

Foraging behavior of pollinators leads to effective pollination in radish *Raphanus sativus* L.

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Abstract

To study the pollinator community of radish (*Raphanus sativus* L.) and the best pollinators for radish production in terms of their foraging behavior, an experiment was performed at the research farm of University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan. A total of 771 individuals of insects were observed, 51 percent of which were the flies (Diptera) and remaining 49 percent were the bees (Hymenoptera). The most abundant species was *Episyrphus balteatus* followed by *Andrena* sp., *Apis florea*, *A. dorsata*, *Eristalinus aeneus* and *E. laetus*. The maximum visitation rate was recorded in case of *E. balteatus* and *Andrena* sp. The maximum stay time was recorded in case of *E. aeneus* and *E. laetus*. The maximum number of pollen grains was deposited on stigma by *A. dorsata* (196.32 pollen grains) followed by *Andrena* sp. (155.47), *E. laetus* (143.47), *A. florea* (108.47), *E. aeneus* (102.74) and *E. balteatus* (62.63). Only *A. florea* and *E. balteatus* exhibited nectar robbing behavior i.e. 86% and 28% nectar robbing events, respectively. All the pollinators except *A. florea* came in contact with stigma and fed either for pollen or for both nectar and pollen. *Apis florea* mostly (82% of its visits) fed for nectar alone. All the bees preferred feeding on both nectar and pollen while all the flies preferred feeding on pollen alone. Conserving and enhancing these pollinators may boost the *R. sativus* production in Pakistan.

Keywords: *Raphanus Sativus*, Pollinator Community, Pollination Behavior, Effective Pollinators

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Introduction

More than 90 percent of approximately 25000 flowering plant species depend on animals for their sexual reproduction (Kearns et al., 1998) including 75% of major crop species of fruits, vegetables, oil seed, nuts etc. (Klein et al., 2007). Insects especially the bees are the crucial pollinators responsible for the sustainability of any ecosystem as they contribute 35% to overall food volume besides providing important nutrients for human survival (Klein et al., 2007). The annual contribution of pollinators to global agricultural economy is estimated to be USD 200

billion (Partap et al., 2012) and for Pakistan it is around US\$ 1.59 billion (Irshad and Stephen, 2013). Pollination in general is not carried out entirely by one single agent. The origin of floral specialization must be associated with the most efficient pollen-dispersal agent present in the region (Frankel and Galun, 2012). Since crops vary considerably in their pollination requirements, their reliance on insect pollinators also varies remarkably (Morse and Calderone, 2000). Vegetables are considered as crops with high economic return in Pakistan (Tunio and Majeedano, 2001). The per capita consumption of vegetables in Pakistan is nearly half than the recommended level of



200g per person per day (Ali et al., 2002). Among vegetables grown in winter, radish is one of the highly profitable crops that can be grown on a range of soil types within shorter plantation time (Mahmood and Mehmood, 2015). The average radish yield at farmers level (i.e. 16.2 t ha) is almost 46% less than that of experimental stations (30 t⁻¹ ha) in Pakistan (GoP, 2012).

Radish is a self-incompatible annual plant species. Being an obligate out-crosser it relies on insect pollinators for successful reproduction (Kercher and Conner, 1996). Managed honey bees (*Apis mellifera*) have been regarded as the most efficient pollinators of radish in many countries (Radchenko, 1966). Supplementary pollination by honey bees (*A. mellifera* and *A. cerana*) can increase the radish seed yield by 22 to 45 percent, respectively (Redchenko, 1966; Partap and Verma, 1994).

Horisaki et al. (2003) concluded that the level of self-incompatibility in radish significantly varied between artificial and insect pollination. This finding suggests that besides the phenomenon of self-incompatibility, reproductive success may also be affected by steps in the pollination process. Therefore, while studying seed production in radish, it is imperative to study both pollinator behavior and their interaction with floral traits (Kobayashi et al., 2009).

