' income in the study area (Bhavani et al., 2022).

CONCLUSION

The Pradhan Mantri Krishi Sinchayee Yojana (PMKSY) has had a measurable positive impact on farmers' income through increased irrigation coverage, productivity gains, and crop diversification**.** There was an increase in cropping intensity among PMKSY beneficiary farmers compared to non-beneficiaries, beneficiaries achieved higher crop productivity in bajra and Bengal gram. The beneficiary farmers saw a higher income from crop production compared to non-beneficiary farmers. The overall income of beneficiaries rose by 23.11 per cent, with a significant 58.62 per cent increase post-PMKSY. The DID analysis infers that the beneficiary farmers realised higher farm income per hectare compared to control farmers. These findings underscore the effectiveness of PMKSY in enhancing crop yields, diversifying farmers' income sources, and improving their financial stability. However, to achieve its full potential in doubling farmers' income, there is a need to strengthen extension services, ensure timely disbursal of subsidy, and scale up adoption of micro-irrigation in rainfed regions.

AUTHOR'S CONTRIBUTIONS

Shreekantha Reddy: Writing-original draft preparation, conceptualization, methodology, figure preparation and editing. Suresh K: Conceptualization, formal analysis, supervision, reviewing and editing. Amrutha T Joshi: Supervision and editing. Prabhuling Tevari: Visualization and editing. Kapil Patil: supervision and reviewing. All authors have read and agreed to the published version of the manuscript.

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Price Integration Analysis of Major Potato Markets in Haryana (Trans-Gangetic Plains), India

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***Abstract*:** The present study examines the market integration of potato prices across selected markets in Haryana which lies in the Trans-Gangetic Plains (predominantly semi-arid to sub-humid climate) using monthly time series data from 2015 to 2024, collected from various published sources such as AGMARKNET and related government databases. To understand the inter-market price relationships, various econometric tools were applied, including correlation analysis, Augmented Dickey-Fuller (ADF) test, Johansen co-integration test, and Granger Causality test. Initially, correlation analysis of monthly wholesale prices revealed strong associations among the markets, with coefficients ranging from 0.616 to 0.913, with significant differences, indicating a high degree of market linkage. The ADF test was employed to examine stationarity showed that most price series were stationary at level under both intercept and intercept-trend models. This allowed further analysis using Johansen's co-integration technique, which indicated the presence of two co-integrating equations at the 1% level, suggesting long-run equilibrium relationships among the markets. Granger Causality results revealed multiple significant unidirectional and bidirectional causal relationships among the markets. The study confirms the existence of strong price linkages and dynamic interactions among potato markets in Haryana.

***Keywords*:** Market integration, Correlation analysis, Co-integration test, Augmented dickey fuller test, Granger causality test

Potato (*Solanum tuberosum*), a perennial species of the Solanaceae family, is one of the most important edible tubers cultivated worldwide. The world potato production was about 383 million tonnes in 2023, placing it fourth among food crops after maize, wheat, and rice (FAOSTAT 2024). India ranks third globally in terms of the area under potato cultivation and stands as the second-largest producer after China. In 2024, the nation's potato output reached around 60.18 million tonnes, reflecting a significant rise compared with the preceding year (National Horticulture Board 2024). This growth highlights the positive impact of advanced cultivation practices and technological progress in agriculture. The marketing of potatoes is a primary concern for farmers because of the volatile nature of their prices (Singh et al., 2017). Potato prices fluctuate both within and between years. The total crop arrivals throughout the year heavily influence how much potato prices fluctuate within year. Seasonal variations in potato production can be attributed to several factors, including fluctuations in the overall cultivated land area, unexpected weather conditions, infestations of pests or diseases, fluctuations in the prices of other vegetables, and variations in demand from major urban areas and the agro-industry (Sreepriya and Sidhu 2020).

Long-term price correlations among different market hubs suggest that prices serve as significant indicators in the market, indicating that all exchange locations are interconnected and integrated (Ghosh 2010). Market integration is a significant economic concept, particularly relevant in the context of India, where analysing agricultural market integration holds particular importance. This is due to the significant share of food in the population's consumption basket. The high degree of market integration reflects the competitiveness among these markets. Integrated markets provide opportunities for farmers to specialize based on their comparative advantage. Conversely, non-integrated markets present misleading price information, leading to distorted production decisions, inefficiencies in agricultural markets, and negative consumer consequences, resulting in reduced production and sluggish growth (Mukhtar & Javed, 2008). Furthermore, market integration is crucial in influencing the pattern and pace of diversification towards high-value crops (Sidhu et al., 2010). Understanding the degree of market integration is crucial for effective resource allocation, price stability, food security, and nutrition (Muhammad & Mirza, 2014; Sonar et al., 2023) and will help in targeting agricultural price policies at specific geographic levels to ensure consistent access to food and price stability (Sharma and Kumari 2021).

Potato prices exhibit significant seasonal and regional variability, which poses challenges for consumers, producers, and policy planners. Accurate price forecasting is therefore crucial for effective market regulation and strategic planning. Numerous studies have previously sought to construct models to forecast prices of agricultural commodities (Paul et al., 2022). To effectively address the issue of price volatility, it is essential to understand the temporal behaviour of prices. Insight into the relationship between market arrivals and price levels helps in determining the extent and pattern of such fluctuations. One effective approach to this analysis is the assessment of market integration, which evaluates how price changes in one market influence prices in other markets-either immediately or after a delay (Saha et al., 2019). The strength and speed of this price transmission serve as indicators of the level of integration among markets across regions. The benefits accruing to producers and consumers largely depend on how well local markets are integrated with broader national or regional markets (Vigila et al., 2021). Studying market integration thus provides a measure of price co-movement across geographically distinct markets and helps farmers make informed decisions regarding the optimal timing, location, and quantity of produce to sell. The study aimed to analyse the price dynamics and degree of market integration within the key potato markets in India.

METHODOLOGY

India's potato production reached a record 601.75 lakh tonnes in 2024, while Haryana produced 843.23 thousand tonnes of potato during 2024 (India stat 2024). This study utilizes monthly time series data on potato prices from the selected markets in Haryana for the period 2015 to 2024 to examine the integration among these markets. The most important potato producing districts i.e. Kurukshetra, Yamuna Nagar, Karnal, Ambala and Sonipat were preferred purposely for the study. These districts together account for nearly 80 per cent of the total area and production of potato in Haryana. From each district, two markets were chosen on the basis of the arrival of potato in these markets. Thus, Thanesar and Shahbad markets from Kurukshetra district, Radaur and Jagadhari markets from Yamuna Nagar district, Karnal and Gharaunda markets from Karnal district, Ambala Cantt and Naraingarh from the Ambala district and Sonipat and Gohana markets from Sonipat district were selected. The time series data on prices and arrival of potato for various potato markets were collected from the Agricultural Produce Market Committees and other official websites for the period 2015 to 2024.

Market Integration: To evaluate the interdependence among potato markets in Haryana, several econometric tools were applied. The goal was to assess whether these markets operate in unison or show signs of spatial disconnection. The following tests were conducted to determine the degree of integration.

Correlation analysis: The preliminary method to assess market integration is the correlation analysis of price movements between markets. Pearson's correlation coefficient was employed to quantify the strength and direction of linear relationships between price series of different market pairs.

Correlation coefficient among two markets prices X and Y

$$r(X,Y) = \frac{Cov(XY)}{\sqrt{Var(x)}\sqrt{Var(y)}}$$

To test the statistical significance of the computed correlation coefficient r, a t-test was applied using the formula:

~t(n-2) degrees of freedom $t = \frac{r}{1-r^2}\sqrt{n-2}$

where n represents the number of observations. The hypotheses tested were:

Null Hypothesis (H_0): p = 0

Alternate Hypothesis (H_1): p ≠ 0

Augmented Dickey-Fuller (ADF) test: The monthly potato price data from the selected markets were examined for stationarity using the Augmented Dickey-Fuller (ADF) unit root test. A time series is considered stationary when its statistical properties-such as mean, variance, and autocorrelation-remain constant over time. If the price series is non-stationary at the level form, the first difference is taken and tested again. The number of differences required to achieve stationarity indicates the order of integration, expressed as I(d).

The ADF test is conducted by estimating the following regression model:

$$\Delta Y_t = \beta_1 + \beta_2 t + \delta Y_{t-1} + \sum_{i=1}^{m} a_i \Delta Y_{t-1} + \varepsilon$$

Where,

Y_t = price of potato in a specified market at time t

$\Delta Y_t = Y_t - Y_{t-1}$

$\mathcal{E}$ = pure white noise error term

m = optimal lag which is chosen based on Schwartz information criterion

Test for unit roots in the price series,

Null Hypothesis (H_0): prices series is non-stationary or unit root exists

Alternate Hypothesis (H_1): price series is stationary

If ADF test statistics (t*)<ADF critical value then accept the null hypothesis, i.e. unit root exists.

If ADF test statistics (t*)>ADF critical value then reject the null hypothesis.

Co-integration test: The Johansen cointegration test is based on the estimation of a Vector Auto-Regressive (VAR) model of order *k* in its error correction form as follows:

$$\Delta Y_t = \mu + \sum_{i=1}^{k-1} \Gamma_i \Delta Y_{t-i} + \prod Y_{t-k} + \varepsilon_t$$

Where,

Y_t = vector of non-stationary price variables at time t

Δ = first difference operator

Γ_i = short run adjustment coefficients

Π = long-run impact matrix that contains information on the cointegrating relationships

ε_t = error term

The rank of matrix Π determines the number of cointegrating vectors (r). If r=0, there is no cointegration; if 0<r<n, there are *r* cointegrating vectors indicating long-run price linkages among markets. This method utilizes the Trace Statistic and Maximum Eigenvalue Statistic to determine the number of co-integrating vectors. The number of such vectors is indicative of the degree of price co-movement across markets where a greater number signifies stronger and more stable price linkages.

Granger Causality Test: The Granger causality test was applied to examine the direction of causality among the market price series. Specifically, it tests whether past values of one variable (e.g., X_t) contribute to predicting another variable (e.g., Y_t) and vice versa. The possible causal linkages may appear as unidirectional (from X_t to Y_t or from Y_t to X_t), bidirectional, or absent. The autoregressive distributed lag (ADL) framework employed for this analysis is represented as follows

$$Y_t = a_0 + \sum_{j=1}^{m} a_j Y_{i-j} + \sum_{j=1}^{m} \beta_j X_{i-j} + \epsilon_{t1}$$

$$X_t = a_0 + \sum_{j=1}^{m} a_j Y_{i-j} + \sum_{j=1}^{m} \beta_j X_{i-j} + \epsilon_{t2}$$

Where,

t is the time period

ϵ_{ti} *and* ϵ_{t2} are the error terms

X and Y are the prices of different markets

RESULTS AND DISCUSSION

To understand the inter-market relationships, a cointegration analysis was performed. Initially, correlation analysis of the monthly wholesale prices of potatoes across the selected markets provided a basic approach to studying market integration. However, correlation analysis offers only rough estimates of price movement, prompting the use of advanced econometric tools such as the Johansen Cointegration Test and Granger Causality Test. Prior to analyzing time series data, stationarity testing is essential to avoid spurious results. Therefore, the Augmented Dickey-Fuller (ADF) test was employed to assess the stationarity of the variables. Once the variables were confirmed to be stationary and of the same order, the integration between the selected markets was further explored using the Johansen Cointegration Analysis method. Additionally, the Granger Causality Test was conducted to determine the direction of causality between the markets.

Correlation analysis: The degree of association between the prices of different markets can be represented using a zero-order correlation matrix. This approach assumes bivariate correlation coefficient of price movement in perfectly integrated markets tending towards unity, whereas, correlation coefficient tends towards zero in non-integrated markets.

The correlation coefficients were close to unity and statistically significant, suggesting strong integration among the potato markets in Haryana (Table 1). The correlation values ranged between 0.616 and 0.913, confirming that price movements in these markets were closely associated with one another. Similar findings on market integration were also reported by Isha Sharma et al. (2023) and Divyanshu et al. (2022).

Augmented-Dickey Fuller test: Augmented Dickey-Fuller (ADF) test indicate that the majority of the markets exhibit stationarity at level. Specifically, Naraingarh, Ambala Cantt, Jagadhari, Thanesar, Sonipat, Gharaunda, and Karnal show strong stationarity under both the intercept-only and intercept plus trend specifications, with p-values well below the 1%

Table 1. Correlation coefficients of potato prices in Haryana markets

Markets	Naraingarh	Ambala Cantt	Radaur	Jagadhari	Thanesar	Shahbad	Gohana	Sonipat	Gharaunda	Karnal
Naraingarh	1									
Ambala Cantt	.903***	1								
Radaur	.866***	.809***	1							
Jagadhari	.898***	.832***	.839***	1						
Thanesar	.706***	.680***	.752***	.689***	1					
Shahbad	.909***	.849***	.913***	.819***	.749***	1				
Gohana	.829***	.785***	.860***	.774***	.687***	.885***	1			
Sonipat	.753***	.786***	.786***	.769***	.769***	.765***	.792***	1		
Gharaunda	.623***	.616***	.810***	.652***	.853***	.741***	.750***	.766***	1	
Karnal	.617***	.650***	.668***	.637***	.653***	.684***	.664***	.707***	.684***	1

*** Indicates significant at 1% level,* * 5% level, * 10% level significance level (Table 2). Shahbad and Gohana also demonstrate stationarity but at a slightly weaker significance level (5% to 10%). Radaur, however, presents relatively weaker evidence of stationarity, with p-values near the 10% threshold, suggesting potential non-stationary behavior. Overall, the findings suggest that most market series are stationary at level, enabling modeling without the need for differencing. Similar findings were reported by Saha et al. (2019).

Table 2. Augmented Dickey-Fuller Test (ADF)

Markets	Particulars	At level t-statistic	p-value
Naraingarh	Intercept	-3.98***	0.0021
	Intercept+ Trend	-4.138***	0.0072
Ambala Cantt	Intercept	-4.26***	0.0008
	Intercept+ Trend	-4.42***	0.0031
Radaur	Intercept	-2.62*	0.0923
	Intercept+ Trend	-3.15*	0.0991
Jagadhari	Intercept	-4.48***	0.0004
	Intercept+ Trend	-4.64***	0.0014
Thanesar	Intercept	-4.48***	0.0004
	Intercept+ Trend	-5.75***	0.0000
Shahbad	Intercept	-3.38**	0.0133
	Intercept+ Trend	-4.013**	0.0108
Gohana	Intercept	-3.19**	0.0227
	Intercept+ Trend	-3.83**	0.0182
Sonipat	Intercept	-4.54***	0.0001
	Intercept+ Trend	-5.639***	0.0000
Gharaunda	Intercept	-3.18**	0.0231
	Intercept+ Trend	-5.06***	0.0003
Karnal	Intercept	-4.41***	0.0005
	Intercept+ Trend	-4.68***	0.0005

*** Indicates significant at 1% level, * * 5% level, * 10% level and NS- Non-Significant

Johansen Co-integration analysis: Both the Max-Eigen statistic and Trace statistic indicate the presence of co-integration (Table 3). The null hypothesis of no co-integration is rejected at the 1% level, with significant values for both statistics (Max-Eigen = 105.67, p = 0.0000; Trace = 355.39, p = 0.0000). Additionally, evidence of a second co-integrating relationship is also observed at the 1% level for "at most 1". Beyond this, the test statistics become insignificant, indicating no further long-run relationships. Therefore, it is concluded that there exist two statistically significant long-run equilibrium relationships among the potato markets under study, suggesting that these markets are integrated and tend to move together over the long term. The findings are in line with Jyoti Chaudhary et al. (2021).

Granger Causality test: The pairwise Granger causality results revealed extensive causal linkages among the selected potato markets in Haryana, indicating strong price interdependence (Table 4). Gharaunda emerged as a major dependent market, being Granger-caused by Ambala Cantt, Gohana, Jagadhari, Karnal, Radaur, Shahbad, and Thanesar, while exhibiting bidirectional relationships with Shahbad and Thanesar. Ambala Cantt showed significant two-way causality with Gohana, Jagadhari, and Naraingarh, and unidirectional linkages with several other markets. Gohana also demonstrated substantial influence, sharing bidirectional relationship with Karnal and Radaur.

Jagadhari and Karnal displayed both bidirectional and unidirectional causal connections with multiple markets, reflecting their key roles in the regional price formation process. Several markets, such as Naraingarh and Radour, show no significant causal relationships with other markets, indicating potential price isolation or lack of market integration. Sonipat and Thanesar were identified as major influencing markets, transmitting price signals to several others. The findings are in line with Shohe et al. (2019).

