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EXPLORING THE BEST NATIVE INSECT POLLINATORS FOR THE REPRODUCTIVE SUCCESS OF *BAUHINIA VARIEGATA* L. (FABACEAE) IN MULTAN, PAKISTAN

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Abstract

Insect pollination is an important ecosystem service for the reproductive success of different plant species. The mountain ebony, *Bauhinia variegata* L. (Fabaceae) is a native tree of Pakistan, and its flowering buds are used as a vegetable. The current study aims to determine the diversity and efficiency of native insect pollinators in the seed set of *B. variegata*. The foraging behavior of the pollinators was assessed in terms of their stay time and visitation rate. Furthermore, most abundant insect pollinators were also tested for their pollination efficiency in terms of single-visit pollen deposition and single-visit seed set. Moreover, different pollination treatments (i.e., open, hand cross, hand self, and natural self-pollination) were also compared. The insect pollinator fauna was composed of four hymenopteran species, four dipteran species, and three lepidopteran species. The managed honey bee, *Apis mellifera* L. (Hymenoptera: Apidae) was the most abundant insect pollinator, followed by the wild honey bee, *A. dorsata* and a hover fly species *Ischiodon scutellaris* (F.) (Diptera: Syrphidae). Based on the efficiency of pollen deposition and single visit seed set, *A. dorsata* and *A. mellifera* were the most efficient insect pollinators followed by *I. scutellaris*. Open pollination resulted in better reproductive success parameters (pod length, pod weight, number of seeds per pod, and seed weight per pod) as compared to other pollination treatments. The study concluded that conserving the most efficient insect pollinators (through providing year-round foraging and nesting resources) can help in improving the reproductive success of *B. variegata* and other cross-pollinated crops in the same region.

Key words: Bees; Syrphid flies; Pollen deposition; Single-visit; Conservation.

Introduction

The mountain ebony, *Bauhinia variegate* L., is commonly known as "Kachnar" in Pakistan famous for their health benefits (Verma *et al*., 2012). It is native to the tropical and temperate Indian subcontinent regions, including Bhutan, India, Nepal and Pakistan, and some other Southeast Asian countries (including Myanmar, Vietnam, Laos, and Thailand), and China (CechinelFilho, 2009). The tree possesses many biological and medicinal properties i.e. anti-inflammatory, antidiabetic, antibacterial, and antimicrobial properties (Kumar *et al*., 2016). The leaves of *B. variegata* are often used as a salad with rice and also improve flavor to different meat dishes (Yadav & Bhadoria, 2001). Floral buds of this plant are rich in fats, proteins, antioxidants, carbohydrates (Verma *et al*., 2012) and also as a vegetable in many parts of the world (Samant *et al*., 2014).

Insect pollinators (entomology) play a critical part in the long-term viability and continuity of a given ecosystem through maintaining plant biodiversity on the planet (Breeze *et al*., 2011; Kearns, 2010). Most of the pollination services are provided by the bees and their absence or inactivity could lead to a 90% reduction in the yield of pollinator dependent crops (Nayak *et al*., 2020; Abrol *et al*., 2019; Quigley *et al*., 2019). Every year pollinators contribute around $577 billion USD to the global economy (Potts *et al*., 2016) and $1.59 billion USD to the economy of Pakistan (Irshad & Stephen, 2013). Around 40% of the world's total nutrients depend upon insect pollinators (Eilers *et al*., 2011) and decrease in the insect pollinators can cause nutrient deficiencies in human beings (Hodge *et al*., 2016). These statistics prove the value of insect pollinators in ensuring food security (Sluijs & Vaage, 2016).

