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# Solitary Bees Are More Efficient Pollinators of Sponge Gourd than Giant Honeybees and Syrphid Flies

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**Abstract**

Cucurbits comprise a vast group of vegetables grown worldwide, including Pakistan. The sponge gourd (*Luffa aegyptiaca*) is a summer vegetable in Asia. Because of its monoecious nature, sponge gourd necessitates more pollination, and fruit production occurs only in the presence of insect pollinators. This study compared the foraging behavior and pollination effectiveness of the most abundant sponge gourd floral visitors, including five solitary bees and a giant honeybee, *Apis dorsata*. The foraging behavior was assessed regarding visitation rate, visitation frequency, and time spent per flower, while pollination effectiveness was assessed regarding pollen deposition and fruit set in a single visit. The bees constituted 91% of total pollinator abundance, followed by the flies (8%). *Apis dorsata* was the most abundant visitor, followed by *Xylocopa* sp., *Lassioglossum* sp., and *Amegilla* sp. Solitary bees, including *Amegilla* sp. and *Nomia* sp., demonstrated greater single-visit efficiency in pollen deposition and fruit set than *A. dorsata* and syrphid fly. Conservation of *Amegilla* sp. and *Nomia* sp. can enhance fruit production for sponge gourd and other vegetables cultivated in Punjab, Pakistan, by providing floral resources and nesting places.

## Introduction

Pollinators offer a crucial ecosystem service of pollination to numerous crops, contributing 35% to the worldwide food supply and supporting the global economy with 577 billion USD per year (Ferrier et al., 2016). However, consensus still needs to be reached regarding implementing management practices to optimize crop pollination services (Isaacs et al., 2017; Rollin & Garibaldi, 2019). The initial step toward optimizing pollination services involves assessing the relative efficiency of available insect pollinators. This assessment enables the implementation of targeted conservation measures for pollinator-dependent crops, supporting these species and their pollination services (Garibaldi et al., 2014). Conversely, there has been a global decline in the populations of solitary bees and honey bees over recent years (Lewis & Cratsley, 2008; Hinkel et al., 2014; Garibaldi et al., 2011; Garibaldi et al., 2013; va Klink et al., 2023), potentially leading to reduced seed set and yield in various pollinator-dependent crops (Steffan-Dewenter et al., 2005; Taylor, 2006; Potts et al., 2010).

Various parameters have been established to evaluate the pollination efficiency of insect pollinators, i.e., pollen carrying capacity, pollen deposition on stigma, fruit set, fruit yield, etc. (Dafni, 1992). Higher pollinator abundance has generally been shown to positively impact the yield of pollinators' dependent crops (Kremen et al., 2002, 2004; Ricketts, 2004). Previous research has also found a link between cucurbit yield and the abundance and diversity of social and solitary bees (Gautam & Kumar, 2018; Knapp & Osborne, 2019), Canto-Aguilar & Parra-Tabla, 2000; Pande & Verma, 2016). It is important to note that a common pollinator may or may not be the most effective one (Freitas & Paxton, 1998; Ivey et al., 2003). Hence, continuous research is essential to uncover efficient pollinators specific to each crop and region.

Pollen deposition and fruit/seed set efficacy from a single visit are essential indicators for assessing the performance of a pollinator species (Ali et al., 2016; Ali et al., 2014). Honeybees have been identified as efficient pollen depositors in several crops (Wist & Davis, 2013; Saez et al., 2014). However, more thorough reports have demonstrated that solitary bees outperform honey bees in cucurbit pollination efficiency in terms of pollen deposition (Garantonakis et al., 2016; Ali et al., 2015; Ali et al., 2014), visitation rate (Mensah et al., 2011; Ali et al., 2014; Hashifah et al., 2020), early foraging hours (Ali et al., 2016; Mensah & Kudom, 2010), and the enhancement of seed set (Garantonakis et al., 2016). To promote sustainable crop pollination, evaluating the pollination potential of an insect pollinator community is essential. This assessment can be the basis for developing robust management guidelines for crop pollination (Garibaldi et al., 2013).

