Journal of King Saud University – Science 33 (2021) 101608

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Standardization of managed honey bee (*Apis mellifera*) hives for pollination of Sunflower (*Helianthus annuus*) crop

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ARTICLE INFO

Article history:
Received 17 June 2021
Revised 10 August 2021
Accepted 13 September 2021
Available online 20 September 2021

Keywords:
Honey bee
Beehives
Standardization
Pollination
Sunflower
Seed yield

ABSTRACT

To determine the effect of honey bee (*Apis mellifera* L.) pollination on sunflower yield using the most appropriate number of beehives per unit area of crops is very important. By comparing the number of hives and yield components, we can satisfy optimal pollination needs and improve economic yields. For this purpose, a series of experiments were conducted at thatha Khalil farmer field Taxila Rawalpindi, under the supervision of Beekeeping and Hill Fruit Pests Research, Station Rawalpindi, Pakistan, with four treatments for comparison: 1 hive acre^{-1}, 2 hives acre^{-1}, 3 hives acre^{-1}, and 0 hives acre^{-1} as a control. The hives were kept inside the experimental area and pollination density, pollinator's diversity, agronomic yield, and economic yield were determined. Two acres of sunflower field were sown and four bee colonies were introduced at 5% to 10% flowering. The maximum visitation frequency of *A. mellifera* was recorded at the weekly interval from 24 to 09-2019 to 24–10-2019, according to which maximum visitation was observed (44.8, 38.4, and 16.5 plant^{-1} 5 min^{-1}) at 10:00, 12:00, and 14:00 hrs, respectively. Data regarding foraging pollinators revealed that *A. mellifera* was the most dominant pollinator of the sunflower crop with the highest abundance 17.11% followed by *A. cerana* (3.46%) and the population of *A. dorsata* was minimum (2.12%). Furthermore, agronomic parameters (number of seeds plant^{-1}, 100 seed weight (g), and economic yield were significantly higher in those fields which were pollinated by more beehives in comparison to fewer beehives.

1. Introduction

Pollination is widely acknowledged as an important ecosystem service and a necessary activity for food security (Klein et al., 2007; Aizen et al., 2009; Khan et al., 2021b). Pollination is an important service in the ecosystem accomplished by pollinators. Insects pollinate about 85%, air and water contribute 10% and self-pollination contributes only 5% (Fattorini and Glover, 2020). Pollinators are valuable species in the animal kingdom necessary for maintaining biodiversity. Pollination increases fruit setting, enhanced capacity of seed germination, and better quality of seed or fruit (Ahmad et al., 2021; Khan and Ghramh, 2021). When quality production increases; it has a direct effect on human health. Nearly 75% of the main crop species of the world rely on pollinators for fruit and seed set (Klein et al., 2007), which include managed pollinators such as honey bees (*Apis mellifera*) and various wild bees (Garibaldi et al., 2013; Kleijn et al., 2015; Ahmad et al., 2021). Furthermore, pollinators supply 35% to global food volume and play a key role

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2021.101608
1018-3647/

in supplying vital nutrients for human subsistence (Klein et al., 2007; Gallai et al., 2009; Holzschuh et al., 2012). The statistical value of insect-pollinated crops of the Himalayan region of Pakistan has reported about 954.59 million USA dollars (Abrol, 2015). Pakistan's pollination-dependent crop production value was estimated to be at 1.59 billion US$ (Irshad and Stephen, 2013). For instance, fruits account for 0.98 billion US$, vegetables for 0.32 billion, nuts for 0.15 billion, oilseeds for 0.13 billion, and spices for 0.004 billion US$ (Irshad and Stephen, 2013). Pakistan is spending millions of dollars on importing edible oil, which is a major drain on the foreign exchange reserves of the country (Shahzad and Rashid, 2006). The native oil manufacture of the country could not match the growing demand of the population. The edible oil utilization was 2.764 million tons (MT) of which 0.857 MT (31%) came from local income and 1.907 MT (69%) were imported (Anonymous, 2006). Rape and mustard groups of crops contribute about 21% of the domestic edible oil but their area is continuously decreasing. However, it has been observed that honeybee pollination enhances the yield by increasing the number of pods and seeds/pod. The pod ration in pollinated and non-pollinated was 815:349, similarly seed per pod was also 15% more in pollinated crop. Seed weight was also more in case of honey bee pollinated crop (Munawar et. al.,2009). But different authors have ranked the value of honey bees vs. other pollinators for agricultural pollination differently (Aebi et al., 2012; Ollerton et al., 2012; Khan et al., 2021a; Saleh et al., 2021).

