Plant Bulletin Vol 2, (1) 2023: 59-68 ISSN: 2959-3379 (Print), 2959-3387 (Online)

Research Article

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Effectiveness of Native Insect Pollinators in Radish (*Raphanus sativus L.*) Seed Production

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ABSTRACT

Bees, being effective pollinators, contribute to increasing the yield of fruits and seeds in a variety of crops. We experimented to evaluate the pollination effectiveness of different pollinators for the successful pollination of radish, *Raphanus sativus L*. The effectiveness of the most prevalent pollinators was measured using parameters such as visitation rate, duration of stay per flower, percentage of stigma contact, as well as the percentage of pod set from a single visit and the number of seeds per pod. Carpenter bees *Xylocopa sp.* exhibited a notably higher visitation rate and stigma contact percentage, surpassing Asian giant honeybee, Apis dorsata Fabricius and European honey bee *A. mellifera L*. (both Hymenoptera: Apidae). *Xylocopa sp*. came out as the most proficient pollinator, followed by *A. dorsata* and *A. mellifera*, when evaluating the effectiveness of a single pollinator visit in terms of pod set percentage and quantity of seeds per pod. Additionally, open-pollinated flowers demonstrated significantly higher pod set percentages and a greater number of seeds per pod compared to self-pollinated flowers. Conserving these efficient native bee species could potentially lead to increased vegetable crop production and greater returns for farmers in other cross-pollinated crops in the Punjab Pakistan.

Keywords: Honeybees, solitary bees, open pollination, visitation rate, single visit efficacy, radish and *Raphanus sativus* L.

INTRODUCTION

Pollination is an essential ecosystem service crucial for achieving sustainable food systems [1, 2, 3]. Animal-mediated pollination is essential to the reproductive success of over 90% of flowering plant species and 75% of key crops, including fruits, oilseeds, nuts, and vegetables [4, 1]. Insects serve as important pollinators, establish a beneficial relationship with flowering plants, and play essential roles towards reproductive success of these species [5]. Insects contribute to cross-pollination in many crops, even though wind and self-pollination predominate worldwide agricultural systems [6, 2]. In addition, 87 out of 124 crops grown for human consumption have reported increased yield due to insect-mediated pollination services [1].

Due to its adaptability to many soil types and rapid development, radish (*Raphanus sativus L.*), an important winter vegetable crop, is popular in temperate and tropical regions [7]. Despite its potential, the average yield for this short-duration crop is considerably lower, around 46% (16.2 t/ha),

Article History

Received: October 30, 2023, Accepted: December 1, 2023, Published: December 12, 2023

compared to experimental stations (30 t/ha) at the farmer level in Pakistan [8]. Radish, originating from Asia and Europe, is a popular and fast-growing vegetable grown in temperate and tropical regions [9, 10]. The flowers of radish are self-incompatible and receptive period of the stigma is limited to specific hours in the day [11]. Radish flowers, measuring 2.5 cm, have four petals, and each petal has a white to purple color. The flower yields six seeds in every developed pod per plant [12]. Radish flowers rely on insect pollinators for successful reproductive phase due to their self-incompatible nature [13].

As insect pollinators, bees contribute 35% to primary food production, supplying crucial nutrients for ecosystems and humans [1]. Introduction of honeybee hives optimizes crop pollination; however, the honeybees could create competition with the wild bee populations [14]. Nevertheless, it remains uncertain whether such competition prevails when honey ees are present in large-scale flowering crops, whether resources are abundant or limited.

As per the optimal foraging theory, honeybees engage in neighboring flower foraging to optimize their net energy intake relative to time [15]. In addition to honeybees, wild insects also confer crop pollination benefits through supplementary pollination [16, 17. Short-tongued syrphid flies, distinct from honeybees, display a preference for plants with easily accessible pollen and nectar due to their short corollas, as observed in canola flowers [18, 19]. An efficient insect pollinator demonstrates the ability to deliver sufficient conspecific pollen grains onto viable stigmas at the right time. This metric of pollinator efficiency often categorizes the significance of diverse pollinator visitors, thereby influencing plant fitness through the contribution of insect pollinators [20, 21, 22]. Successful fertilization depends on the pollen collecting and depositing ability of the insect pollinator [22].

