

From petals to seeds: Understanding the role of hymenopteran and dipteran pollinators in the reproductive success of onion (*Allium cepa* L.)

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ABSTRACT

An experiment was conducted to assess the pollinator community of onion (*Allium cepa* L.) as well to find the pollinators contributing to a higher onion seed production. Fifteen insect pollinator species belonging to two orders and two families were found to be visiting onion flowers. The dwarf honeybee, *Apis florea* F. (Hymenoptera: Apidae) was most frequent pollinator among bees. The maximum visitation rate was recorded in the case of *Eristalinus aeneus* S. (Diptera: Syrphidae), while the maximum stay time was recorded for *A. florea*. For observing the pollination effectiveness, we used pollinator exclusive approach, and four pollination trials were maintained i.e., 100%, 50%, 25% and 0% pollination. Pollinator effectiveness was accessed in terms of yield parameters such as umbel weight, number of seed and seed weight. Compared to 0% pollination, plants with 100% and 50% pollination levels showed 44- and 19-times higher seed production in onion, respectively. Six major pollinators were tested for their pollination efficiency. The single visit efficiency in terms of the number of seeds showed that plants with *A. florea* pollination showed higher seed yield. There was an increase in the number of seeds and seed weight per umbel, proving that *A. florea* is an effective pollinator in onion production. Moreover, syrphid flies also contributed to the onion seed production, though they were less efficient pollinators.

Keywords: pollinator-fauna, pollinator abundance, pollination efficiency, reproductive success, honeybee

INTRODUCTION

Due to agricultural intensification, resulting in the intensive monoculture and loss of habitat, the number of naturally occurring insect pollinators is declining [1]. An important environmental service provided by insect pollinators is the reproductive success of multiple flowering species. Insect mediated pollination is essential for the reproductive success of approximately two-third of the major cultivated crops, with bees being the most vital insects, producing 35 percent

of the global food and supplying nutrients for human diets [2]. Annually, pollinators contribute around \$1.59 billion US dollars to the Pakistani economy [3]. This clearly shows the importance of pollination in agricultural landscapes.

Different crops have varying pollination requirements, so their dependence on the pollinators also varies accordingly [4]. Many cross-pollinated crops, such as onion (*Allium cepa* L.) (Amaryllidaceae), require insect pollination, particularly for seed production (5). Onion, grown in nearly every country globally, is consumed both as raw and cooked forms. With protandrous nature, onion flowers release pollen 2-3 days before the stigma opens [6]. Therefore, pollen from another flower of the same or a different plant is required for successful onion pollination [7]. Onion produces quality seed when insect pollinators are involved in pollination process [8]. Open pollination has been reported to increase seed production by 60-80%, while the absence of insect pollinators could reduce both seed quality and quantity to about 5-14% [7,9,10].

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Another study shows that open-pollinated plants yield 616% higher seed sets than self-pollinated plants [11]. To produce hybrid seeds, male-sterile onions require cross-pollination [12]. After two rounds of self-pollination within a plant, onion suffers inbreeding depression, leading to a decreased bulb size and lower seed production [13]. On the flowering onset in onion, only a few flowers on an umbel open on daily basis, but this number increases until full bloom, when 50 or more florets open in a single day [14].

Other than hymenopterans, some dipterans also visit onion flowers [8]. Previous studies report that various insect pollinators including honey bees, syrphid flies and some other insects, visit onion umbels for pollination. Most studies on onion pollination have reported honey bees as the most abundant pollinators in onion [15, 8, 16, 10]. However, some studies have also identified syrphid flies as abundant pollinators of onion [11, 17].

The reproductive success of crops is dependent upon the visitation frequency of pollinators and their ability to deposit the pollen in a single visit [18]. Single visit efficacy is important to check which pollinator is more efficient in improved seed production. In onion single visits revealed that *Apis dorsata* F. (Hymenoptera: Apidae) produced more seeds per umbel [11]. In the same region, the single visits in canola showed that *A. dorsata*, *A. florea* and *Halictus* sp. (Hymenoptera: Halictidae) were found to be more efficient in canola seed production [19]. Nevertheless, agronomic management techniques have not been fully optimized for underutilized crop production, causing lower productivity [20]. The objective of the current study was to explore the diversity and the pollination efficiency of the common insect pollinators in onion.

