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Original article

## Effectiveness of honeybee (*Apis mellifera*) visit on the pollination of different sunflower cultivars



Atif Idrees<sup>a,b</sup>, Ziyad Abdul Qadir<sup>c,d</sup>, Amin Ul Hasnat<sup>e</sup>, Ayesha Afzal<sup>a,f</sup>, Saboor Ahmad<sup>g</sup>, Muhammad Anjum Aqueel<sup>e,h</sup>, Zhigang Li<sup>a,\*</sup>, Ahmed Rady<sup>i</sup>, Shahbaz Ali<sup>j</sup>, Jun Li<sup>a,\*</sup>

<sup>a</sup> Guangdong Key Laboratory of Animal Conservation and Resource Utilization, Guangdong Public Laboratory of Wild Animal Conservation and Utilization, Institute of Zoology, Guangdong Academy of Sciences, Guangzhou 510260, China

<sup>b</sup> Guizhou Provincial Key Laboratory for Agricultural Pest Management of the Mountainous Region, Scientific Observing and Experimental Station of Crop Pest in Guiyang, Ministry of Agriculture and Rural Affairs, Institute of Entomology, Guizhou University, Guiyang 550025, China

<sup>c</sup> Honeybee Research Institute, National Agricultural Research Centre, Park Road, Islamabad 45500, Pakistan

<sup>d</sup> Department of Entomology and Wildlife Ecology, University of Delaware, Newark, DE 19716, USA

<sup>e</sup> Department of Entomology, College of Agriculture, University of Sargodha, Sargodha 40100, Pakistan

<sup>f</sup> Institute of Molecular Biology and Biotechnology, The University of Lahore, 1-Km Defense Road Lahore 54000, Pakistan

<sup>g</sup> Institute of Apicultural Research/Key Laboratory of Pollinating Insect Biology, Ministry of Agriculture, Chinese Academy of Agricultural Sciences, Beijing 100081, China

<sup>h</sup> Department of Entomology, The Islamia University of Bahawalpur, Bahawalpur-63100, Pakistan

<sup>i</sup> Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

<sup>j</sup> Department of Agricultural Engineering, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Punjab, Pakistan

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### ABSTRACT

**Background:** Utilizing pollinators is one of the most dependable, economical, and environmentally friendly ways to improve cross-pollination, boost crop output, and improve crop quality. Bees are regarded as the best pollinators among all other pollinators.

**Methods:** The current study was conducted to elucidate the impact of honeybee (*Apis mellifera*) visits on the pollination of various sunflower cultivars including Hysun-33, Hysun-38, and FH-106.

**Results:** Our finding revealed that the highest average number of honeybee visits was observed in the morning followed by noon and afternoon on different sunflower cultivars. The maximum number of honeybee visits was recorded on Hysun-38, while the minimum was recorded on the FH-106 cultivar, which was 87.85 and 73.6, respectively. In (Hysun-33) and (FH-106), the maximum average time spent by the honeybee noticed in the morning on the flower was 34.24 and 32.67 min, whereas the maximum average time spent in the evening on the flower of (Hysun-38), which was 36.41 min. Regression analysis demonstrated that a significant effect between the head diameter of sunflower cultivars and the number of honey bee visits ( $p < 0.05$ ). In addition, a significant effect was observed between the number of honeybees visited and the number of seed settings on all cultivars of sunflower ( $p < 0.05$ ). There was a highly strong positive correlation between the number of seed sets and the head diameter of all cultivars.

**Conclusion:** It is concluded that farmers should maintain honeybee colonies close to the desired crop to increase pollination and increase output.

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## 1. Introduction

In terrestrial habitats, insect pollination of blooming plants is a process of major significance that offers essential ecological services for human well-being (Garibaldi et al., 2011a), including as crop production. Agricultural crop species benefit from cross-pollination by producing more fruit and fruit of greater quality, while roughly 75% of crop species rely to some extent on animal pollination (Classen et al., 2014). Additionally, pollination boosts

\* Corresponding authors.

E-mail addresses: [leegdei@163.com](mailto:leegdei@163.com) (Z. Li), [junl@giabr.gd.cn](mailto:junl@giabr.gd.cn) (J. Li).