Pollen grain deposition onto a receptive stigma is commonly regarded as a measure of pollinator efficiency due to its practicality in field settings. Recent studies have suggested that the pollen deposition due to a single pollinator visit to a receptive stigma serves as an effective indicator of pollinator success [23, 24, 25]. However, it's important to note that the reproductive success of female flowers cannot be accurately determined solely by the single visit pollen deposition. *A. dorsata* has been identified as an optimal pollinator for various crops, such as rapeseed Brassica napus [26], onion Allium cepa [27], Egyptian river hemp Sesbania sesban [28], and bitter melon Momordica charantia [29] in the southern Punjab region of Pakistan. The effectiveness of pollinators has been evaluated using single visit efficiency, revealing shifts in pollination traits and preferences for various floral traits among floral visitors [30].

To the best of our knowledge, no study has investigated the diversity and abundance of pollinators on radish grown in Multan, Pakistan. The current study investigates the diversity and abundance of insect pollinators on radish.

MATERIALS AND METHODS

Experimental site

The experiment took place at the research farm of MNS University of Agriculture in Multan (30.1424, 71.4429). Radish, *R. sativus L*. (Brassicaceae), was cultivated within a 505.9 m^2 plot during July 2017. Surrounding vegetation included perennial trees like Indian rosewood *Dalbergia sissoo* and gum arabic tree *Acacia nilotica* (both Fabaceae), alongside crops such as mustard (*Brassica campestris L.*), maize (*Zea mays L.*), cotton (*Gossypium hirsutum L.*), and rice (*Oryza sativa L.*). The region has sub-tropical desert climatic conditions characterized by cold winters and hot summers, with daily mean temperatures ranging from 8 to 12 °C and 38 to 50 °C, respectively. The typical summer monthly mean rainfall stands at approximately 18 mm.

Floral visitor censuses

Throughout the flowering period, we documented the abundance of pollinators on 30 plants selected randomly. We conducted observations at 0800 to 1000 hours, with one-week intervals. For each assessment, we randomly chose 30 plants and observed each plant for one minute to record insect pollinators. In the experimental plot, we tallied the total number of flowers per plant to identify flower-visiting insects. Some representative specimens of pollinators were also gathered for taxonomic classification. The collected specimens were preserved in the laboratory.

Foraging behavior

The foraging activity of various available pollinators were observed, including the count of flower visits within a minute (visitation frequency), the duration of time spent on a flower, and their pursuit of pollen and nectar. Moreover, the lateral feeding behaviors of honeybees were visually noted. These observations were conducted weekly at different times of the day, specifically between 0800 and 1000 hours, acknowledging the distinct variations in seasonal and daily dynamics that are associated with insect pollinators [31].

Single visit efficacy with seed production

To assess the efficiency of pollination in a solitary visit, the deposition of pollen by a specific insect pollinator was measured. For this purpose, unopened buds were enclosed within netting bags. During the peak activity window, which spanned from 1000 to 1400 hours, insect pollinators were observed, and the flowers were subsequently released from the enclosures. Physiological parameters such as pod length, number of seeds per pod, pod weight, and seed weight per pod were measured to evaluate the pollination effectiveness of each pollinator [31]. For checking the effectiveness of pollination services, both caged (self-pollinated) and open-pollinated plants were maintained.

Statistical analyses

The data gathered from various parameters, including pod length, pod weight, the number of seeds per pod, visitation rate, stay time, pollen loads, single visit efficacy, and seed weight, underwent a comprehensive statistical examination employing ANOVA (analysis of variance). To perform this analysis, the XLSTAT software (version 2008) was employed. Further, the means were separated using the Tukey Kramer test. The correlation between different yield parameters was determined using Spearman rank correlation.

