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Comparing the Efficacy of Single and Multiple Visits by Honey and Solitary Bees on Sunflower Seed Production

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Abstract

Sunflower (*Helianthus annuus* L.) is an important oil seed crop with a considerable acreage of cultivated hybrids in Pakistan. Sunflower crops depend on insect pollination for seed setting, as it attracts diverse pollinator fauna, mainly due to pollen and nectar availability. This study was conducted in the agricultural landscape of Multan, Pakistan, to evaluate the role of native and social bees in sunflower seed production. The abundance and diversity of pollinators were assessed. Effectiveness was tested regarding foraging behavior, including visitation rate, stay time, and seed set efficacy for single and multiple visits (5, 10, 15). Additionally, various reproductive success parameters were recorded: flower head (pseudanthium) diameter, flower head weight, seed weight/flower head, and number of seed/flower head. The results showed that the relative abundance of honey bees was the highest (77%) in both years, followed by solitary bees (17%), while syrphid flies were the least abundant (6%) on sunflower. Among honey bees, *Apis mellifera* and *A. dorsata* were the most abundant in both years, followed by the solitary bee *Xylocopa* sp. Furthermore, in single and multiple seed set efficacy (5 and 10 visits), the solitary bee *Xylocopa* sp. produced the maximum seeds, followed by *A. mellifera*. Higher seed production could be achieved with a focused conservation plan of solitary and managed bees on sunflower.

Introduction

Pollinators are crucial for healthy ecosystems, as they facilitate the reproductive process of flowering plants (Lautenbach et al., 2012; Sajjad et al., 2017). Pollination plays an important role in crop production (Klein et al., 2007), with about 84% of commercial crops pollinated by insects, primarily honey bees, accounting for 70-80% of the pollination (Bareke et al., 2019). Approximately 20,000 bee species worldwide have been reported to contribute to pollination (Ollerton, 2011). Insect pollinators contribute an estimated 235-577 billion US dollars annually to global production (Potts et al., 2016). Pollinators significantly improve the crop yield and quality of seeds, enhancing the nutritional value of crops used in human diets (Wietzke et al., 2018; van der Sluijs & Vaage, 2016).

Among bees, solitary bees (excluding honey bees) have been reported to enhance crop seed production (Mallinger & Prasifka, 2017; Ali et al., 2023). Solitary bees are efficient pollinators, depositing more pollen on stigmas than honey bees (Greenleaf & Kremen, 2006; Eeraerts et al., 2020). Honey bees, being generalists, interact strongly with various plant species, while most solitary bees are specialists and, therefore, visit limited flora (Odoux et al., 2012; Aslan et al., 2016).

Honey bees primarily rely on mass-flowering crops for survival, while solitary bees can thrive on less abundant but diverse groups of native flowering plants (Michener, 2007; Odoux et al., 2012). Solitary bees have been reported as efficient pollinators in crops, including strawberry, canola, sunflower, and tomato (Greenleaf & Kremen, 2006; MacInnis

& Forrest, 2019; Mallinger & Gratton, 2015; Mallinger et al., 2018). Solitary bees contribute approximately 150 billion US dollars to global crop production (Gallai et al., 2009).

An important parameter commonly used to estimate the effectiveness of a pollinator in a single visit per flower is a single-visit seed set (Ne'eman et al., 2010; King et al., 2013). Previous studies have shown that solitary bees, including *Xylocopa* spp., *Melissodes* spp., and *Andrena* spp., exhibit greater efficacy in terms of single-visit seed set in sunflower crops, as compared to the social honey bees (Mallinger et al., 2019).