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agricultural output stability and preserves genetic diversity, which prevents inbreeding depression and promotes tolerance to environmental changes (Garibaldi et al., 2011b). That's why pollinators are more crucial to world agriculture (Hünicken et al., 2022). Among pollinators, honeybees are considered one of the most important pollinator (Hung et al., 2018) due to their ideal body size, hairiness, attentiveness, floral regularity, and manageable populations, and bees are regarded as the best pollinators among all other pollinators (Chittka, 2022). They successfully pollinate a lot of different crops. Insect-pollinated plants make up around one-third of the human diet, and honey bees are responsible for 80% of pollination (Altieri et al., 2015). In the case of sunflower, *Helianthus annuus* L., a crop planted all over the world for the manufacture of oil or the direct consumption of seeds, its dependency on animal pollination varies depending on the degree of self-compatibility of such oilseed or confection cultivars (Chambó et al., 2011). Sunflower is a significant cash crop (Fernández-Martínez et al., 2010). In Pakistan, the spring and summer seasons are ideal for growing sunflowers (Shah et al., 2013). *Apis* bees are the insect pollinators that visit sunflowers most frequently. *Apis mellifera* is believed to be the most effective pollinator among *Apis* bees. In comparison to other *Apis* bees and other pollinators, it boosts sunflower yield (Susic Martin and Farina, 2016). Bee presence is crucial for increasing yields, and increasing food security and revenue (Stein et al., 2017). The reproduction of other crops also depends on bee pollination (Khalifa et al., 2021). A significant increase in fruit size and seed quality sunflower oil (Silva et al., 2018), resulted in higher market prices. We eat about one out of every three mouthfuls of food that was pollinated by honeybees (Ingram et al., 1996).

Pollinators rely on their foraging choices on various floral characteristics, such as the availability of nectar and pollen rewards and related visual and olfactory cues (Mallinger and Prasifka, 2017). Most floral visitors in natural settings around the world are *A. mellifera*, accounting for an average of 13% of floral visits across all networks (range 0–85%) while only visiting 5% of plant species. For 49% of plant species and 33% of the networks (Hung et al., 2018). The second and third flowering days usually had a maximum number of visits by *A. mellifera* between the hours of 7 and 8:30. During periods of increased visitation, the average bee density varied from 2.27 to 2.94 bees per capitulum (Chambó et al., 2011). When compared to plants without pollinator visits, sunflower plants with pollinator-insect visits yield 43% more seeds. Bees' foraging behavior can be affected by variations in floral shape in addition to variations in pollen supply and pollinator population makeup (Ishii, 2006). Sunflower breeding programs analyze physical traits including plant height and head size, which affect seed output, in an effort to create high-yielding cultivars (Sapkale et al., 2016). Additionally, it has been noted that honey bees exhibit variance in their foraging behavior toward various genotypes of sunflower for a variety of causes (Estravis Barcala et al., 2019). The genotype of sunflowers' varying attraction was mostly caused by variations in the nectar's sugar concentration (Mallinger and Prasifka, 2017). The capitulum responded favorably to the application of sucrose solution. The height and weight of the capitulum, the flower's sowing and flowering period, and seasonal variability were the other variables that contributed to variations in the pollen's attractiveness to bees (Estravis Barcala et al., 2019). In addition, very few studies examine pollinator visits to other types or the presence of cross-pollen on the bodies of the pollinators. Thus, deeper comprehension of honeybee foraging behavior (honey bee visits to different sunflower cultivars, timing preference, attraction, and impact of honey bee pollination on seed production) in a mixed

system with self-compatible and self-incompatible cultivars could be of assistance to sunflower growers.