Sunflower (*Helianthus annuus* L.), is a globally significant oilseed crop that has essential agronomic qualities, such as drought, cold, and heat resistance. Sunflower is the main source of high-quality edible oil (40% to 47%) and its seed contains 20% to 25% protein (Saleem et al., 2003). Its cultivation is a cost-effective crop rotation option that allows for intercropping and crop succession in seed-producing areas (Porto et al., 2007). Additionally, it requires insect pollinators on flowering, particularly the honeybees for seed production (Ali et al., 2015; Latif et al., 2019). *A. mellifera* is the only most abundant ecologically important introduced pollinator and is mostly managed for honey production. Therefore, there is a vast scope of improving the pollination of crops by designing and implementing strategies to manage economically important insect pollinator's especially native bees for seed and fruit production in the agricultural ecosystem (Delaplane et al., 2013). Only if production is quantified at field scale along a pollinator gradient will an estimate of pollinator contribution be useful to farmers (Vaissière et al., 2011).

Keeping in view its economic importance, the current project was designed to examine the role of the managed honey bee *A. mellifera* L. pollinator in increasing seed yield of sunflower crop by providing the most suitable number of beehive need per unit area of crops to meet optimum pollination needs and better economic yields by comparing the number of hives and yield components. Therefore, the findings of this research will contribute to the definition of general guidelines to maintain or improve sunflower crop pollination concerning the beehive or bee population size.

2. Materials and methods

2.1. Study area and field selection

The study was conducted on sunflower crops in the farmer field area of Taxila (Thatta Khalil) under the supervision of Beekeeping and Hill Fruit Pests Research Station Rawalpindi, Pakistan during 2018–19. The experiments were set up in a completely randomized block design along with four replications of each treatment.

2.2. Abundance of Apis mellifera during the blooming period of sunflower

The hives were kept inside the experimental area marked in the study field. One-acre field of the hybrid variety of sunflower Suncross was sown and four bee colonies were introduced at 10% to 15% flowering. At the time of seedling emergence, sunflower plants were hand-thinned. All recommended agronomic and cultural practices along with irrigation were adopted uniformly to all treatments weakly.

During the blooming period of the crop, no insecticides were applied to the experimental area. The observations were made from the beginning of the 5% flowering session until the end of the crop's flowering session. A comparative visitation pattern of *A. mellifera* was noted irrespective of pollen collection and time spent on each fluorescence. This experiment consists of the following three treatments: T1, 10:00; T2, 12:00, and T3; 14:00 h.

2.3. Comparative frequencies of honeybee species during the flowering period of sunflower

Honeybee species density was measured by scan sampling 200 flower heads in each of the four plots located in each experimental site. Data were recorded weekly during the whole blooming period. The scan sampling method was not involve timing but rather the insect recorded or not depending on whether it is present at the time has given flower is first seen. Sampling was done by walking slowly on 4 set patches in the experimental area. The data was taken at 10:00, 12:00, and 14:00 h weekly throughout the blooming period of sunflower (Said et al., 2017).

2.4. Effect of number of bee colony pollination efficiency on sunflower agronomic parameters

This experiment was carried out to determine the density of *A. mellifera* for maximum crop pollination sunflower fields with two sets of four treatments for comparison: 3 hives acre-1, 2 hives acre-^{1}, 1 hive, and 0 hives acre-^{1} as a control. To investigate total seed yield kg acre-^{1}, sunflower heads from all treatment plots were collected, dried, and threshed. The weight of 100 pod seeds was measured using electric balance and each net treatment yield was later converted into kg acre-1.