Similar observations have been made in other oilseed crops, where a single visit by wild bees, such as *Halictus* spp. in canola (Ali et al., 2011) and *Andrena* spp. and *Bombus* spp. in rapeseed (Phillips et al., 2018) results in relatively higher seed production, compared to the honey bees. Some crops require a greater quantity of pollen for an effective fruit set. There is a higher chance of pollen grains being wasted or not properly deposited in a single visit; therefore, multiple visits may be necessary, increasing the probability of depositing more pollen grains and increasing fruit production (Parker et al., 2016). Multiple visits help measure the efficiency of a pollinator in two or more visits per flower. Multiple-visit efficacy refers to visits of the same pollinator on the same flower (Stavert et al., 2020). In Pakistan, sunflower is an important edible oil crop after cotton and canola, grown across various ecological zones (Tariq et al., 2018). A wide range of pollinator fauna is associated with sunflower crops (Iqbal et al., 2022). Globally, only one study documents that multiple visits by different pollinators (*Melissodes* sp. and *Andrena* sp.) in sunflower yield more seeds than a single visit (Mallinger et al., 2019). However, the role of individual pollinators in multiple-visit seed sets is still unknown. Previous studies on multiple-visit efficacy have shown that multiple visits are required for better seed set production, i.e., around 2.44 visits of *Apis cerana* Fabricius (Hymenoptera: Apidae) in mustard (Stanley et al., 2017), 21 visits of bumblebees in pumpkins (Pfister et al., 2017), and 16 visits of honeybees in watermelons (Walters, 2005).

Understanding the role of solitary bees for multiple-visit seed set in sunflowers is important. While no evidence has previously been reported from Pakistan, fewer studies have documented the efficacy of single and multiple visits by pollinators in sunflower (Martin & Farina, 2016; Mallinger et al., 2019). Therefore, to address this regional research gap, the present study was planned to assess the efficacy of single and multiple visits by honey bees and solitary bees on sunflower seed production. We hypothesized that both honey and solitary bees will significantly contribute to sunflower seed production through single and multiple visits. We also expected that the pollination effectiveness of solitary and social bees would be different.

Materials and Methods

Experimental Setup and Crop Management Practices

The experiment was performed at the research farm of MNS University of Agriculture Multan, Pakistan (30.147047, 71.448326). Multan, situated in the subtropical zone, has hot summers (with temperatures ranging from 35-40ºC) and cold winters (with an average temperature range of 8-10ºC). The mean annual rainfall ranges from 127 to 254 mm, with heavy fog occurring during winter (Iqbal et al., 2022). The soil type was clay loam on the experimental farm. Hybrid sunflower (*Helianthus annus* L.) HYSEN-33 seeds were sown on ridges with a row-to-row distance of 0.76 m. The crop was sown in mid-February and harvested in July, during 2020 and 2021. One and a half bags of diammonium phosphate (18% N and 46% P), one bag of sulfate of potash (46% K_2O), and half bag of urea (46% N) were used at the time of sowing. Furthermore, three split urea applications were done during the first and second irrigation and at flower initiation combined with flood irrigation. Four irrigations were done to ensure optimum water availability for the crop. No pesticide treatment was applied during both years. Weeds were manually removed to ensure clean cultivation. The sunflower crop was surrounded by Berseem *Trifolium alexandrinum* (Fabaceae), cotton *Gossypium hirsutum* L. (Malvaceae), maize *Zea mays* L. (Poaceae) and quinoa *Chenopodium quinoa* (Amaranthaceae). Moreover, perennial trees, including shisham *Dalbergia sissoo* Roxb. (Fabaceae), gum Arabic tree *Vachellia nilotica* (L.) PJH Hurter & Mabb. (Fabaceae), and mulberry *Morus alba* L. (Moraceae) were also present in the field surroundings.

Pollinator abundance and diversity

After the initiation of the flowering of 10% of the total plants, thirty flowering plants were selected randomly from the sunflower field. Each flower head was observed for 60 sec, and every floral visitor was recorded during this time. Observations were made at four-hour intervals between 8:00 am and 4:00 pm in three days.

