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Research Article

Insect pollinator diversity and their foraging behaviour in Radish, *Raphanus sativus* L.

Pratap A. Divekar, Sampat Kumar Patel, Manimurugan C., Vikas Singh

Abstract

Radish is a cross-pollinated crop and honey bees are essential for its pollination. In the present investigation, the diversity and abundance of insect pollinators and additionally their visitation rate and frequency were also studied during 2019 and 2020. The foraging observations were noted at 08:00–10:00 hours (early morning foraging activity), 10:00-12:00 hours (late morning foraging activity), 12:00-14:00 hours (noon foraging activity), and 14:00-16:00 hours (afternoon foraging activity). The results revealed that nine species of bees, five flies, three butterflies, and three wasps species visited the flowers of radish during the study period. *Apis florea*, *Apis mellifera*, and *Apis dorsata* were the dominant pollinator species with 132-201, 110-130, and 90-115 individuals, respectively during the study duration. The maximum foraging activity of the dominant pollinators was observed at 14:00 hrs followed by 12:00 hrs whereas the minimum foraging activity was recorded in the early morning hours at 08:00. The frequency of floral visitors varied greatly, with *A. florea* (14.50-15.80 visits/flower/5 min) being the most frequent, followed by *A. mellifera* (9.80-11.00 visits/flower/5 min) and *A. dorsata* (8.30–9.40 visits/flower/5 min) in both seasons. Similarly, *A. florea* had statistically higher visitation rate (26.93–27.40 flowers visited/min) followed by *A. mellifera* (15.93–16.00 flowers visited/min) and *A. dorsata* (12.62-15.13 flowers visited/min). The findings suggested that *A. florea*, *A. mellifera*, and *A. dorsata* may be efficient radish pollinators. Therefore, on a commercial scale, these three species can be effectively used to increase crop yields.

Keywords foraging behavior, honeybees, pollinator diversity, radish

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Introduction

For sexual reproduction, animals are necessary for more than 90% of the 25,000 species of flowering plants [1], and of which, 75 percent of the major crop species comprise fruits, vegetables, oilseeds, and nuts [2]. Insects, particularly bees, play a key role as pollinators in maintaining the health of any ecosystem because they produce 35% of the total food volume and supply vital nutrients for human existence [2]. Among the vegetables, radish is a self-incompatible annual vegetable. Since radish is an obligate out-crosser, effective reproduction depends on insect pollinators [3]. In several countries, managed honey bees (*Apis mellifera*) are thought to be the best pollinators of radish [4]. By providing supplemental pollination, honey bees (*A. mellifera* and *A. cerana*) can boost radish seed production by 22 to 45 percent [4-5]. Radish is the most commonly grown vegetable in both tropical and temperate climates. Self-incompatibility prevents radish flowers from reproducing, therefore they are completely dependent on insect pollinators [3].

The majority of plant species that need pollination services have evolved to rely on a few individuals of insect species for efficient reproduction. However, studies reported that the insect species pollinating radish include a wide range of honey bees, mining bees, and syrphid species [6]. To prevent any adverse consequences on pollinating bees, it is best to select biopesticides, biocontrol agents, and plant secondary metabolites that are generally safer for crop protection during the flowering phase of development [7-10].

The pollinator diversity noted with radish is extremely fascinating because plant species that only rely on insect pollination usually limit their floral visitors to a small number. In general, the diversity and abundance of pollinators varies with the terrain, latitude, and time [11]. In addition, the presence of different flowering plant species may also alter the shared pollinator abundances and their foraging behaviours, resulting in increased or decreased plant pollination success [12]. Though several studies on the influence of honey bee pollination in terms of yield enhancement have been published [13], the foraging behaviour that favors the efficiency of key pollinators has been investigated poorly. In order to manage and protect the most prolific pollinators, it is necessary to identify the native pollinator community that is specific to a given crop.

Very scanty information is available on the diversity and abundance of insect pollinators and their foraging behaviour in radish from the Eastern Uttar Pradesh region. Therefore, the present investigation was carried out to document the diversity and abundance of insect pollinators; to study the behaviour of major pollinator in terms of visitation rate and visitation frequency in radish.