The Cucurbitaceae comprises nearly 800 species and is the most prominent family of crops (Rolnik & Olas, 2020). In Pakistan, cucurbits are the third most-grown vegetable after potatoes and onions (MINFAL, 2008). includes sponge gourd *Luffa aegyptiaca* Mill., a popular vegetable that thrives in tropical and subtropical regions (Heiser & Schilling, 1988). This plant relies heavily on insect pollinators due to its monoecious nature (Ramirez et al., 2002; Stanghellini et al., 2002; Njoroge et al., 2004). Both male and female flowers provide ample amounts of pollen and nectar and attract a diverse range of floral visitors (Sadeh et al., 2007). The enhanced secretion of nectar renders the female flower particularly appealing to insect pollinators, resulting in effective pollination (El-Kazafy & Mahmoud, 2006).

Previously, the pollination efficiency of only two species, *A. mellifera* and *X. olivacea*, was estimated in terms of fruit set and size. However, the novelty of the current study lies in the documentation of the pollination efficiency and foraging behavior of most abundant floral visitors, including honey bees, solitary bees, and a syrphid fly, to assess their potential contribution to sponge gourd pollination. Pollination performance was gauged by pollen deposition on stigma and fruit set in a single visit (Mensah & Kudom, 2010; Ali et al., 2016). At the same time, foraging behavior was analyzed based on variables like stay time (time spent per flower), visitation rate (number of flowers visited per minute), and the preference for nectar or pollen.

## Materials and Methods

### *Study area*

This study was conducted from April to September 2020 at the research farm of MNS University of Agriculture Multan, Pakistan. Sponge gourd (*Luffa aegyptiaca*) was cultivated on a 0.25-hectare plot surrounded by cotton on three sides and pumpkin on one side. The study area is arid and experiences hot summers and cold winters. The average summer temperature is 40 ± 5 ºC, while the winter temperature is 10 ± 5 ºC. The annual rainfall ranges from 127 to 254 mm (Khan & Mahmood, 2019).

### *Abundance and Diversity of Insect Pollinators*

Live observations were conducted at 6:00 am, 9:00 am, 12:00 pm, and 3:00 pm with three-day intervals throughout the flowering season (July to September 2020), while the peak flowering period was observed in August 2020. Thirty flowers were randomly chosen in each census, and each flower was observed for one minute to document all insect visitors that encountered the stigma or anther of the flower. Moreover, the visitation frequency of each species was calculated as the number of individuals per flower per minute (Ali et al., 2014). We also calculated the abundance, which is the total number of individuals of a species visited throughout the data recording period. A few individuals were also captured using an insect net for taxonomic identification.

### *Diversity indices*

Diversity indices estimate species biodiversity based on the species richness (number of recorded species) and abundance (number of individuals of a species) for a given population. This study calculated diversity indices from the insect pollinators visiting the flowers. Shannon diversity indices (H) and Simpson's diversity indices (D) were calculated for the diversity of pollinators on sponge gourd flowers. The formulas for the diversity indices are given as follows:

$$H = -i = 1Spilnpi$$

$$D = 1i = 1Spi^2$$

Where, H = Shannon diversity index, S = richness of arthropods, *pi* = proportion of individual arthropods, ln = natural logarithm, and D = Simpson diversity index

### *Foraging behavior*

The foraging behavior of the most abundant insect pollinators (80 individuals of each of the six species) was observed in terms of stay time (time spent per individual) and visitation rate (number of flowers visited per minute). We selected a new individual to record the stay time and visitation rate data each time. Observations were conducted at three-hour intervals from 6:00 am to 3:00 pm every week, considering the seasonal dynamics of various insect pollinators (Ali et al., 2014; Ali et al., 2011).