## 2. Materials and Methods

### 2.1. Experimental area and setting

The current study was conducted in January 2013 at the University College of Agriculture, University of Sargodha, Sargodha, Punjab in Pakistan. The experimental land was selected to demonstrate the impact of honey bee visits on the pollination of different sunflower cultivars including Hysum-33, Hysun-38, and FH-106. For this purpose, the six canals of the experimental area were divided into three equal plots, and the plot size was two canals for each cultivar. Each cultivar was sown manually during February with a row-to-row distance (2.25\*2.25 ft) and plant-to-plant distance (9\*9 in.). For each cultivar, the same agronomic procedures such as hoeing, weeding, irrigation, and fertilizer application (NPK) were used. Forty plants were selected randomly from each plot. Twenty plants were treated (opened to allow free visits of bees) and another twenty plants were controlled (enclosed with perforated paper bags) to prevent honeybee visits to the plants. To protect it from water, rain, and termite infestation, honeybee colonies were kept close to the experimental area at one foot above the ground.

### 2.2. Assessment of honeybee preference for daytime and duration of stay on different cultivars of sunflower

At the flowering stage, every treated plant for each cultivar was examined for ten minutes three times per day (morning, noon, and afternoon). During this time, counted the bees that visited the sunflower and recorded their length of stay. Using this information, it was possible to determine when honeybees preferred to visit flowers at the morning, noon, and afternoon. Daily data from the flower's blossom until maturity was collected.

### 2.3. Measure the honeybee visit based on the head diameter of the sunflower cultivars

At maturity, cut the sunflower heads of the treated plants randomly from each cultivar that was propagated in a different plot. A scale was used to measure the diameter of the heads.

### 2.4. Impact of honeybee visits on seed production in sunflower cultivars

When the sunflower seeds were ready, each treated plant was harvested. To ascertain the impact of honeybees on seed setting and eventually on production, the seeds were manually removed from the sunflower head and counted on each chosen plant. In the control plant group, the heads of the sunflower plants were cut from each cultivar. After cutting, separate the seeds from the covered sunflower head and count manually in each control plant of different cultivars. To assess the involvement of honeybees in seed setting, compare the two treatments.

### 2.5. Statistical analysis

Study parameters such as the preference of honey bee visits, size of head diameter, and the effect of bee visits on seed setting are calculated by average number. All statistical data was measured by regression, correlation, and LSD test at  $p < 0.05$  using R software.

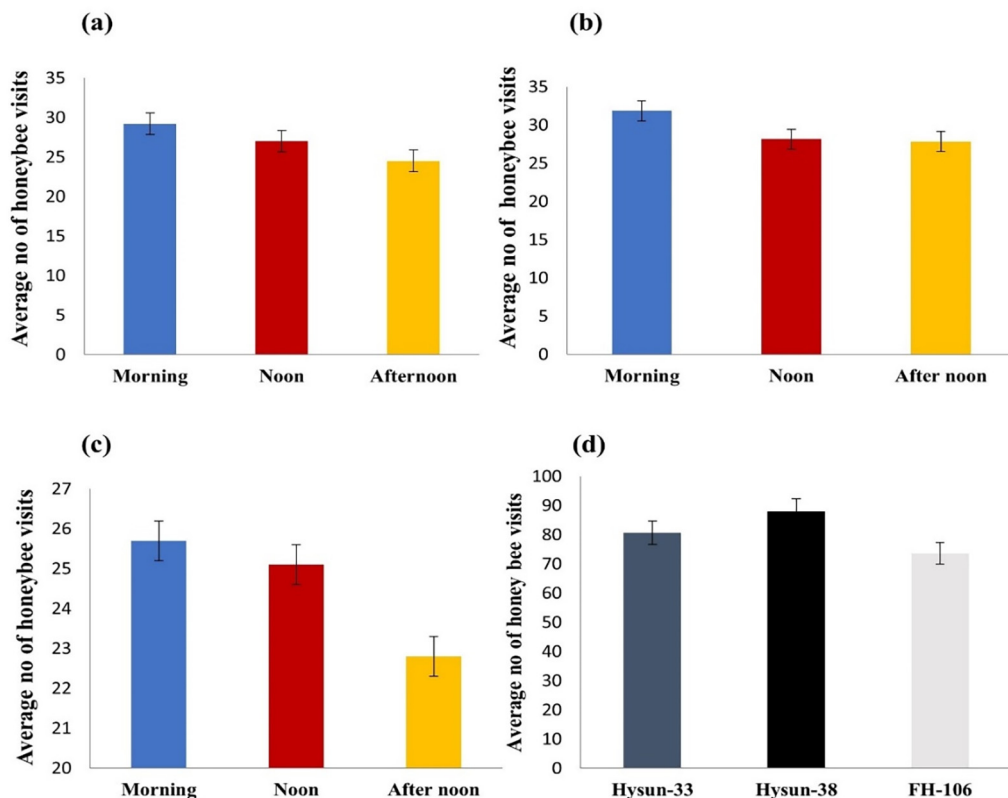


Fig. 1. The average number of honeybees visited different cultivars of sunflowers. (a) Hysun-33, (b) Hysun-38, (c) FH-106, and (d) comparison.