Overall, the analysis suggests that market integration in

Table 3. Johansen co-integration analysis

Co-integrating equations	Max-Eigen statistic	P-value	Trace statistic	P-value
None	105.6696	0.0000	355.3926	0.0000
At most 1	69.9108	0.0088	249.7230	0.0033
At most 2	53.9474	0.0920	179.8122	0.1145
At most 3	35.4426	0.6847	125.8647	0.5107
At most 4	26.4473	0.8861	90.4221	0.6902
At most 5	25.2802	0.6516	63.9749	0.7355
At most 6	15.6043	0.9273	38.6947	0.8875
At most 7	12.4751	0.8418	23.0905	0.8744
At most 8	5.8539	0.9616	10.6154	0.8961
At most 9	4.7615	0.6309	4.7615	0.6309

Table 4. Pair-wise granger causality test results in Haryana markets (Based on 119 observations)

Null hypothesis	F-statistic	Prob.	Relationship
AMB does not Granger Cause GHA	1.0414	0.3096	GHA→AMB
GHA does not Granger Cause AMB	3.4029*	0.0676	
GOH does not Granger Cause GHA	1.8561	0.1757	GHA→GOH
GHA does not Granger Cause GOH	3.9194*	0.0501	
JAG does not Granger Cause GHA	0.0738	0.7863	GHA→JAG
GHA does not Granger Cause JAG	7.9933***	0.0055	
KAR does not Granger Cause GHA	1.9339	0.1670	GHA→KAR
GHA does not Granger Cause KAR	10.5714	0.0015	
NAR does not Granger Cause GHA	0.1848	0.6681	NO CAUSALITY
GHA does not Granger Cause NAR	1.9180	0.1687	
RAD does not Granger Cause GHA	1.7088	0.1937	GHA→RAD
GHA does not Granger Cause RAD	11.3109***	0.0010	
SHA does not Granger Cause GHA	3.8975*	0.0507	SHA↔GHA
GHA does not Granger Cause SHA	2.9097*	0.0907	
SON does not Granger Cause GHA	1.3209	0.2528	NO CAUSALITY
GHA does not Granger Cause SON	1.2932	0.2578	
THA does not Granger Cause GHA	7.1013***	0.0088	THA↔GHA
GHA does not Granger Cause THA	3.8696*	0.0516	
GOH does not Granger Cause AMB	6.6391**	0.0112	GOH↔AMB
AMB does not Granger Cause GOH	6.6481**	0.0112	
JAG does not Granger Cause AMB	4.0955**	0.0453	JAG↔AMB
AMB does not Granger Cause JAG	10.8591***	0.0013	
KAR does not Granger Cause AMB	2.5288	0.1145	AMB→KAR
AMB does not Granger Cause KAR	5.1164**	0.0256	
NAR does not Granger Cause AMB	10.2805***	0.0017	NAR↔AMB
AMB does not Granger Cause NAR	4.7661**	0.0310	
RAD does not Granger Cause AMB	4.8914**	0.0290	RAD→AMB
AMB does not Granger Cause RAD	2.1054	0.1495	
SHA does not Granger Cause AMB	12.2787***	0.0007	SHA→AMB
AMB does not Granger Cause SHA	0.6505	0.4216	
SON does not Granger Cause AMB	19.7633***	2.E-05	SON→AMB
AMB does not Granger Cause SON	0.4768	0.4913	
THA does not Granger Cause AMB	10.6075***	0.0015	THA→AMB
AMB does not Granger Cause THA	0.1826	0.6699	
JAG does not Granger Cause GOH	1.3386	0.2497	NO CAUSALITY
GOH does not Granger Cause JAG	2.4065	0.1236	
KAR does not Granger Cause GOH	3.4996*	0.0639	KAR↔GOH
GOH does not Granger Cause KAR	5.8812**	0.0168	
NAR does not Granger Cause GOH	3.4528*	0.0657	NAR→GOH
GOH does not Granger Cause NAR	0.9107	0.3419	
RAD does not Granger Cause GOH	9.0277***	0.0033	RAD↔GOH
GOH does not Granger Cause RAD	2.9037*	0.0911	
SHA does not Granger Cause GOH	9.6689***	0.0024	SHA→GOH
GOH does not Granger Cause SHA	0.9276	0.3375	
SON does not Granger Cause GOH	16.1805***	0.0001	SON→GOH
GOH does not Granger Cause SON	0.1662	0.6842	
THA does not Granger Cause GOH	10.6772***	0.0014	THA→GOH
GOH does not Granger Cause THA	0.0371	0.8477	
KAR does not Granger Cause JAG	4.2961**	0.0404	KAR↔JAG
JAG does not Granger Cause KAR	4.3502**	0.0392	
NAR does not Granger Cause JAG	7.1170***	0.0087	NAR→JAG
JAG does not Granger Cause NAR	0.0643	0.8003	
RAD does not Granger Cause JAG	4.4313**	0.0374	RAD→JAG
JAG does not Granger Cause RAD	0.0102	0.9198	
SHA does not Granger Cause JAG	7.5585***	0.0069	SHA→JAG
JAG does not Granger Cause SHA	0.6706	0.4145	
SON does not Granger Cause JAG	17.4196***	6E-05	SON→JAG
JAG does not Granger Cause SON	2.0366	0.1562	

Cont...

Table 4. Pair-wise granger causality test results in Haryana markets (Based on 119 observations)

Null hypothesis	F-statistic	Prob.	Relationship
THA does not Granger Cause JAG	18.1322***	4E-05	THA→JAG
JAG does not Granger Cause THA	4.3832**	0.0385	
NAR does not Granger Cause KAR	2.9806*	0.0869	NAR↔KAR
KAR does not Granger Cause NAR	4.1619**	0.0436	
RAD does not Granger Cause KAR	8.0579***	0.0054	RAD→KAR
KAR does not Granger Cause RAD	2.4856	0.1176	
SHA does not Granger Cause KAR	6.0800**	0.0151	SHA→KAR
KAR does not Granger Cause SHA	2.4162	0.1228	
SON does not Granger Cause KAR	11.4302***	0.0010	SON↔KAR
KAR does not Granger Cause SON	3.5471*	0.0622	
THA does not Granger Cause KAR	7.1280***	0.0087	THA→KAR
KAR does not Granger Cause THA	1.6561	0.2007	
RAD does not Granger Cause NAR	0.5337	0.4665	NO CAUSALITY
NAR does not Granger Cause RAD	0.1267	0.7225	
SHA does not Granger Cause NAR	3.3450*	0.0700	SHA→NAR
NAR does not Granger Cause SHA	1.0179	0.3151	
SON does not Granger Cause NAR	9.3000***	0.0028	SON→NAR
NAR does not Granger Cause SON	0.0019	0.9648	
THA does not Granger Cause NAR	10.3857***	0.0016	THA→NAR
NAR does not Granger Cause THA	1.4873	0.2251	
SHA does not Granger Cause RAD	7.7146***	0.0064	SHA→RAD
RAD does not Granger Cause SHA	0.0257	0.8730	
SON does not Granger Cause RAD	11.8798***	0.0008	SON→RAD
RAD does not Granger Cause SON	0.2233	0.6374	
THA does not Granger Cause RAD	16.1383***	0.0001	THA→RAD
RAD does not Granger Cause THA	0.2596	0.6114	
SON does not Granger Cause SHA	9.3777***	0.0027	SON↔SHA
SHA does not Granger Cause SON	2.8803*	0.0924	
THA does not Granger Cause SHA	9.8389***	0.0022	THA→SHA
SHA does not Granger Cause THA	0.0219	0.8827	
THA does not Granger Cause SON	0.9048	0.3435	NO CAUSALITY
SON does not Granger Cause THA	2.4438	0.1207	

Significance at ***1% level, ** 5% level and *10% level

Haryana is not uniform, with some markets playing more central roles in price transmission, while others may have more localized price behaviour. The presence of strong integration among certain market pairs indicates efficient information flow and quick price adjustments, whereas weaker linkages in others may be attributed to differences in infrastructure, market arrival patterns, or distance from major consumption centers. These findings provide important insights for policymakers and market participants looking to understand price dynamics and improve market efficiency in Haryana's potato sector. Promoting better storage, transportation, and real-time market information systems could further improve price transmission and reduce spatial price disparities across markets.

CONCLUSION

The study concludes that potato markets in Haryana are significantly integrated, as evidenced by strong correlations, confirmed cointegration, and dynamic causal linkages among the major markets. The correlation analysis suggested a strong positive association in price movements, Johansen's cointegration test established long-term equilibrium relationships, indicating that despite short-term fluctuations, prices tend to converge across markets over time. Granger causality results further highlighted the presence of both unidirectional and bidirectional influences, with markets like Sonipat and Thanesar emerging as key price leaders, while others such as Gharaunda showed greater dependence on external signals. Collectively, findings affirm the existence of a unified market system with efficient price transmission across regions, underscoring the importance of strengthening market infrastructure, improving information dissemination, and formulating supportive policies to enhance market efficiency and ensure better returns for farmers.

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Livelihood Security of Migrant and Non-Migrant Labour Households in Northern Dry Zone of Karnataka

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Abstract: The study examines the nature of labour migration and its influence on the livelihood security of migrant and non-migrant labour households in the Northern dry zone of Karnataka. A multistage purposive random sampling technique was employed to select migrant and non-migrant labour households. Primary data were collected through well-structured interview schedules from total of 320 sample respondents, comprising of 160 migrants and 160 non-migrant labour households from Vijaypur in Gadag districts respectively. The analytical tools such as descriptive statistics and livelihood security indices were used for the analysis. The Gadag district pendular migration (movement for less than a day) was predominant (55%), whereas seasonal migration (up to 3 months) was most common in Vijaypur district (47.5%). Temporary and permanent migration were observed to a lesser extent in both districts. Migrant households generally have higher economic, educational and overall livelihood security in both the districts. In Vijaypur district, migrants secured significantly higher food security (0.852) and the overall livelihood security index (0.835). whereas, non-migrants showed stronger livelihood security indices in health, habitat and social network dimensions, indicating a trade-off between mobility and stability. Similar patterns were observed in Gadag district, with more pronounced differences. These findings underscore the complex, multidimensional impact of labour migration and provides valuable inputs for targeted policy interventions, strengthening support systems for migrants while addressing the vulnerabilities of non-migrants is essential for enhancing rural livelihood resilience and promoting balanced regional development.

Keywords: Livelihood security index, Multidimensional impact, Pendular migration and seasonal migration

In India, a large share of the population is directly or indirectly dependent on agriculture and allied activities, as farming forms a core part of daily life in the subcontinent. The sector employs nearly 70 per cent of the country's workforce, supplying food for the people, raw materials for industries, fuelwood and timber for shelter, as well as herbs used in traditional medicine. More importantly, agriculture continues to be the backbone of rural livelihoods providing both sustenance and income (Bose and Dey 2007, Upadhyay and Palanivel 2011). For developing economies like India, agriculture ensures survival not only in farm-based occupations but also in non-farm sectors that rely on it. Strengthening this sector is essential to improving rural livelihoods (Shyamali and Saini 2010). Livelihood security further emphasizes stable access to resources, income-generating activities and assets that helps reduce risks, cope with shocks and handle uncertain situations (Ijarotimi and Oyeneyin 2005, Salim et al., 2013). Rural households earn their livelihoods from a variety of sources. Many depend directly on agriculture, others find work in the rural labour market, engage in self-employment within the non-farm sector or migrate to towns, cities and even abroad in search of better opportunities. Agriculture remains the dominant source of livelihood not only in India but across the Asia-Pacific region, although in some countries the rural non-farm sector also makes a significant contribution (Bhuvaneshwari 2008, Aliber and Tom 2009). Migration, in particular has become a vital means of income generation as the remittances sent by migrants help households to cope with financial shocks and safeguard their productive assets. Household livelihood security refers to stable and sustainable access to resources and income that can meet essential needs such as food, safe drinking water, healthcare, education, housing and opportunities for social participation. Livelihoods are often built on a combination of farm and non-farm activities, which together create different strategies for earning cash and securing food (Baiphethi and Jacobs 2009, Akter and Rahman 2012). Each household therefore relies on multiple sources of entitlement shaped by its assets and its position within the broader social, political and legal systems it operates in (Conelly and Chaiken 2000).

The extent to which households remain vulnerable depends on the likelihood of as its impact on food security, income stability, health, and nutrition. A livelihood can be considered secure only when households have reliable ownership or access to resources and income-generating opportunities, alongside reserves and assets that allow them to handle risks, overcome shocks and manage unexpected crises (Ellis 2000). Migration is able to withstand and recover from stress and shocks, maintain its assets and capabilities, and create opportunities for future generations. However, not all households possess the same capacity to cope with such challenges. In particular, poorer families often face difficult trade-offs as they must juggle between preserving assets, ensuring immediate food needs, and generating income for both present and future requirements (Bagchi and Majumdar 2011). The present study was undertaken with objective of labour migration and its influence on the livelihood security of migrant and non-migrant labour households in the Northern dry zone of Karnataka.

MATERIAL AND METHODS

The present study was undertaken in Vijayapur and Gadag districts of Karnataka. Vijayapur District, located in northern Karnataka at 16.8302° N latitude and 75.7100° E longitude, lies at an elevation of about 606 meters above sea level and forms part of the semi-arid Deccan Plateau region. Gadag District, situated in central-north Karnataka at 15.4334° N latitude and 75.6387° E longitude with an elevation of around 655 meters, is characterized by dryland agriculture and a transitional climate between semi-arid and dry tropical zones. The multi-stage purposive random sampling technique was employed for the selection of the sample respondents. Primary data were collected through well-structured interview schedules from total of 320 sample respondents comprising of 160 migrants and 160 non-migrant labour households from Vijaypur and Gadag respectively. The analytical tools such as descriptive statistics and livelihood security indices were used.

Conceptual frame work: Migration was classified into four categories pendular, seasonal, temporary and permanent based on the duration of stay away from the native village. The household livelihood security index (HLS) uses a balanced weighted average approach with a large number of indicators, where each indicator assumed to contribute equally to the overall index. The indicators are grouped into different domains representing the security areas such as economic, nutrition, health, education, habitat and socio-network security.

Economic security: This includes annual income earned, value of land, value of livestock, value of household farm assets and household savings

Food security: This consists of annual consumption expenditure and quantity consumed

Education security: This is based on number of years of schooling of adult males, number of years of schooling of females and number of years of schooling of children.

Health security: comprises yearly expenditure on health problems and availability of health care centers.

Habitat Security: This includes type of house (Pakka house, semi pakka and kaccha house) availability of safe drinking water and presence of toilet facility.

Social–network security: This refer to number of members participating in institutions.

Since each indicator is measured on a different scale, indicators are standardized following the approach adopted in measuring 'Life Expectancy' in Human Development Reports (Akter and Rahman 2012).

Standardized indicator j is given by:

$$\text{zindj} = \frac{\text{indicatorj} - \min \text{j}}{\max \text{j} - \min \text{j}}$$

Where minimum and maximum values of the indicators are from the same community to which the household belongs. Once each indicator representing a particular livelihood security domain is standardized, then the relevant household livelihood security index for the particular domain is constructed by averaging the standardized indicators:

$$HLSj = \frac{\sum^{j}_{j=1} z\ ind\ j}{j}$$

Where: J is the number of indicators used to construct the index. The composite overall livelihood security (CLS) index for the household is constructed by using the formula.

$$\text{CLS} = \frac{\sum^{n}_{i=1} \text{wi HLSi}}{\sum^{n}_{i=1} \text{wi}}$$

Where:

w - Indicates the weights determined by the number of indicators used to construct each HLS index. Weights vary between households, because of the variation in the number of indicators at the household level.

RESULTS AND DISCUSSION

Vijaypur district, pendular migration (less than 24 hours or within a day) accounted for 31.25 per cent of the respondents (Table 1). This implies that, nearly one-third of the households were engaged in short distance commuting, largely to nearby towns or villages for wage employment. In contrast, pendular migration was considerably higher in Gadag district 55.00 per cent**.** The relatively higher incidence of this type of migration in Gadag suggests greater availability of employment opportunities in close proximity, thereby enabling labourers to return home on a daily basis without incurring relocation costs. Seasonal migration (up to three months) emerged as the dominant form in Vijaypur district 47.50 per cent while only 22.50 per cent of Gadag district households fell into this category. Seasonal migration is typically associated with agricultural operations such as sowing, weeding and harvesting. The higher share in

Vijaypur district, attributed to the district's dependence on rainfed agriculture which often compels labour households to seek supplementary employment during lean periods.

Temporary migration (less than one year) was observed among 12.50 per cent of the respondents in Vijaypur district and 10.00 per cent in Gadag district. This form of migration generally involves movement to nearby cities for construction, industrial work or non-farm activities. The similarity in percentages across both districts indicates that, households in both areas resort to such migration when local employment opportunities are insufficient. Permanent migration (more than one year) was relatively less prevalent with 8.75 per cent of in Vijaypur district and 12.50 per cent in Gadag district respectively. Permanent migration often reflects a structural shift where households relocate in search of stable employment, improved living conditions and better access to social amenities. The higher proportion in Gadag district could be indicative of greater urban pull factors such as better non-farm job prospects. The findings highlighted district-level contrast in the nature of labour migration. While Vijaypur district households were more inclined towards seasonal migration due to the agrarian nature of the local economy, Gadag district households exhibited a higher tendency for pendular and permanent migration, possibly reflecting relatively better connectivity, diversified employment opportunities and urban influence. Deshingkar and Start (2003); Srivastava (2011) also emphasize that the nature and extent of migration are shaped by local economic structures, availability of wage opportunities and household strategies for livelihood security. Distinct-wise variations in livelihood security between migrant and non-migrant labour households across Vijaypur and Gadag districts of Karnataka highlights the composite livelihood security index and its sub-components shows that, migration plays a dual role in enhancing certain dimensions of livelihood while constraining others (Table 2).

Food security: In Vijaypur district, both migrant (0.852) and non-migrant households (0.847) exhibited high food security index, with a marginal but significant difference. This indicates that, migration has not markedly influenced food access and availability, as both groups benefit from government food welfare schemes such as the public distribution system (PDS) ensuring minimum food support to all categories. In Gadag, food security was comparatively lower but remained higher among migrants (0.510) than non-migrants (0.473. The slightly better performance of migrant households may be attributed to remittance income, which supplements food expenditure during lean agricultural periods. Rao and Veena (2018) and Pingali et al. (2019)**,** also observed that universal food subsidy programs reduced rural food insecurity across income categories.

Economic security index: The economic security index was significantly higher for migrants (0.450) than non-migrants (0.310). This clearly demonstrates that, migration enhances household income and savings capacity. Migrants gain access to regular employment in construction, transport and service sectors leading to higher cash inflows and financial resilience. Conversely, non-migrants depend heavily on seasonal agricultural labour, which is uncertain in dryland regions. Similar conclusions were reported by Deshingkar and Farrington (2009) and Keshri and Bhagat (2012) and Rao and veena (2018), emphasizing migration as a key income diversification strategy in drought-prone areas.

Education security: Education security showed a substantial difference between migrant (0.642) and non-migrant households (0.451. The improved educational performance among migrants can be attributed to higher household earnings and greater awareness of education gained through urban exposure. Remittance income allows migrant families to afford better schooling and educational materials. Stark and Taylor (1991) and Haan (1999) also observed that remittance-receiving households invest more in education and skill development, thereby improving future livelihood prospects.

Health security: Health security remained relatively low for both groups, but non-migrants (0.226) performed better than

Table 1. Labour migration pattern of the sample respondents in study area

Type of migration	Vijaypur district (n=80)	Gadag district (n=80)	Pooled (160)
Nature of migration (Based on the number of days)			
Pendular migration (< 24 hours or < 1 day)	25 (31.25)	44 (55.00)	69 (43.12)
Seasonal migration (up to 3 months)	38 (47.50)	18 (22.50)	56 (35.00)
Temporary migration (< 1 year)	10 (12.50)	08 (10.00)	18 (11.25)
Permanent migration (>1 year)	07 (8.75)	10 (12.50)	17 (10.63)

Note: Figures in parentheses represent percentage to respective sample total

Table 2. Livelihood security of migrant and non-migrant labour households in Northern dry zone of Karnataka

Districts	Particulars	Migrants	Non-migrants	t-value	Range of livelihood securities	
					Low	High
Vijaypur District (n=160)	Food security Index	0.852	0.847	1.882*	0.638	0.999
	Economic security Index	0.470	0.345	2.187**	0.332	0.508
	Education security Index	0.585	0.445	3.429***	0.367	0.785
	Health security Index	0.227	0.287	3.149***	0.234	0.356
	Habitat security Index	0.340	0.463	5.504***	0.350	0.565
	Social network security Index	0.285	0.309	4.411***	0.192	0.389
	Overall livelihood security Index	0.835	0.774	2.143**	0.731	0.925
Gadag District (n=160)	Food security Index	0.510	0.473	3.209***	0.382	0.630
	Economic security Index	0.429	0.275	2.696***	0.342	0.585
	Education security Index	0.698	0.456	1.844*	0.423	0.854
	Health security Index	0.133	0.165	2.534***	0.109	0.283
	Habitat security Index	0.172	0.210	1.816*	0.115	0.275
	Social network security Index	0.198	0.282	2.062**	0.147	0.336
	Overall livelihood security Index	0.811	0.645	2.692***	0.670	0.821
Pooled (n=320)	Food security Index	0.681	0.660	1.252*	0.382	0.999
	Economic security Index	0.450	0.310	5.350***	0.332	0.585
	Education security Index	0.642	0.451	4.395***	0.367	0.854
	Health security Index	0.180	0.226	2.746**	0.109	0.356
	Habitat security Index	0.256	0.337	4.837***	0.115	0.565
	Social network security Index	0.242	0.296	3.228***	0.147	0.389
	Overall livelihood security Index	0.823	0.710	2.124**	0.670	0.925

*** Significant at 1 per cent, ** significant at 5 per cent and *significant at 10 per cent

migrants (0.180). The lower health index among migrants can be explained by poor occupational conditions, lack of sanitation at workplaces and limited access to healthcare facilities in urban areas. Migrant workers often lack insurance coverage and depend on informal medical services. Mosse et al. (2005) and Srivastava (2011) also noted that migrant labourers face exclusion from organized healthcare systems and are vulnerable to work-related illnesses.