Foraging behavior

The foraging behavior of pollinators was also assessed in terms of visitation rate, stay time, and floret visited per minute. Visitation rate refers to the number of flower heads visited by an individual in one minute. For this purpose, the bee (one individual) was followed for one minute, and the number of heads the bee visited was recorded. Only five readings were taken per day for each individual to avoid uncertainty. Similarly, stay time refers to time spent by an individual pollinator per flower. Florets visited by an individual per stay on one flower head were also counted to assess pollinator efficiency.

Pollination effectiveness

Prior to the opening of any floret, sixty sunflower flower heads were randomly wrapped with white nylon fine mesh bags (1 mm mesh width) to prevent insect visitors, promoting self-

pollination. Simultaneously, another sixty heads were randomly selected and tagged as open heads, making them accessible to natural pollinators (Open pollination). After harvest, reproductive success parameters were compared between open and cage pollination, including flower head weight, diameter, number of seeds/flower head, and seed weight/flower head.

The pollination effectiveness was assessed by observing both single and multiple visits of specific insect pollinators. During the bud stage, flower heads were enclosed with fine mesh bags. At peak bee activity hours, the mesh bags were removed to allow a single pollinator visit, after which the flower head was re-caged until flower senescence. Subsequently, the heads were harvested, and various reproductive success parameters were recorded. Similarly, the mesh bags were uncaged for multiple visits, and pollinators were allowed to visit the flower head 5, 10, or 15 times. The abovementioned reproductive success parameters were compared with the results obtained from single-visit efficacy.

Harvesting

Harvesting was performed manually in July, and it was done when the back of the flower head became light yellow, the yellow florets turned dry, and the green florets turned brown. After the harvesting, the flower heads were wrapped in the mesh and placed on the cemented floor for drying. After drying, the individual heads were beaten using bamboo sticks, and the seeds were separately packed in brown paper bags. The seeds were stored in the laboratory at room temperature (~25-27°C).

Data analyses

The data for foraging behavior, i.e., visitation rate, stay time, and florets visited per minute, as well as single and multiple visits, were subjected to analysis of variance (ANOVA). Mean comparisons were conducted using the LSD test at a significant level of $P = 0.05$. All statistical analyses were done using the computer software XLSTAT (2008).

Results

Pollinator community

Eight bee species were observed during the flowering season, consisting of five solitary and three honey bee species and two syrphid flies. In both seasons, honey bees exhibited the highest relative abundance (77%), followed by solitary bees (17%), while syrphid flies were the least abundant (6%) (Fig 1). The identified honey bee species were *A. dorsata*, *A. mellifera*, and *A. florea*. In contrast, the observed solitary bee species were *Xylocopa* sp., *Lasioglossum* sp., *Pseudapis* sp., *Amegilla* sp., *Ceratina* sp., and *Megachile* sp.

In both seasons, the honey bee *A. mellifera* was the most abundant bee species, comprising 60% in 2020 and 33% in 2021 of the total abundance, followed by *A. florea* (18% in 2020, 15% in 2021) and *Xylocopa* sp. (5% in 2020, 12% in 2021). The syrphid fly *Eristalinus latus* was the least abundant. In both years, Apidae dominated, while Syrphidae was the least abundant (Table 1).

Foraging behavior

Significant differences were observed for the visitation rate ($F = 14.1$, df = 3.0, $P < 0.001$) of insect pollinators. The visitation rate of *Xylocopa* sp. was the highest (7.2 ± 0.95 number of flower heads/minute), followed by *Apis dorsata* (5.3 ± 0.38 number of flower heads/minute) and *A. mellifera* (4.3 ± 0.56 number of flower heads/min), while *A. florea* visited flowers the least (1.04 ± 0.22 number of flower heads/min).