MATERIALS AND METHODS

Experimental Site

The field experiment was conducted at the research farms located in Muhammad Nawaz University of Agriculture, Multan, Pakistan (30.146095, 71.448804). Observations were made from November 2019 to April 2020. With hot summers and cold winters, Multan district has been classified as a sub-tropical desert. Temperature range remains 8-12°C minimum and 38-50°C maximum. Mean monthly rainfall during summers remains ~18 mm.

Planting Material

Onion variety, red phulkari, was purchased from the open market and planted on a plot, which measured 45.7 m × 45.7 m. The plot was isolated from the surrounding plots by borders 1.0 m dug into the ground. In early November, a variety of onion, red phulkari was sown directly in the soil of experimental site at the depth of 3.0 cm depth with the 0.46 m distance of row-to-row and 0.15 m of plant-to-plant distance. Plots were irrigated immediately after

sowing to support seed germination and plant development. Plant density was similar across the plot. Experimental plots were checked daily to avoid over irrigation and as well as drought stress. Four irrigations were applied in the plot. First irrigation was applied immediately after sowing, second was done 5 weeks after germination, third during flowering and last on seed setting. The recommended nitrophos fertilizer (phosphorus 20% and nitrogen 22%) 150 kg/acre was applied. To ensure the equal distribution of fertilizer per row, fertilizer was measured per row accordingly. Fertilizer applied in the form of granular in three doses at ~7-10 cm of plant growth, 20% flowering and 60% of flowering respectively, right after the irrigation. Control or unfertilized plots (0 kg N/acre) received similar amounts of water. Moreover, no pesticides were applied to manage pests.

Diversity and Abundance

Thirty flowering plants were randomly selected and the number and species of pollinators were counted for 1 minute per umbel using a stop watch [21, 17, 6]. These observations were done daily from 10:00 am to 12:00 pm for 15 days. This window was selected based on the peak activity of pollinators. All the insect pollinator specimens were identified to the lower taxonomic level possible using identification keys [22]. Pollinator foraging behavior was calculated by the number of umbellule visited by the pollinators per minute and their stay time per umbel [21, 17, 6]. These observations were recorded during day time between 10:00 am to 12:00 pm.

Pollination Effectiveness

The effectiveness of pollinators was recorded by recording pollen deposition by pollinators in a single visit. To record this, 36 umbels of the same age, size and vigor were selected, based on visual observation. Before the opening of floral buds, umbels were caged using nylon mesh bags. During their peak activity time period (10:00 am-12:00 pm) [21] the bags were removed when 50% of flowers were opened. One individual of the pollinator was allowed to sit on the umbel and the umbel was covered again after the pollinator flew [21,17].

Open pollinated plants and caged plants (with no insect visitation) were also kept for the yield comparison. Different yield parameters were recorded i.e., weight of the umbel, number of seeds per umbel and weight of seed per umbel (g), using digital weighing balance.

Pollination Treatments

By using the methodology of Tamburini et al. [23], plants with similar height and vigor were selected randomly and were exposed to four levels of pollination (0, 25, 50 and 100%). The levels were as follows: no pollination (0%, complete exclusion), one day of pollination and three days of exclusion (25%),

one day of pollination and one day of exclusion (50%), and open pollination allowed on all days (100%). Exclusion was done by covering the umbels before opening with fine mesh net bags. The net bags were covered and removed on daily basis during peak foraging time in the morning from 10:00 am to 12:00 pm.

STATISTICAL ANALYSIS

The visitation rate, stay time and abundance of pollinators were subjected to the paired sample t-test. Two-way analysis of variance (ANOVA) was used to analyze the umbel weight, number seed per umbel and seed weight per umbel. Tukey's test was used as a

post-hoc test. Minitab (16) was used for all statistical analysis.

RESULTS

Diversity and Abundance

The Pollinator community visiting onion umbels comprised of three hymenopteran and twelve dipteran species. The bee species from the family Apidae included *A. florea*, *A. dorsata* and *Xylocopa* sp. Five dipteran families visiting onion flowers included Calliphoridae, Muscidae, Sarcophagidae, Syrphidae and Stratiomyidae. *A. florea* was most abundant among bee species while *E. aeneus* dominated the fly species (Table 1).