Habitat security: The Habitat Security Index revealed that non-migrants (0.337) had significantly better housing conditions than migrants (0.256). Non-migrant households usually reside in their own dwellings with basic amenities, whereas migrants live in temporary or rented accommodations near their work sites, often without proper sanitation or water facilities. Mosse et al. (2005) and Kundu (2009), also documented inadequate housing conditions among urban migrants due to the informal nature of employment and residence.

Social network security: The social network security index was higher among non-migrants (0.296) compared to migrants (0.242), with significant difference. Migration disrupts traditional community linkages, social participation and mutual support systems. Migrants, being geographically distant, have less interaction with local institutions and community activities. Non-migrants, on the other hand, maintain stronger ties with village networks and self-help groups. Mukherjee and Dutta (2017), also highlighted the weakening of traditional social safety nets among migrant households, particularly in rural India, where social capital plays a critical role in times of distress.

Overall livelihood security: The overall livelihood security index showed a significant difference, with migrants (0.823) outperforming non-migrants (0.710). This indicates that despite facing disadvantages in health, habit and social dimensions, migration ultimately contributes positively to overall livelihood security through improved income and education. Rao and Veena (2018) also observed that migration enhances household resilience and asset creation, helping rural families adapt to environmental and economic uncertainties.

CONCLUSION

Migration has emerged as an important livelihood strategy for rural households, particularly in regions experiencing persistent agrarian distress, underemployment and climatic uncertainties. The process of migration significantly influences livelihood security across multiple dimensions including food, economic, educational, health, habitat and social well-being. The, migration plays a dual role in shaping household livelihoods in the Northern Dry Zone of Karnataka. Migrant households achieved higher levels of economic, educational and overall livelihood security, primarily due to diversified income sources and exposure to urban opportunities. However, they lagged in health, housing and social connectivity because of insecure living conditions and limited access to welfare services at destination areas. Hence, while migration acts as a significant livelihood diversification strategy in semi-arid regions, it also creates new vulnerabilities that need policy attention. The welfare of migrant workers necessitates a multidimensional policy approach that integrates health, housing, skill development, employment generation and social protection. State governments, in collaboration with the central government reinforce health insurance initiatives such as Ayushman Bharat and E-Shram portal to ensure comprehensive healthcare access for migrant populations across origin and destination regions. Enhancement of housing facilities through the effective implementation of the Pradhan Mantri Awas Yojana (PMAY) and the development of state-level rental housing frameworks, with specific provisions for seasonal, temporary and permanent migrants residing in informal settlements. Furthermore, targeted interventions under the Deen Dayal Upadhyaya Grameen Kaushalya Yojana (DDU-GKY) can facilitate skill development and education, thereby improving the employability and job security of migrant workers in diverse sectors. Strengthening rural employment alternatives through the expansion of the Mahatma Gandhi National Rural Employment Guarantee Act (MGNREGA) and the promotion of non-farm rural enterprises can mitigate distress migration and enhance livelihood resilience among non-migrant households. In addition, the establishment of inclusive social protection networks via local institutions, cooperatives and self-help groups is vital for integrating migrant families within community systems, reducing social exclusion and fostering sustained social cohesion.

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Engineering Interventions for Increasing Farm Income and Natural Resources Conservations in Upper Krishna Command Area in North Eastern Dry Zone of Karnataka

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Abstract: Engineering interventions helps to maximizing agricultural productivity and profitability with a minimum drudgery to farm workers, especially women. In the present study, nine interventions were demonstrated at Yadgir district by ICAR-Krishi Vigyan Kendra from 2019 to 2024. The direct seeded rice (DSR), drum seeder (DS) and laser levelling (LL) has achieved the water productivity (kg/ha/mm) of 5.90, 4.50 and 4.66 compared to 4.28, 3.39 and 4.66 by saving irrigation water (%) of 22, 15 and 23.17 as compared to transplanting paddy. Use of nipping machine in pigeonpea and chickpea increased yield up to 10.25 (%) and 8.29 (%) compared to farmers practices. Cotton shredder saved 80 (%) and 55.45 (%) of labour and management cost in cotton crop. Compartment bunder in greengram helps to achieve water productivity (kg/ha/mm) and yield (q/ha) of 10.06 and 19.80 compared to 7.5 and 14.75 in farmers practices. Raised bed with plastic mulch in watermelon increased water productivity (kg/ha/mm) and yield (q/ha) of 144.45 and 650 compared to 64.44 and 388 in farmers practices by saving 33.33 (%) of irrigation water. Adaptation of engineering interventions helps to increase water productivity and crop yield by saving irrigation water, time and labour cost.

Keywords: Crop yield, Drudgery, Irrigation, Water productivity

Engineering interventions are multidisciplinary approach which integrates principles of engineering of various other disciplines with agricultural sciences for the development of innovative solutions which aims for enhancing agricultural practices for natural resources management (Sreedevi et al., 2024, Madhusudan et al., 2025). Demonstrations of such pivotal technologies provides solutions to the challenges faced by the agriculture sector, like increasing global food demand, limited land resources, and environmental sustainability. The need for engineering arises to address the growing population and the need to produce more food efficiently with limited land and other resources (Jerzak and Śmiglak-Krajewska 2020). Traditional farming methods had limitations like less productivity, un judicious use of resource utilization and environmental impact (Patel et al., 2020, Thakur and Sidana 2024,). Engineering interventions provides solutions to these challenges by applying engineering principles and technologies for development of sustainable and efficient farming systems. The aim of engineering technologies is to increase the availability of machinery, irrigation systems, and post-harvest processing techniques to optimize agricultural production. They focus on the areas like improving crop yields, minimizing waste, conserving resources, and reducing the environmental footprint of farming operations.

Mechanization in agriculture is the use of various machinery and equipment to ease farming operations, which reduces the dependence on manual labour and drudgery (Mentsiev et al., 2020). Agricultural machinery involves a variety range of equipment, including tractors, harvesters, planters, sprayers, and tillage tools. These machines are designed to perform specific tasks efficiently, which saves time and labour and improves productivity (Apoorv et al., 2025). The development of agricultural machinery has greatly affected farming practices in positive ways, which enable farmers to accomplish operations quickly and effectively. These modern machineries are equipped with modern advanced technologies such as GPS, computer control systems, and sensors, which allow precise and efficient operation. With population growth in progress, the global agriculture industry has also to progress, which is currently at 10 percent of the global GDP, to grow at least 60 percent by 2030 in order to keep the demand in check (Mentsiev et al., 2020).

Water is the fundamental resource for agriculture, and most important input for crop growth, development, and yield (Prikxit et al., 2025). Irrigation is the artificial application of water to the plants for meeting the evapotranspiration need of the plant. If there is lack of adequate water supply during various growing season of the plant optimal plant health and productivity are severely affected which also increase susceptibility to pests and diseases, and overall farm productivity decreases by many folds (Tamboli et al., 2025). Irrigation plays an important role in the productivity of the framing system and growth of agriculture, where the consumption of fresh water no less than 75 percent globally (Ramachandran et al., 2022). Water unavailability can be a major factor for the production and productivity of the agriculture sector. There are various water conservation techniques available which are essential for sustainable agriculture, particularly in regions facing water scarcity or drought conditions (Umesh et al., 2020). The study presents results of demonstration of different engineering interventions at farmers field for reducing cost of production, labour management, resource conservation and increase the crop yield under different cropping system.

MATERIAL AND METHODS

Study area: The present study was conducted at Yadgir district of Kalyan Karnataka region. The normal rainfall of the district is 710 mm and major crops in the district are paddy (148167 ha), cotton (167471 ha) and groundnut (21244 ha) of total cultivable area of 723601 ha with 222828 ha of net irrigated area. The major soil group is medium deep red clay soils covering an area of 153000 ha in the district.

Villages and farmers selection: The study was conducted at six villages of Yadgir district in Karnataka state located at 16°46'12" N and the longitude of 77°8'15" E (Fig. 1) through participatory rural appraisal (PRA) procedure. Parameters considered for selecting villages and farmers were major crop growing and area, percent of small and marginal farmers, farmers interest, soil type, farmers education and other socio-economic levels. Two farmers were selected from each village based on their interest to adopt the new technologies. Data related to crop inputs used, cost of production, labour used and market rates were recorded for both farmers practices and front-line demonstrations.

Technology selection: The technologies selected for demonstration based on cropping system, identified field problems, rainfed and irrigation situation, labour management and farmer's demand. Suitable technologies were selected and demonstrated to overcome these problems and increased farm income (Table 1).

Data collection and calculation: The field data were collected time to time under demonstration and farmers

Table 1. Technologies demonstrated at farmers field under UKP command area

Name of the technology	Crop	Year/season
Direct seeded rice method (DSR)	Paddy	2019-2020
Drum seeded rice in paddy (DS)	Paddy	2019-2020
Laser levelling technology (LLT)	Paddy	2021-2022
Nipping machine	Pigeonpea	2019-2020
Solar operated nipping (foliage collector) machine	Chickpea	2022-2023
Compartment bunding as soil moisture conservation practices	Greengram	2022-2023
Groundnut harvester & BBF for resource conservation	Groundnut	2022-2023
Tractor operated cotton shredder for cotton straw management	Cotton	2022-2023
Raised bed & plastic mulch for water conservation	Watermelon	2022-2023

Number of demonstration – 10, Area-2.5 ha

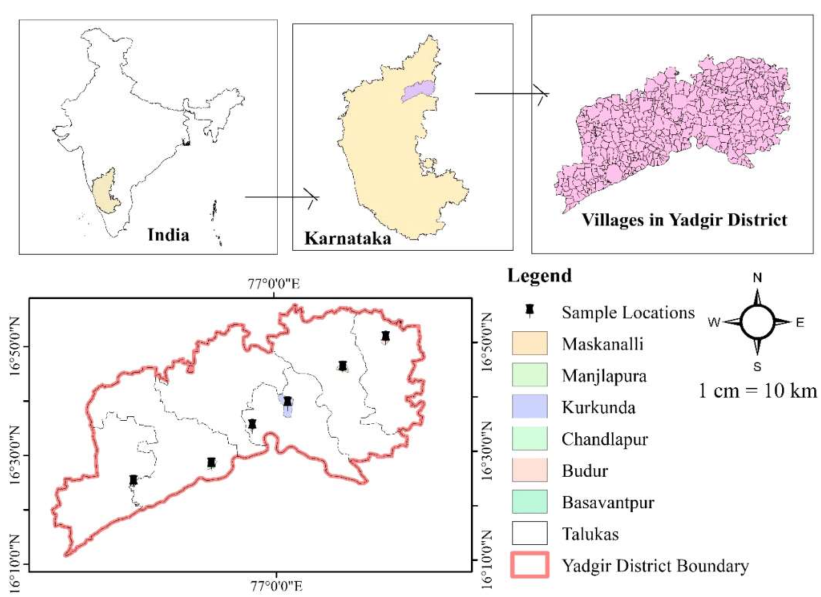

Fig. 1. Location map of study area

practices field at different crop stages to study the effect of technologies. The water productivity, water saving, technology gap, extension gap and BC ratio were calculated (Umesh et al., 2020).

RESULTS AND DISCUSSION

Paddy yield and water saving (%) under different technologies and traditional transplanting method: The average paddy yield increased by 11.29, 12.58 and 15.50 percent by saving irrigation water by 12.77, 22.05 and 23.52 percent in DS, DSR and LLT as compared to traditional transplanting method. The BC ratio increased from 1.62 to 2.58, 2.77 and 2.26 in DS, DSR and LLT as compared to traditional transplanting method (TTM) during 2019 to 2022. The yield is significantly increased by saving irrigation water 12.77 to 23.52 percent. It is observed that, the technology gap ranges from 1168 to 1469 (kg/ha) which showed huge yield gap between technologies and farmers practices. This gap can be minimised through extension programmes like demonstration, training programmes and skill enhancing programmes. The BC ratio increased from 1.62 to 2.26, 2.58 and 2.77 under LLT, DS and DSR respectively. The findings were in close agreement with earlier findings (Bista 2018, Kuchanur et al., 2018, Umesh et al., 2020, Singh and Ranguwal 2024, Tanu Oinam et al., 2025)

Yield of chickpea and pigeonpea under farmers practice and nipping operation: The nipping operation was performed through nipping machine during 45 to 55 days and 35 to 45 days after sowing in pigeonpea and chickpea respectively. The average chickpea yield increased up to 8.29 (%) from 11.10 to 12.02 q ha^{-1} in farmers practices to technology demo field (Table 3). The average pigeonpea

Table 2. Yield, water saving and water productivity of DS, DSR and LLT in paddy cultivation during 2019-2022

Parameters	Technology assessed			
	TTM	DS	DSR	LLT
Crop yield (kg/ha)	5350	6031 (+11.29)	6120 (+12.58)	6332 (+15.50)
Water used during crop growth period (mm)	1245	1104	1020	1008
Water productivity (kg/ha/mm)	4.28	5.46 (+27.57)	6.00 (+40.18)	6.28 (+46.73)
Water saving (%)	-	12.77	22.05	23.52
Technology gap (kg/ha)	-	1469	1380	1168
Extension gap (kg/ha)	-	681	770	982
Technology index (%)	-	19.58	18.40	15.57
Total expenditure (Rs/ha)	59210.00	42158.00	39784.00	50233.00
Grass income (Rs/ha)	96300.00	108558.00	110160.00	113976
Net income (Rs/ha)	37090.00	66400.00	70376.00	63743.00
B:C	1.62	2.58	2.77	2.26

Table 3. Yield parameters and BC of nipping operation and farmers practices in chickpea and pigeonpea production

Parameters	Chickpea		Pigeonpea	
	Farmers practices	Nipping operation	Farmers practices	Nipping operation
No. of pods/plant	67.84	73.60	131.56	168.13
No. of branches/plant	17.00	19.00	13.08	15.96
Average yield (q/ha)	11.10	12.02	10.50	13.60
Per cent increase	-	8.29	-	29.52
Technology gap (q/ha)	-	1.98	-	2.4
Extension gap (q/ha)	-	0.92	-	3.1
Technology index (%)	-	14.14	-	15
Gross return (Rs/ha)	49950.00	54090.00	69300	89760
Gross cost (Rs/ha)	26000.00	23400.00	21000	22000
Net profit (Rs/ha)	23950.00	30690.00	48300	67760
B:C Ratio	01.92	02.31	3.30	4.08

yield increased up to 29.52 (%) from 10.50 to 13.60 q /ha in technology demo field as compared to farmers practices. The technology gap between farmers and demon field was 1.98 and 2.4 q/ha which has to be reduced through extension activities like awareness programme and training programmes. The BC ratio was increased from 1.92 to 2.31 and 3.30 to 4.08 in chickpea and pigeonpea in nipping operation field as compared to farmers practices. The increase in yield is due to nipping operation in both chickpea and pigeonpea which reduced the growth of plant and enhanced the number branches which subsequently increase the number pods and crop yield. The use of nipping machines for operation also reduced the labour requirement and reduced the cost of production. The similar results were obtained in earlier studies on irrigation levels, sowing methods and nipping operation helps to increase the yield in pigeonpea and chickpea crop (Manjunatha et al*.,* 2019, Devaranavadagi et al., 2021 Ammaiyappan et al., 2023, Aditi Agrawal et al., 2024).

Crop yield and moisture content under compartment bunding and farmers practices in greengram crop: The compartment bunding technology was demonstrated in greengram crop for enhancing soil moisture during crop growth period (Table 4, 5). The soil moisture was reduced from 28.10 to 07.06 percent after 10 days of rainfall, however under compartment bunding field from 32.85 to 18.58 percent. This indicated that, good amount of soil moisture was retained in demo field as compared to farmers field which subsequently increase the greengram yield from 7.5 to 10.06 (q/ha). The rainwater productivity increased from 14.75 to 19.80 (kg/ha/mm) in demo field as compared to farmers practices through saving 34.23 percent of rainwater during crop growth period. The similar results were also reported in arlier studiex (Patil et al., 2016, Kalbande Devaranavadagi et al., 2021, Vishal Dashrathrao et al., 2023, Rathod Digvijay Singh et al., 2025), The studies indicated that, use of compartment bund under rainfed situation enhance the soil moisture for longer duration and helps to achieve higher crop yield.

Table 4. Compartment bunding as soil moisture conservation practices soil moisture content and rainwater use efficiency

Technology details	Days after rainfall	Soil moisture (%)
Farmers practices	2	28.10
	5	19.25
	7	12.18
	10	07.06
Compartment bunder	2	32.85
	5	27.51
	7	21.12
	10	18.58

Watermelon yield and water productivity under raised bed and plastic mulch and farmers practices: The watermelon yield increased up to 40.30 % from 388 to 650 q/ha in raised bed & plastic mulch as compared to farmers practices by saving 33.33 (%) (Table 5). The BC ratio increased from 2.15 to 2.5 in demo field as compared to farmers practices. The use of raised bed and plastic mulch minimizes the number of irrigations, enhance soil moisture and increases the water productivity from 0.64 to 1.45 q/ha/mm as compared to farmers practices. Similar results were observed in earlier studies (Kanak Lata et al., (2025), Nodar A et al., (2016), Dadheech S et al., (2018), Pawar et al., (2019) and Rao et al., (2017). The studies indicated that using plastic mulch as a soil cover increased the vegetative growth and yield of watermelon by retaining soil moisture for longer duration.