Significant differences were also observed for the stay time of pollinators ($F = 2.8$, df = 3.0, $P < 0.001$). *A. florea* had the highest stay time (66.34 ± 11.25 seconds), followed by *A. mellifera* (33.08 ± 8.63 seconds) and *A. dorsata* (31.54 ± 6.22 seconds), while *Xylocopa* sp. had the lowest stay time per flower head per visit (8.11 ± 3.02 sec). There was a significant difference in the number of florets visited per flower head ($F = 4.27$, df = 3.0, $P < 0.001$). *Xylocopa* sp. visited the highest number of florets per flower head (14.65 ± 2.25), followed by *A. dorsata* (13.47 ± 1.85), while the least number of florets were visited by *A. florea* (2.4 ± 0.97) (Table 2).

Reproductive Success

No significant differences were observed in flower head diameter ($F = 1.98$; df = 8, $P = 0.079$) and flower head weight ($F = 0.81$, df = 8, $P = 0.595$) in single and multiple insect visits. However, multiple *Xylocopa* sp., *A. mellifera*, and *A. florea* visits produced more seeds ($F = 4.11$, df = 8, $P = 0.002$) than a single visit (Table 3). During both years, there was a significant difference between open and cage pollination regarding the number of seeds per flower head and seed weight per flower head. The number of seeds in open flower heads was 61% higher in 2020 and 65% higher in 2021 than in caged heads. Similarly, seed weight in open flower heads was 55% higher in 2020 and 53% higher in 2021 than in caged heads (Fig. 2).

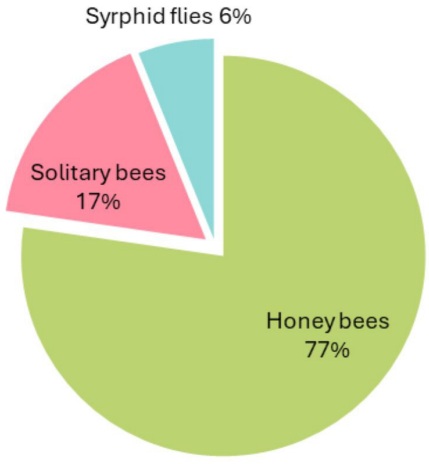

Figure 1 - Proportional abundance of native pollinators in Sunflower.

Table 1: Abundance and relative proportion of floral visitors on sunflower

Order	Family	Genus/Species	Total Abundance		Proportion (%)	
			2020	2021	2020	2021
Hymenoptera	Apidae	*Apis dorsata*	63	52	5.21	11.11
		A. mellifera	734	157	60.66	33.55
		A. florea	218	73	18.02	15.60
		Xylocopa sp.	68	57	5.62	12.18
		Lasioglossum sp.	45	38	3.72	8.12
		Pseudapis sp.	24	47	1.98	10.04
Diptera	Syrphidae	*Eristalinus aeneus*	22	32	1.82	6.84
		E. latus	36	12	2.98	2.56

Table 2. Pollination effectiveness pollinators in sunflower: visitation rates, number of florets visited, and stay time.

Pollinator Species	Stay time/flower head	Number of flower heads visited/minute	Number of florets /flower heads visited
Xylocopa sp.	8.11 ± 3.02 c	7.2 ± 0.95 a	14.65 ± 2.25 a
Apis dorsata	31.54 ± 6.22 b	5.3 ± 0.38 b	13.47 ± 1.85 b
A. mellifera	33.08 ± 8.63 b	4.3 ± 0.56 b	12.0 ± 2.17 b
A. florea	66.34 ± 11.25 a	1.04 ± 0.22 c	2.4 ± 0.97 c

Means in a column followed by the same letters are not statistically different based on LSD at a 5% significance level (± S.E.).