The relative efficiency of floral visitors in terms of plant reproductive success depends upon three factors i.e. 1) how many compatible pollen grains they drop (in a single visit) on a stigma during peak visitation hours, 2) how efficiently they visit flowers in a unit time (visitation rate), and 3) how many times a specific flower receives visits by that species (visitation frequency) (Faegri and van-der-Pijl, 1971; Fenster et al., 2004). Pollinators differ considerably towards their pollination effectiveness on account of their inherent pollination behavior i.e. honey bees can be the best as well as the poor pollinator as they can either rob the nectar without depositing pollen grains on stigma or can pollinate effectively (Free, 1960). The most abundant floral visitors, therefore, may not always be the most efficient pollinator (Ivey et al., 2003). In a recent account Willcox et al. (2017) emphasized on considering the community level effectiveness of pollinators as a key property of pollination effectiveness rather than considering species level effectiveness in order to fully account for the factors known to influence successful total pollination.

The importance of conserving and exploiting efficient native pollinators in southern part of Punjab, Pakistan

can be justified in two perspectives i.e. (1) pollinators are declining across the world due to intensification in agriculture and habitat loss and Pakistan is no exception (Sajjad et al., 2008). (2) Although managed honey bees are known to be the major pollinators of the world crops (Free, 1993) however, in southern Punjab, this industry has been poorly adopted mainly because of unrestricted use of pesticides, very hot summer and socioeconomic conditions of farmers (Kremen et al., 2007; Sajjad et al., 2012).

The present study was planned to identify the most effective native pollinators of radish on the basis of their foraging behaviors i.e. diurnal dynamic pattern, abundance, visitation frequencies, stay time on flowers, nectar robbing, stigma contact events, thrust for nectar and/or pollen and number of pollen grains deposited in a single floral visit.

Materials and Methods

Study Area

The study was conducted at Horticultural research farm of University College of Agriculture and Environmental Sciences, Baghdad-ul-Jadeed Campus, The Islamia University of Bahawalpur, Punjab, Pakistan (29°22'16.3"N 71°45'52.9"E; 181 meter above sea level). The experimental material was Radish, *Raphanus sativus* L. (Brassicaceae). The crop was sown in an area of half an acre on 3rd December, 2016 and started flowering in 2nd week of February, 2017; the study started on 15th February, 2017. Nearby crops included canola, some medicinal herbs, some ornamental herbs and a citrus orchard.

The district Bahawalpur is situated in southern Punjab which is featured by cold winters and hot summers. The climate is subtropical with mean daily minimum and maximum temperature range of 15°C to 20°C and 30°C to 35°C, respectively. The maximum temperature (45°C and 51°C) is recorded during May to July while the minimum temperature (3°C to 0°C) is recorded during January (Khan et al., 2010). The average annual rainfall is 300 to 500 mm (PARC, 1980).

Floral visitor censuses

Number of visits in a flower during one minute (Visitation frequency) and pollinator abundance were recorded all along the flowering season i.e., mid of February to mid of March. Observations were made at 08:00, 10:00, 12:00, 14:00, and 16:00 hours with one



week interval. In each census, twenty plants were randomly selected and each plant was observed for one minute.

We also observed some flower visiting insects in experimental plot which did not come under systematic observation. Such insects were also collected for their taxonomic identification. Insect were identified to species level where possible. Bee and syrphid specimens were identified to generic level by using keys of Michener (2000) and Vockeroth (1996), respectively. To reach species level we sought help from relevant taxa specialists (see acknowledgements). One of the bee species could not be named due to unavailability of local taxonomic keys. Therefore, we morphotyped it at genus level on the basis of its strong interactive morphological features i.e. *Andrena* sp. The studied specimens were deposited to the Insect Repository of Entomological Laboratory of the University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan.

Foraging behavior

Certain parameters of foraging behavior were recorded i.e., thrust for pollen and/or nectar, stay time per flower and side feeding (nectar robbing). Stay time on a single flower (during a specific visit) by an individual insect was recorded with the help of a stop watch. For this purpose, fifty observations were made for each insect species on virgin flowers (i.e. by caging with nylon mesh bags) during peak floral visitation activity timing (10:00 -12:00 hours) of the day. It was also observed that whether pollinators actually came in contact with stigma or not.