Table 6. Watermelon yield and water productivity under raised bed & plastic mulch and farmers practices

Parameters	Farmers practice	Raised bed & plastic mulching
Crop yield (q/ha)	388.00	650.00
Water used during crop growth period (mm)	600.00	450.00
Water productivity (q/ha/mm)	0.64	1.45
Water saving (%)	-	33.33
Technology gap (q/ha)		150.00
Extension gap (q/ha)		262.00
Technology index (%)		187.50
Total expenditure (Rs/ha)	1,80,000.00	2,62,000.00
Grass income (Rs/ha)	3,88,000.00	6,50,000.00
Net income (Rs/ha)	2,08,000.00	3,88,000.00
B:C	2.15	2.50

Table 5. Greengram yield, water saving and rainwater productivity under compartment bunding and farmers practice during 2022-23

Technology details	Grain yield (q/ha)	Rainwater used to during crop growth period (mm)	Rainwater productivity (kg/ha/mm)	Water saving (%)
Farmers practices	7.5	508.30	14.75	-
Compartment bunder	10.06	508.30	19.80	34.23

Table 7. Cost involved and labour requirement under cotton shredder and farmers practices

Particulars	Working hours (h/ha)		Total labour required per ha		Cost involved (Rs/ha)	
	Demo	Check	Demo	Check	Demo	Check
Cutting and uprooting of cotton straw	01	08	01	08	2340.00 (10 litre*94 Rs/lit) + Machine Hiring charges Rs.1400)	2000.00
Collection and Buring of cotton straw	-	05	-	05		1250.00
To cover one hectare of area	01	13	01	08		2000.00
Total					2340.00	5250.00

Tractor operated cotton shredder for reducing labour cost: Cotton shredder were used to shred the cotton stalks after the complete cotton picking. In farmers practice, eight-man hours is required for cutting and uprooting of cotton plants and five-man hours for collection and burning of cotton plants total of 13-man hours per hector. The same work can be completed by one cotton shredder by reducing 80 (%) of labour and time. The cost for the same work can be reduced from Rs. 5250 to Rs. 2340 per hector (Table 7). The main advantage of the cotton shredder was to incorporation of shredded materials of 2-2.5 t/ha cotton stalks per ha in to soil which could increase the soil fertility through decomposing process. The, incorporation of shredded cotton plants in to soil could enhance 1.43:0.78:0.82 % of NPK as compared to 0.5:02-04:0.5 % of NPK in adding 12.4-20 kg of N/ha, 1.6 kg of P_2O_5/ha, 12.2-13.6 kg of K_2O/ha.

CONCLUSION

The new engineering intervention reduced the cost of cultivation and increased the farm income through natural resource conservation. The study results highlighted the necessity of educating farmers through a wide range of methods to encourage the adoption of better agricultural production methods and stop the current trend of a large extension gap. The demonstrations lead the farmers to adopt new technologies through self-confidence and horizontal spread of technologies among farming community.

AUTHORS CONTRIBUTION

Barikara Umesh: Study implementation, data collection, funding acquisition, writing, review & editing, resources, conceptualization. J B Kambale: Data curation and statistical analysis, writing, review & editing. Shreevani G N: Methodology, investigation, and data curation. Jaiprakah Narayan R P: Writing – review & editing, visualization, supervision, study administration, investigation, funding acquisition, formal analysis, data curation, conceptualization. A R Kububar: Writing – review & editing, validation, supervision, resources. D K Hadimani: Writing – review & editing.

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Papaya Epicarp Powder Meal as Natural Carotenoid Source for Pigmentation and Growth in Indigenous Ornamental Fish, Rosy Barb (*Pethia conchonius*)

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Abstract: Experiment was conducted to observe the efficacy of experimental feeds supplemented with graded levels of papaya epicarp powder (PEP) meal as a natural source of carotenoid on growth and colouration in the muscle and skin of indigenous ornamental fish, rosy barb (*Pethia conchonius*). PEP was incorporated in the control feed (T0) at four different levels (1.5, 3, 4.5, and 6%) to prepare the experimental feeds. During the experimental period of 120 days, water quality parameters remained in optimum range for fish culture. Fish growth in terms of total body length gain was significantly higher in treatment with PEP @ 3% and @ 6%, whereas net weight gain and SGR was significantly higher in T2 (PEP @ 3%), FCR also significantly reduced in T2, whereas PER and condition factor did not show any significant differences. Fish muscle and skin colour in terms of carotenoid content and digital analysis showed significant improvement in diet with PEP @ 6%. Histological changes observed in muscle tissue in terms of increased muscular striations and number of nuclei were also coincides with improvement in growth in PEP @ 3%. The fish growth in terms of weight gain significantly improved in T2, but colouration was significantly enhanced in PEP @ 6%. Based upon these results and considering the value of ornamental fish in terms of colouration, papaya epicarp powder meal can be incorporated @ 6% in diet of ornamental fish, rosy barb.

Keywords: Ornamental fish, Papaya epicarp meal, Rosy barb, Carotenoid, Pigmentation

Ornamental fish keeping is 2^{nd} most popular hobby after photography worldwide due to their high aesthetic value. It is a well-known fact that ornamental fish have highly attractive skin colouration and physical appearance, which is responsible for their high commercial value (Khairnar and Kaur 2021). The ornamental fish industry faces a major challenge when replicating fish colours in captivity. To achieve acceptable pigmentation of ornamental fish integuments, carotenoids (natural or synthetic) need to be added in the regular feed. Further, the colour of the integument is determined by three significant pigments: melanins, pterins, and carotenoids; although fish synthesize pterins and melanins, they cannot synthesize carotenoid pigments (Grether et al., 2001). Therefore, fish rely on dietary carotenoids in natural or synthetic forms to achieve their optimal pigmentation. In addition to pigmentation, the carotenoids also play significant role in growth, reproduction and disease resistance due to antioxidant and anti-inflammatory properties (Nakano and Wiegertjes 2020). Number of natural carotenoid pigments are present in the fruits, flowers, seeds, roots, and leaves of higher plants and several studies have shown an increased interest of researchers in exploiting carotenoid pigments of plant origin (Sathyaruban et al., 2021). Among various plant pigment sources, papaya is one such source, which hold great potential as natural source of carotenoids. India accounts for around 43% of papaya output, producing more than 13.9 million tonnes per year (Sharma et al., 2022). After peeling, papaya fruit is typically served as a salad. The peel accounts for 12.5% of papaya waste and has a high concentration of protein, fat, fibres, phenolic compounds, vitamin C, potassium and other nutrients (Pathak et al., 2019). The orange and red colours of papayas are due to carotenoids such as beta-carotene and beta-cryptoxanthin (Jha et al., 2010). Considering nutritional value of papaya and its pigments status, the study was designed to evaluate the efficacy of papaya epicarp meal as a potential dietary carotenoid supplement for colour enhancement in rosy barb, *Pethia conchonius*, one of the indigenous colourful ornamental fish having great market demand.

MATERIAL AND METHODS

Experimental Details

Preparation of the experimental fiber reinforced plastic (FRP) pools: Experiment was conducted in triplicate in FRP pools (1000 litres capacity) for 120 days at Instructional cum Research Farm of College of Fisheries. Prior to the fish stocking, FRP pools were thoroughly cleaned. One-inch-thick layer of soil was spread at the bottom of each tank followed by liming to disinfect the tanks and maintain the water pH in the optimum range (7.5-8.5) for fish culture. All the tanks were manured (pre-stocking) with cow dung @20, 000 kg ha^{-1} yr^{-1}. The tube well water was used for filling and maintaining the water level in the pools during the experimental period.

Procurement, acclimatization and stocking of the experimental fish: Experimental fish, rosy barb, *Pethia conchonius,* was procured from ornamental fish culture and breeding facility of the College of Fisheries. Fish were kept on a control feed and acclimated under indoor conditions for a month in order to decolorize them. After proper acclimatization, twenty fish were stocked (average initial weight - 0.9-1 g and length - 2-4 cm) in each pool.

Preparation of experimental feeds: Five experimental feeds were tested including one control (T0). In the four experimental feeds, dried papaya epicarp meal powder (PEP) was added @ 1.5% (T1), 3% (T2) 4.5% (T3) and 6% (T4), respectively in control feed composed of mustard meal (23.75%), soybean meal (23.75%), rice bran (47.75%), tapioca flour (3.0%), vitamin –mineral mixture (1.5%) and salt (0.5%). For preparation of experimental feeds, feed ingredients were dried and finely grounded before mixing. Carotenoid source i.e. papaya epicarp was procured from local market and fruit chat shops. Papaya epicarp was separated from other fruit and vegetable wastes and dried in sunlight for 3-4 days followed by oven drying at temperature of 60±5^{0}C. Dried papaya epicarp then finely grounded to form the meal and mixed with other ingredients to make crumbled feed. Following AOAC (2000) guidelines, the proximate analysis (crude protein, crude fat, crude fibre, moisture, and ash) on dry matter (DM) basis of feed ingredients/ experimental feeds was done (Table 1). The values of crude protein, crude fat (ether extract), crude fibre, crude ash and moisture were subtracted from 100 to determine the nitrogen free extract.

Fish feeding: For whole experimental duration (120 days), fish were fed twice a day at 9:30 and 16:30 hr. with control and experimental feeds @ 3% of their body weight (BW). At each sampling (monthly interval), the amount of feed was adjusted based on the increase in fish weight.

Water quality parameters: Water samples were taken every two weeks in the morning hours to analyse physico-chemical characteristics viz. water temperature, pH, dissolved oxygen (D.O.), total Alkalinity (TA), total hardness (TH), ammoniacal - nitrogen (NH3-N), nitrite and nitrate as per standard methods (APHA 2005).

Fish survival and growth: By comparing the number of live fish collected at the end of the experiment with the total number of fish stocked, the survival rate of each treatment was ascertained. Fish were sampled every month to measure body weight and total body length. The total length gain (TLG), net weight gain (NWG), percent total length gain (% TLG), percent net weight gain (% NWG), specific growth rate (SGR), condition factor (K), feed conversion ratio (FCR), and protein efficiency ratio (PER) were calculated.

TLG = Average final total body length (cm) – Average initial total body length (cm)

%TLG = Final total body length (cm)-initial body length (cm) /initial total body length (cm) x100

NWG = Average final body wt. (g) – Average initial body wt. (g)

%NWG = Final body weight (g) – initial body weight (g) /initial body weight (g) x 100

SGR (% increase in weight /day) = [(*ln* final body wt. – *ln* initial body wt.)/ Culture days] x 100

ln – Natural logarithm

$$\text{·Condition Factor (K)} = \frac{\text{Body weight}}{(\text{Body length})^3} \times 100$$

Table 1. Proximate composition (% DM basis) and gross energy (Kcal g^{-1}) of different feed ingredients and experimental feeds

Ingredients/Experimental feed		Crude protein	Ether extract	Crude fibre	Ash	Moisture	NFE	Gross energy
Ingredients	Rice bran	19.49	1.46	14.27	9.67	4.43	50.68	3.32
	Mustard meal	45.54	2.04	11.31	7.00	1.34	32.77	4.11
	Soybean meal	43.27	2.05	5.10	5.67	3.27	40.64	4.30
	Papaya Epicarp Meal (PEP)	17.68	2.03	8.74	8.34	3.67	59.54	3.63
Experimental Feeds	T0	32.34	1.66	10.68	7.63	3.20	44.49	3.81
	T1	32.77	1.69	10.81	7.76	3.26	43.71	3.80
	T2	33.20	1.72	10.94	7.88	3.31	42.95	3.80
	T3	33.63	1.75	11.07	8.01	3.37	42.17	3.79
	T4	34.06	1.78	11.20	8.13	3.42	41.41	3.79

FCR Weight gain (g) = Feed given (g) / Weight gain (g)

PER = Weight gain (g) / Protein intake (g)

Colouration in Fish

Total carotenoid content: Carotenoid analysis in fish muscle and skin colour analysis was analysed by following method Olson (1979) at initiation and termination of the experiment.

Digital analysis of fish skin: Sony full HD camera (DSC-HX 300) was used to take digital pictures of the experimental fish once a month. At least three fish were taken from each replicate to record their skin colour at three different locations: i) directly above the opercular region, ii) at the base of the dorsal fin, and iii) at the caudal peduncle. All camera settings were maintained during the photo shoot. The digital pictures were analysed in CIE and RGB colour scales

CIE colouration scale: Using digital photos, the CIE (Commission International de l'Eclairage) Lab is used to measure the colour of the fish skin. The "Lab colour mode" colour space in Adobe Photoshop is based on CIE Lab with L*, a *, and b* are its three parameters. L* stands for luminosity, which is the lightness that ranges from 0 for black to 100 for white. The balance between red and green is represented by the a* channel, while the balance between yellow and blue is described by the b* channel.

RGB colouration scale: Using RGB (Red, Green, and Blue) values, photographs were examined in Adobe Photoshop to assess the colour intensity of fish skin from various treatments (Hancz et al., 2003, Tlusty 2005).

Histological studies: Three muscle samples per tissue per treatment were taken and were processed as per method of Kong et al., (2008). Every tissue was sliced into 4-6 μm serial pieces using a rotary microtome (Leica RM2125RT, Germany). After being placed on frosted slides (Abdo's Labtech Pty., Ltd., India), the sections were dried for 15 to 20 hours at 35 °C. Hematoxylin and eosin was used to stain tissue sections, which were then examined using a compound microscope (Shanthanagouda et al., 2014). The NIKON 80i microscope was used to view the tissues' histomorphology at a 40× objective, and a digital camera mounted on the microscope was used to take images.

Statistical analysis: The SPSS statistical package (v16.0 for Windows, SPSS Inc., Richmond, CA, USA) was used to do the statistical analysis of the data with Duncan's multiple range test.

RESULTS AND DISCUSSION

Water quality: During the experimental period, temperature, pH, dissolved oxygen, total hardness, total alkalinity, ammonia-nitrogen, nitrite, and nitrate varied from 27.67 to 31.68°C, 6.43 to 7.69, 4.93 to 6.40 mg l^{-1}, 385.33 to 248.67 $CaCO_3$ mg l^{-1}, 254 to 404 $CaCO_3$ mg l^{-1}, 0.005 to 0.049 mg l^{-1}, 0.007 to 0.174 mg l^{-1}, and 0.003 to 0.042 mg l^{-1}, respectively, in the various treatments (T0-T4). Variations were observed for all of the water quality parameters during the culture period, but they remained within the optimum range for ornamental fish growth. Supplementation of feed with PEP for rosy barb had no negative effect on water quality.

Fish survival and growth: After completion of the experiment, fish survival ranged between 75 to 78.33% in treatments and 70% in control diet. Although maximum fish survival was recorded in T3, however the differences were insignificant, for all the treatments (Table 2). Fish growth in terms of total body length gain (TBLG) was significantly higher in T2 & T4 i.e. Papaya epicarp Powder @ 3 % & 6 %, whereas net weight gain (NWG) & specific growth rate (SGR) were significantly higher in T2 i.e. PEP @ 3.0 %. FCR also

Table 2. Survival and growth parameters in different treatments

Parameters	Treatments				
	T0	T1	T2	T3	T4
Survival (%)	70.00^{a}	76.67^{a}	75.00^{a}	78.33^{a}	76.67^{a}
Final total body length (cm)	5.60^{ab}	5.50^{b}	5.90^{a}	5.67^{ab}	5.73^{ab}
% TBLG	31.09^{b}	39.96^{ab}	42.07^{a}	37.55^{ab}	44.11^{a}
Final body weight (g)	3.87^{cd}	3.70^{d}	5.20^{a}	4.20^{b}	4.00^{bc}
NWG	2.91^{c}	2.77^{c}	4.25^{a}	3.24^{b}	3.05^{ab}
%NWG	301.62^{c}	298.32^{c}	445.86^{a}	338.39^{b}	318.91^{c}
SGR	1.16^{c}	1.15^{c}	1.41^{a}	1.23^{b}	1.19^{ab}
K-Value	2.21^{a}	2.32^{a}	2.58^{a}	2.31^{a}	2.14^{a}
FCR	3.60^{a}	3.01^{ab}	2.91^{b}	2.88^{b}	3.10^{ab}
PER	0.62^{a}	0.56^{a}	0.78^{a}	0.73^{a}	0.76^{a}

TLG = Total length gain, NWG = Net weight gain, FCR = Feed conversion ratio, PER = Protein efficiency ratio, SGR= Specific growth rate, CF = Condition factor
Values with same superscript in row do not differ significantly ($P \leq 0.05$)

showed significant improvement in T2 and T3 i.e. PEP @ 3 and 4.5%. Values for condition factor (K value) and PER showed insignificant differences (Table 2). The growth parameters revealed that feed having PEP @ 3% resulted in growth increment in rosy barb with efficient feed utilization. Das and Biswas (2020) observed that supplementing ripe papaya meal @ 4% can be one of the effective and cost-efficient way to promote growth and survival of banded gourami (*Trichogaster fasciata*).

Pimpimol et al. (2020) also revealed that pineapple juice (PA) and dried papaya peel (PP) @ 5% resulted in improved growth performance of channel catfish in a recirculating aquaculture system. Hamid et al., (2022) reported that papaya leaf extract acted as a growth promoter in seabass (*Lates calcarifer*) and red hybrid tilapia (*Oreochromis mossambicus* X *Oreochromis niloticus*) besides reduced hatching time in seabass. Papaya fruit and its parts including leaves, roots, bark, peel, seeds and flesh are rich source of protein, fat, vitamins and pigments, due to which it must have resulted in improved growth of rosy barb in the present experiment (Rodrigo and Perera 2018).

Muscle and skin colouration: PEP supplementation in rosy barb feed at all levels, i.e. 1.5 - 6% enhanced total carotenoid content of skin and muscle significantly as compared to control feed. After completion of the experiment, significantly higher carotenoid (μg g^{-1} wet weight basis) content (13.21) was in fish fed with feed having PEP @ 6% followed by T3, T2 and T1 (Table 3).

Pigmentation is the one of the most important factors in determining the value of ornamental fish. Digital parameters i.e. red, green and blue colouration on RGB and L*a*b* values on CIE scale also reflected corresponding results (Table 4, 5). The pigmentation in fish body is affected by different factors like concentration of carotenoid, dietary lipid, fish species, environment and feeding period (Lee et al., 2010). In the present study, papaya epicarp meal @ 6% resulted in significantly enhanced carotenoid content, which is also reflected in intense colouration of fish skin. One of the earlier studies too reported that incorporation of ripe papaya meal (RPM) @ 4% resulted in enhanced colouration in banded gourami (*Trichogaster fasciata*) without any adverse effect (Das and Biswas 2020). The major component of the papaya epicarp powder meal used in the study is beta-carotene and beta-cryptoxanthin. The colour improvement in rosy barb might be due to the effective utilization of beta-carotene and beta-cryptoxanthin by the fish and revealed in terms of intense orange-red colour.