Table 3. Comparison of yield parameters for different visits of three bee species on sunflower

Visitation by pollinator species	Flower head diameter (mm)	Flower head weight (g)	No. of seeds /flower head	Seed weight (g)
1 visit				
Xylocopa sp.	10.100 ± 1.17	38.60 ± 9.35	443.8 ± 70.3 abc	16.2 ± 2.9 ab
A. mellifera	8.825 ± 1.09	34.50 ± 6.06	359.0 ± 65.7 bc	13.0 ± 1.9 ab
A. florea	9.080 ± 0.5	33.60 ± 2.8	305.0 ± 31.2 c	11.6 ± 1.7 b
5 visits				
Xylocopa sp.	10.260 ± 0.41	40.20 ± 3.2	569.4 ± 44.0 abc	19.0 ± 1.7 ab
A. mellifera	9.420 ± 0.73	37.40 ± 6.84	470.6 ± 51.1 abc	16.6 ± 2.34 ab
A. florea	9.980 ± 0.66	39.40 ± 2.8	400.2 ± 29.0 bc	14.2 ± 1.2 ab
10 visits				
Xylocopa sp.	11.200 ± 0.76	48.00 ± 5.0	715.0 ± 86.9 a	23.800±3.6 a
A. mellifera	12.440 ± 0.76	48.00 ± 3.9	657.4 ± 55.5 ab	19.800±1.46 ab
A. florea	9.620 ± 1.03	36.80 ± 7.9	484.2 ± 117.4 abc	15.600±3.6 ab
	$P = 0.079$ df = 8 $F = 1.98$	$P = 0.595$ df = 8 $F = 0.81$	$P = 0.002$ df = 8 $F = 4.11$	$P = 0.04$ df = 8 $F = 2.93$