### *Pollination Efficiency*

a) Pollen deposition

Floral buds were caged with nylon mesh bags 24 hours before opening. Subsequently, the bags were removed during the peak activity time of insect visitors (10:00 am to 11:00 am), and following the visitation of a specific insect

pollinator, the stigmas were excised. These stigmas were then fixed in a solution of alcohol-acetic acid, followed by staining with safranine and aniline blue (Dafni, 1992). Pollen grain counts deposited by an individual pollinator (30 individuals of the same species) were recorded using a 40x magnification stereoscopic microscope.

b) Single-Visit Fruit Set

To determine the efficacy of single-visit fruit set by insect pollinators, floral buds were caged with nylon mesh bags before their opening and uncaged during the peak activity period of insect pollinators. After the single visit of an individual insect pollinator (contacting the floral stigma), the flower was labeled with the type of insect pollinator visited and caged again until senescence. The initial fruit set was evaluated 5-6 days post-pollination when the difference in ovary swelling or abscission was evident (Vidal et al., 2010). Additionally, fifty floral buds each for open pollination (free insect visits) and caged pollination (no insect visits) were reserved for pollination assessment.

**Data analyses**

To compare the diversity of pollinators in different months, individual-based rarefaction curves of Shannon (H) and Simpson's (D) diversity indices were used (Chao et al., 2014). To compare pollinator species' foraging behavior and pollination efficiency, the data regarding pollen deposition, single-visit fruit set efficacy, visitation rate, and stay time were analyzed using analysis of variance (ANOVA). Means were compared using Fisher's test at P = 0.05. To know the effect of pollen deposition on stigma and visitation rate of pollinator species on the reproductive performance of sponge gourd (in terms of fruit set), we used simple linear regression analysis. Diversity analysis was performed using PAST software (Hammer et al., 2001), and other statistical analyses were performed using XLSTAT (XLSTAT, 2012).

**Results**

In our study, seven bee species and one syrphid fly species visited the sponge gourd flowers. Among the bee species, *A. dorsata*, *Xylocopa* sp., *Lasioglossum* sp., *Amegella* sp., *Nomia* sp., *Megachile* sp., and *Ceratina smaragdula* were observed. In contrast, *Halictus* sp., *Thyreus* sp., *Agapostemon* sp., and *Pseudapis* sp. were rarely seen.

Solitary bees (as a group) were more abundant (58%) compared to honey bees (33%) and syrphid flies (8%). *Apis dorsata* was the most abundant visitor with the highest visitation frequency, followed by the solitary bees *Xylocopa* sp., *Lasioglossum* sp., and *Amegilla* sp., while the syrphid fly *Eristalinus aeneus* was the least abundant. The primary foraging task of all the bee and syrphid fly species was nectar and pollen, except *Xylocopa* sp. and *A. dorsata*, which foraged for nectar alone (Table 1).

**Table 1.** Total abundance, visitation frequency, and foraging behavior of insect pollinators visiting sponge gourd flowers.

| Order | Family | Genus/Species | Total Abundance | Visitation frequency (Individuals/flower/minute) | Foraging Task Nectar (N) / Pollen (P) |
|---|---|---|---|---|---|
| Hymenoptera | Apidae | *Apis dorsata* | 1903 | 0.66 | N |
| | | *Xylocopa sp.* | 1050 | 0.37 | N |
| | | *Ceratina smaragdula* | 68 | 0.02 | N/P |
| | | *Amegilla* sp. | 683 | 0.24 | N/P |
| | Halictidae | *Lasioglossum* sp. | 900 | 0.31 | N/P |
| | | *Nomia* sp. | 541 | 0.19 | N/P |
| | Megachilidae | *Megachile* sp. | 105 | 0.04 | N/P |
| Diptera | Syrphidae | *Eristalinus aeneus* | 492 | 0.17 | N/P |

Individual-based rarefaction curves showed that Shannon and Simpson's diversity indices did not change with the increasing sampling efforts. However, both had the highest values in September, followed by July and August (Fig 1). There was a significant difference among the insect pollinators regarding the visitation rate (F = 49.1, df = 5.0, $P < 0.0001$). The visitation rate of *Amegilla* sp. was higher (8.11 ± 0.33), followed by *Nomia* sp. (6.37 ± 0.31) and *Xylocopa* sp. (4.37 ± 0.31), while that of *A. dorsata* was lower than the solitary bees (3.18 ± 0.43). The stay time also varied among the insect pollinators ($F = 40.8$, df = 5.0, $P < 0.0001$). The stay time of *A. dorsata* (30.48 ± 1.95) was higher compared to solitary bees *Amegilla* sp. (24.3 ± 1.89) and *Nomia* sp. (22.2 ± 2.64) (Table 2).