Histomorphological changes in muscle tissue: The histomorphological observations of control and treated groups were recorded and compared at 40x (Plate I). In control, the muscle tissue of Rosy barb showed normal histological characteristics as each muscle bundle was uniform in shape and possessed peripheral nuclei, with muscle fibres slightly spaced and showed striations as

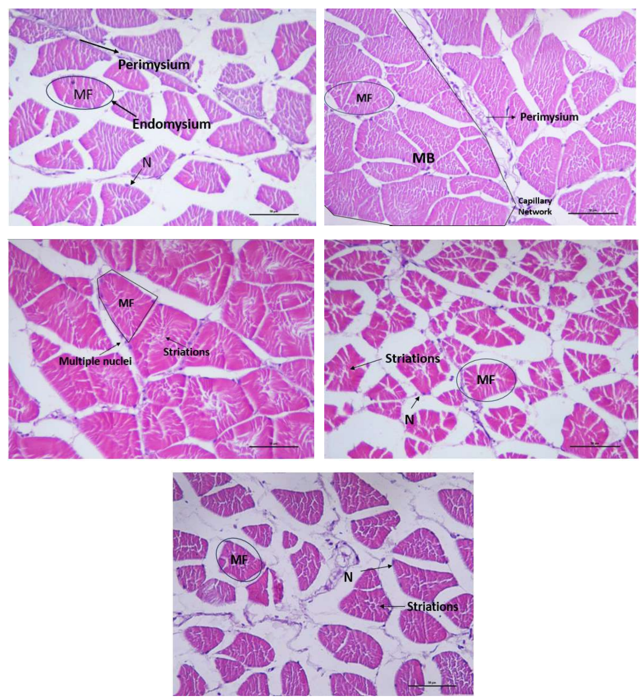

Plate I. Histomorphological changes in muscle tissue of rosy barb at 40x in control and different treatments after completion of the experiment

Table 3. Changes in total carotenoid content (μg g^{-1} wet weight basis) in skin and muscle of rosy barb in different treatments at initiation and termination of experiment

Days	Treatments				
	T0	T1	T2	T3	T4
0	6.79^{a}	6.80^{a}	6.72^{a}	6.58^{a}	6.77^{a}
120	7.385^{c} -----	8.17^{c} (10.70)	8.87^{bc} (20.19)	10.13^{b} (37.26)	13.21^{a} (79.00)

Values with same superscript in row do not differ significantly (P≤ 0.05)
Figures in parenthesis represent % change over control

compared to other treatments. Signs of improvement in muscle tissue quality were observed in T1 and T2 (feed with PEP @1.5 and 3.0%) with enhanced muscle fibres, perimysium and capillary network in muscle fibre along with reduced muscle fibre gapping as compared to control. However, the size of muscle fibre was maximum in T2 (feed with PEP @3%) with increased muscular striation. In this treatment, the number of nuclei in the fish muscle also showed an increase along with less gapping in muscle fibre (Plate I). Similarly, presence of prominent nuclei further supports the notion that PEP promoted cellular activity and growth. The increased muscle fibre size observed in T1 and T2 is a positive indicator of enhanced muscle growth and development. This improvement due to incorporation of PEP

Table 4. Changes in RGB values in skin of rosy barb (*P. conchonius*) in different treatments during the experimental period

Days	RGB value	Treatments				
		T0	T1	T2	T3	T4
0	R	166.67^{a}	173.33^{a}	170.33^{a}	170.33^{a}	173.33^{a}
	G	148.00^{a}	155.00^{a}	151.33^{a}	151.33^{a}	154.33^{a}
	B	105.67^{a}	114.33^{a}	110.67^{a}	111.67^{a}	112.00^{a}
30	R	170.33^{b}	174.67^{b}	187.00^{ab}	182.33^{ab}	197.00^{a}
	G	156.67^{b}	153.67^{b}	160.00^{ab}	163.00^{ab}	174.00^{a}
	B	131.67^{a}	120.67^{a}	111.33^{a}	124.33^{a}	138.67^{a}
60	R	180.33^{a}	189.33^{a}	198.00^{a}	188.33^{a}	200.67^{a}
	G	156.67^{a}	159.00^{a}	163.33^{a}	156.67^{a}	173.33^{a}
	B	112.00^{a}	112.33^{a}	119.33^{a}	110.67^{a}	123.00^{a}
90	R	186.00^{a}	176.00^{a}	196.00^{a}	191.00^{a}	200.00^{a}
	G	156.00^{a}	158.33^{a}	157.33^{a}	156.00^{a}	167.33^{a}
	B	114.67^{a}	127.00^{a}	118.33^{a}	103.33^{a}	124.33^{a}
120	R	195.67^{c}	199.00^{c}	210.33^{b}	214.67^{b}	225.00^{a}
	G	163.33^{c}	168.67^{bc}	170.33^{bc}	173.67^{ab}	180.67^{a}
	B	116.67^{a}	118.33^{a}	120.67^{a}	120.67^{a}	122.67^{a}

Values with same superscript in a row do not differ significantly (p≤0.05)

Table 5. Changes in Lab values in skin of rosy barb (*P. conchonius*) in different treatments during the experimental period

Days	Lab value	Treatments				
		T0	T1	T2	T3	T4
0	L*	61.96^{a}	64.59^{a}	63.23^{a}	63.40^{a}	64.45^{a}
	a*	0.67^{a}	0.67^{a}	0.89^{a}	0.94^{a}	0.63^{a}
	b*	24.96^{a}	23.75^{a}	23.79^{a}	23.49^{a}	24.89^{a}
30	L*	65.23^{b}	64.48^{b}	67.21^{ab}	67.81^{ab}	72.28^{a}
	a*	1.17^{a}	2.84^{a}	3.25^{a}	1.26^{a}	3.22^{a}
	b*	14.84^{a}	19.95^{a}	29.11^{a}	22.89^{a}	21.25^{a}
60	L*	65.76^{a}	67.34^{a}	69.32^{a}	66.50^{a}	72.13^{a}
	a*	2.20^{a}	5.60^{a}	6.91^{a}	5.62^{a}	3.29^{a}
	b*	26.63^{a}	28.43^{a}	27.73^{a}	28.70^{a}	29.31^{a}
90	L	66.00^{a}	66.00^{a}	67.56^{a}	66.40^{a}	70.51^{a}
	a*	5.28^{a}	1.53^{a}	9.00^{a}	6.42^{a}	6.09^{a}
	b*	25.82^{ab}	18.90^{b}	25.94^{ab}	32.37^{a}	26.68^{ab}
120	L	68.96^{d}	70.70^{cd}	72.26^{bc}	73.44^{b}	76.31^{a}
	a*	5.45^{a}	4.49^{a}	8.20^{a}	8.13^{a}	8.95^{a}
	b*	28.49^{a}	29.87^{a}	31.36^{a}	32.78^{a}	35.60^{a}

Values with same superscript in a row do not differ significantly (p≤0.05) is likely due to the presence of essential nutrients required for muscle growth along with bioactive compounds like carotenoids, which might have promoted muscle growth and improve overall health, which can be positively correlated with reduced oxidative stress leading to enhanced cell cellular activity (Chandran et al., 2024). Additionally, the nutritional composition of floral waste, including vitamins and minerals, supports the synthesis of proteins required for muscle development.

CONCLUSION

Colouration is most important parameters to decide the value of ornamental fish. Hence in view of all the results, PEP can be incorporated @ 3% and above in the feed of Rosy Barb with 6% inclusion giving intense colouration. The present study is a step towards valorization of papaya waste in the form of papaya epicarp meal. However, further studies are warranted to explore the potential of papaya epicarp meal through a holistic approach by considering other aspects including breeding performances, fecundity, gut health etc., so that an unexplored nutrients rich (especially carotenoids) source can be utilized for cost effective feed formulation, which will also be a step towards waste to wealth.

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Assessment of Epilithic Microalgae along Longitudinal Gradient in Freshwater Bodies of South India

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Abstract: Epilithic microalgae are associated with hard substrates of freshwater environments and they play a vital role in primary production, nutrient cycling and contributing habitat for other organisms in aquatic environments. Thus, the present study was focused to assess the diversity and distributional pattern of epilithic microalgae along longitudinal gradient in lentic freshwater bodies, Tamil Nadu, India. Nine freshwater bodies (lakes, ponds and temple tanks) were examined in Madurai district. Microalgae were collected from submerged substrates of bedrock, boulders and pebbles in lentic waters. In total, 14 genera of microalgae were collected. *Fragilaria* was the highest percentage. The high diversity was observed in site 9 revealed by Shannon diversity index. Abundance of microalgae was high in lakes when compared to temple tanks and ponds. Of twelve environmental variables, total dissolved solids, conductivity and salinity were significant factors for the distribution of microalgae. This finding suggests that microalgae in lentic freshwater bodies are not influenced with longitudinal gradient whereas chemical parameters of water such as total dissolved solids, dissolved oxygen, electrical conductivity and salinity are the determining factors.

Keywords: Diversity, Distribution, Environmental variables, Longitude, Freshwater

Microalgae are unicellular aquatic organisms and they exist in variety of environments ranging from ponds, lakes, brackish water and oceans. Microalgae perform photosynthesis in aquatic environment and participate in the role of energy transmission in the ecosystem (Thore et al., 2023). In addition, microalgae used for pollution indicators, nutrition dynamics, sewage treatment, food industry, and synthesis for novel compounds. About eight lakhs species of microalgae are distributed in the world, of which 50,000 species described (Ampofo and Abbey 2022). Several types of microalgae are commonly found in hard substrates (bed rocks, boulders, pebbles) of freshwater environments known as epilithic, including Bacillariophyta, Chlorophyta, Cyanophyta and Chrysophyta) (Arulraj et al., 2022). Epilithic microalgae play an important role in primary production, nutrient cycling and habitat for other organisms in aquatic environments.

Studies showed the epilithic microalgae research that diversity of microalgae from building surfaces during monsoonal period (Samad and Adhikary 2008), biodiversity of microalgae in Western and Eastern Ghats rivers, India (Suresh et al. 2012), microalgae diversity in unexplored freshwater bodies associated with industry region (Severes et al. 2018), periphytic microalgae colonization and litter decomposition in an intermittent stream (Arulraj et al. 2019), seasonal variation of microalgae and cyanobacteria in Komuki dam, Tamil Nadu (Selvaraj et al., 2021) and periphytic microalgae colonization in mosquito breeding stream puddles of Western Ghats (Arulraj et al., 2022). Due to increasing anthropogenic impacts in freshwater bodies like waste disposal, urbanization, habitat destruction and sewage integration, necessity to study the ecology of freshwater environments, in particular urban regions. Therefore, the present study focused to study the diversity and distribution of epilithic microalgae along a longitudinal gradient in freshwater bodies of Tamil Nadu, South India.

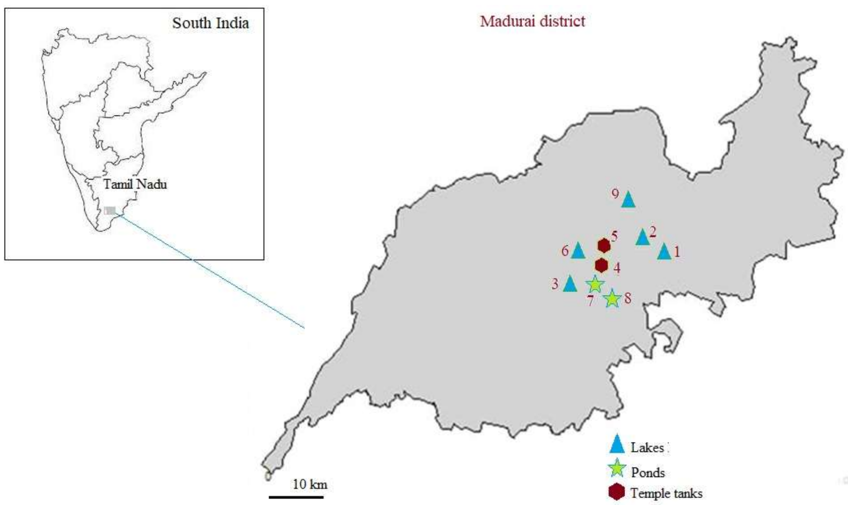
MATERIAL AND METHODS

Sampling was collected in three seasons September 2023 (South-west monsoon), November 2023 (North-east monsoon) and pre-summer (February 2024) in the submerged substrates of boulders, gravels and pebbles from nine freshwater bodies in triplicates from Madurai district of Tamil Nadu, India (5 lakes, 2 ponds and 2 temple tanks, Fig. 1) Measurements of water temperature, pH, electrical conductivity, total dissolved solids and salinity were done with portable digital tester (PCS Testr 35, Eutech Instruments, India). Dissolved oxygen was measured by Winkler method in the laboratory. Water circumference and average water depth were measured with the help of meter tape. Water color is also recorded by visual observation. The canopy cover is measured with Densiometer. The submerged substrates were taken in a plastic tray and they were rinsed with filtered pond water. The surface biofilms were scrapped from the substrates by using a hard tooth brush. The scrapped biofilm was immediately stored in 25 ml plastic vial. Then, it brought to the laboratory and preserved at−4°C until analysis. In the laboratory, 1ml aliquot of algal sample taken each site was counted by zig zag slide observation method and identified with standard identification manual up to genus level.

Data analyses: All data were sorted out in MS-Excel. All statistical analyses including diversity indices and principal component analysis were calculated by using statistical software Biodiversity Pro and PAST 4.0. Diversity indices for microalgae distributed different sites were calculated with Biodiversity Pro 2.0 software.

RESULTS AND DISCUSSION

The physical and chemical parameters differed with sampling sites (Table 1). The high dissolved oxygen, pH, conductivity and salinity were observed in site 1 and low total dissolved solids were at site 1 (lake), while the low dissolved oxygen, pH, conductivity and salinity were at site 7 (pond). A



Vandiyur-L1, Kannenthal-L2, Seventhikulam-L3, Pannaikudi-L4 and Madakulam-L5; two ponds: Piramalai nagar A-P6 and B-P7; two temple tanks: Saravanapoikai-T8 and Tiruparankundram-T9

Fig. 1. Sampling sites of Madurai district, Tamil Nadu province, India

Table 1. Physico-chemical analysis (mean) of different water bodies of sampling sites for three seasons

Site	L1	L2	L3	L4	L5	P6	P7	T8	T9
Latitude (N)	9.933	9.963	9.872	8.319	9.885	9.522	9.522	9.877	9.525
Longitude (E)	78.15	78.14	78.07	77.57	78.07	78.04	78.04	78.74	78.04
Water temperature (°C)	32	27.7	33.4	32	32.5	35.6	34.2	28.7	36.3
Atmosphere temperature (°C)	35.1	31.3	31.4	38.0	33.4	40.0	32.8	32.6	39.3
Salinity (ppm)	850	742	560	97	502	115	98.5	401	621
Total dissolved solids (ppt)	128	110	850	625	743	177	146	599	926
Conductivity (µmhos)	1757	1559	1180	216	1054	248	208	830	1342
pH	7.6	8.2	8.2	6.4	6.8	7.2	6.2	7.5	7.4
Dissolved oxygen (mgL^{-1})	12	8	8	8	8	4	4	8	8
Water circumference (ft)	2077	800	150	800	3150	100	80	1020	120
Water depth (ft)	15	7	5	40	45	52	20	15	30
Number of riparian species	3	4	3	3	4	3	3	2	2
Riparian cover	Open	Open	Open	Open	Open	Open	Open	Open	Open
Water colour	Light green	Light green	Dark green	Light green	Green	Light green	Light green	Green	Light green

Five lakes: Vandiyur-L1, Kannenthal-L2, Seventhikulam-L3, Pannaikudi-L4 and Madakulam-L5; two ponds: Piramalai nagar A-P6 and B-P7; two temple tanks: Saravanapoikai-T8 and Tiruparankundram-T9 total of 2,271 individuals of microalgae were collected under 14 genera in 11 orders and 6 phyla for three seasons of sampling sites (Table 2). The sites 1 and 9 recorded higher number of taxa whereas other sites 11 to 12 species. Microalgae are influenced with several environmental factors and anthropogenic impact. They have the capability of producing and storing desired products as cell metabolites, and adapting themselves when there is a change in the environmental conditions (pH, temperature, light, carbon dioxide, salinity, and nutrients (Gatamaneni et al., 2018). Of 14 microalgal genera collected, *Fragilaria* was occupied the highest percentage (25%) followed by *Chlorococcum* and *Scenedesmus*. The higher number of *Fragilaria* was at site 1 followed by sites 4 and 6. The site 1 had the high numbers of *Chlorococcum* whereas *Scenedesmus* was high at site 4 (Table 2). The freshwater araphid diatom of *Fragilaria* is a dominant species of the cosmopolitan planktonic diatom, widely distributed in oligotrophic and mesotrophic water reservoirs across the northern hemisphere (Galachyants et al., 2019) and they distributed in all seasons (Deb et al., 2024). *Scenedesmus* and *Chlorococcum* are a dominant genus of algae commonly found in wastewater ponds and it adaptability allows for direct cultivation in wastewater environments (Lyon et al., 2015, Hashmi et al., 2024).

The site 1 and 9 had the higher number of taxa (14) whereas other sites hold 11 to 12 numbers of taxa (Table 3). Shannon and Margalef indices showed that the high diversity was in site 9. Simpson diversity index revealed that the high diversity value was occurred at site 8 and low diversity at site 2. Evenness index was high at site 8 and low at site 2. Microalgae sampled in three freshwater bodies of lakes, ponds and temple tanks showed that 14 taxa found in lakes and temple tanks and 12 taxa in ponds were observed. Abundance of microalgae constituted the higher percentage (63.5%) in lakes, followed by 23.6% in temple tanks and 12.7% in ponds. When compared to generic-wise distribution among sampling sites, temple tanks and lakes had the higher percentage of taxa rather than ponds (Fig. 2) and generic composition had high similarity revealed by ternary plot analysis (Fig. 3).