### 3. Results

#### 3.1. Preference of day timing and time staying on sunflower cultivars

The highest average number of honeybee visits was recorded in the morning followed by noon and afternoon on different sunflower cultivars (Fig. 1). In the morning and noon, the mean number of honeybee visits to the sunflower crop (Hysun-33) was 29.2 and 27, whereas the minimum average number of honeybee visits was 24.5 in the afternoon (Fig. 1 a). The highest number of honeybee visits on (Hysun-38) was in the morning (31.85) followed by noon (28.5) and afternoon (27.85), respectively (Fig. 1 b). Similarly, the maximum average number of honeybee visits in the morning time (25.7) whereas fewer visits were (22.8) in the afternoon

(Fig. 1 c). In addition, the maximum number of honeybee visits was observed on Hysun-38, while the minimum was recorded on the FH-106 cultivar, which was 87.85 and 73.6, respectively.

In the present study, sunflower cultivar (Hysun-33) and (FH-106), the maximum average time spent of the honeybee was noticed in the morning which was 34.24 and 32.67 min, respectively. While on the flower of (Hysun-38), was 36.41 min.

#### 3.2. Effect of head diameter size of sunflower cultivars on bee visits

The role of the honeybee's visit on the head diameter of the different sunflower cultivars is shown in Fig. 2. Regression analysis revealed a significant effect between the head diameter of sunflower cultivars and the number of honey bee visits ( $p < 0.05$ ). In

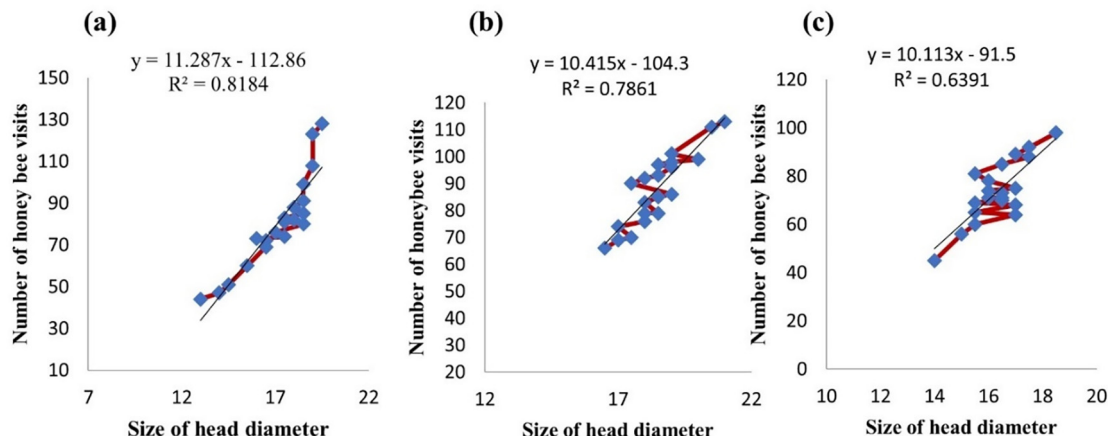


Fig. 2. The average number of bees visits is based on the size of the head diameter of sunflower cultivars. (a) Hysun-33, (b) Hysun-38, (c) FH-106.

Hysun-33 cultivar, when the head diameter was large “19.5 cm”, the number of bee visits was maximum “128”. Whereas the head diameter was minimum “13 cm” then bee visit was also decreased “44” (Fig. 2 a). In Hysun –38 cultivar, the maximum head diameter was “21 cm” and the number of honeybee visits was “113”. The honeybee visits were “66” on the “16.5 cm” head diameter (Fig. 2 a). While in the FH-106 cultivar, the largest head diameter was “18.5 cm”; the maximum number of honey bee visits was “98”. But head diameter was decreased “14” then the number of bee visits also decreased “45” (Fig. 2 c).