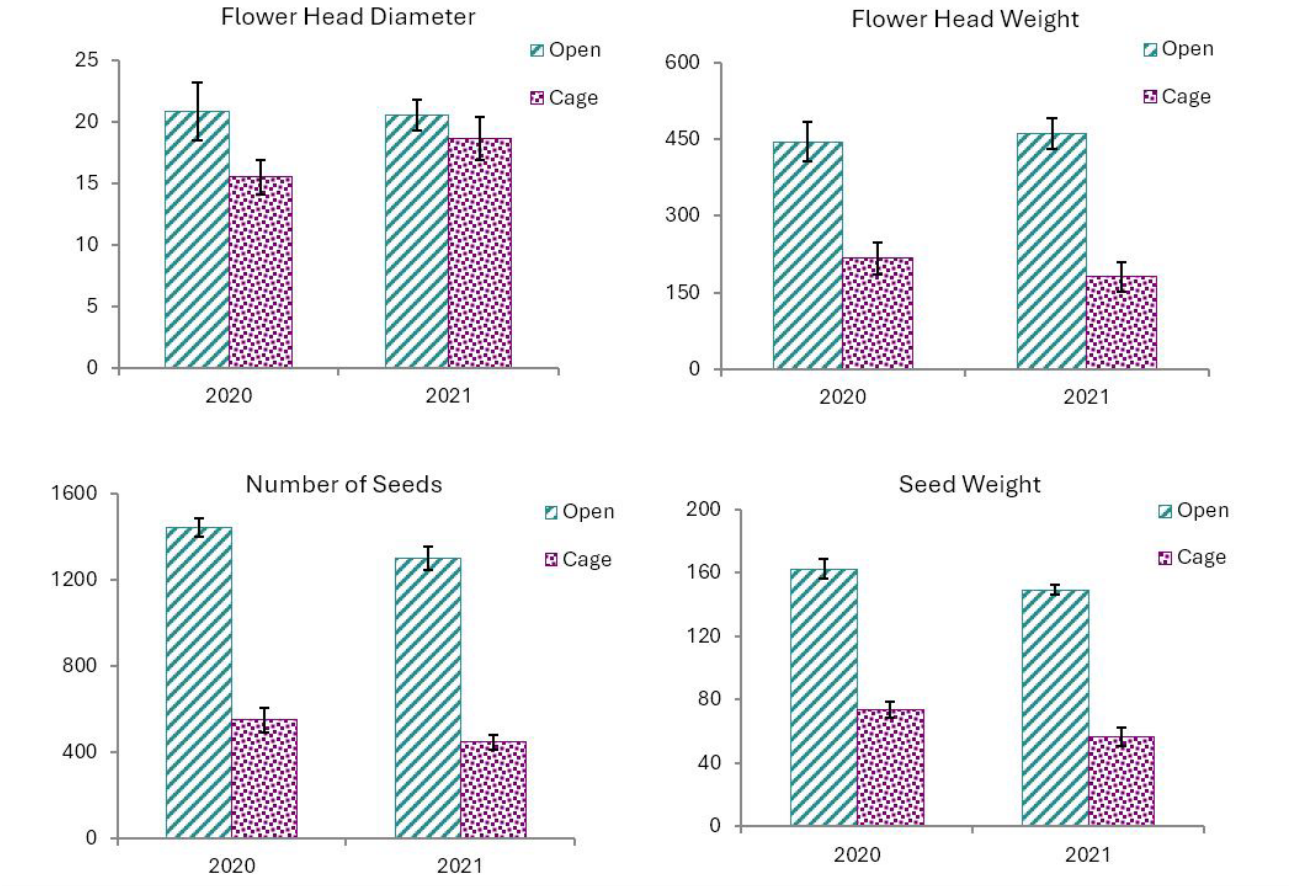

Fig 2: Reproductive success parameters in open and cage pollination (2020-2021)

Discussion

In our study, *Apis florea* and *A. mellifera* were the most abundant pollinators, followed by the solitary bee *Xylocopa* sp., while syrphid flies were the least abundant. Previous studies in Pakistan indicated that honeybees, specifically *A. dorsata*, were the most abundant pollinators among all floral visitors in sunflower (Muhammad et al., 2021; Ali et al., 2017). Other studies have also reported honey bees, including *A. dorsata* and *A. mellifera*, as frequent floral visitors in sunflower (Estravis Barcala et al., 2019; Said et al., 2018; Chambó et al., 2011; Jadhav et al., 2011; Nderitu et al., 2008). Honey bees have been reported to be abundant in mustard (Shakeel et al., 2019; Devi et al., 2019; Parbat, 2019; Atmowidi et al., 2007), canola (Akhtar et al., 2018; Amro, 2021), and sesame (Pashte et al., 2013). While social bees have been abundant pollinators in multiple studies, solitary bees have also been reported to dominate in sunflower, such as *Xylocopa* sp., *Melissodes* spp., and *Andrena* sp. (Mallinger et al., 2019; Mallinger et al., 2015; Greenleaf & Kremen, 2006). Various factors may contribute to differential distribution. Honey bee populations could fluctuate yearly due to variable food availability and environmental factors. Changes in land use, including urbanization or agricultural practices, can significantly influence insect pollinators (Wenzel et al., 2020). The availability of flowering plants, especially the preferred food sources for different pollinators, is an important factor influencing the abundance of a particular pollinator species (Cohen et al., 2021; Ahmad et al., 2023).

The visitation rate is an important parameter for estimating the effectiveness of insect pollinators (Albano et al., 2009). In the current study, we observed that solitary bees, specifically *Xylocopa* spp., exhibited a higher visitation rate than honey bees. *Xylocopa* spp. may prefer specific types of flowers and are potentially attracted to the nectar, pollen, or scent of sunflowers, resulting in higher visitation rates (Iqbal et al., 2022). Solitary bees do not live in colonies like honey bees, and they often nest individually or in small, non-interacting groups. This signature nesting behavior may assist them in foraging actively and visiting flowers more frequently, as they are not dependent on group foraging behavior for getting food (Danforth et al., 2019). In sunflower, a higher visitation rate of the solitary bee *Lasioglossum* spp. compared to honey bee *A. mellifera* has also been reported (Mallinger & Prasifka, 2017). A similar domination of solitary bees has also been documented from rapeseed (Stanley et al., 2013) and canola (Ali et al., 2011).