For meeting their energy requirements, insect feed on nectar and/or pollen. Careful visual observations were made to see if pollinators visited for collecting nectar, pollen or both. For this purpose fifty observations were made for each species during the peak activity timing (10:00-12:00 hours) of pollinators. Prevalence of phenomenon of nectar robbing (i.e. insects sometime tend to feed on nectar without coming in contact with stigma e.g. side feeding of nectar) in pollinators was assessed separately. For this purpose fifty careful visual observations were made for each pollinator during peak activity timing (10:00-12:00 hours) of pollinators.

Pollen deposition

Pollen deposition potential was measured by caging floral buds (20 for each pollinator species) with nylon mesh bags 24h before their opening. The flowers were unveiled during the peak activity timing (9:00-11:00 hours) of pollinators. Once the flowers had been visited by an insect species, stigmas were removed with the help of sharp blade. Alcohol-acetic acid solution was used to fix the stigmas followed by their staining with aniline blue, acetic acid and safranin (Dafni, 1992). A stereoscopic microscope with 40× magnification was used to count stained pollen grains.

Data analysis

The data regarding visitation frequencies, stay time on flowers and pollen deposition were subjected to one-way ANOVA followed by Least Significant Difference (LSD) test at alpha 0.05. Percentages were applied to visualize the relative abundance of different pollinators in the community and different parameters of pollination behavior i.e. nectar robbing, stigma contact events and thrust for nectar and/or pollen.

Results

The community of pollinators in radish was composed of 15 insect species i.e. 5 bees (order Hymenoptera), 7 flies (Diptera) and 3 butterflies (Lepidoptera). Only six of these species were recorded during our systematic observations, i.e., *Apis dorsata* (Apidae), *A. florea* (Apidae) *Andrena* sp. (Andrenidae), *Eristalinus laetus* (Syrphidae), *E. aeneus* (Syrphidae) and *Episyrphus balteatus* (Syrphidae).

The remaining 9 species which did not come in our systematic observations included 2 bee species (*Lasioglossum* sp., *Halictus* sp.), 4 fly species (*Syrpita pipens*, *Eupeodes corollae*, *Ischiodon scutellaris*, *Spharophoria* sp.) and 3 butterfly species *Papilio demoleus*, *Danaus chrysippus* and *Utetheisa pulchella*.

A total of 771 individuals of all the insect species were recorded, of which 51 percent were the flies and remaining 49 percent were the bees. The most abundant species was *E. balteatus* (217 individuals) followed by *Andrena* sp. (158), *A. florea*, (140) *A. dorsata* (99), *E. aeneus* (98) and *E. laetus* (59); comprising 28, 20, 18, 13, 13 and 8 percent of the total pollinator abundance, respectively (Table 1).



There was a statistically significant difference among pollinators in terms of their visitation rates (d.f.=5, f=12.93, p<0.0001). The maximum visitation rate was recorded in case of *E. balteatus* (0.73 individuals/flower/60 seconds) followed by *Andrena* sp. (0.53) and *A. florea* (0.47). *Apis dorsata* (0.33 individuals/flower/60 seconds) and *E. aeneus* (0.33) were statistically not significant while the minimum visitation rate was recorded for *E. laetus* (0.20) (Table 1).

Stay time on a flower during a single visit also differed significantly among the pollinators (d.f.=5, f=13.45, p<0.0001). The maximum and statistically non-significant stay time was recorded in case of *E. aeneus* (39.64 seconds) and *E. laetus* (41.76) followed by *Andrena* sp. (34.28). The minimum and statistically non-significant stay times were recorded in case of *A. dorsata* (26.58 seconds), *E. balteatus* (24.12) and *A. florea* (22.78) (Table 1).

There was a statistically significant difference (d.f.=5, f=3.456, p=0.006) between the numbers of pollen grains deposited on stigma by different species. The maximum number (196.32) of pollen grains was deposited by *A. dorsata* followed by *Andrena* sp. (155.47) and *E. laetus* (143.47). This was followed by *A. florea* (108.47) and *E. aeneus* (102.74) which also showed statistically non-significant difference. The minimum number (62.63) of pollen grains was deposited by *E. balteatus* (Table 1).