RESULTS

The visiting pollinator groups for radish flowers included seven bees (Hymenoptera) and four true flies (Diptera) (Table 1). Among the bee species, systematic observations covered *A. florea*, *A. dorsata*, *A. mellifera*, *Halictus sp.*, *Lasioglossum sp.*, and *Nomia sp.*, while *Xylocopa sp.* and flesh flies were observed less frequently. Apidae emerged as the prevailing family with four species, followed by three species from Halictidae. Notably, *A. florea* and *A. dorsata* exhibited the highest frequency as floral visitors, totaling 197 and 145 individuals, respectively. Out of a total of 763 recorded insect individuals, 77 percent constituted bees, while the remaining 23 percent were flies (Table 1). Visitation frequency of *A. florea* and *A. dorsata* was highest among all the observed bee species, at 0.82 and 0.60 individuals per plant per minute, respectively (Table 1). Among true flies, the hover flies dominated, including two species with hoverfly *Eristalinus aeneus* (Scopoli) and *E. laetus* (both Diptera: Syrphidae) being the most abundant. They displayed the highest visitation frequencies at 0.29 and 0.24, respectively. The two remaining Diptera families featured merely one to two species each. Calliphoridae (*Euphumosia sp.*) held dominance, both in terms of abundance (8 individuals) and visitation frequency (Table 1). The sarcophagids (*Sarcophaga sp.*) occasionally visited the flowers.

Table 1. Insect diversity visiting radish flower, their abundance and foraging task

Order	Family	Insect pollinator	Abundance	Relative Percentage (%)	Visitation frequency (flowers visited/plant /min)	Foraging task
Hymenoptera	Apidae	*Apis florea*	197	25	0.82	N
		Apis dorsata	145	19	0.60	N
		Apis mellifera	102	13	0.42	N
		Xylocopa sp.	83	10	0.34	N
Hymenoptera	Halectidae	*Halictus sp.*	67	08	0.27	N
		Lasioglossum sp.	14	01	0.05	N/P
		Nomia sp.	12	01	0.05	N
Diptera	Syrphidae	*E. aeneus*	71	09	0.29	N/P
		E. laetus	59	07	0.24	N/P
	Calliphoridae	*Euphumosia sp.*	08	01	0.03	N/P
	Sarcophagidae	*Sarcophaga sp.*	05	06	0.02	N/P
			763			

Significant variations were evident in the reproductive parameters. Specifically, in the presence of pollinators, there was a substantial increase of 3.43 times in pod length and 2.62 times in hand pollination treatments, compared to instances where no influence of pollinators was observed. Moreover, pod weight exhibited a remarkable augmentation of 10.33 times in open pollination and 333 percent in hand pollination. The number of seeds per pod saw an increase of 6.98 times in open pollination and 3.63 times in hand pollination, while seed weight per pod showed a striking boost of 13 times in open pollination and 4.00 times in hand pollination, all in contrast to self-pollinated flowers across various pollination modes (Table 2). Conversely, no notable differences were detected in these parameters within self-pollinated plants.

Table. 2 Comparison of reproductive parameters in open, hand and self-pollinated plants

Pollination Modes	Pod length (cm)	Pod weight (g)	No. of seeds/pod (g)	Seed weight/pod (g)
Pollinators influence	12.01 ± 0.46 a	0.31 ± 0.01 a	8.10 ± 0.22 a	0.13 ± 0.005 a
Caged or no Pollinators influence	3.50 ± 0.47 b	0.03 ± 0.006 b	1.16 ± 0.16 c	0.01 ± 0.002 b
Hand pollination	9.18 ± 1.26 a	0.13 ± 0.03 b	4.22 ± 0.92 b	0.04 ± 0.014 b

Pod length showed significant differences based on individual visits from various pollinators ($F = 4.1$, $df = 8$, $P < 0.001$) (Table 3). A greater pod length resulted from visits by *A. dorsata* and *Xylocopa sp*., followed by *A. mellifera*. In terms of pod weight ($F = 9.2$, $df = 8$, $P < 0.001$), the highest values were recorded after interactions with *A. dorsata* and *Xylocopa sp.*, followed by *A. florea*, and finally *A. mellifera* (Table 3). Notably, open-pollinated flowers exhibited greater pod length and weight in comparison to self-pollinated flowers.