Table 1: Insect pollinator groups foraging on onion flowers in Multan, Pakistan

Pollinator group	Order	Family	Scientific name	No. individuals
Honeybees			<i>Apis florea</i>	28
Wild bees	Hymenoptera	Apidae	<i>Apis dorsata</i>	3
			<i>Xylocopa</i> sp.	3
			<i>Calliphora</i> sp.	8
		Muscidae	<i>Musca domestica</i>	13
		Sarcophagidae	<i>Sarcophaga</i> sp.	2
Flies	Diptera	Syrphidae	<i>Eristalinus aeneus</i>	146
			<i>Eupeodes corollae</i>	8
			<i>Sphaerophoria bengalensis</i>	19
			<i>Eupeodes corollae</i>	3
			<i>Episyrphus balteatus</i>	43
			<i>Eristalis tenax</i>	10
			<i>Mesembrius</i> sp.	9
			<i>Eristalinus megacephalus</i>	3
Stratiomyidae	<i>Stratiomyidae</i> sp.	3		

Foraging Behavior

Stay time of *A. florea* was longest followed by *E. aeneus*, *Musca domestica* and *Eristalis tenax* While least stay time was found of *M. bengalensis* (Table 2).

Visitation rate of *E. balteatus* was found highest followed by *A. florea* and least was observed for *Sphaerophoria. bengalensis* (Table 2).

Table 2: Mean (\pm SE) stay time and visitation rate of insect pollinators observed on onion flowers.

Insect pollinators	Stay time (n=50) (individuals/plant/min)	Visitation rate (n=50)
<i>Apis florea</i>	1.99 \pm 0.25a	1.06 \pm 0.10a
<i>Sphaerophoria bengalensis</i>	1.62 \pm 0.05d	0.78 \pm 0.03e
<i>Eristalinus aeneus</i>	1.81 \pm 0.12b	0.82 \pm 0.04d
<i>Episyrphus balteatus</i>	1.58 \pm 0.14e	0.70 \pm 0.04f
<i>Mesembrius bengalensis</i>	1.73 \pm 0.16c	0.86 \pm 0.20b
<i>Calliphora</i> sp.	1.73 \pm 0.36c	0.83 \pm 0.59c
<i>Musca domestica</i>	1.99 \pm 0.25a	1.06 \pm 0.10a
<i>Eristalis tenax</i>	1.62 \pm 0.05d	0.78 \pm 0.03e

Means within the columns with the different lowercase letters significantly different, as determined by the Tukey-Kramer's post hoc test ($P < 0.05$).

Pollination Treatments and Yield

Maximum umbel weight, number of seeds and seed weight was recorded in 100% pollination treatment followed by 50% pollination treatment (Table 3). Among these pollination treatments,

maximum umbel weight (8.04 g), number of seeds (45.22 g) and seed weight (0.14 g) was observed at 100% pollination. While least reproductive success was found at 0% pollination treatment.

Table 3: Mean (S.E.) umbel weight, seed number, and seed weight in different pollination treatments.

Pollination treatment	Umbel weight (g)	No. Seeds (no.)	Seed weight (g)
100%	8.04 ± 0.96a	45.22 ± 6.0a	45.22 ± 0.02a
50%	6.74 ± 0.68a	20.89 ± 2.18b	20.89 ± 0.01b
25%	4.29 ± 0.32b	9.78 ± 0.16b	9.78 ± 0.00c
0%	1.93 ± 0.27c	1.2 ± 0.49c	1.22 ± 0.00c

Means within the columns with the different lowercase letters significantly different, as determined by the Tukey-Kramer's post hoc test ($P < 0.05$).

Single Visit Efficacy

Significant differences were found between different insect species in seeds produced because of single visits. *A. florea* demonstrated maximum

reproductive success after the open pollination (visited by different pollinators) (Table 4).

Table 4: Comparison of different insect pollinators in terms of their single visit efficacy in onion reproductive success.