Table 2. Distribution of epilithic microalgae collected from sampling sites during three seasons

Order	Class	Family	Genus	Sampling site								
				S1	S2	S3	S4	S5	S6	S7	S8	S9
Desmidiales	Zygnematophyceae	Closteriaceae	*Closterium*	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	-	-	ǂ
Zygnematales	Zygnematophyceae	Peniaceae	*Penium*	ǂ	-	-	-	-	-	-	-	ǂ
Zygnematales	Conjugatophyceae	Mesotaeniaceae	*Roya*	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ
Chlamydomonadales	Chlorophyceae	Chlamydomonadaceae	*Chlamydomonas*	ǂ	-	-	-	-	-	-	-	ǂ
Chlamydomonadales	Chlorophyceae	Chlorococcaceae	*Chlorococcum*	Φ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ
Sphaeropleales	Chlorophyceae	Scenedesmaceae	*Scenedesmus*	ǂ	ǂ	ǂ	Φ	ǂ	ǂ	ǂ	ǂ	ǂ
Ulotrichales	Ulvophyceae	Ulotrichaceae	*Ulothrix*	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ
Oscillatoriales	Cyanophyceae	Microcoleaceae	*Platensis*	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ
Chroococcidiopsidales	Cyanophyceae	Aliterellaceae	*Gloeocapasa*	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ
Euglenida	Euglenoidea	Euglenaceae	*Euglena*	ǂ	Φ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ
Fragilariales	Bacillariophyceae	Fragilariaceae	*Fragilaria*	Φ	Φ	Φ	Φ	Φ	Φ	ǂ	ǂ	Φ
Cymbellales	Bacillariophyceae	Cymbellaceae	*Cymbella*	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ
Naviculales	Bacillariophyceae	Amphipleuraceae	*Frustula*	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ
Naviculales	Bacillariophyceae	Naviculaceae	*Navicula*	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ

-:No individuals; ǂ: 1 to 100 individuals; Φ: >100 individuals

Table 3. Diversity analyses for epilithic microalgae collected from sampling sites for three seasons

	S1	S2	S3	S4	S5	S6	S7	S8	S9
No. of taxa	14	12	12	12	12	12	11	11	14
Simpson index	0.8752	0.8458	0.8858	0.8704	0.8637	0.8587	0.8883	0.8926	0.8824
Shannon index	2.371	2.103	2.303	2.26	2.205	2.213	2.269	2.305	2.403
Evennes index	0.7649	0.6827	0.8339	0.7983	0.7562	0.7622	0.8795	0.9115	0.7897
Margalef index	2.73	2.483	2.503	2.359	2.581	2.457	2.473	2.473	2.848

The eigen value and percentage of variance were1.215 and 71.66 for PC1 and 35061 and 20.65 for PC2. The scores of principal component Analysis are given table 4. Result of the PCA indicates that total dissolved solids, conductivity and salinity were significantly correlated with microalgae distribution rather than other environmental variables (Fig. 4). Among sampling sites, the lake sites of L1 and L2 were important sites for the distribution of microalgae (Fig. 4). The different longitudinal and lateral gradients found in the aquatic system underpins the composition of biological communities, given that species tends to occur predominantly in the patches that most favor their development. Total dissolved solids are an important factor for determining the abundance and growth of phytoplanktons (Li et al., 2013). Environmental variables of pH, conductivity and nutrient concentrations are related to

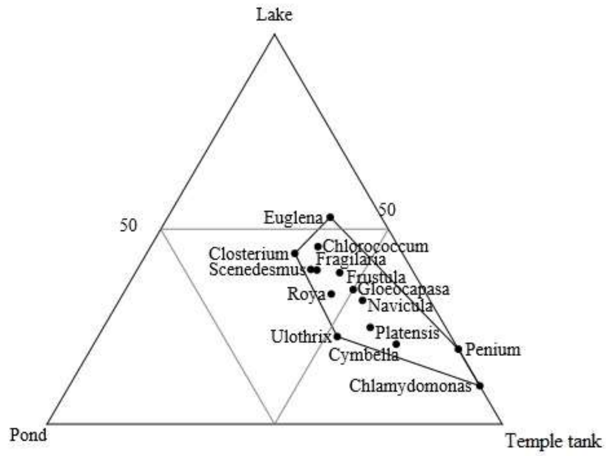


Fig. 3. Distribution of taxa among sampling sites in three freshwater bodies

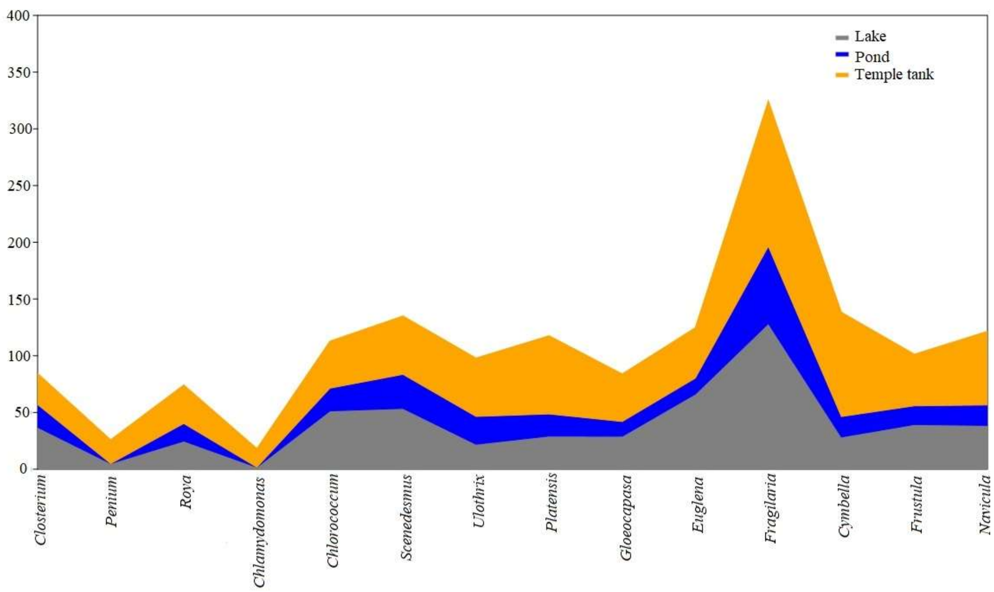


Fig. 2. Generic composition of microalgae (no. of individuals) collected from three freshwater bodies

Table 4. Loading score and loading values of Principal component analysis

	PC 1	PC 2	Site	PC 1	PC 2
LAT -Latitude	0.00017	0.00044	L1	1391	480.76
LON -Longitude	0.000042	0.00012	L2	115.45	690.9
WAT-Water temperature	-0.0009	-0.0007	L3	-647.06	512.1
ATT-Atmosphere temperature	-0.0008	-0.0012	L4	-356.19	-685.24
SAL-Salinity	0.14168	0.40293	L5	2153.1	-590.18
TDS-Total dissolved solids	0.01613	0.04509	P6	-1039.3	-474.21
CON-Conductivity	0.28955	0.84822	P7	-1074.7	-511.46
pH	0.000043	0.001	T8	54.874	-135.36
DO-Dissolved oxygen	0.00144	0.00209	T9	-597.21	712.69
WC-Water circumference	0.94363	-0.3284			
WD-Water depth	0.00087	-0.0196	Eigen	1.215	71.66
NR-No. of Riparian species	0.00029	-0.0002	Covariance	35061	20.65
Microalgae	0.07342	0.08872			

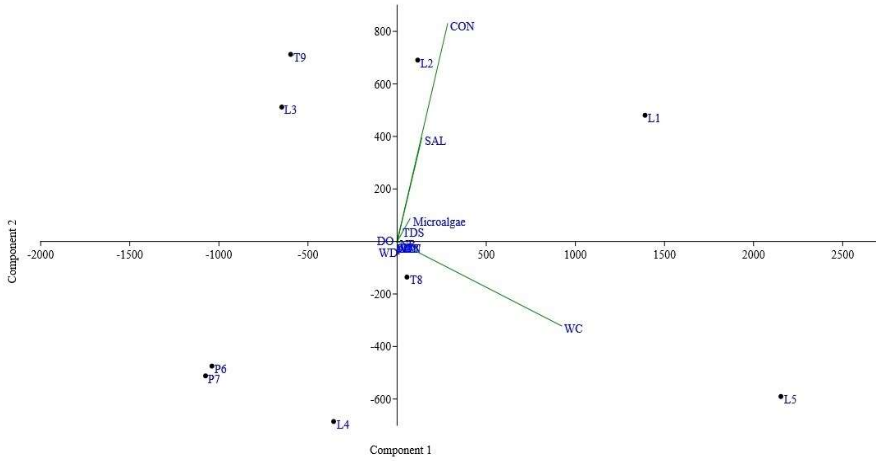


Fig. 4. Principal component analysis (PCA) shows the relationship between environmental variables and microalgae distribution

the richness and abundance of phytoplanktons (Moura et al., 2021).

CONCLUSION

The present study was carried out the distribution of microalgae with longitudinal gradient in lentic freshwater bodies. In total, 14 genera of microalgae were collected. Among genera, *Fragilaria* had the highest percentage followed by *Chlorococcum* and *Scenedesmus*. The highest diversity value was observed in both lakes and temple tanks sites and ponds had low diversity. Abundance of microalgae was high in lakes when compared to temple tanks and ponds. Total dissolved solids, conductivity and salinity were significant factors for the distribution of microalgae rather than other environmental variables. Overall, microalgae in lentic freshwater bodies are determining by chemical characters of water (total dissolved solids, dissolved oxygen, electrical conductivity and salinity) and not be influenced with longitude.

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Therapeutic Efficacy of Novel Herbal Formulation in Protecting $PM_{2.5}$-Induced Lung Tissue Degeneration

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***Abstract*:** Particulate matter (PM) poses a major threat to human health, with lung damage evident even in early stages. Effective and low-risk treatments are essential. This study evaluated a novel herbal formulation comprising eight plants: *Angelica archangelica, Dioscorea bulbifera, Fagopyrum dibotrys, Myrtus communis, Nasturtium officinale, Perilla frutescens, Catharanthus roseus, and Solanum xanthocarpum*. The formulation was tested against PM-induced lung tissue damage in Wistar albino rats at doses of 100, 200, and 400 mg/kg over 30 days. Terminal blood samples were analyzed for hematology and differential cell count, including bronchoalveolar lavage fluid (BALF), showing significant reductions in inflammatory cells. Histopathological findings revealed PM-induced inflammation, alveolar deformation, and edema, which were progressively reduced across treatment groups, with the 400 mg/kg dose showing the greatest therapeutic effect.

***Keywords*:** Particulate matter, Lung tissue, Herbal formulation, Therapeutic actions, Wistar albino rats, Lung necropsy, Hematology

Air pollution is harmful to human health because it comprises both gaseous components and particulate matter (PM) constituents. The main components present in PM include carbonaceous material, reactive metals, nitrates, polycyclic aromatic hydrocarbons (PAHs), sulphates, endotoxins, and metals such as iron, nickel, zinc, vanadium, and copper. The primary source of PM is the combustion of fossil fuels. PM is classified based on particle size, such as PM_{10} (particles with a diameter less than 10 µm) and $PM_{2.5}$ (particles with a diameter less than 2.5 µm). Ultrafine particulate matter, referred to as $PM_{0.1}$, includes particles smaller than 0.1 µm (Hamanaka and Mutlu 2018). PM exhibits strong oxidative properties and exerts toxic effects on the human respiratory and circulatory systems. When inhaled by susceptible individuals, particulate matter triggers oxidative stress within lung cells, which contributes to the early stages of pathogenesis and poses significant risks to pulmonary health. It disrupts the balance of inflammatory cells in the lungs and stimulates the excessive production of free radicals. This overproduction, in the absence of adequate antioxidants, can lead to lung injury due to the oxidative damage inflicted on lung tissue components (Albano et al., 2022). Following PM exposure, numerous pro-inflammatory cytokines contribute to oxidative stress in lung tissues and epithelial cells, as PM acts as a potent oxidant. This leads to increased levels of inflammatory markers such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha (TNF-α) in lung tissues (Chang et al., 2019). Inflammation is also characterized by elevated neutrophil counts in bronchoalveolar lavage fluid (BALF), particularly in samples from the lower respiratory tract (Clinquart et al., 2023).

Several therapeutic alternatives exist for the treatment of respiratory and pulmonary diseases. In allopathic medicine, corticosteroids such as budesonide and formoterol are commonly used but may result in side effects with prolonged use (Garg et al., 2024). In contrast, traditional medical systems including Ayurveda, Siddha, Unani, Yoga, Naturopathy, and Homeopathy, offer treatment options that are typically associated with fewer side effects and are more cost-effective. The Ayurvedic approach, which emphasizes a holistic treatment philosophy, has received increasing attention compared to allopathic treatments (Verma et al., 2024). In alignment with the 2030 Agenda for Sustainable Development, adopted by all United Nations member states in 2015, this study was conducted to formulate and evaluate the efficacy of a novel herbal formulation against particulate matter-induced lung tissue degeneration under Sustainable Development Goal (SDG) 3.9.1. The efficacy of novel herbal formulation was evaluated using complete blood count (CBC), bronchoalveolar lavage fluid (BALF) analysis, and histopathological examination of lung tissues in Wistar albino rats.

MATERIAL AND METHODS

For this study, 24 healthy adult Wistar albino rats were used. These rats were housed in an acrylic exposure chamber, and air containing particulate matter was introduced using a diesel generator. The concentration of diesel exhaust was measured using an Air Quality Monitor (GRIMM Portable Aerosol Spectrophotometer Model 11-D), which assessed PM_{10} and $PM_{2.5}$ concentrations. Lung toxicity was induced by exposing the animals to diesel exhaust for three months, 3 hours per day. The animals were randomly assigned to four groups, with six rats in each group.

Dose administration schedule: Twenty four Wistar albino rats were divided into four groups, with six animals in each group. Group 1 (control) received distilled water orally at a dose of 5 mL/kg for 30 consecutive days, while Groups 2, 3, and 4 were administered with the test formulation orally at doses of 100, 200, and 400 mg/kg, respectively, for the same duration.

Blood sample collection and lung isolation: Blood samples were collected from the retro-orbital plexus of each animal on day 30 for complete blood count (CBC) analysis. CBC was performed using a hematology analyzer (Genrui Biotech KT-6400). Following euthanasia on day 30, the lungs were isolated for histopathological examination and bronchoalveolar lavage fluid (BALF) analysis.

Histopathological analysis: The isolated lungs were placed in buffered formalin for 24 hours. After fixation, the tissues were washed in running water for 12 hours, then processed using a tissue processor (Fig. 1). The tissue underwent a 12-step processing sequence across 12 stations, as outlined in the flow chart (Fig. 1). Tissue processing was performed to preserve structural integrity and prevent degradation. Fresh tissue was fixed in a formaldehyde solution to harden and preserve morphology. The dehydration step involved graded alcohol series in a two-step process to remove water. This was followed by clearing with acetone and benzene to extract lipids, facilitating wax infiltration. Subsequently, the tissue was infiltrated with paraffin wax. The processed tissues were embedded in L-shaped molds, filled with paraffin wax, and allowed to solidify. These tissue blocks were mounted on metal holders for sectioning. Staining was conducted as per the schedule using hematoxylin and eosin (H&E) staining (Fig. 2).

Staining procedure: Dewaxing was performed using xylene to remove residual wax. Blueing was carried out with an alkaline solution, while acid alcohol was used for differentiation, removing background staining. The sections were then stained with aqueous or alcoholic eosin, followed by rinsing, dehydration, and clearing. Finally, the slides were mounted with DPX (distrene dibutyl phthalate xylene) for microscopic examination (Bai et al., 2023).

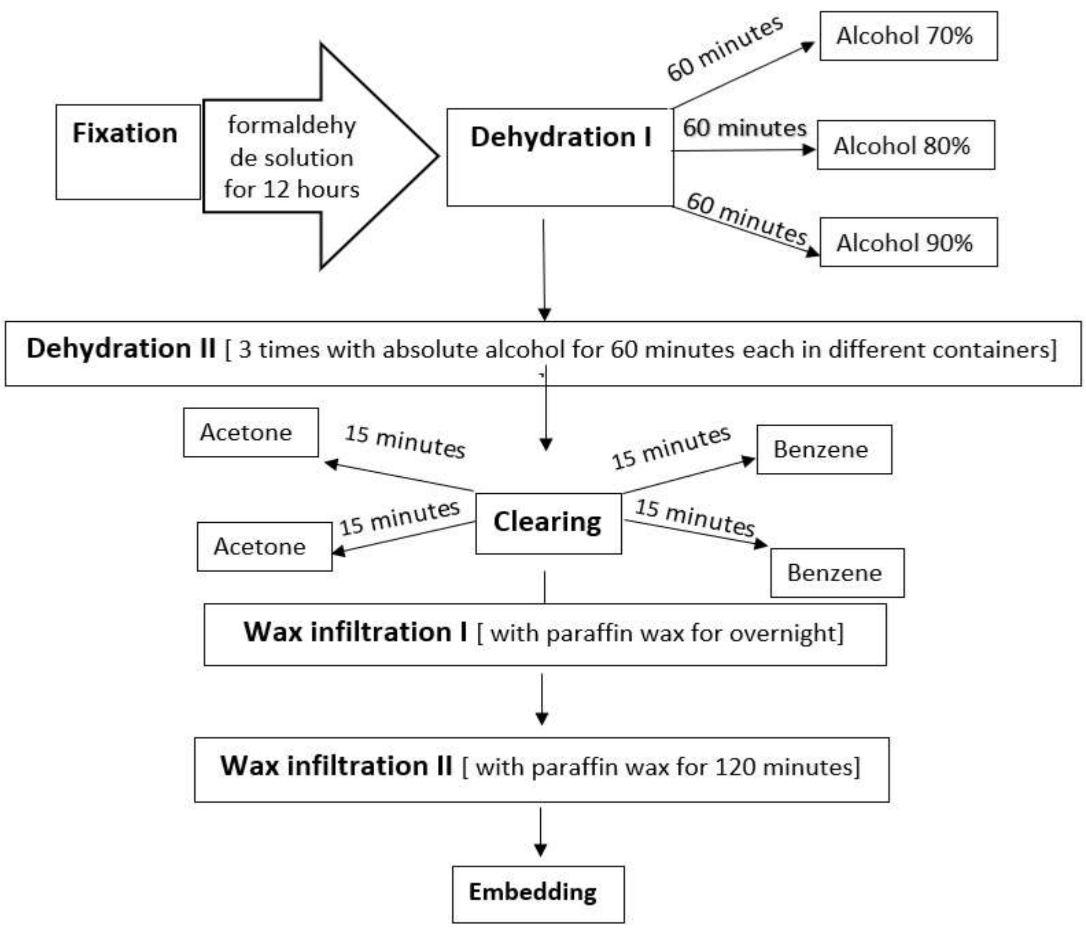

Fig. 1. Tissue processing schedule (12-step flow chart)

RESULTS AND DISCUSSION

After the 30-day dosing period, all four experimental groups were evaluated using complete blood count (CBC), leukocyte profiling in bronchoalveolar lavage fluid (BALF), and histopathological examination of lung tissues, with comparisons made relative to Group 1 (control).