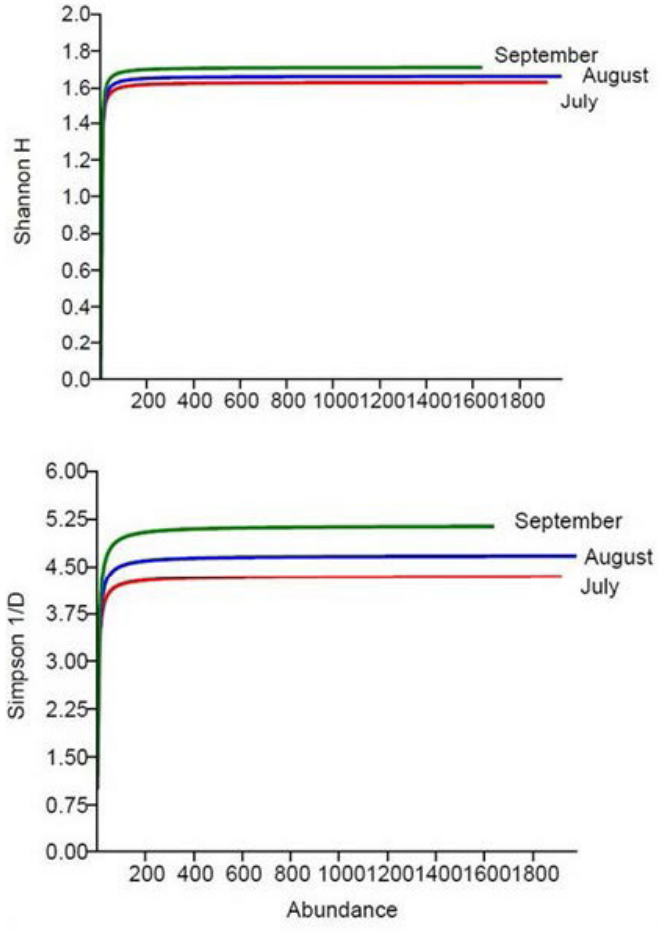


**Fig 1.** Comparison of Shannon (H) and Simpson's (D) diversity indices using individual-based rarefaction curves of July to September 2020.

**Table 2.** Effectiveness of six insect pollinators based on stay time and visitation rate.

| Insect Pollinator | Stay time (time spent per flower in seconds) (N = 80) | Visitation rate (Number of flower visited/minute) (N = 80) |
|---|---|---|
| *Apis dorsata* | 30.48 ± 1.95 a | 3.18 ± 0.43d |
| *Xylocopa* sp. | 3.22 ± 0.30 e | 4.37 ± 0.31 c |
| *Lasioglossum* sp. | 28.9 ± 1.85 ab | 2.36 ± 0.20 d |
| *Amegilla* sp. | 24.3 ± 1.89b | 8.11 ± 0.33 a |
| *Nomia* sp. | 22.20 ± 2.64 c | 6.37 ± 0.31 b |
| *Eristalinus aeneus* | 16.45 ± 1.95 d | 1.57 ± 0.24 e |

According to Fisher's test, means sharing the same letters are not statistically different at the 5% significance level (± S.E.).