Different types of pollination have been observed in *Bauhinia* species such as chiropterophily, melittophily, sphingophily, ornithophily, and psychophily (Bobrowiec & Oliveira, 2012). The flowers of *Bauhinia* species provide enough food sources for both nectar and pollen foragers. The solitary bees *Xylocopa* sp. and honey bees *Apis dorsata* have been the most frequent visitors of *B. variegata* flowers (Solomon *et al*., 2008). Previously, free visit of bees (*A. dorsata*, *Xylocopa latipes*, *X. pubscens*, *Ceratina simillima*, *Apis florea*, *A. cerana* and *Trigona iridipennis*) have resulted in 60-82% more pod set as compared to 8% in caged plants (Solomon *et al*., 2008; Rao, 2020). Furthermore, previously from the same region, the honey bees (*A. dorsata* and *A. florea*), solitary bees (*Amegilla* sp., *X. aestuans*, *Megachile bicolar* and *Nomia oxybeloides*) and the syrphid flies (*I. scuterallis* and *Paragus serratus*) have been reported as abundant insect foragers on the flowers of *Albizia lebbeck* (L.) (Latif *et al*., 2019) and *Callistemon viminalis* (Sol ex. Gaertn.) (Latif *et al*., 2016).

Pollen deposition and single visit fruit set are important parameters to determine the efficiency of an individual insect pollinator (King *et al*., 2013). Previously, from the same region, *A. dorsata* has been reported as the most efficient pollinator in terms of single visit fruit set and pollen deposition in different crops i.e. canola (Ali *et al*., 2011), pumpkin (Ali *et al*., 2014), Luffa gourd (Ali *et al*., 2016) and bitter gourd (Saeed *et al*., 2012) and also for a tree (*A. lebback*) pollination (Latif *et al*., 2018).

Previously, few studies have reported the diversity of the insect pollinators in *B. variegata*; however, none of the studies reported the single visit seed set and pollen deposition ability of individual insect pollinators in the pollination mechanism. Therefore, the current study was performed to evaluate the most efficient insect pollinators in terms of single visit seed set and pollen deposition ability towards the reproductive success of *B. variegata*.

Materials and Methods

Study site: The study was carried out at the research farm of MNS-University of Agriculture (30°9′54.79″N 71°29′48.72″E), Multan during the vegetative season (March- April) 2021. Multan is located in the subtropical region with hot summer (35 to 40ºC), cold winter (8 to 10ºC), annual rainfall is about 127-254 mm annually, and heavy fog in winters season (Khan & Hassan, 2019). The selected area consisted of 50 plants of *B. variegata*, surrounded by few mulberry plants (01 Kanal) & wheat fields (25 acres).

Abundance and diversity of pollinators visiting *B. variegata* flowers: The abundance and diversity of the insect pollinators was observed at four different time intervals (0700, 1000, 1300, and 1600 hrs) during the flowering period (first week of March to the first week of April). For this purpose, 15 plants were selected, and each plant was observed for four minutes and all the insect pollinators visiting the flowers were recorded. The data was recorded on clear sunny days in order to avoid any effects of abiotic factors on the activity of insect pollinators. The insect pollinators were identified with the help of taxonomic keys of Vockeroth (1969) and Michener (2000).

Foraging behavior of insect pollinators: Foraging behavior of the most abundant insect pollinators was observed in terms of stay time and visitation rate. The stay time referred as the time spent by an individual pollinator on a flower, whereas the visitation rate was noted as the number of flowers visited in one minute by an individual pollinator. These observations were taken during the peak activity time of the pollinators (Latif *et al*., 2019). Nectar robbing and pollen foraging activity were also observed through visual observations. Only those insect visitors were considered as pollinators that contacted the reproductive parts (stigma/anther) of the plant.

Pollination treatments: The assessment of the reproductive success in four different pollination treatments was done by using 30 floral buds for each treatment. These treatments include; (a) hand cross pollination (pollen grains from one flower were deposited on the stigma of another flower) (b) hand self-pollination (each caged flower was hand pollinated with its own pollen) (c) open pollination (flowers were left open to be freely visited by the insect pollinators) (d) wind pollination (the buds were caged to avoid insect visits but not the wind) (Etcheverry *et al*., 2003). The reproductive success was measured in terms of the following parameters, i.e., pod length (cm), pod weight (g), number of seeds, and seed weight per pod (g).

Single visit pollen deposition: Pollination effectiveness was recorded in terms of the number of pollen grains deposited on the stigma of the flower in a single insect visit. Single buds were caged a day before their opening, followed by their un-caging during the peak activity time of insect pollinators. After the contact of an individual insect pollinator to the stigma in its single visit, the individual stigma was removed and fixed in alcohol acetic acid solution followed by staining with safranine and aniline blue (Dafni, 1992). Pollen grains deposited by an individual pollinator species were counted by using a stereoscope with 40X magnification.