We further revealed that all the pollinators except *A. florea*, fed either for pollen or for both nectar and pollen. *Apis florea* mostly (during 82% of its visits) fed for nectar alone. All the bees preferred feeding on both nectar and pollen while all the flies preferred feeding on pollen alone (Table 2).

Table - 1: Foraging behavior (visitation rate, stay time and pollen deposition) of different pollinator species on flowers of *R. sativus*. Mean values sharing similar letters in respective columns show non-significant differences (P<0.05) by using LSD test.

Insect species	Abundance	Visitation rate (n=300)	Stay time (n=50)	Pollen deposition (n=20)
<i>Andrena</i> sp.	158	0.53±0.06 b	34.28±2.50 b	155.48±21.18 ab
<i>A. dorsata</i>	99	0.33± 0.05 cd	26.58±2.61 c	196.32±33.18 a
<i>A. florea</i>	140	0.47± 0.06 bc	22.78±2.00 c	108.47±28.60 bc
<i>E. aeneus</i>	98	0.33± 0.05 cd	39.64±1.76 ab	102.76±22.68 bc
<i>E. balteatus</i>	217	0.73± 0.03 a	24.12±2.10 c	62.63±12.95 c
<i>E. laetus</i>	59	0.20± 0.06 d	41.76±2.24 a	143.46±22.75 ab
ANOVA results	d.f	5	5	5
	f	12.93	13.52	3.456
	p	< 0.0001	< 0.0001	0.006

Table - 2: Foraging behavior of different pollinator species associated with nectar and pollen feeding on flowers of *R. sativus*.

Insect species	Nectar robbing (%) ; n= 50	Stigma contact (%) ; n=50	Nectar or pollen feeding (%); n=50		
			N	P	N/P
<i>Andrena</i> sp.	0	100	0	18	82
<i>A. dorsata</i>	0	100	0	24	76
<i>A. florea</i>	86	50	82	8	10
<i>E. aeneus</i>	0	100	0	88	12
<i>E. balteatus</i>	28	100	0	98	2
<i>E. laetus</i>	0	100	0	98	0



Discussion

The flowers of radish are actinomorphic with exposed nectarines. They offer a landing platform of white petals along with pollen and nectar. Due to this generalized structure of the radish flower, almost all types of pollinator groups can feed from it, e.g. bees, flies, beetles, butterflies and wasps, (Kunin, 1993). Both the short tongued and the long tongued insects can easily get access to the nectar and floral rewards of radish flowers. Five bees, 7 flies and 3 butterflies were observed foraging in the focal plot. As a matter of fact, pollinator abundance, richness and composition vary across different geographical areas, latitudes, and times (Ollerton and Louise, 2002).

In this study, 51 percent of floral visitors were comprised of flies and remaining 49 percent were the bees. The most abundant species was *Episyrphus balteatus* followed by *Andrena* sp., *Apis florea*, *A. dorsata*, *Eristalinus aeneus* and *E. laetus*. The managed honey bees (*Apis mellifera*) are typically reported as the sole pollinators of about one third of cultivated crops; however, its potential of efficient pollinator has been the subject of controversy (Westerkamp, 1991; Williams, 1995; Allen-Wardell et al., 1998).

In this study, the maximum visitation rate was recorded in case of *E. balteatus* (0.73 individuals/flower/60 seconds) followed by *Andrena* sp. (0.53) and *A. florea* (0.47), *A. dorsata* (0.33) and *E. aeneus* (0.33) and *E. laetus* (0.20). Both the visitation frequency and foraging rate are thought to be the most important predictors of pollination effectiveness. As a common perception, the pollination percentage of plant species is directly proportional to visitation frequencies and foraging rates of pollinator species (Singh et al., 2006); however, no any sharp rule has been established in this regard so far. Many previous accounts have shown that apparently good pollinators with high visitation rates many not necessarily be the good pollen depositors (Engel and Irwin, 2003). For example, Ali et al., (2011) showed that although *Halictus* sp. showed to have low visitation rate on canola flowers yet they showed the highest pollen deposition among other tested insects.