Pollen deposition also differed significantly among the insect pollinators ($F$ = 15.3, df = 5.0, $P$ < 0.0001). *Amegilla* sp. was the most efficient regarding pollen deposition (465.6 ± 32.38), followed by *Nomia* sp. and *Xylocopa* sp. (423.0 ± 16.43). At the same time, there was no difference in pollen deposition between *Xylocopa* sp. and *A. dorsata*. Single visit fruit set percentage also revealed significant differences among the tested insect pollinators ($F$ = 62.1, df = 5.0, $P$ < 0.0001). *Amegilla* sp. proved to be the most efficient insect pollinator based on single visit fruit set percentage (32.2 ± 2.05), followed by *Nomia* sp. (26.8 ± 1.23). At the same time, *Xylocopa* sp. and *A. dorsata* did not differ significantly in fruit set percentage (Table 3).

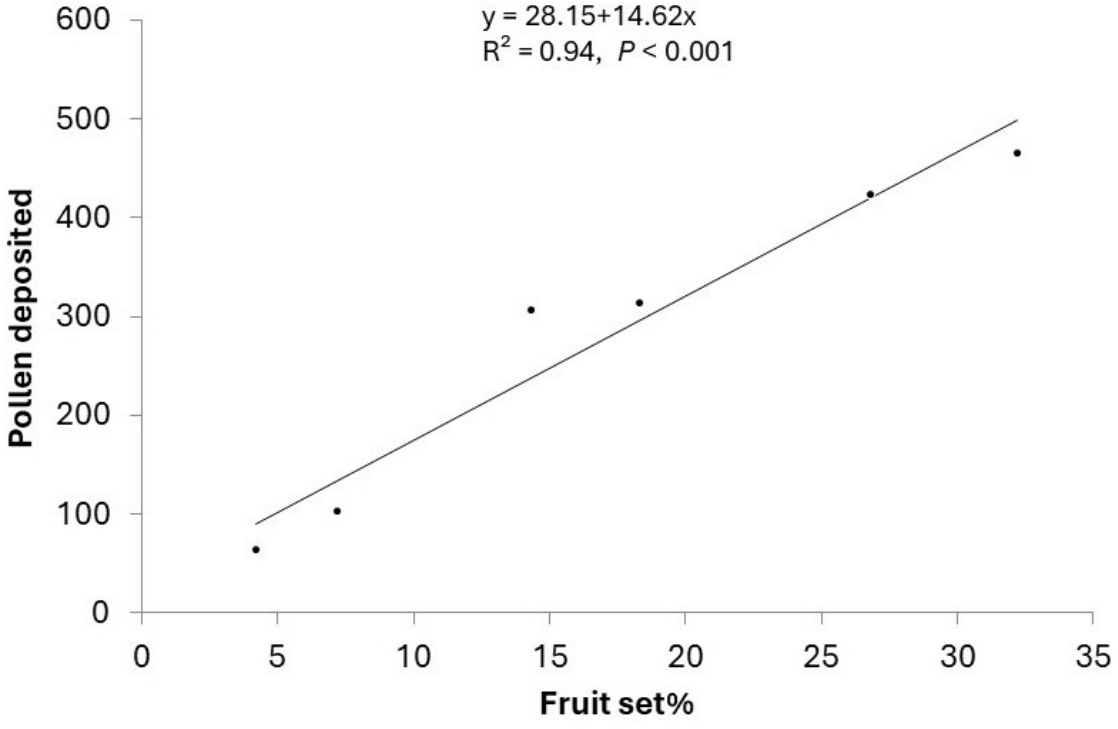


**Fig 2.** Relationship between pollen deposition and fruit set (%) in sponge gourd.

Most flowering buds (80%) receiving free insect visits set fruit, while no fruit was set in caged buds due to flower abortion. There was a significant positive relationship ($R^2$ = 0.94, f = 64.28, p = 0.001) between pollen deposition and fruit set percentage (Fig 2). Similarly, visitation rate had a strong positive relationship with fruit set percentage ($R^2$ = 0.98, f = 215.29, p = 0.000) (Fig 3).