Significant differences were observed in the number of seeds per pod ($F = 10.2$, $df = 8$, $P < 0.001$) across the seven different pollinator visits (Table 3). *A. dorsata* and *Xylocopa sp.*, showed higher pollinator effectiveness, yielding more seeds per pod in a single visit compared to the flies, as assessed by correlation values. *A. mellifera*, *A. florea*, and *E. laetus* followed this trend, respectively. The ranking of pollinator effectiveness, as indicated by correlation values, aligned with the order observed for the number of seeds per pod.

Table 3. Pollination effectiveness in terms of single visits by different pollinators

Pollinators	Pod length (cm)	Pod weight (g)	No. of seeds/pod (g)	Seed weight/pod (g)
Open pollination	12.01± 0.46 a	0.31± 0.01 ab	8.10 ± 0.22 ab	0.13 ± 0.005 b
Apis florea	4.90 ± 1.21 bc	0.07 ± 0.008 c	3.00 ± 1.52 cd	0.02 ± 0.02 c
Apis dorsata	11.53 ± 0.62 ab	0.38 ± 0.07 a	9.50 ± 1.19 a	0.23 ± 0.05 a
Apis mellifera	7.00±1.17 abc	0.07±0.01 c	4.75±1.49 bc	0.04±0.01 c
Xylocopa sp.	10.86±1.61 ab	0.32±0.04 ab	8.33±0.88 ab	0.19±0.026 ab
E.aeanus	4.10±0.20 bc	0.03±0.01 c	1.50±0.50 cd	0.005±0.005 c
E.laetus	4.15±1.85 bc	0.03±0.03 c	1.50±0.50 cd	0.01± 0.005 c
Self-pollination	3.50 ± 0.47 c	0.03 ± 0.006 c	1.16 ± 0.16 d	0.01 ± 0.002 c

The Pearson correlation matrix revealed a significant positive correlation between the number of seeds per pod (0.773) and seed weight per pod (0.945). Furthermore, a positive correlation was found between seed weight per pod and the number of seeds per pod (0.793) (Table 4).

Table 4: Pearson correlation matrix among yield parameters

	Pod length (cm)	Pod weight (g)	No. of seeds/pod (g)
Pod weight (g)	0.473		
No. of seeds/pod	0.558	0.773	
Seed weight/pod	0.437	0.945	0.793

DISCUSSION

The study investigated the diversity and abundance of pollinator species on radish flowers in the tropical desert climate of Multan, Pakistan. In this study, a total of eleven insect pollinators from five various families and two various orders visited radish flowers. Honeybees, solitary bees, and syrphid flies all had the highest abundance. Radish flowers, with their radially symmetrical structure and accessible nectarines, provide a white landing platform along with nectar and pollen. The flowers of radish are radially symmetrical and possess accessible nectarines. These flowers offer a white landing platform, along with nectar and pollen. Nectar serves as a primary sugar source for visiting insects [32], and its total sugar volume and content provides energy to the pollinators [33, 34]. Given their generalized structure, radish flowers attract and nourish various pollinators, including flies, bees, butterflies, wasps, and beetles [35].