Pollinator	Umbel weight (g)	No. Seeds (no.)	Seed weight (g)
<i>Apis florea</i>	7.90 ± 0.69a	31.66 ± 2.02a	0.16 ± 0.03a
<i>Episyrphus balteatus</i>	5.13 ± 1.27b	16.33 ± 2.18d	0.05 ± 0.01b
<i>Mesembrius</i> sp.	3.87 ± 0.78d	20.66 ± 7.21c	0.06 ± 0.02b
<i>Sphaerophoria bengalensis</i>	3.47 ± 0.75e	22.33 ± 5.78f	0.07 ± 0.02b
<i>Eristalinus aeneus</i>	4.22 ± 1.05c	12.66 ± 2.33b	0.23 ± 0.03b
<i>Musca domestica</i>	3.88 ± 0.25d	15.00 ± 1.73e	0.05 ± 0.00c

Means within the columns with the different lowercase letters significantly different, as determined by the Tukey-Kramer's post hoc test ($P < 0.05$).

Discussion

Compared to honey bees (20%), syrphid flies (80%) were the most common pollinators in the current study, as also indicated by other studies in onion [6,17,21]. *E. aeneus* was observed as the most abundant pollinator followed by *A. florea*. This contrasts with the findings of Sajjad et al. [21], who found that after *A. florea* and *A. dorsata*, were the most abundant onion

pollinators. However, in another study from Asia, *A. dorsata*, was the most prevalent pollinator of onion umbels, whilst syrphid flies were the least common pollinators [8]. While *A. dorsata* was the least common pollinator in our study [8], the variation may result from a different data recording time [24], cropping strategy, or from pollinator species competing for the same resources [17].

The visitation rate stands as a crucial parameter for assessing the effectiveness of pollinators [25, 26]. In the current study, *E. balteatus* and *A. florea* had the maximum visitation [21] and stay time rate on onion respectively. The foraging strategy of visiting several flowers may not be beneficial for self-incompatible species and could reduce seed set [27, 28, 29]. Previous evidence from southern Punjab indicates *A. mellifera* [30] and *A. dorsata* as the most effective pollinators in terms of visiting rate for various pollinator-dependent crops [31,32,19]. There have also been reports of the combined pollination efficacy of syrphid flies and honey bees for bitter melon [32], canola and carrot [19].

Single-visit efficacy is crucial for assessing the ability of a pollinator and its impact on crop yield. Studies have shown that a single bee visit could increase the yield of onion by up to 2.5 times [8]. While it has been widely accepted that the *A. mellifera* serve as the sole pollinator and directly or indirectly pollinates approximately one-third of the world's crops [33], its efficacy has been questioned by some studies [34, 35]. Numerous studies have showed that wild bees prove to be more efficient pollinators than honey bees [19]. Current study shows that single visits by honey bees result in more seeds per umbel compared to single visits by dipteran syrphid flies. A previous study, Ali et al. [14], proved that the two syrphid flies, *E. balteatus* and *E. corollae*, deposited fewer pollen grains than bees because they primarily fed on anthers and rarely encountered the stigma. The *A. florea* was identified as a more efficient pollinator in onions. Although *A. florea* is not always considered an efficient pollinator due to its nectar robbing nature in canola [19] and radish [36], this variability might be due to the effect of nectar or pollen quantity in flowers on pollinator behavior, consequently affecting the efficacy of pollination [37].

In the current research, compared to the caged treatment, open pollination increased the number of seeds and umbel weight in onions by 36 and 4.0 percent, respectively. Open pollination yielded the highest quantity of onion seeds compared to self-pollination. A previous study also indicated that the presence of syrphid flies and honey bees increased seed set per umbel by 90% [25] and seed germination by 616% [30] in open-pollinated onion, compared to the caged

treatment [30]. Garibaldi et al. [38] showed that an increase in wild insect visitation enhanced fruit set by twice as much as an equivalent increase in honeybee visitation in 41 crop systems worldwide.

Future studies may explore the impact of environmental variables, such as temperature shocks, drought, and precipitation, on pollinator activity and subsequent changes in onion seed yield. Additionally, exploring habitat management and modifications, such as planting conservation strips or flowering plants near onion fields, could offer insights into the long-term changes in native pollinator communities. Moreover, the effects of pesticides and other management practices on local pollinator populations and honey bees should be considered for the development of practical and effective integrated pest and pollinator management plan in onion.

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