Methodology

Study location and experimental layout

The research was done at ICAR-Indian Institute of Vegetable Research, Regional Research Station, Sargatia, (Latitude NS 26˚ 43’ 56.61 and Longitude EW 84˚11’ 12.95) Kushinagar, India. Radish var. Kashi Hans (area: one acre) was raised throughout the spring of 2019 (season 1) and 2020 (season 2) for the field experiment. The crop was grown in accordance with the specified practices and plant protection measures were not taken once the flowering of radish initiated. The observations were taken during the flowering period of the radish (February-March). The abundance of floral visitors (the total number of individuals of a particular species in a given area), was observed from 100 plants in the research field by randomly placing a quadrat of 1 m during season 1 and season 2. From each quadrat, 10 plants were selected for recording in situ observations on the abundance of insect pollinators. Non-destructive method of sampling was deployed for studying the insect pollinators. Species diversity was worked out by using different indices such as Margalef’s index of richness (MI), Shannon-Wiener Diversity index (H), Simpson’s index (D), Pielou’s evenness index (J), and Berger-Parker index of dominance (d) by using the standard formula [14, 15, 16]. Engelmann’s scale of dominance was used to evaluate the dominance structure as elaborated and used by earlier researchers [17].

Abundance, visitation frequency, and rate of floral visitors

The visitation frequency (Numbers of visits/flower/2 min) and visitation rate (Numbers of flowers visited/2 min) of insect pollinators were determined during January-February months in 2019 and 2020 by following the standard methodology [18-19]. The insects that visit flowers were caught using a hand net, killed in a killing bottle, and kept in ethanol for further identification. Using the keys used by earlier researchers [20-21], the insects were identified to the genus level and to the species level by specialists.

Data analysis

Three-way analysis of variance (ANOVA) was used to analyze the data on visitation frequency and visitation rate, with species, time period, and season being the main explanatory variables. The statistical analysis was performed in Minitab version 18.00.

Results and Discussion

Diversity, percent Abundance and Engelmann abundance of insect pollinator

The results of the present investigation revealed that nine species of bees, five flies, three butterflies, and three wasp's species visited the flowers of radish during the study period at Sargatia, Kushinagar. The pollinator species *A. florea* (132–201 individuals), *A. mellifera* (110-130 individuals), and *A. dorsata* (90-115 individuals) were the most prevalent (abundant) on the flowers of radish during the study period. The least abundant species were wasps, *Vespula vulgaris* (5-8 individuals), *Vespa cincta* (4-9 individuals), and *Polystes* spp. (6-8 individuals) (Table 1).

Table 1. Diversity and Abundance of pollinators in Radish at Sargatia, Kushinagar

Group of insect pollinator	Insect Pollinator	Season 1			Season 2		
		No. of individuals	Percent Abundance	Engelmann abundance class	No. of individuals	Percent Abundance	Engelmann abundance class
Apis	*Apis mellifera*	110	14.95	D	130	13.35	D
	Apis dorsata	90	12.23	D	115	11.81	D
	Apis florea	132	17.93	D	201	20.64	D
	Apis cerana	30	4.08	SD	48	4.93	SD
	Tetragonula spp	28	3.80	SD	46	4.72	SD
	Xylocopa spp	46	6.25	SD	54	5.54	SD
	Andreina spp	21	2.85	R	21	2.16	R
	Megachile spp	26	3.53	SD	18	1.85	R
Scoliidae	*Scolia affinis*	34	4.62	SD	40	4.11	SD
Wasps	*Vespula vulgaris*	5	0.68	SR	8	0.82	SR
	Vespa cincta	4	0.54	SR	9	0.92	SR
	Polystes spp	6	0.82	SR	8	0.82	SR
Flies	*Episyrphus balteatus*	24	3.26	SD	44	4.52	SD
	Eristalinus aeneus	12	1.63	R	30	3.08	R
	Ischiodon scutellaris	14	1.90	R	18	1.85	R
	Stomorhina discolor	16	2.17	R	26	2.67	R
	Chrysomya megacephala	28	3.80	SD	26	2.67	R
Lepidoptera	*Danaus chrysippus*	32	4.35	SD	34	3.49	SD
	Euploea core	38	5.16	SD	46	4.72	SD
	Eurema blanda	40	5.43	SD	52	5.34	SD