2.5. Economic yield of sunflower

After assessing the agronomic yield of sunflower, the economic yield was calculated. The economic yield was obtained from the net income, which was found out by using the following formula (Delaplane et al., 2013):

$$\Delta NI = P \times \Delta Y - CY \times \Delta Y - Ch \times \Delta H$$

Where:
ΔNI = Increase in number of hives
P = Price that farmer obtains from each metric tons of the crop
ΔY = Yield increased in metric tons because of addition of hives
CY = Cost of producing each ton of yield
Ch = Cost of renting hive
ΔH = Addition of hives

2.6. Statistical data analysis

All statistical data were analyzed using the SPSS statistical package (version 26). The significance of the difference between the two groups was determined using the Student's-test. Differences between means were considered statistically significant at the 95% ($p < 0.05$) confidence level, while differences at the 99% ($p < 0.01$) confidence level were considered highly significant.

3. Results

3.1. Visitation rate of A. Mellifera during the blooming period of sunflower

Our results indicated that the maximum visitation frequency of *A. mellifera* was recorded at the weekly interval from 26 to 09-2019 to 24–10-2019 during the whole blooming period. According to which maximum visitation frequency was observed (44.8, 38.4, and 16.5 plant^{-1} 5 min^{-1}) at 1000, 1200, and 1400 hrs, respectively on October 17, 2019 (Table 1). The visitation rate of *A. mellifera* differed significantly at 1000, 1200, and 1400 hr, respectively on 24th September, 11th, 17th· and 24th October. Whereas no significant difference was observed visitation rate on 4th October (Table 1).

3.2. Foraging pollinator rate of honey bee species on sunflower

Data regarding the foraging rate of honey bee species have been taken during the blooming period of sunflower. The maximum honey bee pollinators were observed on 17 October 2019 with a maximum population of *A. mellifera* (17.11) followed by *A. cerana* (3.46), *A. florea* (2.95), and population of *A. dorsata* was minimum (2.00) (Fig. 1).

The foraging rate of *A. mellifera* differed significantly in comparison to *A. cerana*, *A. dorsata*, and *A. florea* on 24th September, 4th, 11th, 17th· and 24th October during the blooming period of sunflower (Table 2).

3.3. The effect of the number of honeybee colonies on yield parameters of sunflower

The mean comparison of yield parameters is given in (Table 3) that illustrated the effect of sunflower plants exposed to *A. mellifera* along with other insects, and sunflower plants kept under 1 hive acre^{-1}, 2 hives acre$^{-1\cdot}$ and 3 hives acre^{-1} on agro- morphological parameters of *Helianthus annuus* var Hysun-33. Our results reported that the number of seeds per plant was significantly in 3 hives acre^{-1} (1472) as compared to 2 hives acre^{-1} (1165), 1 hive acre^{-1} (934), respectively (Table 3). While a smaller number of seeds per plant were recorded in control (880). Similarly, 100 seed weight (g) was observed significantly more in 3 hives acre^{-1} (5.34 g) as compared to 2 hives acre^{-1} (4.87), 1 hive acre^{-1} (3.98), respectively (Table 3). At the same time, <100 seed weight (g) was noted in control (2.84 g).

3.4. The effect of honeybee colonies on economic yield of sunflower

After assessing the agronomic yield, the economic yield calculated. The economic yield was obtained from the net income, which was found out by using the following formula:

1 hive acre^{-1}	**= P × ΔY – CY × ΔY – Ch ×ΔH**
	= 80,000 × 0.2 – 62,500× 0.2 – 1000 ×1
	= 16,000 – 12,500 – 1000
Net income	= **2,500**/ =
2 hives acre^{-1}	**= P × ΔY – CY × ΔY – Ch ×ΔH**
	= 80,000 × 0.48 – 62,500× 0.48– 1000 ×2
	= 38,400 – 30,000 – 2000
Net income	= **6,400**/ =
3 hives acre^{-1}	**= P × ΔY – CY × ΔY – Ch ×ΔH**
	= 80,000 × 0.84– 62,500× 0.84 – 1000 ×3
	= 67,200 – 52,500 – 3000
Net income	= **11,700**/ =

Economics is the key factor to do everything. Our findings revealed that economic yield was increased as well as the number of hives acre-1 was increased (Table 4). The results showed that 3 hives acre-^{1} pollinated sunflower plants had maximum economic yields (11700) than 2 hives acre-^{1} (6400) and 1 hive s acre-^{1} (2500) sunflower plants, respectively.