### 3.3. Impact of honeybee visit on seed setting

The effect of honeybee visits on the seed setting of various sunflower cultivars is mentioned in Fig. 3. A significant effect was observed between the number of honeybees visited and the number of seed settings on all cultivars of sunflower ( $p < 0.05$ ). Our findings indicated that the highest number of seed sets (1510) was recorded when honeybee visits were also maximum, which was “128” (Fig. 3 a). Similarly, in Hysun –38 cultivar, the maximum seed set was “1546” and the maximum number of bee visits was “113” (Fig. 3 b). In FH-106, the maximum seed set and honey bee visits were “532” and “98” (Fig. 3 c). The maximum average number of seed sets were “1297.9” and “1183.5” in honey bees treated and covered with perforated bags plants of Hysun –38 cultivar (Fig. 3 d). The typical number of seed sets in treated plants for the FH-106 cultivar was “1232” and “1086.9” in the control group. In Hysun-33cultivar, the minimum average number of seed sets was “1142.6” and “986” in treated and controlled plants, respectively (Fig. 3 d).

### 3.4. Correlation between size of head diameter and seed set

The correlation between the number of seeds set and the size of the head diameter of different cultivars is shown in Fig. 4. In Hysun-33 cultivar, there was highly strong positive correlation between the number of seed sets and head diameter ( $r = 0.957$ ). The maximum head diameter size was “19.5 cm” and the maximum number of seed set were “1510”. There was a positive correlation ( $r = 0.789$ ) between head diameter size and seed set in Hysun-38 cultivar. When the head diameter size was large “21 cm” then the seed set was “1546” (Fig. 4 b). Similarly, a positive correlation ( $r = 0.755$ ) was found between the head diameter size and seed sets of the corresponding flower of FH-106 cultivar (Fig. 4 c). The head diameter size was “18.5 cm” and the number of seed sets was “1532”.

## 4. Discussion

The foraging behavior of pollinators on the various cultivars is typically ignored, despite its importance for successful pollination. Our findings showed that different sunflower cultivars (Hysun-33, Hysun-38, FH-101 and a comparison) have varied *A. mellifera* foraging habits. The most honeybee visits occurred in the morning for all three sunflower cultivars that were the research subject: Hysun-33, Hysun-38, and FH-106. In comparison to the morning, there were fewer honeybee visits at noon. Fewer honeybee visits in the afternoon compared to noon. Different authors that reported on the honeybee’s visits to various crops agreed that their activity peaked in the morning. The possible explanation can be that for diurnal bees it may be possible to take use of the hours most effective