D: Dominant; SD: Subdominant; RD: Recedent; SR: Subrecedent

On the basis of Engelmann abundance classification, the insect pollinators namely, *A. mellifera*, *A. dorsata* and *A. florea* were found to be in the dominant class, *A. cerana*, *Tetragonula* spp. and *Xylocopa* spp., *Scolia affinis*, *Episyrphus balteatus*, *Danaus chrysippus*, *Euploea core*, and *Eurema blanda* were observed in the Subdominant class. Pollinators namely, *Andreina* spp., *Eristalinus aeneus*, *Ishiodon scutellaris* and *Stomorhina discolour* were in Recedent class. However, *V. vulgaris*, *V. cincta*, and *Polystes* spp. were found in Subrecedent class in season 1 as well as season 2. In the present investigation, honeybees, syrphids, flies, lepidopteran butterflies, and wasps were observed as insect pollinators in radish. The pollination of the radish is carried out by honeybees, syrphid flies, wild bees, and other insect pollinators (butterflies) [22]. Among different pollinators, honeybees *A. florea*, *A. mellifera* and *A. dorsata* were the dominant and abundant insect pollinators in radish. The maximum number of honeybees was observed followed by syrphid flies and other insect pollinators (other wild bees). In onion, it was found that *A. dorsata*, *A. cerana indica*, *A. mellifera* and *A. florea* were the frequent insect pollinators [23]. Of which, *A. dorsata* was the predominant one. Similarly, there are reports that *Apis dorsata* was most dominant

species (44.48%) followed by *Apis cerena indica* (41.86%) and *A. florea* (4.59%) [24]. Our findings differ from the findings of [6], who reported that *Andrena* sp. and *Episyrphus balteatus* were the most prevalent floral visitors of radish. This variation in diversity of pollinators is mostly brought about by variations in geographic factors including topography, flora and fauna, and climate. However, bees play an important role in pollinating cruciferous vegetables, especially radish [13]. Through mutual compensation and complementarity, many pollinator groups serve as essentials for seed setting [25].

Our results indicated that honey bees, *A. florea*, *A. mellifera* and *A. dorsata* were major pollinators in radish seed production. Similarly, several researchers [26-27] found that bees were the major pollinators of cauliflower. Hymenopterans (family Apidae namely; *A. dorsata*, *A. mellifera*, *Trigona irridipennis*) were the major floral visitors on mustard (*Brassica campestris* var. toria) with *A. dorsata* and *A. mellifera* being the most frequent visitors [28]. There were 13 insect species reported on Indian mustard out of which 6 species belonged to the order Hymenoptera (*A. dorsata*, *A. mellifera*, *A. cerana indica*, *A. florea*, *Ceratina* spp., and *Halictus* spp.), 5 species to order Diptera (*Episyrphus* spp., *Eristalinus tabanoides*, *Musca domestica*, *Chrysomya* spp. and *Sarcophaga* spp.) and 2 species to order Lepidoptera (*Amata bicincta* and *Pieris* spp.) [29]. In mustard (*Brassica napus*), 15 insect species from 4 orders and 7 insects were found to visit the flowers [30]. The majority of the species turned out to be Hymenoptera.

Diversity indices of insect pollinators in Radish

The results of the assessment of insect pollinators using the different diversity index in Kushinagar was summarized in Table 2. The index of alpha diversity including Shannon-Weiner, Berger-Parker, Margalef richness, and Pielou's eveness index values were recorded as 1.15, 0.18, 6.63, and 0.88 during season 1 and 1.29, 0.18, 6.36 and 0.99 during season 2. The pooled diversity indices of insect pollinators in the present investigation were observed as Margalef's Richness Index (2.25), Shannon-Weiner Diversity index (1.02), Pielou's Evenness Index (0.37), and Berger-Parker dominance index (0.24). Similar types of findings also reported the diversity indices of insect pollinators in onions as follows: Shannnon-Weiner (1.805), Berger-Parker (0.288), and Margalef Richness (1.846) [23].

Table 2. Diversity indices of insect pollinators in Sargatia, Kushinagar

Diversity indices	Season 1	Season 2	Pooled
Margalef's Richness Index	6.63	6.37	6.50
Shannon-Weiner Diversity index	1.15	1.29	1.22
Pielou's Evenness Index	0.88	0.99	0.94
Berger-Parker dominance index	0.18	0.21	0.20

Visitation frequency and Visitation rate

The source of variation are presented in Table 3 for visitation frequency. The source of variation for species, time interval, and season was found highly significant for the dominant *Apis* pollinators however the interactions of species*season, time interval* season, and species*time interval*season were found non-significant. Similarly, the source of variation was presented in Table 3 for visitation rate. The source of variation for species, time interval, and season was found highly significant for the dominant *Apis* pollinators however the interactions of species*season, time interval * season, and species*time interval*season were found non-significant.