**Table 3.** Effectiveness of six pollinators based on pollen deposition and fruit set (%).

| Insect Pollinator | Pollen Deposition (N = 30) | Fruit Set (%) (N = 30) |
|---|---|---|
| *Apis dorsata* | 306.3 ± 28.22b | 14.3 ± 1.29c |
| *Xylocopa* sp. | 313.5 ± 29.23b | 18.3 ± 1.42c |
| *Lasioglossum* sp. | 102.2 ± 12.43c | 7.2 ± 0.78d |
| *Amegilla* sp. | 465.6 ± 32.38a | 32.2 ± 2.05a |
| *Nomia* sp. | 423.0a ± 16.43ab | 26.8 ± 1.23b |
| *Eristalinus aeneus* | 63.5 ± 7.80c | 4.22 ± 0.48d |

According to Fisher's test, means sharing the same letters are not statistically different at the 5% significance level (± S.E.).

## Discussion

Sponge gourd attracts diverse insect pollinators due to its abundant nectar and pollen production (Nicodemo et al., 2009). In the current study, eight species of insect pollinators from two orders and three families were attracted. The total abundance of solitary bees was higher than that of honey bees. Some previous studies have also reported a higher abundance of solitary bees in various cucurbits, such as *Luffa aegyptiaca* (Mensah & Kudom, 2010; Ali et al., 2016), *Cucurbita moschata* (Canto-Aguilar & Parra-Tabla, 2000), and *C. pepo* (Pande & Verma, 2016). Contrarily, some other studies have reported a higher abundance of wild honey bees in different cucurbits, including pumpkin (Ali et al., 2014), bitter gourd (Dorjay et al., 2017), cucumber (Prakash, 2002), and ridge gourd (Gautam & Kumar, 2018). This variation in the proportion of honey bees and wild bees might be due to climatic conditions, seasonal fluctuations, landscape type, and host preference of bees (Zattara & Aizen, 2021).