Complete blood count (CBC) analysis: CBC parameters including WBC, neutrophils, eosinophils, lymphocytes, monocytes, platelets, and ESR were analyzed across groups (Table 1). No significant differences were observed in total WBC and platelet counts, suggesting that PM exposure did not markedly influence these parameters and that treatment exerted no direct effect on them. In contrast, neutrophil counts were significantly reduced in Groups 2, 3, and 4, with the maximum decrease in Group 4 (23.63%), reflecting a dose-dependent therapeutic effect. As neutrophilia is a hallmark of PM-induced pulmonary inflammation, the observed reduction suggests that the herbal formulation exerts antioxidant and anti-inflammatory activity.

Eosinophil also declined significantly in the treated groups, particularly in Group 4 (2.98%) compared with the control (6.78%), indicating a reduced requirement for eosinophil-mediated immune defense. Conversely, lymphocyte counts progressively increased from Group 2 to Group 4 (42.96% in control vs. 64.93% in Group 4), suggesting immunomodulatory effects of the formulation. Monocyte counts were moderately reduced in Group 3 and significantly reduced in Group 4, supporting attenuation of inflammatory responses. A progressive decline in ESR values across treatment groups further confirmed the suppression of systemic inflammation. Taken together, the higher lymphocyte and platelet counts in Groups 3 and 4, along with lower neutrophil, eosinophil, monocyte, and ESR levels relative to Group 1, demonstrate the dose-dependent efficacy of the formulation in restoring hematological homeostasis (Table 1).

BALF leukocyte profiling: BALF analysis was performed to directly assess pulmonary inflammation (Table 2). Neutrophil percentages were markedly reduced across treated groups, with the steepest decline observed in Group 4 (22.20%) compared to Group 1 (43.42%). Eosinophil and monocyte counts followed a similar decreasing trend, whereas lymphocyte levels increased progressively with dose, reaching 70.84% in Group 4. Statistical analysis confirmed that monocyte reductions were non-significant in Group 2, moderately significant in Group 3, and highly significant in Group 4. These findings reinforce the immunomodulatory potential of the formulation, highlighting its capacity to suppress pro-inflammatory cell infiltration while enhancing lymphocyte-mediated responses. Correlation analysis further validated the dose-dependent therapeutic effects, with Group 4 demonstrating the most consistent improvements across BALF parameters.

Histopathological examination of lung tissue: Histopathological analysis revealed distinct pathological changes in the control group (Group 1), including

Table 1. Complete blood count (CBC) parameters following 30-Day treatment across experimental groups*

Parameters	Group 1	Group 2	Group 3	Group 4
WBC (10^9/L)	4.97±0.33	5.72±0.36	5.28±0.39	4.22±0.24
Neutrophils (%)	51.04±1.66	40.31±0.57	35.96±1.22	23.63±2.52
Eosinophils (%)	6.78±0.19	5.95±0.28	4.77±0.34	2.98±0.11
Lymphocytes (%)	42.96±1.13	48.40±1.45	53.25±2.14	64.93±2.28
Monocytes (%)	3.85±0.28	3.38±0.24	2.93±0.18*	2.63±0.15
Platelet count (10^9/L)	323.33±29.71	388.00±25.18	405.00±28.52	369.50±20.86
ESR (mm/hr)	10.16±1.13	8.47±0.62	6.16±0.45	4.15±0.32

*Mean±SE

Table 2. Evaluation of leukocyte count in BALF

Parameters	Group 1	Group 2	Group 3	Group 4
Parameters	Group 1	Group 2	Group 3	Group 4
Neutrophils (%)	43.42 ± 0.94	38.54 ± 1.14 *	30.28 ± 2.38 **	22.20 ± 0.88 ****
Eosinophils (%)	9.24 ± 0.53	7.35 ± 0.29 *	5.04 ± 0.29 **	3.48 ± 0.25 ****
Lymphocytes (%)	45.45 ± 1.56	54.01 ± 1.36 *	62.94 ± 1.52 **	70.84 ± 1.10 ****
Monocytes (%)	3.94 ± 0.31	3.33 ± 0.24 ns	2.41 ± 0.12 *	2.09 ± 0.22 **

Mean±SE, Statistical significance was determined using one-way ANOVA followed by post hoc analysis. ns = not significant;*=p value <0.05, **=p value <0.01, ***= p value <0.001, ****= p value <0.0001 Mean±SE , Statistical significance was determined using one-way ANOVA followed by post hoc analysis. ns = not significant;*=p value <0.05, **=p value <0.01, ***= p value <0.001, ****= p value <0.0001

inflammatory cell infiltration, alveolar distortion, edema, and thickening of intrabronchial walls, classic indicators of PM-induced lung damage. These features are closely associated with the activation of neutrophils, lymphocytes, and eosinophils, which release inflammatory mediators such as TNF-α, IL-1β, IL-4, IL-5, IL-6, histamine, leukotrienes, and nitric oxide. In contrast, Groups 2, 3, and 4 exhibited progressive histological improvements, including reduced inflammatory cell infiltration, restoration of alveolar structure, and thinning of intrabronchial walls (Fig. 3). The degree of recovery was dose-dependent, with Group 4 showing the most substantial improvements after 30 days of treatment. These findings suggest that the formulation effectively suppresses the excessive production of pro-inflammatory cytokines and reactive oxygen species (ROS), thereby protecting against PM-induced pulmonary injury and promoting structural recovery of lung tissue (Sharma and Upadhyay 2023).

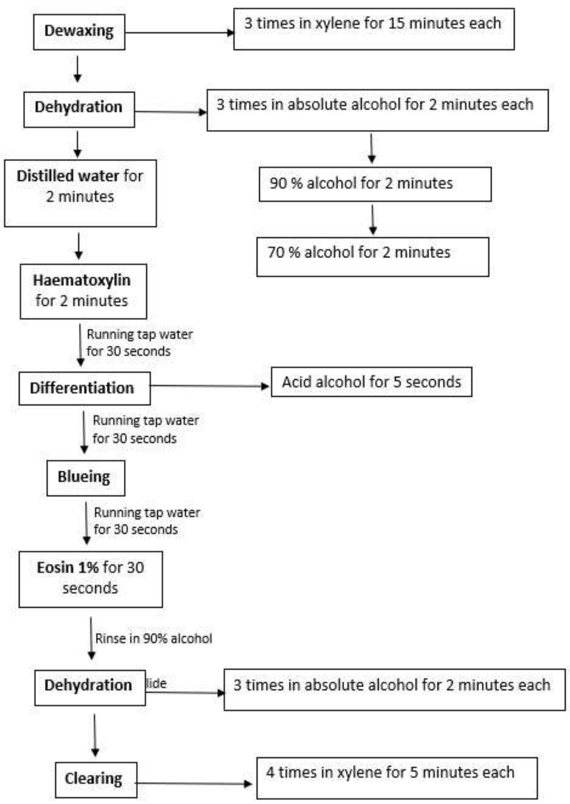

Fig. 2. Staining process schedule

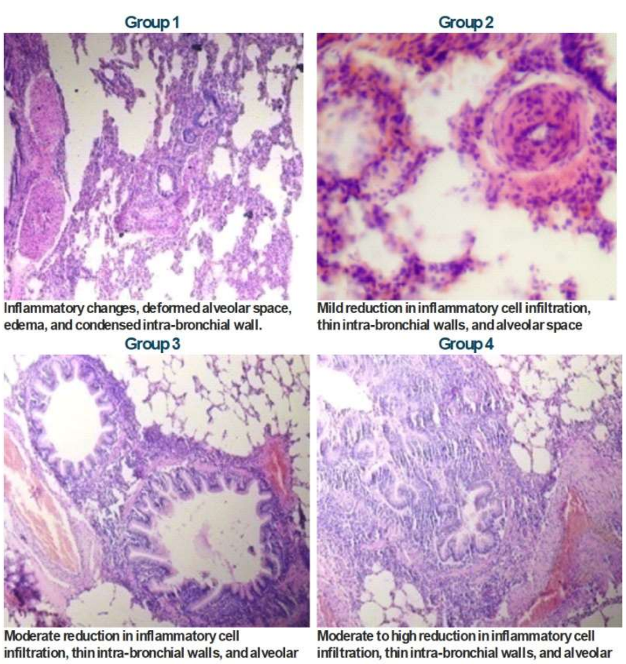

Fig. 3. Histopathological examination of lung tissues across experimental groups

CONCLUSION

Following the completion of the 30-day dosing schedule, the evaluation of hematological parameters (CBC), leukocyte profiling in bronchoalveolar lavage fluid (BALF), and histopathological examination of lung tissue served as reliable indicators of the therapeutic efficacy of the novel herbal formulation. The administered doses of 100, 200, and 400 mg/kg demonstrated significant improvements across these parameters compared to the control group.

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Seasonal Variations in Air Pollution Tolerance Index (APTI) of Selected Avenue Tree Species in Bhubaneswar, Odisha

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Abstract: The study examines spatial and seasonal variations in the air pollution tolerance index (APTI) of four tree species (*Ficus religiosa*, *Polyalthia longifolia*, *Syzygium cumini,* and *Lagerstroemia speciosa)* across three sites (industrial, traffic, and natural forest area) in Bhubaneswar city of Odisha. Significant variations in leaf biochemical parameters (leaf pH, ascorbic acid, total chlorophyll, and relative water content) were observed among species, depending on site conditions and seasons. The calculated APTI value (using four biochemical parameters) inferred *P. longifolia* as tolerant (33.97), *F. religiosa* moderately tolerant (22.07 to 25.46) towards air pollution. Conversely, *S. cumini* and *L. speciosa* showed lower APTI value, indicating their sensitivity to the polluted environment. Irrespective of species, pollution tolerance was higher in the rainy season than in the winter and summer seasons. Pearson's correlation study revealed that leaf relative water content and ascorbic acid enhance the tree's defence against pollution-induced oxidative stress. Study suggest *P. longifolia* and *F. religiosa* are best for urban afforestation, avenue plantation, and air pollution control management in Bhubaneswar.

Keywords: Leaf pH, Ascorbic acid, Chlorophyll, Relative water content, Urban green belt, Avenue plantation

Air pollution has emerged as a significant environmental and public health concern in India, affecting not only major metropolitan centers but also medium and smaller towns with increasing severity. This pervasive degradation in air quality is an inevitable result of rapid industrialization and unplanned urban expansion. Rapid population growth, trade liberalization, rising vehicular emissions, and industrialization have collectively intensified atmospheric pollutants, especially oxides of sulphur (SO_x), nitrogen (NO_x), volatile organic compounds (VOCs), and trace metals (Shaheen et al., 2025). The relentless emission of harmful gases and fine particulates poses a significant threat to air quality, profoundly impacting human health, ecosystem stability, and overall quality of life on Earth (Lohwasse et al., 2025). Addressing this environmental crisis is imperative for protecting public health and ensuring a sustainable future for posterity.

Trees play a crucial role in improving air quality by removing pollutants through impingement, absorption, and accumulation (Manasa et al., 2023). They absorb gaseous pollutants through photosynthesis while simultaneously capturing particulate matter on their leaf surfaces, effectively reducing greenhouse gas levels in the atmosphere during the process. Although pollutants generate reactive oxygen species (ROS) that induce stress, plants produce ROS scavengers and detoxification agents to tolerate this damage (Escobedo et al., 2008). Leaves metabolize and assimilate pollutants into various tissues, acting as sinks that lower environmental contamination. Moreover, trees regulate microclimates through shading and transpiration, which reduce ambient temperatures, limit smog formation, and enhance pollutant dispersion (Paoletti et al., 2004).

Plant responses to air pollutants vary significantly based on their intrinsic tolerance capacity, which is influenced by a combination of internal traits and external environmental conditions. These intrinsic factors comprise physiological traits such as stomatal conductance, transpiration, and chlorophyll content; biochemical traits including antioxidant activity, ascorbic acid and proline; and morphological traits like leaf size, texture, and cuticle thickness. Seasonal variations further affect both the pollutant load and the physiological responses of plants.

The APTI quantifies a plant's ability to withstand toxic pollutants, calculated using four biochemical parameters: leaf extract pH, ascorbic acid content, total chlorophyll, and relative water content (Chauhan, 2010). Plants with higher APTI values exhibit greater tolerance and are suitable for pollution mitigation, while those with lower values are more sensitive and serve as bio-indicators of air quality (Chauhan and Joshi, 2008). Additionally, external leaf symptoms, *viz.* colour changes, shape alterations, necrotic patches, and stomatal characteristics are important indicators of a plant's response to pollution stress (Seyyednejad et al., 2011, Leghari et al., 2013).

In urban areas, dust from vehicular exhaust, unpaved roads, construction, and industries poses a significant problem. Trees play a vital role in mitigating this issue by effectively capturing dust. However, their dust retention capacity varies with height, canopy structure, phyllotaxy, and leaf morphological traits including size, pubescence, surface roughness, and presence of wax layer (Chauhan and Sanjeev 2008, Singh et al., 2002). Therefore, selecting tree species for green belts and avenue plantation should carefully consider both their pollution tolerance and dust retention abilities to maximize air quality improvement.

Bhubaneswar stands as one of the fastest-expanding smart cities in eastern India, characterized by rapid infrastructure development, burgeoning small to medium-scale industries, enhanced street transport networks, and a significant surge in vehicular traffic concurrent with its growing population. These developments have collectively contributed to the emission of substantial quantities of toxic pollutants, adversely impacting the city's air quality. Despite this, Bhubaneswar remains one of the few planned urban centers in the country where residents exhibit a strong commitment to environmental stewardship, with trees and green spaces deeply embedded in the city's cultural and social fabric. Among various avenue tree species observed along streets of the city, *Ficus religiosa*, *Polyalthia longifolia*, *Syzygium cumini,* and *Lagerstroemia speciosa* were very common because of their aesthetic morphological traits (Satapathy and Das 2021) and tolerance to cyclonic damages (Bhola and Sinha 2006). Understanding these variations is essential for selecting the most resilient and pollution-tolerant tree species can be effectively used in urban greening strategies to enhance air quality throughout the year.

MATERIAL AND METHODS

To assess the pollution tolerance of selected avenue tree species, three distinct zones were identified in Bhubaneswar. These included: Site 1 – the Mancheswar Industrial Area (MIA), Site 2 – the high-traffic corridor along National Highway 16 from Rasulgarh to Patrapara (NHTZ), and Site 3 – the Natural Forest area within the City Forest Division (NFCFD). Four common avenue tree species found in each zone (*Ficus religiosa*, *Polyalthia longifolia*, *Syzygium cumini*, and *Lagerstroemia speciosa*) were selected . These site, has a tropical savannah climate with a mean annual rainfall of 1,498 mm and an average temperature of 27.4 °C. Three distinguishable seasons, summer (March-June), rainy (July-October), and winter (November-February) can be observed. The pedogenic mass primarily consists of red laterite and alluvial soils. The morphometric characteristics and location details of the studied trees are provided in Table 1. To study the APTI of tree species, 15 plants of each species were randomly selected within the study area. From each plant, five leaf samples (each consisting of 4-6 fully developed leaves) were collected at an accessible height during the morning hours, between 7:00 and 8:00 a.m.

Biochemical analysis of leaf samples: The freshly collected leaf samples were analyzed for leaf extract pH, relative water content, total chlorophyll content, and ascorbic acid using standard procedures. The pH was determined using a pH meter (Systronics Model 361) following the protocol of Kaur and Nagpal (2017). The leaf relative water content (LRWC) was estimated according to the method described by Liu and Ding (2008). The concentrations of total chlorophyll pigments were estimated using Arnon's 80% acetone method (Arnon 1949). Ascorbic acid content was estimated colorimetrically using 3% metaphosphoric acid for extraction and 2,6-dichlorophenol-indophenol as the indicator dye, following the protocols of Ranganna (1977) and Sadasivam and Manickam (1992).

Estimation of air pollution tolerance index: The air pollution tolerance index (APTI) was calculated based on the concentrations of ascorbic acid (mg g^{-1}), total chlorophyll (mg g^{-1}), leaf extract pH, and relative water content (%) of leaf samples following the equation proposed by Singh and Rao (1983).

$$APTI = [Aa \times (Tcc + P) + R]/10$$

Table 1. Location coordinates and characteristics of selected plants at study site

Tree name	Leaf shape	Average height (m)	Average GBH* (cm)	Site 1 (MIA)	Site 2 (NHTZ)	Site3 (NFCFD)
FR	Cordate	30.25±5.0	115.25±8.7	20º30'78.1"N 85º84'60.5"E	20º23'18.2"N 85º74'75.4"E	20º34'87.5"N 85º66'18.4"E
PL	Lanceolate	18.70±6.2	75.81±4.5	20º30'78.5"N 85º84'60.8"E	20º23'17.6"N 85º74'75.9"E	20º34'87.8"N 85º66'18.9"E
SC	Elliptic-oblong and ovate	27.52±4.0	85.90±2.9	20º30'78.4"N 85º84'60.7"E	20º23'16.9"N 85º74'74.7"E	20º34'86.7"N 85º66'18.1"E
LS	Oval to elliptic	20.64±2.4	80.33±2.6	20º30'77.6"N 85º84'61.3"E	20º23'18.9"N 85º74'75.3"E	20º34'87.1"N 85º66'18.6"E

Tree species FR- *Ficus religiosa* L., PL- *Polyalthia longifolia* (Sonn.) Thwaites, SC- *Syzygium cumini* (L.) Skeels, and LS- *Lagerstroemia speciosa* (L.) Pers; sites: S1-Mancheswar industrial area (MIA), site2-Rasulgarh to Patrapara national highway (number 16) traffic zones including bus stand (NHTZ), and site3- natural forests in city forest division (NFCFD); GBH: girth at breast height (1.37 m from ground)

Where Aa represents the ascorbic acid content (mg g^{-1}) of the leaf sample, Tcc is the total chlorophyll content (mg g^{-1}), P denotes the pH of the leaf extract, and R is the relative water content (%) of the leaf. After obtaining the APTI values, tree species were classified into different categories based on their tolerance levels as: very sensitive (APTI < 1), sensitive (APTI 1 to 16), intermediate/moderately tolerant (APTI 17 to 29) and tolerant (APTI 30 to 100) (Kaur and Nagpal 2017).

Statistical analysis: To evaluate the spatial and temporal variations in the air pollution tolerance index (APTI) of the selected tree species, the significance of differences was tested at 5% level (P < 0.05) and the critical difference (CD) was calculated for pairwise comparisons. All calculations were performed using MS Excel 2011 and IBM SPSS 2020 package.