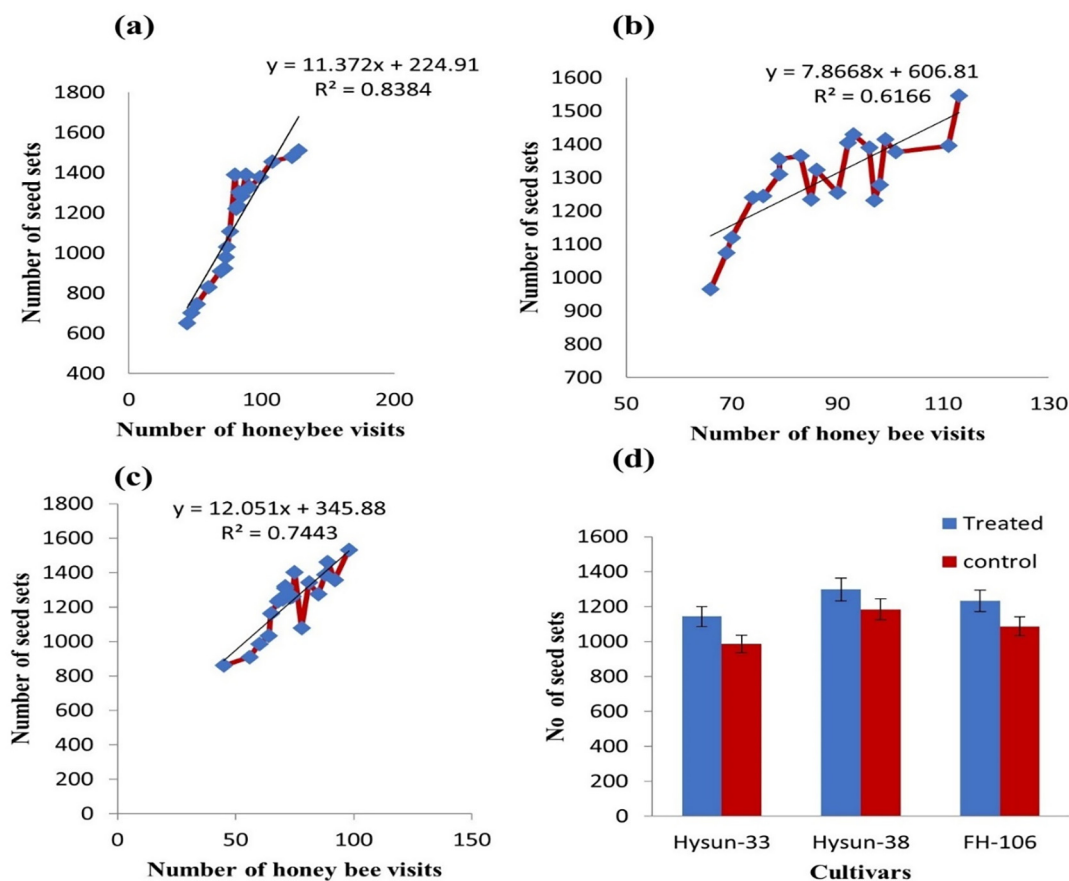


Fig. 3. The impact of the number of honey bee visits on seed setting of sunflower cultivars. (a) Hysun-33, (b) Hysun-38, (c) FH-106, and (d) Comparison.

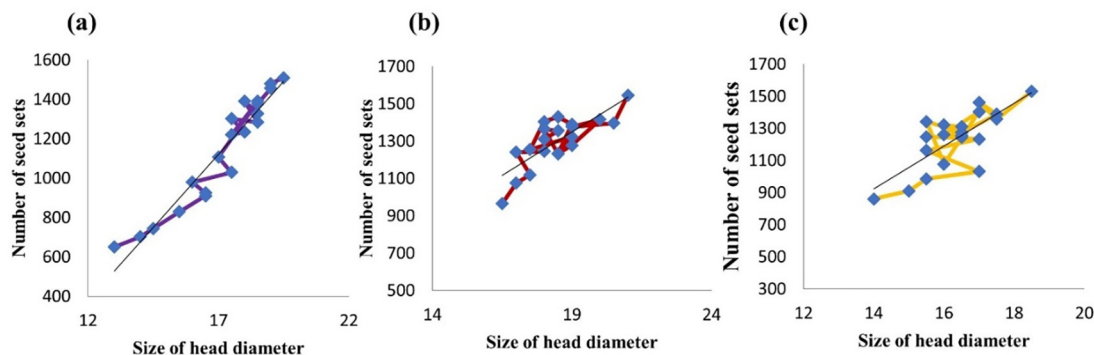


Fig. 4. Correlation between head diameter size and the number of seed sets of sunflower cultivars. (a) Hysun-33, (b) Hysun-38, (c) FH-106.