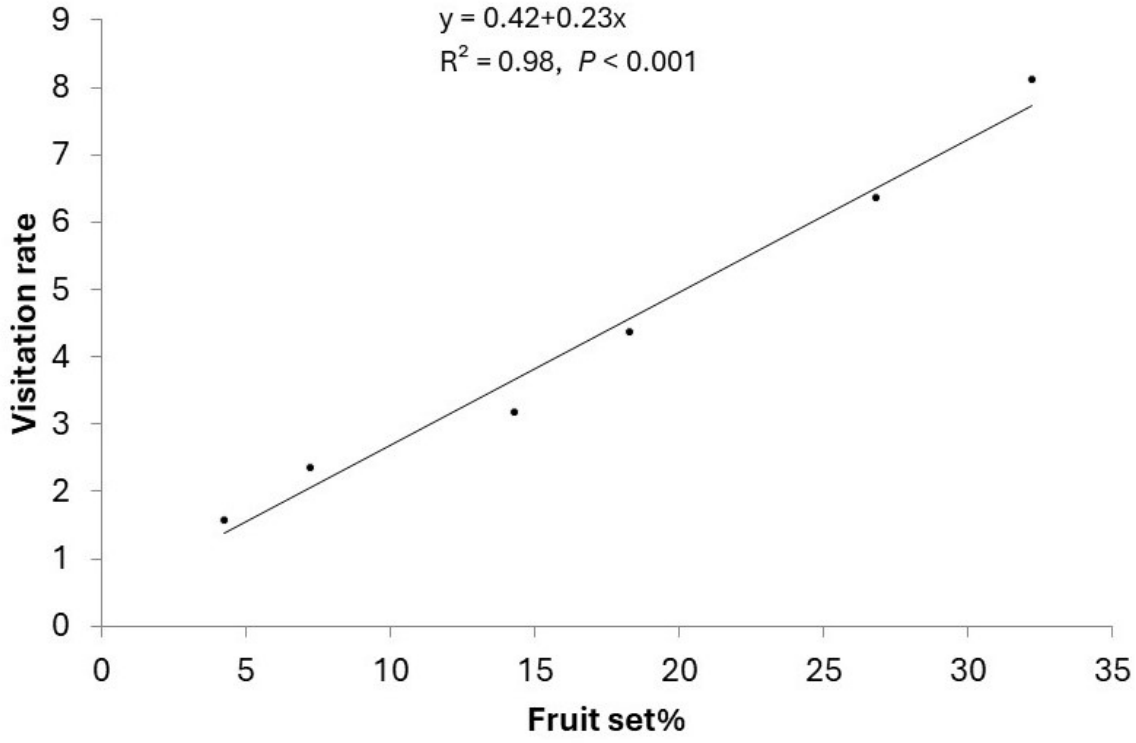


**Fig 3.** Relationship between visitation rate and fruit set (%) in sponge gourd.

Visitation rate is a crucial criterion for assessing the effectiveness of insect pollinators, and increased visitation has been positively linked with fruit set in cucurbits (Walters, 2005; Nicodemo et al., 2009; Vidal et al., 2010). In our study, solitary bees (*Amegilla* sp. and *Nomia* sp.) visited more flowers than honey bees. Previously, *Xylocopa* sp. was reported to be more efficient regarding visitation rate than honeybees (*Apis cerana*) in cucumber (Hashifah et al., 2020). Conversely, honey bees exhibited a higher visitation rate than solitary bees (*Xylocopa* sp. and *Amegilla* sp.) in *L. cylindrica* (Mensah & Kudom, 2010).

In the present study, *Amegilla* sp. deposited the maximum pollen on the stigma, followed by *Nomia* sp., while *A. dorsata* deposited a few pollen grains. Most studies indicate that pollen deposition and single-visit seed or fruit set are crucial parameters for assessing the pollination efficiency of

