Research Article

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Role of Insects in Cross-Pollination and Yield Attributing Components of Fenugreek

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ABSTRACT

Fenugreek (*Trigonella foenum-graecum*) is a self-pollinated crop however insect pollination can improve the seed and fruit set. The present study was carried out at the agricultural research farm of The Islamia University of Bahawalpur, Pakistan to enlist the pollinator community of fenugreek, their foraging behavior, and identify the most effective pollinator species in terms of plant reproductive success. The floral visitor community of fenugreek was comprised of six bees, four flies, one moth, and one butterfly species. The highest visitation frequency was observed for *Apis florea* (3.86 individuals/meter^2/120 seconds) and the visitation rate for *Apis dorsata* (19.90 flower/120 seconds). The highest stay time on a single flower was observed for *Ceratina smaragdula* (3.94 seconds/flower). The maximum pod weight, pod length, and seed weight were recorded in *C. smaragdula* pollinated pods. Results suggest that *C. smaragdula* is an efficient pollinator of fenugreek in Bahawalpur in terms of plant reproductive success. Future studies should focus on the conservation of *C. smaragdula* and other allied bee species for enhanced crop pollination.

Keywords: Cross-pollination, insects, seed set, yield, fenugreek

INTRODUCTION

Bees are considered as the potential pollinators of many crop species in arid and semi-arid regions because of high richness, abundance and favorable foraging behavior [1]. The bees vary in their foraging behavioral attributes such as visitation rate, visitation frequency, pollen deposition, nectar robbing and pollen and/or nectar foraging that affect the key function of crop pollination [2, 3, 4]. For example, the number and length of floral visits can raise the amount of deposition on the stigma [5]. Bees are regarded as one of the most essential groups of pollinators in the world [6]. Bees are declining due to the degradation of natural habitats and the intensification of agriculture [7]. The protection and management of wild pollinators, particularly honeybees, is critical for sustainable ecological services [7,8]. Depending on the cultivar, pollinators especially honeybees can improve fruit or seed production and its quality [9, 10. 11]. It has been reported that *Apis mellifera* L. is responsible for precise pollination in agro-ecosystem than other *Apis* species with respect to their role as pollinators, learning performance, and foraging behavior [12, 13]. Honeybees are not always the efficient pollinator for all the crops and beyond an optimum visitation rate they can even be detrimental to crop productivity [14]. Therefore, crop-specific recommendations regarding A. mellifera should be followed to increase the crop yield in terms of quantity and quality. Fenugreek, *T. foenum-graecum* L. (Fabaceae) requires floral visitor insects for efficient pollination, healthier seed, and fruit setting [15,16]. Fenugreek is a culinary and medicinal plant. Its leaves are consumed as vegetables and seeds employed as a spice [17]. Fenugreek is rich in choline, tryptophan, ascorbic acid, niacin, and potassium, all of which are vital for various aspects of health [18]. In cross-pollinated crops, flowers receiving no pollinator species do not produce any fruit. It has been reported that honeybees i.e., *A. mellifera* and *Apis cerana* F. and some solitary bees i.e., *Trigona* sp. and *Halictus* sp. are major floral visitors of fenugreek [19]. Ecological factors such as relative humidity, temperature, light intensity, and wind velocity influence the foraging behavior of pollinators on flowers [2, 3, 20, 21, 22]. This dependence of insects on abiotic conditions

Article History

Received: September 12, 2023, Accepted: December 11, 2023, Published: December 28, 2023.

significantly limits crop cross-pollination [23, 24]. Certain recent local level studies have shown that wild honeybees i.e., *Apis dorsata* F. and *Apis florea* F. constitute 30 to 80 percent of the total pollinators' abundance in various crops and play a significant role in crop pollination [3, 25, 26, 27, 28]. It seems to be an excellent choice to compare the foraging proficiency of different native bees with special focus on the biology and ecology of most efficient ones for future conservation [29]. With all of these considerations in mind, the current study was planned to enlist the insect pollinator community of fenugreek and to compare pollination potential of the different native insect pollinator species in terms of single visit effectiveness aiming to find the most efficient one for future conservation.