Single visit seed set: To assess insect pollinator effectiveness after a single visit, floral buds of *B. variegata* were caged a day before their opening, followed by their un-caging during the peak activity time of insect pollinators. After the single visit of an individual pollinator, the flowers were caged again (Canto-Aguilar & Parra-Tabla, 2000). Pods were harvested at maturity and the following parameters were recorded, i.e. pod length (cm), pod weight (g), number of seeds, and seed weight per pod (g).

Statistical analyses

The data of pollinator stay time, visitation rate, and plant yield parameters (pod length, pod weight, no. of seeds per pod, seed weight per pod) was analyzed by analysis of variance (ANOVA). The means were compared using the Fischer test at $p<0.05$. The statistical analysis was performed by using the computer software XLSTAT (XLSTAT, 2018).

Results

Pollinator community of *B. variegate*: The current study found that insect pollinator community of *B. variegata* consisted of different pollinators belonging to the orders Hymenoptera, Diptera and Lepidoptera. In Hymenoptera, 4 bee species belonged to the family Apidae were recorded i.e. *Apis mellifera* (Fig. 1), *A. dorsata* (Fig. 2), *A. florea* (Fig. 3), and *Xylocopa* sp. The Diptera order included three species from family Syrphidae i.e. *Ischiodon scutellaris* (Fig. 4), *Episyrphus balteatus* (Fig. 5) and *Eristalinus aeneus* (Fig. 6). The Lepidoptera order was consisted of three species of butterflies i.e. *Danaus plexippus*, *Papilio demoleus*, and *Pieris rapae*. Bees were the most abundant insect pollinators (63%) followed by syrphid flies (35%) and butterflies (2%) (Fig. 7). Among the bees, *A. mellifera* was the most abundant insect pollinator (41.3%) followed by *I. scutellaris* (13.9%) and *A. dorsata* (11.8%) of the total population (Table 1).

Different foraging patterns were observed in the insect visitors. Honey bee, *A. florea* was observed robbing the nectar primarily by side feeding. Those insect visitors that foraged for both nectar and pollen were *A. mellifera*, *A. dorsata*, and *P. demoleus*. The syrphid flies (*I. scutellaris* and *E. balteatus*) foraged pollen, whereas other Dipterans foraged nectar (Table 1).

Stay time & visitation rate: There was a significant difference among the insect pollinators in terms of stay time ($F = 107$, df = 4, $p<0.001$). Syrphid fly, *I. scutellaris* spent the highest time per flower (184.68 sec) followed by *A. florea* (50.54 sec) while it was lowest for *A. mellifera* stayed (7.51 sec). Pollinator species also varied significantly in terms of visitation rate ($F = 177$, df = 4, $p<0.001$). Managed honey bee (*A. mellifera*) visited the highest number of flowers per minute (7.12) followed by *A. dorsata* (4.80) while it was lowest for *Ischiodon scutellaris* i.e. 1.68 (Table 2).

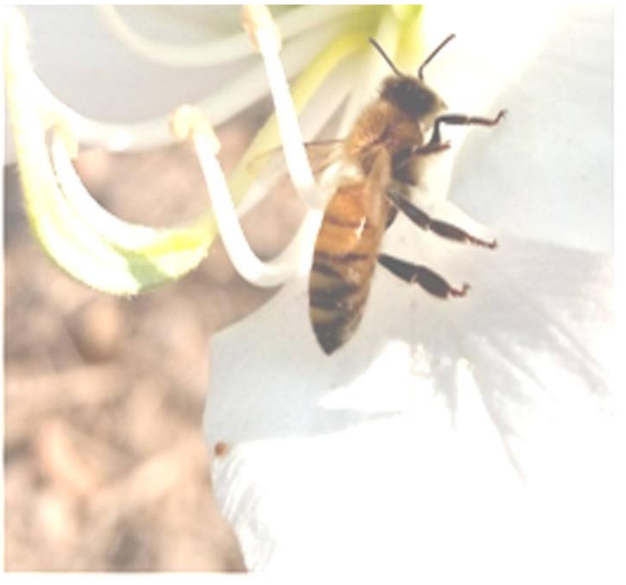

Fig. 1. *Apis mellifera* foraging from kachnar flowers.