Table 3. Source of variation for the parameters visitation frequency and visitation rate

Source of Variation	Visitation Frequency		Visitation Rate	
	F	P	F	P
Species	60.19	0.000**	112.20	0.000**
Time Interval	130.29	0.000**	338.12	0.000**
Season	11.94	0.001**	3.35	0.069NS
Species *Time Interval	3.32	0.002**	14.80	0.000**
Species *Season	0.66	0.518NS	0.88	0.416NS
Time Interval* Season	2.62	0.037NS	0.15	0.961NS
Species *Time Interval* Season	0.19	0.992NS	0.86	0.550NS

**: Highly Significant; *: Significant, NS: Non-Significant

Significant differences were observed for the visitation frequency (no. of bees visited per flower per 2 min) at different time intervals (*A. mellifera*: F = 16.76, p<0.001; *A. dorsata*: F = 12.54, p<0.001; *A. florea*: F = 37.73, p<0.001) during season 1. The data revealed that the visitation frequency of the *Apis* pollinators during different day hours varied between 2.87 to 9.80 for *A. mellifera*; 2.20-8.30 for *A. dorsata* and 4.33-14.50 for *A. florea* during season 1.

Similarly, the treatments were significantly different in terms of visitation frequency for all the dominant insect pollinators during season 2 (*A. mellifera*: F = 19.05, p<0.001; *A. dorsata*: F = 16.61, p<0.001; *A. florea*: F = 37.01, p<0.001). The data revealed that the visitation frequency of the *Apis* pollinators during different day hours varied between 3.40-11.00 for *A. mellifera*; 2.13-9.40 for *A. dorsata* and 5.33-15.80 for *A. florea* during season 2 (Table 4).

Table 4. Mean values of visitation rate of dominant *Apis* pollinators in Radish

Time interval	Season 1			Season 2		
	Apis mellifera	*Apis dorsata*	*Apis florea*	*Apis mellifera*	*Apis dorsata*	*Apis florea*
8.00	2.43±0.37	2.50±0.22	4.37±0.36	3.00±0.36	1.67±0.20	6.43±0.68
10.00	8.20±0.74	7.03±0.58	9.47±1.07	8.67±1.04	6.20±0.69	11.73±1.15
12.00	14.13±0.43	11.60±0.89	18.10±1.14	13.83±1.12	12.90±1.32	18.80±1.23
14.00	15.93±0.57	12.62±0.82	26.93±1.66	16.00±1.06	15.13±1.41	27.40±1.86
16.00	4.27±0.56	3.13±0.41	5.10±0.56	4.50±0.61	2.93±0.22	5.67±0.48
LSD	**1.53**	**1.38**	**2.64**	**2.24**	**2.31**	**2.62**
F	116.35	53.72	81.85	40.41	41.78	59.96
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

The visitation frequency was relatively low in the early morning, 08:00-10:00 h (2.87-5.20 for *A. mellifera*; 2.20-4.47 for *A. dorsata* and 4.33-7.17 for *A. florea*) but rapidly increased throughout the afternoon and reached to its peak at 12:00-14:00 h (7.53-9.80 for *A. mellifera*; 6.70-8.30 for *A. dorsata* and 10.43-14.50 for A. florea) which differed significantly with other times of the day. Similar findings were observed in the second season. The highest visitation frequency was observed for A. florea followed by *A. mellifera* followed by A. dorsata. The higher visitation frequency indicates the higher efficiency in pollination of the crop. There was a significant difference in the visitation rate (no . of flowers visited per 2 min) of Apis pollinators at different time intervals during season 1 (*A. mellifera*: F = 116.35, p<0.001; *A. dorsata*: F = 53.72, p<0.001; *A. florea*: F = 81.85, p<0.001) (Table 5). The data revealed that the visitation rate of all the *Apis* species varied at different day hours between 2.43-15.93 for *A. mellifera*; 2.50-12.62 for *A. dorsata* and 4.37-26.93 for *A. florea* during season 1. Significant differences were noted in the visitation rate of *Apis* pollinators varied at different daytime intervals during season 2 (*A. mellifera*: F = 40.41, p<0.001; *A. dorsata*: F = 41.78, p<0.001; *A. florea*: F = 59.96, p<0.001). The visitation rate of all the *Apis* species was varied at different day hours between 3.00-16.00 for *A. mellifera*; 1.67-15.13 for *A. dorsata* and 6.43-27.40 for *A. florea* during season 2. The visitation rate was relatively low in the early morning, 08:00-

10:00 h (2.43-8.20 for *A. mellifera*; 2.50-7.03 for *A. dorsata* and 4.37-9.47 for *A. florea*) however, it quickly increased throughout the entire afternoon and peaked at 12:00-14:00 h (14.13-15.93 for *A. mellifera*; 11.60-12.62 for *A. dorsata* and 18.10-26.93 for *A. florea*) which varied greatly from different times of the day. Similar findings were observed in the second season. The highest visitation rate was observed for *A. florea* followed by *A. mellifera* followed by *A. dorsata*. The higher visitation rate indicates a higher efficiency in pollination of the crop.