MATERIALS AND METHODS

Study Area

The present study was conducted at the agricultural research farm of The Islamia University of Bahawalpur (IUB), Punjab, Pakistan (29°22'20" N 71°46'01" E; 122 meters a.s.l.). Fenugreek *T. foenum- graecum* (Fabaceae) was sown in the last week of October 2022 in an area of 47 square meters. Bahawalpur is a district in southern Punjab that is blessed with hot summers and cold winters. The climate of the region is classified as arid sub-tropical. The average annual rainfall ranging from 83 to 218 mm while average daily maximum and minimum temperatures are 33.5 ˚C and 18.8 ˚C, respectively [30].

Floral Visitor Censuses

To measure the pollinator community of fenugreek, visitation frequency (i.e., number of individuals per one square meter per 120 seconds) of pollinators was recorded. Visitation frequency was recorded on a weekly basis all over the flowering period i.e., from third week of February to second week of March. The observations were made at 09:00, 11:00, 13:00, 15:00 and 17:00 hours. In each census, 20 plants were chosen at random, and each plant was observed for 120 seconds and counted the number of visits of each floral visitor.

Wherever feasible, insects were identified to the species level. For generic level identification, keys of Michener [31] for bees and Thompson and Vockeroth, [32] for syrphid flies were employed. Species were identified by the relevant experts (acknowledgements). Due to lack of local taxonomic literature on native bees, two of the bees could not be identified to species level. Therefore, based on strong interactive morphological features, we morphotyped them at genus level i.e., *Sphecodes* sp. and *Megachile* sp. The voucher specimens were deposited at the Insect Repository of Entomological Laboratory of the Department of Entomology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur.

Foraging Behavior

Foraging behavior of the four most abundant floral visitors i.e., *A. dorsata*, *A. florae*, *A. mellifera* and *C. smaragdula* F. was recorded in terms of stay time (time spent on a single flower by an individual during single visit; n=120) and visitation rate (number of flowers visited by an individual in 120 seconds; n=40). Floral visitors were also monitored for contact with the stigma. The stay time and visitation rate of bees were recorded all through the flowering season with weekly intervals. These observations were made at 09:00, 11:00, 13:00, 15:00, and 17:00 hours using a stopwatch.

Pollination Effectiveness

Pollination effectiveness was also recorded for the four most abundant floral visitors i.e., *A. dorsata*, *A. florae*, *A. mellifera* and *C. smaragdula*. Ten floral buds were confined with nylon mesh bags 24 hours before they opened. They were un-caged when the pollinators activity was maximum i.e., 11:00 to 12:00 hours and then re-caged after a single visit of the experimental species had been made. Ten such observations were made for each bee species. As a function of pollination effectiveness, the resultant pod weight, pod length, number of seeds per pod, and seed weight per pod were measured. Twenty open and caged buds (remain closed throughout the flowering period) were also maintained as control treatments.

Statistical Analysis

The data regarding visitation frequencies, visitation rates, stay times, pollination efficiencies in terms of pod weight, pod length, number of seeds per pod and seed weight per pod for different pollinator species were subjected to one-way ANOVA. The means were compared using Least Significant Difference (LSD) test at alpha 0.05.

RESULTS

Twelve species of insects i.e., six bees (Order: Hymenoptera), four true flies (Order: Diptera), one moth and one butterfly (Order: Lepidoptera) with a total of 1253 individuals visited the flowers of *T. foenum-graecum* (Table 1). Bees were the most prevalent flower guests comprised of 99.16% followed by flies (0.52%) and butterflies (0.32%). Among bees, *A. florea* was found to be the most persistent floral visitor and comprised 57.28% of the total visitors followed by *C. smaragdula* (19.33%) and *A. dorsata* (18.17%). Among true flies, *Eupeodes corollae* F. was the most abundant floral visitor (0.19%) followed by *Musca domestica* L. (0.13%) and *Episyrphus balteatus* G. (0.13%). Among lepidopterans, *Helicoverpa armigera* H. was more abundant (0.26%) than *Vanessa cardui* L. (0.06%) (Table 1).