Insect pollinators exhibit a stronger attraction towards flower rewards that offer both higher quantity and quality [36, 37, 38, 39, 40, 41, 42]. According to a previous study, honeybees are the most frequent insect visitors to radish flowers, with a preference for white blooms over those with pinkish pigmentation. Conversely, syrphid flies consistently favor pink petals over other colors. The ability of pollinators to differentiate among different morphs of radish flowers is likely to affect their vigor by altering male reproductive success [43]. Notably, the abundance and diversity of pollinators tend to vary across different latitudes, geographical regions, and time periods [44]. Most bees primarily consume pollen, with the added benefit of nectar sticking to their bodies as an additional reward. The energy needs of insect pollinators and the resources that plants can provide have an impact on plant-pollinator interactions [45, 46]. The pollen-to-nectar ratio within flowers can influence the behavior of pollinators and, consequently, impact pollination efficiency. This suggests that a nectar feeder has the potential to modify its level of engagement in nectar robbing [47]. Several authors have reported similar groups of pollinators visiting radish flowers. For instance, Bhatia et al. [48] documented twelve insect pollinators on radish flowers, comprising five from the Hymenoptera group, four from Diptera, and three species from Lepidoptera. These findings align with Sihag [49], who highlighted nine species of hymenopterans and three species of dipterans as major visitors to radish flowers. Similarly, Priti et al. [50] reported analogous findings regarding radish flowers. Significant variations in stay time and visitation rate were observed among pollinator groups, including honeybees, solitary bees, and flies visiting radish flowers. *A. dorsata* and *Xylocopa sp*. visited the most flowers, displaying the highest visitation rate. Pollinators adjust their foraging behavior based on floral rewards, favoring more flowers with greater nectar incentives [51, 36, 52]. Proboscis length, natural behavior [53], nectar sugar concentration [54], floral rewards, and abiotic factors like wind speed, humidity, light, and temperature [55], all have an impact on insect pollinator visitation and foraging rates. Successful pollination relies on compatible pollen delivery and visitation frequency, both linked to nectar and pollen rewards and influenced by factors like soil moisture [56].

As per the optimal foraging theory, the rate of flower visitation might be higher in dense plantations, while a larger number of flowers need to be examined per plant visit in sparser plants [57, 58]. Some studies have shown a positive correlation between plant density and visitation rate [59, 60, 61], although several others have not observed this relationship [62, 63]. Conversely, one study demonstrated a lower visitation rate with increased flower density [64]. The notion of greater visitation rates has also been corroborated in the case of less accessible flowering plants by multiple researchers [59, 65, 66, 67].

Several prior investigations have been conducted on various crops, such as sarson, which showed a substantial difference in seed production between self-pollination (79.96% reduction) and open-pollination conditions (88.05%) [68]. In a study by Goswami and Khan [69] focusing on mustard, different pollination methods were examined. They found that pod setting was highest in open-pollinated plots (83.42%), followed by cage-pollinated plants (62.80%). Additionally, it was reported that the number of pods per plant was greater in insect-pollinated plants compared to caged plants [70].

The effectiveness of insect pollinator groups exhibited significant variations in terms of single-visit efficacy on reproductive parameters (Table 3). *A. dorsata* and *Xylocopa sp.* emerged as the most efficient pollinators, as a single visit from them yielded a greater number of seeds and seed weight. *A. dorsata* has already been identified as the best pollinator for certain crops, including rapeseed *Brassica napus L.* (Brassicaceae) [26], onion *Allium cepa L.* (Amaryllidaceae) [27], and Egyptian riverhemp *Sesbania sesban (L.)* Merr. (Fabaceae) [28] in southern Punjab, Pakistan. The utilization of single-visit efficiency effectively gauged pollinator effectiveness and highlighted shifts in pollination efficiency and preferences for various flower traits among floral visitors [30].

Recognizing the effectiveness of aligning the dimensions of flowers with those of their pollinators has gained notable attention as a strategy to amplify the triumph of pollination (70). Prior investigations have employed both seed production and pollen deposition following individual pollinator visits as benchmarks for assessing pollinator efficiency [22]. Within natural ecosystems, blooming plants frequently allure an array of pollinators, resulting in the receipt of pollen from both members of the same species and other species [72, 73].

The transfer of pollen between diverse species is a prevalent phenomenon, leading to a circumstance where the pollen grains settled on a stigma might not always be of the same species or compatible [73, 74, 75, 76, 77]. Previous studies have shown that pollen grains from different species may hinder germination and deposition of same-species pollen. Additionally, they can prevent the development of seeds, pollen tubes, and ovules [76, 77, 78]. When flowers are cross-pollinated, they produce more seeds than flowers that are self-pollinated. This is because inbreeding depression makes seeds more likely to get damaged during the maturation process [79].

Conserving native, effective insect pollinators has the potential to significantly strengthen seed production of the pollinator dependent crops. Future research should investigate nesting sites and supplementary nectar sources for these insect pollinators. Moreover, future studies should also focus on pollinator diversity and their relative contribution to seed production in different ecological zones.

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