4. Discussion

Plants with appealing floral characteristics may attract more pollinators and have a better reproduction rate, resulting in more fruit and seed sets (Mallinger and Prasifka, 2017). Notably, mass-flowering crops, such as sunflower, cannot support a persistent pollinator population. It is ideal to have both managed honey bees and wild pollinators in the agricultural environment to ensure pollination (Lajos et al., 2021). Honey bees are the most important flower visitors (Hung et al., 2018), and were primary pollinators of sunflower in our study. In this study, *A. mellifera* was the main insect pollinator involved in increasing the agronomic yield as well as the economic yield of sunflower. Following previous findings that honey bees (*A. mellifera*) is the most dominant pollinator of sunflower (Riedinger et al., 2014; Sardiñas and Kremen, 2015).

On the other hand, very low foraging rates by *A. dorsata* was recored. Unexpectedly, we did not measure the percentage of edible oil and protein contents in the seed of sunflower in our experiments. However, a recent study by Lajos et al. (2021) revealed that foraging rates of honey bees in sunflower fileds can increase the seed set and oil content.

Dušanic et al. (2004) suggested that the diameter of head sunflower be strongly correlated with seed mass and the number of fertilized seeds. Our results elucidated that bee visitors were found throughout the day but the visitation rate of *A. mellifera* was significantly higher at 1000 AM in comparison to 1200 and 1400 during the blooming period of sunflower. In contrast, (Said et al., 2017) reported that frequent visitation of *A. mellifera* and *A. florea* was recorded at 1200 noon during the flowering period of sunflower. Currently extensive use of insecticide decreased honey bee visits in sunflower (Pashte and Patil, 2017).

Further, among the honeybee species, the foraging rate of *A. mellifera* was statistically more than *A. cerana*, *A. dorsata*, and *A. flo-*

Table 1
Foraging rate of honeybee species recorded on a sunflower on different dates of observation (2019).

Pollinators	24th Sep.	4th Oct.	11th Oct.	17th Oct.	24th Oct.
Apis mellifera	9.41a	11.41a	15.37 a	17.11a	10.78a
A. cerana	2.14b	3.3b	2.55b	3.46b	2.77b
A. dosata	2.90b	3.41cb	2.12 cd	2.00 bd	1.64c
A. florea	3.89b	3.92 d	4.44 bc	2.95 cbd	2.26b
LSD	0.53	0.36	0.44	0.68	0.47

Means sharing the same letter are not similar at 5% probability of LSD.