In this study, the maximum stay time was recorded in case of *E. aeneus* (39.64 seconds) and *E. laetus* (41.76) followed by *Andrena* sp. (34.28) while the minimum and statistically non-significant stay times were recorded in case of *A. dorsata* (26.58), *E. balteatus*

(24.12) and *A. florea* (22.78). These findings are in accordance with Ali et al., (2011) who reported the minimum stay time by *A. dorsata* and *A. florea* on canola flowers however, contrary to our findings they reported *E. balteatus* as having the maximum stay time on canola flowers. It has been rationalized that the foraging behavior of pollinators is governed by a number of biotic and abiotic features. The biotic features include length of the proboscis of pollinators (Inouye, 1980), quantity of floral rewards i.e. nectar and pollen (Rao and Suryanarayana, 1990) and sugar concentration in nectar (Abrol, 2007). The abiotic factors include light intensity, relative humidity, wind speed and ambient temperature (Vicens and Bosch, 2000).

In the present study, the maximum number (196.32) of pollen grains was deposited by *A. dorsata* followed by *Andrena* sp. (155.47), *E. laetus* (143.47), *A. florea* (108.47), *E. aeneus* (102.74) and *E. balteatus* (62.63). Although number of pollen grains harvested during a single visit matters a lot towards effective pollination (Canto-Aguilar and Parra-Tabla, 2000), yet pollen deposition is rather more favorable. Moreover, behavior of pollinators in flowers is also important, i.e., stigma contact duration. For example, Ali et al., (2011) showed that although *Halictus* sp. was not a good pollen harvester (148 pollen grains) than *A. florea* (326 pollen grains), but it deposited more number of pollen grains than *A. florea*. In the present study, *E. laetus* and *E. aeneus* proved to be better pollen depositor than *E. balteatus* may be because of their larger size. The single visit efficiency of pollinators has not previously been reported for radish. Managed honey bees (*A. mellifera*) have not successfully been adopted in southern Punjab because of high summer temperature and the indiscriminate use of insecticides (Sajjad et al., 2008); therefore, conserving alternative native pollinators is seems to be an appropriate choice. Of the three most effective pollinators described in this study, *A. dorsata* is a social bee and makes its nests in large trees in wild areas, whereas *Andrena* spp. species mostly burrow in soil.

Present study revealed that *A. florea* exhibited 86% and *E. balteatus* exhibited 28% nectar robbing events. Moreover, all the pollinators except *A. florea* came in contact with stigma during 100% of their visits. Data further revealed that all the pollinators except *A. florea*, fed either for pollen or for both nectar and pollen. *A. florea* mostly (during 82% of its visits) fed for nectar alone. All the bees preferred feeding on both



nectar and pollen while all the flies preferred feeding pollen alone. Most of the bees show thrust for nectar alone while pollen attached to their body is an extra reward. Plant and pollinator interaction primarily depends on the energy requirements of the pollinators in shape of nectar and pollen (Heinrich, 1975; Abrol, 1986).

In many flowers, the availability of nectar and pollen rewards decreases with time of the day (Meyerhoff, 1954). Ali et al., (2011) showed that *A. florea* tended to robe nectar more in the afternoon in canola crop, possibly because nectar robbing is regarded as a short cut way to find nectar. They also showed that the two of syrpid flies, *E. corollae* and *E. balteatus* mostly foraged from anthers and rarely became in contact with stigma, therefore they deposited less pollen grain than that of bees.

Conserving and enhancing native pollinators - especially the bees- can promote the radish productivity in Pakistan. Furthermore, findings of current study can be widespread over large geographical range, because *A. dorsata* and many *Andrena* sp. are distributed widely across India, Oman, Sri Lanka, Iran, Afghanistan, Indonesia, Philippines, and southern China (Akratanakul, 1990). Further studies should focus other hundreds of native pollinators for their pollination potential coupled with basic investigations on their life cycle and biology e.g. nesting biology and host plant range.

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