Fig. 2. *A. dorsata* foraging from kachnar flowers.

Fig. 3. *A. florea* foraging from kachnar flowers.

Fig. 4. *Ischiodon scutellaris* foraging from kachnar flowers.

Fig. 5. *Episyrphus balteatus* foraging from kachnar flowers.

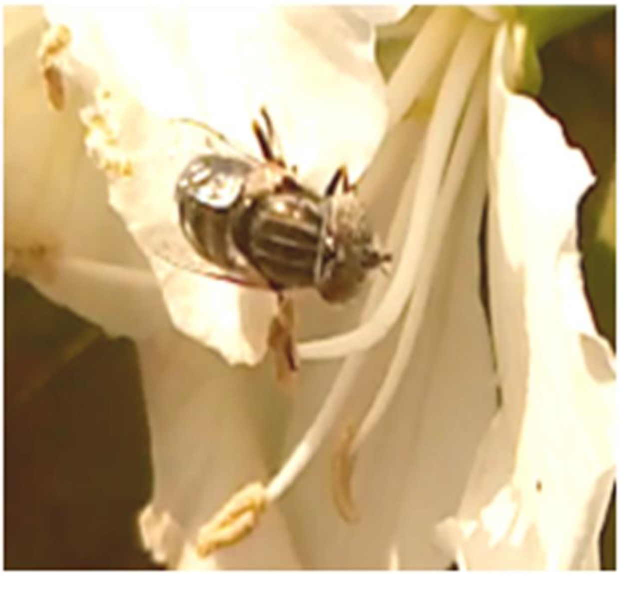

Fig. 6. *Eristalinus aeneus* foraging from kachnar flowers.

Table 1. Insect pollinators in kachnar flowers with their total abundance, visitation frequency and foraging task.

Order	Family	Genus/Species	Total abundance	Visitation frequency (individuals/umbel/min)	Foraging task (N/P)*
Hymenoptera	Apidae	*Apis mellifera*	1679	0.69	N/P
		Apis dorsata	482	0.20	N/P
		Apis florea	228	0.09	N
		Xylocopa sp.	47	0.01	N
Diptera	Syrphidae	*Ischiodon scutellaris*	568	0.23	P
		Episyrphus balteatus	368	0.15	P
		Eristalinus aeneus	227	0.08	N
	Calliphoridae	*Lucilia sericata*	213	0.09	N
Lepidoptera	Nymphalidae	*Danaus plexippus*	25	0.01	N
	Papilionidae	*Papilio demoleus*	30	0.01	N/P
	Pieridae	*Pieris rapae*	28	0.01	N

*N/P, Nectar/Pollen

Table 2. Pollination effectiveness of five insect pollinators in terms of stay time rates and pollen deposition.

Insect pollinators	Stay time/Flower (seconds) (N=100)	Visitation rate /Flower/60s (N=100)	Pollen deposition (N=20)
Apis dorsata	8.74 ± 0.60^{c}	4.80 ± 0.21^{b}	281.00 ± 15.50^{a}
A. mellifera	7.51 ± 0.52^{c}	7.12 ± 0.18^{a}	276.33 ± 59.90^{a}
A. florea	50.54 ± 2.22^{b}	1.88 ± 0.12^{d}	137.33 ± 31.89^{b}
Ischiodon scutellaris	184.68 ± 15.60^{a}	1.68 ± 1.68^{d}	154.33 ± 52.00^{b}
Episyrphus balteatus	25.13 ± 3.69 ^{bc}	2.94 ± 0.20^{c}	152.33 ± 59.65^{b}

The mean values sharing the same letter are not different significantly as per Fischer Test at 5% level (±SE)

Table 3. Single visit seed set efficacy of insect pollinators in terms of reproductive success parameters.