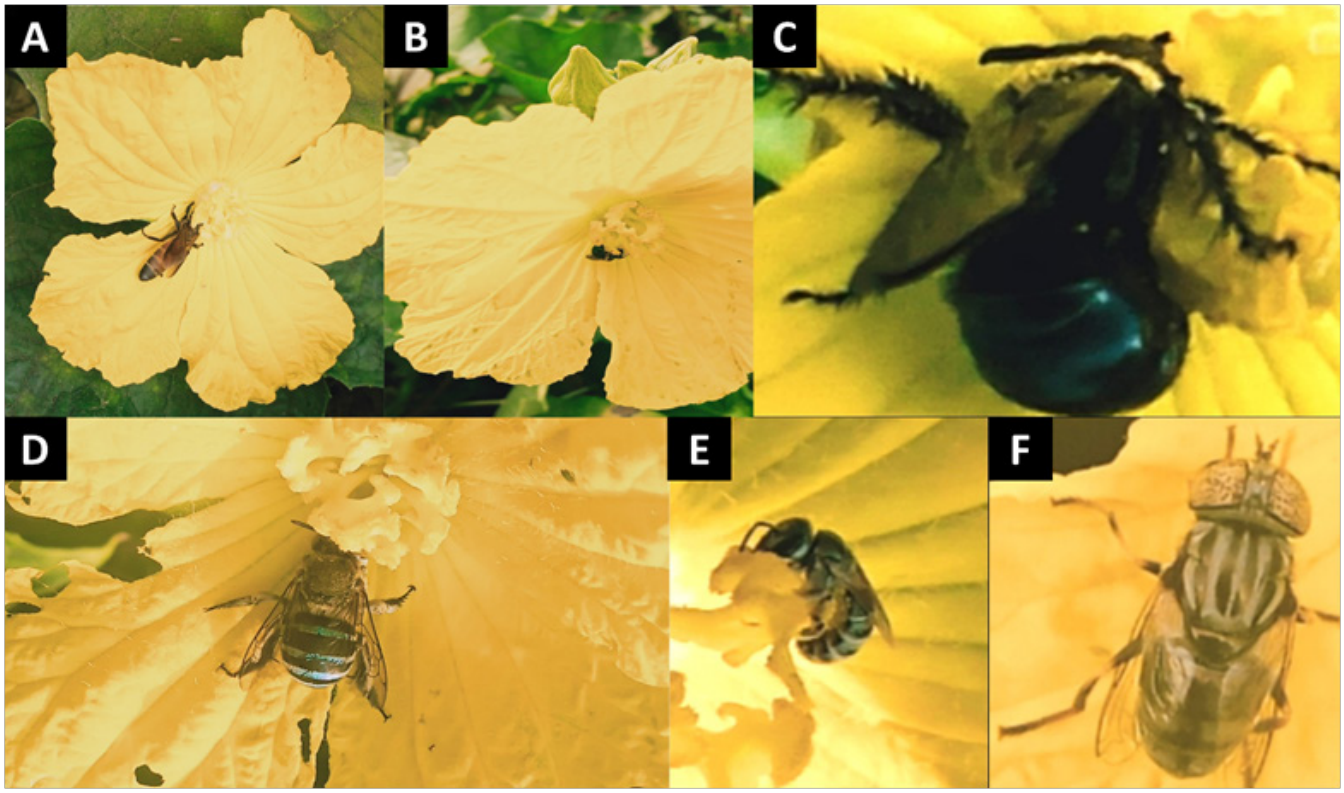


**Fig 4.** Pollinators visitation on sponge gourd flowers: (A) *Apis dorsata* (B) *Lasioglossum* sp. (C) *Xylocopa* sp. (D) *Amegilla* sp. (E) *Nomia* sp. and (F) *Eristalinus aeneus.*

individual insect pollinators (Fenster et al., 2004; Ne'eman et al., 2010). Previous studies have also reported a higher pollen deposition ability of solitary bees compared to honey bees in various cucurbit crops, such as watermelon (Garantonakis et al., 2016; Ali et al., 2015; Njoroge et al., 2010), pumpkin (Ali et al., 2014), and giant pumpkin (Pfister et al., 2018). However, no difference in pollen deposition ability was found between *Bombus impatiens* and *A. mellifera* in melon (Goodell & Thomson, 2007). Pollen deposition on stigma is primarily the function of body size (Cruden & Miller, 1981; Willmer & Finlayson, 2014), grooming behavior (Martins et al., 2015; Parker et al., 2015), and hairiness (Stavert et al., 2016). Despite collecting sufficient pollen, honey bees may exhibit less pollen deposition due to certain morphological and physiological traits (Willmer, 2011; Ollerton et al., 2011; King et al., 2013). Moreover, pollen deposition on the stigma is also closely associated with the size of the bee (Solis-Montero & Vallejo-Marin, 2017; Everaars et al., 2018).

Based on a single visit initial fruit set, *Amegilla* sp. proved to be the most effective pollinator, followed by *Nomia* sp., while it was approximately half for *A. dorsata*. Studies conducted elsewhere have also reported higher single-visit fruit set efficiency of solitary bees compared to managed honeybees in different cucurbit crops, such as *L. aegyptiaca* (Mensah & Kudom, 2010) and *Cucurbita pepo* (Ali et al., 2014).

Solitary bees may have much higher pollination efficiency than managed honeybees due to their richness and diversity. However, this can be affected by the landscape. Identifying the relative pollination efficiency of the available insect pollinators and implementing targeted conservation measures can support these species and their pollination services, ultimately leading to increased crop yields for sponge gourd and other pollinator-dependent crops. Moreover, providing year-round floral and nesting resources could further improve the area-wide diversity of native pollinators.

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**Authors' Contributions**

Sibbaha Abdullah: Investigation.
Mudssar Ali: Conceptualization, methodology, validation, resources, writing-original draft, writing-review & editing, visualization, supervision, project administration, funding acquisition.
Fawad Zafar Ahmad Khan: Software, formal analysis, writing-original draft, writing-review & editing, visualization.
Asif Sajjad: Writing-review &editing.
Mirza Abdul Qayyum: Writing-review & editing.
Nadeem Ahmad: Writing-review & editing.

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