tively with enough sunlight for foraging if they rely on their circadian clock to predict when the sun will set and rise. Additionally, bees may benefit from a competitive edge by arriving early at blooms with abundant nectar and pollen. Other researchers also reported that maximum foraging peak time is in the morning and concluded that most visits were recorded in sunflower crops between 7:00 and 8:30 (Chambó et al., 2011). *A. mellifera* spent the entire day foraging in sunflowers, with the peak times for visits to obtain pollen and nectar being at 9:00 and 14:00, respectively (José de Paiva et al., 2002). After 10:00 and before 16:00, Morgado et al. (2002) observed a decrease in the foraging activity of Africanized honeybees in sunflower crops. However, it was found that *A. mellifera* was most prevalent at 8 h, with only slight variations over the rest of the day (Santana et al., 2002). While according to Aslan and Yavuksuz (2006), honeybee activity on sunflower heads increased between 10:00 am and 12:00 pm and subsequently decreased between 12:00 pm and 14:00 pm. On the other hand, during the sunflower blossoming season, regular visits from *A. mellifera* and *A. florea* were noted at 12:00 noon (Said et al., 2017). At present, extensive insecticide use has reduced honeybee visits to sunflowers (Pashte and Patil, 2017). In the present study, sunflower cultivar (Hysun-33) and (FH-106), the maximum average time spent by the honeybee was noticed in the morning, which was 34.24 and 32.67 min, respectively. While on the flower of (Hysun-38), it was 36.41 min. This difference in time can be explained, and the reason may be the nectar and pollen; that's why honey bees spent more time on the flower of Hysun-38 and less time on the flowers of Hysun-33 and FH-101. Other factors include the quantity and makeup of nectar sugars as well as the length of the corolla varied widely across sunflower inbred lines. Honey bee visits rose dramatically as nectar sugar concentration increased and declined as corolla length decreased (Mallinger and Prasifka, 2017). The crop arrangement might potentially be a factor in the persistent foraging behavior observed. When bees find a match with their current flower objective within a brief window of time of flight, previous investigations have shown that continual foraging happens with a high likelihood (Marden and Waddington, 1981).

In the present study, a significant relationship between sunflower cultivar head diameter and honey bee visits was found by regression analysis ( $p < 0.05$ ). Our results are in agreement with other researchers as Chambó et al. (2011) reported that the average number of bees per capitulum for *A. mellifera* during periods of high visitation ranged from 2.27 to 2.94 as the diameter increased. According to Moreti et al. (1996), there are typically 5.3 visits from *A. mellifera* per capitulum throughout the Anhandy flowering period of sunflowers. The same authors claim that as compared to plants that were only accessible to pollinators, the density obtained resulted in an 86% increase in the amount of lib-

erated seeds in pollinators. However, there is a direct correlation between seed mass and the quantity of fertilized seeds, and the diameter of the head of the sunflower (Dušanic et al., 2004) as with the increase in diameter number of honey bee visits was also increased.

In the current study, a significant effect was observed between the number of honeybees visited and the number of seed settings on all cultivars of sunflower ( $p < 0.05$ ). These findings support those made by Du Toit (1990), Nderitu et al. (2008), and Oz et al. (2009), who noted increase in sunflower seed output of 38%, 53%, and 206%, respectively, compared to areas without bees, in areas where Africanized bees were introduced. Additionally, the fields with the most beehives for pollinating sunflowers had much more seeds per plant and 100 seed weight (g) than other fields (Abbasi et al., 2021). According to numerous earlier research, depending on the cultivar of the crop, pollination services from different species of honey bees greatly enhanced sunflower seed size (10%–15%) and overall seed output (18%–100%) (Nye and Anderson, 1974). In the present study, positive correlation was found between the number of seed sets and head diameter, which agrees with a study conducted by Khaleghizadeh (2011) and concluded that sunflower head and seed density had a positive correlation ( $r = 0.320$ ). The association analysis results between seed yield per plant showed a highly significant and positive link between head diameter and seed production per plant (Pandya and Narwade, 2015).

## 5. Conclusions

The highest average number of honeybee visits was noticed in the morning followed by noon and afternoon on different sunflower cultivars. The maximum number of honeybee visits was recorded on Hysun-38 whereas the minimum was recorded on the FH-106 cultivar. The maximum average time spent of the honeybee was noticed in the morning on the flower of (Hysun-33) and (FH-106). Regression analysis demonstrated that a significant effect between the head diameter of sunflower cultivars and the number of honey bee visits and between the number of honeybees visited and the number of seed settings on all cultivars. In addition, there was highly strong positive correlation between the number of seed sets and head diameter of all cultivars. It has been determined that farmers should keep honeybee colonies close to the targeted crop in order to boost pollination and productivity. However, more research is needed to check the efficacy of honeybee pollination on various agricultural crops to increase yield.

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### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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