Insect Pollinators	Pod length (cm)	Pod weight (gm)	No. of seeds	Seed weight/Pod (gm)
Apis mellifera	21.69 ± 0.57^{a}	7.49 ± 0.35^{a}	8.80 ± 0.72^{a}	2.61 ± 0.28^{a}
A. dorsata	20.73 ± 2.02^{a}	6.96 ± 2.06^{a}	9.00 ± 1.00^{a}	2.93 ± 0.20^{a}
A. florea	18.56 ± 1.42^{b}	4.15 ± 0.48^{c}	4.67 ± 0.81^{c}	1.35 ± 0.23^{b}
Ischiodon scutellaris	19.00 ± 1.27^{b}	6.15 ± 0.31^{b}	7.00 ± 0.63^{b}	1.60 ± 0.17^{b}
Episyrphus balteatus	18.85 ± 1.06^{b}	5.86 ± 0.53^{ab}	6.83 ± 0.86^{b}	1.56 ± 0.17^{b}

Average values showing the same letters are not statistically different as per Fischer LSD Test at 5% (±SE)

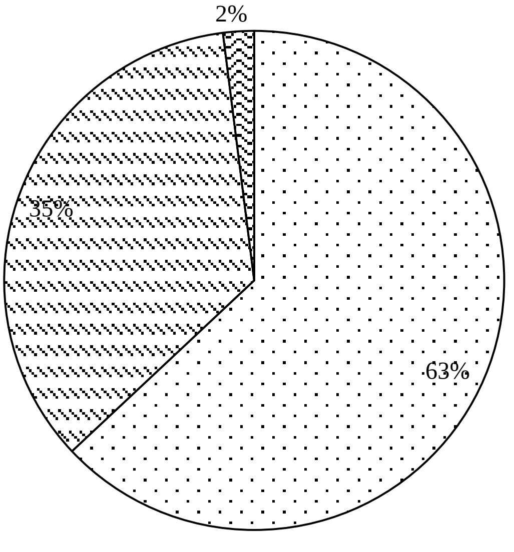

Fig. 7. Proportional abundance of Hymenoptera, Diptera and Lepidoptera in *B. variegate.*

Pollen deposition & single visit efficacy: In terms of pollen deposition, *A. dorsata* and *A. mellifera* deposited the highest number of pollen grains per their single visit (281.00 and 276.33 pollen grains respectively) while it was lowest for *A. florea* (137.33) and both syrphid fly species i.e. *I. scutellaris* and *E. balteatus* (154.33 and 152.33 respectively) (Table 2).

The single visit seed set efficacy of insect pollinators revealed significant differences among the insect pollinators in terms of pod weight (F = 6.13, df = 4, p<0.001), pod length (F= 2.29, df= 4, p<0.001), number of seeds per pod (F = 5.22, df = 4, p<0.001) and seed weight per pod (F = 8.05, df = 4, p<0.001). After one visit of the insect pollinators, *A. mellifera* and *A. dorsata* proved to be the most efficient insect pollinators in terms of all reproductive success parameters while *A. florea* and both syrphid fly species (*I. scutellaris* and *E. balteatus*) were proved less efficient in terms of their single visit efficacy (Table 3).

Pollination treatments: Pollination treatments also varied significantly in terms of reproductive success parameters i.e. pod length (F = 31, df = 3, p<0.001), pod weight (F = 27.2, df = 3, p<0.001), number of seeds (F = 16.5, df = 3, p<0.001) and seed weight per pod (F = 0.02, df = 3, p<0.001). Open pollination (free insect visits) resulted in better reproductive success of *B. variegata* followed by hand cross and hand self-pollination treatments whereas the lowest values were recorded for wind pollination (Table 4).

Table 4. Reproductive success of *B. variegata* in different pollination treatments.