The efficiency of a pollinator is generally determined by the percentage of fruit set in a single visit and multiple visits (Klein et al., 2007). A single visit also determines the

efficiency of a pollinator in one visit per flower (Ne'eman et al., 2010; King et al., 2013). Solitary bees, i.e., *Xylocopa* spp., *Halictidae* spp., and *Andrena* spp., have been reported as efficient single visitors in oil seed crops (Greenleaf & Kremen, 2006), including rapeseed (Phillips et al., 2018), and canola (Ali et al., 2011; Hoyle et al., 2007). *Xylocopa* bees carry more pollens than honey bees due to their larger sizes, making them efficient single-visit pollinators (Wousla et al., 2020). Solitary bees, including *Xylocopa* spp., are known for their signature "buzz pollination," which involves vibrating their flight muscles at a specific wing beat frequency, ultimately helping release pollen from certain plants like sunflower and tomatoes. Buzz pollination allows solitary bees to extract and transfer more pollen in a single interaction with the flower (Portman et al., 2019).

A study compared pollination services by honey bees and native bees in vegetables, documenting that despite low efficiency in single visits, the high number of honey bees compensated for their individual pollination effectiveness (Rocha et al., 2023). Another study in sunflower reported that solitary bees, *Andrena* sp., resulted in a higher seed set (51%), followed by *Melissodes* spp. (Mallinger et al., 2019). Multiple pollen visits may be necessary for optimum fruit development in some crops. Multiple pollinator visits could increase the chances of fruit set by depositing more pollen than a single visit (Stavert et al., 2020). Our study showed that multiple visits by *Xylocopa* sp. resulted in a higher seed set, which shows a high degree of flower constancy, i.e., repeated visits to the same flower species. This consistency in flower choice improves the chances of cross-pollination, contributing to a higher seed set (Grüter & Hayes, 2022). Moreover, in watermelon, after 2, 4, 8, and 16 visits of honeybees, it was determined that the highest fruit set (53%) occurred with maximum visits (Walters, 2005). In pumpkin, a study showed that bumblebees required 21 visits, and honeybees required 123 visits, contributing to the maximum fruit set (Pfister et al., 2017).

Solitary bees, like *Xylocopa* sp., are crucial for sunflower pollination for a healthy seed set. Moreover, *Xylocopa* sp. has been reported as an efficient pollinator in melons (Sadeh et al., 2007), common beans (Kingha et al., 2012), pigeon pea (Fohouo et al., 2014), sponge gourd (Mensah & Kudom, 2011), blueberry (Sampson et al., 2004), and watermelon (Azo'o et al., 2020). Conservation efforts should focus on conserving nesting habitats suitable for *Xylocopa* sp., i.e., dead tree trunks may serve as suitable nesting sites. Moreover, planting year-round blooming floral plants is necessary to provide food resources (Glatz et al., 2015; Santos et al., 2020; Kline & Joshi, 2020).

Our study reports effective sunflower pollinators that emphasize the need to improve conservation efforts on key pollinators such as honey bees and the solitary bee *Xylocopa* sp. This conservation improvement can be achieved by implementing strategies that enhance their populations. Farmers could attract solitary bee pollination by planting diverse floral and nesting resources. In conclusion, our study on sunflowers reveals the diversity of pollinators in the agricultural landscape of a tropical desert region. The study highlights the importance of considering single and multiple visitation rates when assessing pollinator efficiency. Our findings provide valuable insights into pollinator abundance dynamics, visitation rates, and efficiency in sunflower fields, with practical implications for promoting pollinator conservation and sustainable agricultural practices.

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Authors' Contribution

MA: Conceptualization, Methodology, Supervision, Software, Validation, Writing – Original Draft, Visualization, Project Administration, Funding Acquisition;
FZAK: Writing – Original Draft, Writing – Review & Editing, Visualization;
QA: Investigation, Data Curation;
THA.: Formal Analysis, Resources, Project Administration.

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