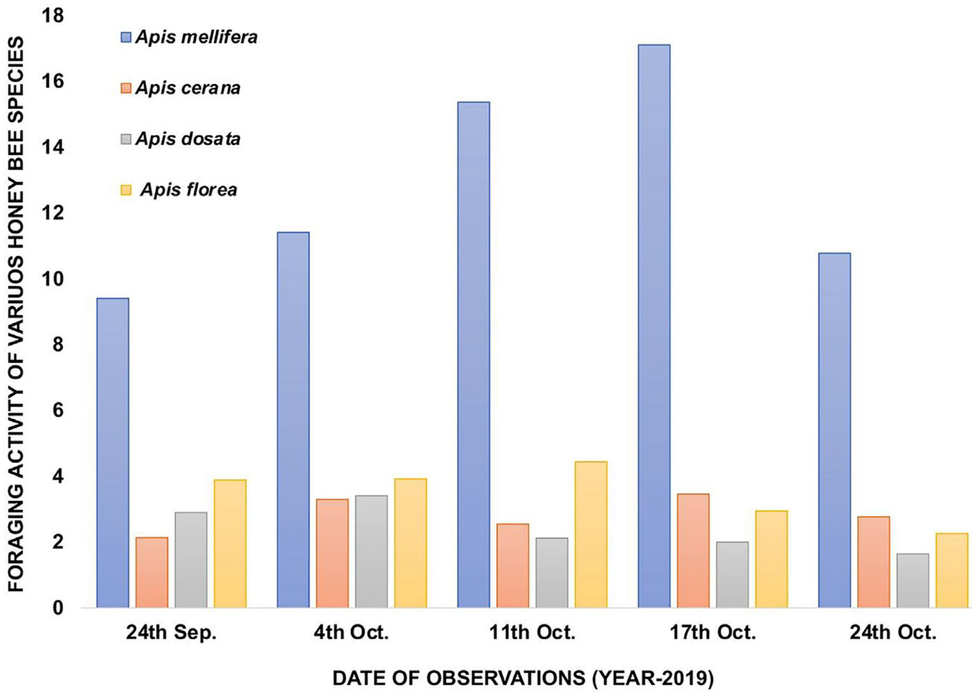

Fig. 1. Foraging activity of different honey bee species during the blooming period of sunflower on different observation dates (2019).

Table 2
Foraging rate of honeybee species recorded on a sunflower on different dates of observation (2019).

Pollinators	24th Sep.	4th Oct.	11th Oct.	17th Oct.	24th Oct.
Apis mellifera	9.41a	11.41a	15.37 a	17.11a	10.78a
A. cerana	2.14b	3.3b	2.55b	3.46b	2.77b
A. dosata	2.90b	3.41cb	2.12 cd	2.00 bd	1.64c
A. florea	3.89b	3.92 d	4.44 bc	2.95 cbd	2.26b
LSD	0.53	0.36	0.44	0.68	0.47

Means sharing the same letter are not similar at 5% probability of LSD.

Table 3
Means comparison of agro- morphological parameters of sunflower (*Helianthus annuus*).

Modes of Pollination					
Parameters	**1 hive acre^{-1}**	**2 hives acre^{-1}**	**3 hives acre^{-1}**	**Control**	**LSD**
No. of seeds plant^{-1}	934c	1165b	1472 a	880 cd	201.246
100 Seed weight(gm)	3.98c	4.87b	5.34 a	2.84 d	0.5653

Means sharing the same letter are not similar at 5% probability of LSD

Table 4
Net income obtained (In Pakistani Rupees, PKR) from different modes of pollination in sunflower.

Mode of Pollination	Net Income (PKR)
I hive **acre^{-1}**	2,500
2 hive **acre^{-1}**	6,400
3 hive **acre^{-1}**	11,700

rea, respectively during the flowering period of sunflower. Our results are inline with Silva et al. (2018), *A. mellifera* frequently visited sunflower field as compared to wild pollinators. Similarly, other studies reported that *A. mellifera* is the most abundant pollinator of several crops, especially sunflower (Greenleaf and Kremen, 2006; Carvalheiro et al., 2011). Moreover, the number of seed per plant and 100 seed weight (g) were significantly higher in those filed, containing a greater number of beehives for sunflower pollination. Many previous studies reported that pollinated service in sunflower provided by various types of honey bee species significantly increased the seed size (10%-15%) and the total number of seed yield (18%-100%) depending on the cultivar of the crop (Nye and Anderson, 1974).

Similarly, the economic rate was also higher in pollinated fields by more beehives than less beehives. However, more studies are needed in the future to better understand the effectiveness of the different types of honeybee species to increase the agronomic characteristic of various cultivars of sunflower.

5. Conclusions

This present study concluded that *A. mellifera* was the vital insect pollinator involved in increasing the agronomic parameters as well as the economic yield of sunflower. The foraging rate of *A. mellifera* was significantly higher than *A. cerana*, *A. dorsata*, and *A.*

florea during the blooming period of sunflower. Furthermore, agronomic parameters and economic yield were significantly higher pollinated fields by more beehives in comparison to less beehives. This study may help the local framers to understand the importance of bee pollination and maintain insect pollinators near to sunflower to ensure good productivity.

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.

Acknowledgments

We thank the PARB to provide the chance of this study, thank the scientist and the staff of beekeeping station .The facilitation provide by the farmer from Taxila, the scientist help in statistical analysis from Millet Research Station and Natural Resource Division, PARC, Islamabad. The authors appreciate the support of the Research Center for Advanced Materials Science (RCAMS) at King Khalid University Abha, Saudi Arabia through project number RCAMS/KKU/002-21.

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