There was a statistically significant difference in terms of visitation frequency (df = 3, F = 92.1, p-value = <0.0001), visitation rate (df = 3, F = 218, p-value = <0.0001) and stay time (df = 3, F = 57.2, p-value =

<0.0001) among *A. dorsata*, *A. mellifera*, *A. florea* and *C. smaragdula*. The highest visitation frequency was observed in *A. florea* (3.86 individuals/meter^2/120 seconds) followed by *C. smaragdula* (1.30 individuals/meter^2/120 seconds), *A. dorsata* (1.23 individuals/meter^2/120 seconds), and *A. mellifera* (0.28 individuals/meter^2/120 seconds).

Table 1. Floral visitors of fenugreek and their abundance with order proportion

Order	Family	Insect Visitor	Specie Percent Proportion	Order Proportion (%)
Hymenoptera	Apidae	*Apis dorsata*	18.17	99.16
		Apis mellifera	4.25	
		Apis florea	57.28	
		Ceratina smaragdula	19.33	
	Halictidae	*Sphecodes* sp.	0.06	
	Megachilidae	*Megachile* sp.	0.06	
Diptera	Muscidae	*Musca domestica*	0.13	0.52
	Syrphidae	*Episyrphus balteatus*	0.13	
		Eupeodes corolla	0.19	
		Ischiodon scutellaris	0.06	
Lepidoptera	Noctuidae	*Helicoverpa armigera*	0.26	0.32
	Nymphalidae	*Vanessa cardui*	0.06	

Whereas *A. dorsata* showed the highest visitation rate in (19.9 flower/120 seconds) followed by *A. mellifera* (12.77 flower/120 seconds), *A. florea* (12.23 flower/120 seconds), and *C. smaragdula* (8.78 flower/120 seconds). The highest stay time was observed in *C. smaragdula* (3.94 seconds/flower) followed by *A. florea* (1.95 seconds/flower), *A. dorsata* (1.82 seconds/flower), and *A. mellifera* (1.37 seconds/flower) (Table 2).

Table 2. Comparison of the foraging behavior of four bee species

Species	Visitation Frequency (Indi./m^2/120 sec.)	Visitation Rate (Flowers/120 sec.)	Stay Time (Sec./flower)
A. dorsata	1.23 ± 0.08 b	19.90 ± 0.62 a	1.82 ± 0.09 c
A. mellifera	0.28 ± 0.03 c	12.77 ± 0.55 b	1.37 ± 0.10 bc
A. florea	3.86 ± 0.29 a	12.23 ± 0.53 b	1.95 ± 0.11 b
C. smaragdula	1.30 ± 0.10 b	8.78 ± 0.34 c	3.94 ± 0.19 a

Lettering on the basis of LSD, $\alpha = 0.05$

Table 3. Comparison among *A. dorsata*, *A. mellifera*, *A. florea*, *C. smaragdula* and open-pollinated pods in terms of different parameters

Species	Pod Weight (g)	Pod Length (cm)	No of Seeds/Pod	Seed Weight/Pod(g)
A. dorsata	0.009 ± 0.002 b	1.57 ± 0.09 c	4.73 ± 0.84	0.006 ± 0.001 b
A. mellifera	0.011 ± 0.002 b	2.08 ± 0.10 ab	4.40 ± 0.86	0.008 ± 0.002 ab
A. florea	0.010 ± 0.001 b	1.79 ± 0.10 bc	4.67 ± 0.43	0.007 ± 0.001 b
C. smaragdula	0.019 ± 0.002 a	2.24 ± 0.19 a	4.44 ± 0.38	0.012 ± 0.001 a
Open pollination	0.014 ± 0.001 ab	1.61 ± 0.04 c	5.60 ± 0.60	0.008 ± 0.001 ab