RESULTS AND DISCUSSION

Leaf extract pH: Leaf extract pH varied significantly with species, site, and season. Significant interactions were for species × site, and species × season (Table 2). The pH ranged from 3.72 (at traffic zone in *Lagerstroemia speciosa) to* 7.23 (at traffic zone in *Ficus religiosa*), peaking at 7.83 during the rainy season in the natural forest area, with overall means ranging from 3.72 to 7.61. *Polyalthia longifolia* and *Syzygium cumini* showed moderate pH ranges, from 5.01 (summer season, industrial area) to 6.53 (rainy season, natural forest area), and from 4.38 (summer season, traffic zone) to 6.40 (rainy season, natural forest area), respectively. *Lagerstroemia speciosa* had the most acidic values, with a low of 3.44 during the winter season in the industrial area and a high of 5.00 in the rainy season at the natural forest site, with overall means ranging from 3.72 to 4.38. Based on overall leaf extract pH values, the species were ranked as: *Ficus religiosa* > *Polyalthia longifolia* > *Syzygium cumini* > *Lagerstroemia speciosa*. These trends reflect the combined influence of inherent genetic traits and environmental stressors, particularly pollutants. The lower pH values observed in industrial and traffic zones can be attributed to higher concentrations of sulfur oxides (SOx), nitrogen oxides (NOx), and volatile organic compounds (Swami et al., 2004, Singare and Talpade 2013). Conversely, the higher pH levels recorded during the rainy season are likely due to the washing off and dilution of acidic particulate pollutants by rainfall (Das and Prasad, 2010, Dash and Sahoo, 2017).

Leaf total chlorophyll contents (TCC): The TCC of different trees exhibited significant variations with respect to species, site, and season . Significant interactions were also noted for species × site and species × season, while the interactions between season × site and species × season × site were not significant (Table 3). The total chlorophyll content ranged from lowest 2.82 mg g^{-1} in *L. speciosa* (traffic zone) to 7.83 mg g^{-1} in *F. religiosa (natural forest area)*. Based on overall total chlorophyll content values species were grouped in the order: *Ficus religiosa* > *Syzygium cumini* > *Polyalthia longifolia* > *Lagerstroemia speciosa*. Spatial variation also revealed significant differences. Trees growing in the natural forest area recorded the highest chlorophyll content, followed by those in the industrial area, with the lowest values in traffic zones. Jyoti and Jaya (2010) and Mir et al. (2008) also observed reduced chlorophyll levels in urban plants due to higher concentrations of automobile emissions and dust load from construction activities. The seasonal variation in total leaf chlorophyll content among selected tree species exhibited a consistent pattern, with peak values during the rainy season, a decline in winter, and subsequent increase in summer. The reduction in chlorophyll content during winter can be attributed to lower temperatures and reduced photoperiod, which limit enzymatic activity and photosynthesis, subsequently affecting chlorophyll

Table 2. Seasonal and spatial variations in leaf extract pH of tree species in Bhubaneswar

Species	Season and location											
	Rainy			Winter			Summer			Overall mean		
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
FR	7.55	6.79	7.83	7.28	6.66	7.60	6.87	6.51	7.38	7.23	6.65	7.61
PL	5.67	5.27	6.53	5.91	5.99	6.04	5.01	5.59	6.09	5.53	5.61	6.22
SC	5.31	5.30	6.40	5.19	5.46	6.09	4.64	4.38	5.12	5.05	5.05	5.87
LS	4.21	4.34	5.00	3.44	3.52	4.25	3.52	3.59	3.90	3.72	3.82	4.38
C D (p=0.05)												
Species				0.30	Site			0.26	Seasons			0.26
Species × Site				0.52	Species × Seasons			0.52	Seasons × Site			NS
Species × Seasons × Site				NS								

biosynthesis (Jyoti and Jaya 2010). Additionally, increased dust deposition during winter has been shown to correlate negatively with chlorophyll content, further contributing to the decline (Singh et al., 2008). The rainy season supports optimal chlorophyll accumulation owing to adequate water availability, nutrient uptake, and favourable temperatures, whereas the moderate recovery in summer may be attributed to longer daylight hours despite heat and water stress. Sharma et al. (2017) also observed similar seasonal patterns in chlorophyll content in tree species exposed to urban particulate pollution. Significant interactions were noted between species × site and species × season. These variations may be attributed to the species' differential tolerance to air pollutants.

Relative water content of leaf (LRWC): Spatial and seasonal variations in LRWC among four tree species in Bhubaneswar showed significant differences influenced by species, season, and site (Table 4). *Ficus religiosa* consistently maintained high RWC (68.37% to 74.38%), indicating strong drought tolerance, while *Lagerstroemia speciosa* had the lowest values (41.15 to 51.71%), reflecting higher sensitivity to stress. LRWC was highest during the rainy season (75.60% in *P. longifolia* at the traffic zone) and declined through winter and summer seasons likely, due to reduced soil moisture and increased evapotranspiration. Natural forest sites generally supported better LRWC, likely due to lower pollution and better soil conditions, consistent with the observations of Joshi and Swami (2007). Significant interactions were also observed for the species × site and species × season, while the three-way interaction was insignificant.

Leaf ascorbic acid content (LAA): Spatial and seasonal variations in leaf ascorbic acid (LAA) content of the four tree species were observed, and the differences were significantly influenced by species, season, and site. Significant interactions were also noted for species × site and species × season, while the interactions between season × site and species × season × site were not significant (Table 5). Generally, LAA accumulation is often used as a physiological marker for oxidative stress resistance and pollution tolerance in plants (Khan et al., 2011). Among all species, *Polyalthia longifolia* demonstrated the highest

Table 3. Spatial and temporal variations in leaf chlorophyll content (mg g^{-1}) of trees in Bhubaneswar

Species	Season and location											
	Rainy			Winter			Summer			Overall mean		
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
FR	7.89	6.10	8.18	6.61	4.51	7.53	7.28	5.30	7.78	7.26	5.31	7.83
PL	5.88	4.78	7.18	4.32	3.07	5.26	3.58	3.27	6.16	4.59	3.71	6.20
SC	7.04	5.48	8.39	4.66	3.67	6.16	6.30	4.23	6.91	6.00	4.46	7.15
LS	4.65	3.70	5.58	2.26	2.04	3.22	3.45	2.71	4.30	3.45	2.82	4.37
C D (p=0.05)												
Species				0.22	Site		0.19	Seasons				0.19
Species × Site				0.38	Species × Seasons		0.38	Seasons × Site				NS
Species × Seasons × Site				NS								

Table 4. Spatial and temporal variations in leaf relative water content (%) of trees in Bhubaneswar

Species	Season and location											
	Rainy			Winter			Summer			Overall mean		
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
FR	73.38	74.38	71.71	71.97	73.40	69.80	69.70	71.49	68.37	71.68	73.09	69.96
PL	74.60	75.60	73.2	71.42	74.13	72.35	69.8	72.0	47.22	71.95	73.91	64.20
SC	50.42	54.09	49.84	49.02	55.82	47.22	47.99	48.97	45.56	49.14	52.96	47.54
LS	46.38	51.71	45.3	43.37	49.12	44.26	41.8	41.1	41.33	43.87	47.3	43.6
C D (p=0.05)												
Species				1.29	Site		1.12	Seasons				1.12
Species × Site				2.24	Species × Seasons		2.24	Seasons × Site				NS
Species × Seasons × Site				NS								

overall LAA content, with its maximum accumulation during winter season at site 2 (25.71 mg g^{-1}), which is a zone of high vehicular emissions. This trend implies that *P. longifolia* possesses a robust antioxidative defence mechanism capable of countering air pollution-induced oxidative stress, especially in colder months when pollution levels are high due to atmospheric inversion. Conversely, *Lagerstroemia speciosa* recorded the lowest LAA values across all sites and seasons, with the lowest mean (4.38 mg g^{-1}) observed in the summer at Site 3 (natural forest area), suggesting limited stress tolerance and metabolic responsiveness to environmental variations. It was also observed that the LAA content for most species was higher at traffic zones (site 2) compared to the relatively less polluted natural forest area (site 3), supporting the hypothesis that elevated pollution levels trigger enhanced antioxidant production in trees (Gill and Tuteja 2010). The findings are in line with the earlier reports of Mir et al. (2008), who pointed out that higher concentrations of automobile pollutants are one of the prime causes for the elevated level of leaf ascorbic acid content of plants grown along roads. Seasonal differences were also evident, with the winter season consistently showing elevated LAA concentrations, likely attributable to a combination of stress-induced higher antioxidant production and slower biochemical degradation processes under cooler climatic conditions.

Air pollution tolerance index (APTI): The significant difference in APTI was observed among species, sites, and seasons, along with significant interaction effects for species × site and species × season. *P. longifolia* consistently recorded the highest APTI across all sites and seasons, with a peak of 33.97 at Site 1 (MIA) during the rainy season. Its overall mean APTI across sites (30.50 at S1 and 31.48 at S2) classifies as tolerant (T) species. This high APTI score can be attributed to its efficient physiological and biochemical adaptations, such as higher chlorophyll content, stable pH, and robust ascorbic acid levels, which collectively enhance its resilience against oxidative stress induced by atmospheric pollutants. The ability to maintain relatively high APTI even in polluted environments makes it an ideal candidate for urban

Table 5. Spatial and temporal variations in leaf ascorbic acid content (mg g^{-1}) of trees

Species	Season and location											
	Rainy			Winter			Summer			Overall mean		
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
FR	13.36	15.58	11.24	12.69	13.58	13.58	13.36	13.35	10.36	13.14	14.17	11.73
PL	20.85	23.71	15.36	20.18	25.71	16.71	19.51	22.38	8.60	20.18	23.94	13.56
SC	10.66	17.60	9.25	9.86	16.60	8.60	10.3	11.6	7.32	10.28	15.2	8.39
LS	7.38	7.48	6.49	6.38	6.48	5.48	5.38	5.48	4.38	6.38	6.48	5.45
C D (p=0.05)												
Species				0.53	Site			0.46	Seasons			0.46
Species × Site				0.92	Species × Seasons			0.92	Seasons × Site			NS
Species × Seasons × Site				NS								

Table 6. Spatial and temporal variations in air pollution tolerance index (APTI) of selected trees in Bhubaneswar

Species	Season and location												APTI class
	Rainy			Winter			Summer			Overall mean			
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	
FR	25.27	25.46	24.0	21.93	20.56	24.44	20.93	20.20	19.18	22.71	22.07	22.56	I
PL	33.97	33.04	30.2	28.46	32.19	27.63	29.06	29.20	12.74	30.50	31.48	23.53	T
SC	15.67	21.24	16.1	12.24	18.04	12.74	13.14	13.13	11.43	13.68	17.47	13.41	S
LS	12.61	11.78	12.7	11.17	10.63	11.01	9.35	8.07	9.28	11.04	10.16	11.00	S
C D (p=0.05)													
Species				0.62	Site			0.54	Seasons			0.54	
Species × Site				1.07	Species × Seasons			1.07	Seasons × Site			0.93	
Species × Seasons × Site				NS									

APTI class: I-intermediate/ moderately tolerant, S-sensitive and T-tolerant greening and pollution mitigation efforts (Bala et al., 2022). *Ficus religiosa*, categorized as intermediate or moderately tolerant (I), showed moderate APTI values with an overall mean ranging between 22.07 and 22.71, and a maximum of 25.46 at the highway traffic zone during the rainy season. These values suggest a reasonable level of pollution tolerance, although not as robust as *P. longifolia*. Its performance indicates some capacity to withstand environmental stress, likely due to moderate antioxidant responses and reasonable maintenance of leaf metabolic functions in polluted environments. The range of observed APTI value (22.07 to 25.46) of *F. religiosa* is well within the reported value of APTI 21.62 to 25.77 (Sharma et al., 2017). *Syzygium cumini* and *Lagerstroemia speciosa* were classified as sensitive (S) species, with low APTI values across all sites and seasons. *S. cumini* had a particularly low APTI during the summer at Site 3 (11.43), while *L. speciosa* displayed its lowest tolerance during the same season at Site 2 (8.07). These low scores imply poor biochemical buffering capacity under polluted conditions, possibly due to lower chlorophyll content, reduced leaf water retention, or less efficient antioxidant defence mechanisms. Their marked sensitivity suggests these species are less suited for plantation in urban or industrially affected zones, although they might still play a role in aesthetic landscaping in cleaner environments. Seasonal variation in APTI was also evident. Most species recorded higher APTI values during the rainy and winter seasons compared to summer (Fig. 1). This trend may be attributed to seasonal changes in ambient pollutant concentrations, plant physiological responses, and

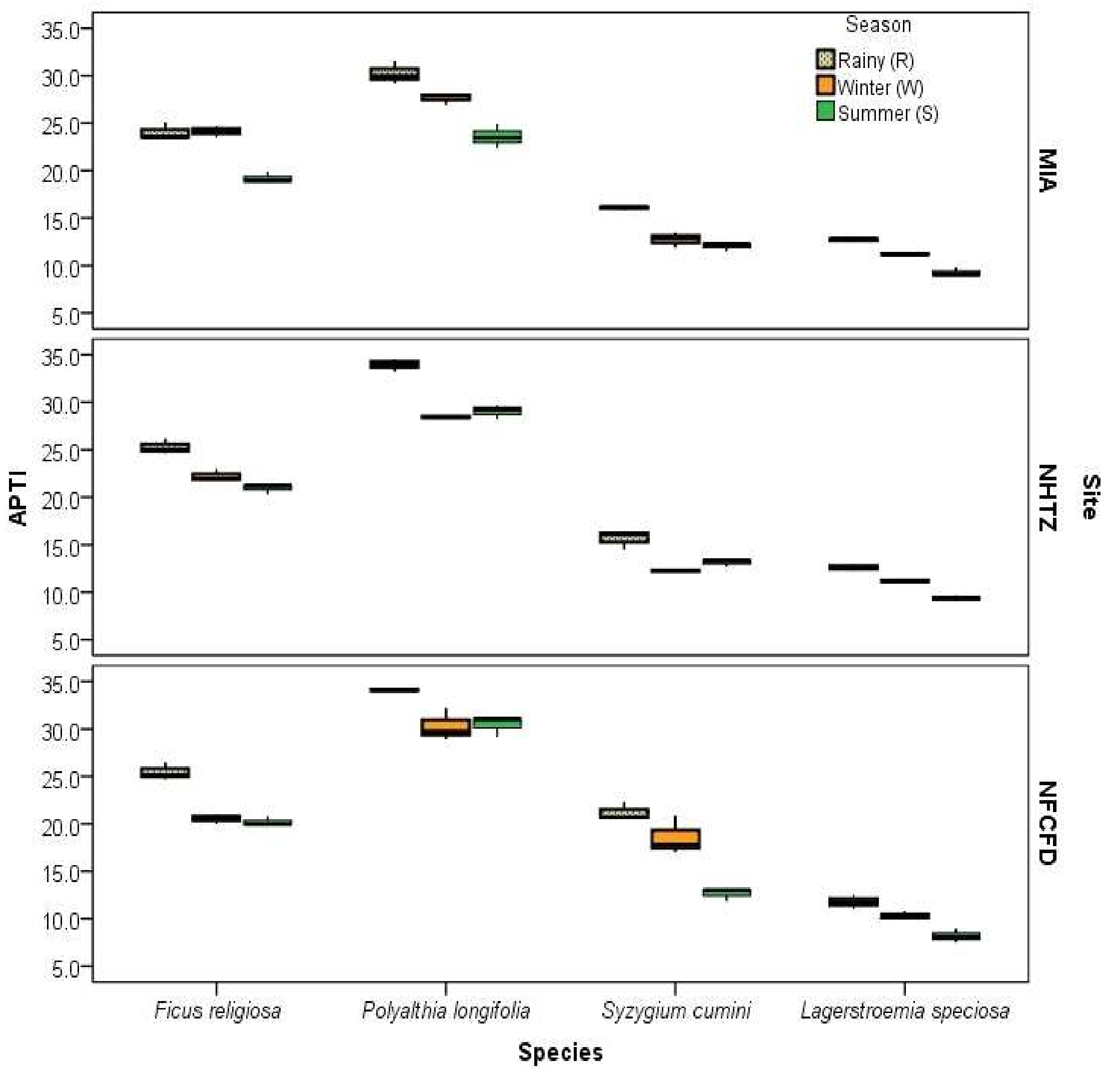

Fig. 1. Seasonal variations in air pollution tolerance index (APTI) of four tree species in different sites (MIA- Mancheswar industrial area, NHTZ- Rasulgarh to Patrapara national highway traffic zones, and NFCFD- natural forests in city forest division) of Bhubaneswar

Table 7. Pearson's correlation between leaf biochemical parameters and air pollution tolerance index (APTI)

Parameters	Leaf pH	TCC	LRWC	LAA
Total chlorophyll content (TCC)	0.760**			
Relative water content (LRWC)	-0.102	-0.405		
Ascorbic acid content (LAA)	-0.492	-0.695*	0.904**	
APTI	0.095	-0.046	0.915**	0.741**

* Correlation is significant at P<0.05
**Correlation is significant at P< 0.01

meteorological conditions. The rainy season, with its higher humidity and frequent precipitation, likely dilutes pollutant concentration, leading to reduced stress levels. In contrast, the summer season, characterized by elevated temperatures and drier conditions, exacerbates plant stress and reduces tolerance levels (Tripathy and Neema 2023).

Correlation between leaf biochemical parameters and APTI: The correlation between leaf relative water content (LRWC) and APTI was positive and significant indicating that tree species with higher water retention capacity exhibit greater tolerance to air pollution (Table 7). This emphasises the role of cell turgidity in maintaining cellular functions and mitigating the detrimental effects of pollutants. Similarly, leaf ascorbic acid (LAA) showed a strong positive correlation with APTI) suggesting that elevated levels of ascorbic acid enhance the plant's defence mechanisms against oxidative stress induced by air pollutants. Positive correlations between APTI and both LAA and LRWC content in various plant species were reported by Kour and Adak (2024). In contrast, total chlorophyll content (TCC) and leaf pH exhibited weak and non-significant correlations with APTI, implying that chlorophyll content and leaf pH are not directly associated with a plant's pollution tolerance. However, TCC showed a significant positive correlation with leaf pH, suggesting that a more alkaline leaf environment may stabilize chlorophyll molecules, thereby supporting photosynthetic activity. The strong correlation between LRWC and LAA infers that sufficient leaf turgidity enhances the synthesis and accumulation of antioxidants, thereby protecting plants from pollution-induced damage (Mehmood et al., 2024).

CONCLUSION

The study revealed significant spatial and seasonal variations in the air pollution tolerance index (APTI) among selected tree species in Bhubaneswar. *Polyalthia longifolia* exhibited the highest tolerance, suggesting its strong potential for urban area afforestation and pollution mitigation. *Ficus religiosa* showed moderate tolerance, while *Syzygium cumini* and *Lagerstroemia speciosa* were identified as sensitive species, more suitable for less polluted environments. Seasonal trends revealed higher APTI values during the rainy and winter seasons, likely due to favourable meteorological conditions. Higher leaf relative water content and ascorbic acid levels significantly enhance plant tolerance to air pollution, while chlorophyll content and leaf pH have an indirect association with tolerance. These findings highlight the importance of APTI-based species selection in urban plantation programmes aimed at enhancing environmental resilience and air quality.

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