Table 5. Mean values of visitation frequency of dominant *Apis* pollinators in Radish

Time Interval	Season 1			Season 2		
	Apis mellifera	*Apis dorsata*	*Apis florea*	*Apis mellifera*	*Apis dorsata*	*Apis florea*
8.00	2.87±0.35	2.20±0.48	4.33±0.66	3.40±0.37	2.13±0.49	5.33±0.44
10.00	5.20±0.31	4.47±0.56	7.17±0.78	5.07±0.70	4.37±0.55	6.60±0.55
12.00	7.53±0.92	6.70±0.72	10.43±1.26	10.63±0.89	8.10±0.50	13.07±0.87
14.00	9.80±1.13	8.30±1.02	14.50±1.26	11.00±1.32	9.40±1.21	15.80±1.20
16.00	3.50±0.36	3.77±0.47	4.97±0.69	4.57±0.48	3.90±0.74	6.33±0.53
LSD	**1.90**	**1.56**	**1.65**	**1.71**	**1.87**	**1.41**
F	16.76	12.54	37.73	19.05	16.61	37.01
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Honeybees were the most active at 12:00-14:00. On the other hand, minimal foraging was noted in the early morning at 08:00 hrs. Our findings are corroborated by those of [31], who discovered that bees visited more flowers per plant in the afternoon hours (17.79) than in the early hours of the morning (15.16). The results of the present investigation are strongly supported by [32] observation, which stated that nectar foragers in *B. campestris* were only around 16 percent in the morning (09:00 h) but swiftly increased during the afternoon to an average of over 40 percent. The current findings are consistent with those of [33], who noted that *A. cerana indica* nectar collection peaked between 12:00 and 14:00 hours. Several researchers reported that due to the afternoon's greater sugar concentration on Brassica crops, many workers have observed that *A. mellifera* and *A. cerana indica* collect nectar around that time of day [34-35]. Similar findings were reported by Rana et al., [36] who found that during the *B. campestris* bloom, the largest percentage of *A. cerana indica* and *A. mellifera* nectar gatherers were observed around 15:00 h and the lowest at 9:00 h. Highest temperature has a favourable impact on pollinator foraging behaviour [37].

The presence of insect pollinators is one of the most crucial elements in plant pollination. Insect pollinators are effectively used to boost crop production in both self-compatible and incompatible plants. Based on their higher abundance, visitation frequency, and visitation rate, *A. florea*, *A. mellifera*, and *A. dorsata* were regarded as the most effective pollinators of radish. The most effective pollinators of chickpea were found to be *A. florea*, *A. dorsata*, *Amigella* sp., and *E. aeneus* owing to their increased abundance, visiting frequency, visitation rate, and pollen load [38]. Similarly, *A. dorsata* and *A. florea* were the most prevalent pollinators in bitter gourd [19]. Another study reported that *A. florea* and *A. dorsata* bees were the second and third most abundant bee species on *Brassica napus*, respectively [39]. Our findings concur with those of [40-41], who found that *A. florea* and *A. dorsata* were the most prevalent species on canola crops, respectively. A floral visitor's frequency and rate of visits are typically key indicators of how effective it is at pollinating [6]. Effective pollinators are those species with high visitation rates and frequency [42]. The maximum visitation rate and visitation frequency were observed for *A. florea* and *A. mellifera* followed by *A. dorsata* in the present investigation. The maximum foraging was noticed 36.90 to 45.56 bees/m2/5min of *Apis dorsata* in between 10:00 to 16:00 hrs. of the day [43].

Conclusion

Several insects, including bees, flies, butterflies, and wasps, visit radish flowers, but only a few of them are efficient pollinators. In comparison to all other groups, bees were the most abundant floral visitors. The three most common and dominating insect pollinators that frequently visited flowers of radish were *A.*

florea, *A. mellifera*, and *A. dorsata*. *A. florea* was regarded as the most effective pollinator of radish owing to its higher visitation rate and frequency. These predominant insect pollinators can be effectively deployed to improve the radish seed production qualitatively and quantitatively.

Author Contributions

Conceptualization: PAD; formal analysis: PAD; investigation: PAD and SKP; data curation: PAD and MC; writing-original draft preparation: PAD and SKP; writing review and editing: PAD, MC and VS; supervision: MC, VS. All authors have read and agreed to the published version of the manuscript.

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