Lettering on the basis of LSD, $\alpha = 0.05$

There was a statistically significant difference in case of pod weight (df = 4, F = 6.36, p-value = 0.0003), pod

length (df = 4, F = 7.52, p-value = <0.0001) and seed weight per pod (df = 4, F = 3.54, p-value = 0.0118) among *A. dorsata*, *A. mellifera*, *A. florea*, *C. smaragdula* and open-pollination. The highest pod weight (0.019g) was observed in *C. smaragdula* pollinated pods followed by *A. mellifera* (0.011g), A. florea (0.010g), and *A. dorsata* (0.009g). The maximum pod length (2.24cm) was observed in C. smaragdula pollinated pods followed by *A. mellifera* (2.08cm), *A. florea* (1.79cm), and *A. dorsata* (1.57cm). The highest seed weight per pod (0.012g) was observed in *C. smaragdula* pollinated pods followed by *A. mellifera* (0.008g), *A. florea* (0.007g), and *A. dorsata* (0.006g). Approximately the same number of seeds per pod was recorded among *A. dorsata*, *A. mellifera*, *A. florea*, *C. smaragdula* and open-pollinated pods (Table 3).

DISCUSSION

Fenugreek requires floral visiting insects for efficient pollination, healthier seed and fruit setting [33]. In cross-pollinated crops, flowers which are not visited by any pollinator do not set fruit. In the present study, the floral visitor community of fenugreek was composed of six species of bees, four species of flies, one species of moth and one species of butterflies. Deyto & Cervancia reported two honeybees i.e., *A. mellifera* and *A. cerana* and few solitary bees i.e., *Trigona* sp. and *Halictus* sp. as the major floral visitors of fenugreek [20].

In the present study, a significant difference in terms of visitation frequency, visitation rate and stay time was observed among all the four species of bees. The maximum visitation frequency was observed in *A. florea* followed by *C. smaragdula* while the visitation rate of *A. dorsata* was greater than *A. florea*. In the same study area, the highest visitation frequencies have been reported for *A. dorsata* and *A. florea* in canola [2] and bitter [34]. Some previous studies also confirm that *A. dorsata* exhibited a much higher visitation rate than *A. florea* in pumpkin, canola and onion crops [2, 3. 35]. In radish crop, however, *A. dorsata* had a considerably lower visitation rate than *A. florea* [36]. Moreover, no significant difference is reported in visitation rate between *A. dorsata* and *A. florea* in bitter gourd crop [34].

The duration of stay on a flower and the foraging speed, both are governed by energy optimization of pollinators aiming to reduce energetic investment whenever possible [24]. In the current study, the highest stay time was observed in *C. smaragdula* but showed lowest visitation rate. Bee species which forage on flowers intensively i.e., having high foraging rates, generally collect pollen and nectar at a quick pace but their stay time on flowers is shorter than other species with less foraging rates [2, 3, 35, 37].

In the present study, *C. smaragdula* was recorded as the most effective pollinator in terms of single visit efficacy i.e., the maximum pod weight, pod length and seed weights were recorded. Recently, wild solitary bees have recently gained attention for their contribution to stabilizing and enhancing crop-pollination services, because fruit set increases significantly with species richness and visitation rate of wild pollinators [38,39]. Solitary bees usually create specialized mutualisms with certain plant species, whereas honeybees are thought to be opportunistic foragers. When foraging on the same floral resources, it is reasonable to assume that solitary bees are more effective pollinators than honeybees [40]. However, this phenomenon may vary with the type of plant and available pollinator species. No significant difference was found among the solitary bee *Lipotriches sp.* and social bee *A. mellifera* in terms of the significances of single visits for seed and fruit set of a weed, *Wahlenbergia* (Campanulaceae) [41].

It was concluded that *C. smaragdula* is the more efficient pollinator of fenugreek than *A. florea*, *A. dorsata*, and *A. mellifera* in terms of pod weight, pod length and number of seeds produced per pod. Therefore, future studies should focus on the conservation and management of *C. smaragdula*.

ACKNOWLEDGEMENT

The study was funded by the Agricultural Linkages Program (ALP) of Pakistan Agriculture Research Council (PARC) under the project “Conservation of native bees through ecosystem approach for enhanced crop pollination, NR-191”.

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