Treatments	Pod length (cm)	Pod weight (g)	No. of seeds per pod	Seed weight(g)
Open pollination	24.30 ± 0.512[a]	6.99 ± 0.29[a]	8.25 ± 0.27[a]	2.47 ± 0.12[a]
Hand cross pollination	18.29 ± 0.71[b]	5.01 ± 0.31[b]	6.86 ± 0.39[b]	2.17 ± 0.15[a]
Hand self-pollination	18.27 ± 1.04[b]	4.58 ± 0.31[b]	6.52 ± 0.34[b]	2.06 ± 0.14[a]
Wind pollination	11.03 ± 0.79[c]	1.82 ± 0.36[c]	3.66 ± 0.25[c]	1.01 ± 0.09[b]

Averages with the same letters are not statistically different as per Fischer LSD test at 5% level (±SE)

Discussion

The flowers of *B. variegata* are large, bisexual and zygomorphic and contain ample amount of nectar and pollen to attract a variety of insect visitors (Solomon *et al*., 2008). In our study, bees were proportionally more abundant compared to flies and butterflies. Moreover, *A. mellifera* was most abundant followed by *I. scutellaris* and *A. dorsata*. Some previous studies have also reported higher abundance of wild honey bees (*A. dorsata*, *A. florea*) and solitary bees (*A. cerana* and wild *Xylocopa* sp.) visiting *B. variegata* flowers (Solomon *et al*., 2008; Hokche & Ramirez, 1990). However, syrphid flies were reported as the visitors of *B. variegata* flowers. Moreover, previously from the same region, insect pollinator fauna of other two tree species (*Albizia lebback* and *Callistemon viminalis*a) was composed of honey bees (*A. dorsata, A. florea*), solitary bees (*Amegilla* sp., *Megachile bicolar, Nomia oxybeloides*) and syrphid fly species (*Ischidon scuterallis*, and *P. serratus*) (Latif *et al*., 2018; Latif *et al*., 2016). The abundance and diversity of the insect visitors varies with the time, area and latitude (Olletron & Cranmer, 2002).

Visitation rate is an important parameter to assess the pollination efficiency of insect pollinators (Zameer *et al*., 2017). *A. mellifera* was most efficient in terms of visitation rate followed by *A. dorsata* and *E. balteatus*. Previously, this behavior has not been assessed fpr *B. variegata* or in other *Bauhinia* species. However, from the same region, wild bee *A. dorsata* has been reported as the most efficient insect pollinator in terms of visitation rate in canola (Ali *et al*., 2011), bitter gourd (Saeed *et al*., 2012), and pumpkin (Ali *et al*., 2014).

Pollen deposition and single visit seed set efficacy are among the important parameters to assess the pollination ability of an individual insect pollinator (King *et al*., 2013; Ali *et al*., 2016). Based on pollen deposition and single visit seed set efficacy, *A. dorsata* followed by *A. mellifera* were proved as most efficient. None of the previous studies evaluated these two parameters for the reproductive success of *B. variegata*. However, from the same region, *A. dorsata* has been reported as the most efficient pollinator in terms of pollen deposition and single visit seed set efficacy in other important crops i.e. canola (Ali *et al*., 2011), pumpkin (Ali *et al*., 2014), luffa gourd (Ali *et al*., 2016) and radish (Zameer *et al*., 2017). In the current study, *A. florea* was found as least effective in terms of pollen deposition and single visit seed set efficacy along with other two tested syrphid fly species. It could be attributed to the nectar robbing/side feeding behavior of *A. florea* that limits its pollination potential as earlier reported in canola from the same region (Ali *et al*., 2011).

Open pollination (flowers receiving free insect visits) resulted in nine times higher seed set as compared to wind pollination (caged flowers). A previous study in *B. variegata* showed that open pollination resulted in 60-82% higher seed set on flowers visited freely by insect pollinators. Yet other study showed self-incompatibility and no seed set in insect excluded flowers of *Bauhinia forficata*. Therefore insect pollinators are vital for the reproductive success of different species of Bauhinia (Solomon *et al*., 2008; Neto, 2013).

The hymenopteran species, *A. dorsata* and *A. mellifera* proved to be the most efficient pollinators as the seed set and pollen deposition were greater than other pollinator taxa. Therefore, conserving these insect pollinators can lead to higher reproductive success of *B. variegata* and other cross-pollinated crops in Southern, Punjab Pakistan. Future studies should consider bio-ecology of these bees along with other solitary bee species keeping in view the vital role of insect pollinators towards the food security worldwide and in Pakistan.

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