

Determination of the Pollination Efficacy in *Apis mellifera* L. Colony Level on Cucumber (*Cucumis sativus* L.)

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Abstract

The series of trials were carried for this initiative at Barani Agricultural Research Institute (BARI), Chakwal (Pakistan) to examine the impact of honey bee (*Apis mellifera* L.) pollination efficacy on cucumber (*Cucumis sativus*) crop and discussed three main studies. i. Pollen transported by honey bees and foraging rate on cucurbit crops. For this purpose two bee colonies were placed in study plot. In both colonies the maximum no. of pollen load and maximum mean weight of pollen load 37% and 36 % obtained from early hours of the day from 10 am to 12 pm while study revealed that foraging activities of bees reduced after noon ii. Figuring out of substitute honeybee pollen sources on cucurbit crops revealed that ten distinct plant types, containing *Cucumis sativus* were recognized as main exploration basis for *Apis mellifera*. Out of these 10 fluorescent resources half were found to be crops plant and other half were weed plants. It has also been realized that when the most of the plants are in flowering, bee foraging activities are at their maximum (From the end of March. to start of May). Throughout this time, *C. sativus* was the most important bee forager in terms of pollen and nectar. iii. Impact of weather conditions on foraging rate of bees on cucurbits. This study revealed that in all significant pair-wise comparisons for March to July 2019, where *A. mellifera* visits to Cucumber flowers was found to be the greatest during the month of April and May 2019 and lowest in the month of June and July 2019. From April (22.45 °C) to July (30.25 °C) 2019, the cumulative increasing temperature was considerably higher at BARI, Chakwal, Pakistan, where the study was carried out.

Keywords: *Honeybee, Pollination, cucurbits, Seed quality, Forager, pollen, transport, weather impact.*

INTRODUCTION

The agriculture sector is a unique industry in which our means of survival and business ventures coexist. This sector is the most significant part of the economy, accounting for nearly 23.4% of Pakistan's GDP. The agriculture sector, as a major source of income in the country, prioritizes food security for the people over production optimization. Approximately 70% of Pakistan's total population reside in rural areas, and agriculture employs nearly 45% of the labor force. Agriculture is also critical to industrial growth. Around 60% of Pakistan's 5,000 industrial establishments are agriculture-based (FAO,

2020) Crop production advancement is a major component of the agricultural industry, with insect pollination, among many other factors, playing an important role. Pollination is essential for keeping the regular equilibrium of biomes and is the foundation of agricultural making, serving as a connection among both cultivation and the life cycle. As a result of the improved quality and quantity, pollination plays a role in the economic sector (Gill et al., 2016). Honeybee *Apis mellifera* L. serves as the utmost ample biologically chief familiarized pollinator, and it is primarily organized for crop and honey production. This European honeybee is very important

economically because it increases the produce and value of conventionally grown insect pollinated crop production. It also benefits self-pollinated crops around the world (Keith et al., 2013). Regardless of the presence of honeybees in a few cases, the practice of bees for crosspollination persists a missing component in crop production in Pakistan.

The commercial value of insect implantation in Pakistan's Himalayan region is US\$ 954.59 million (Partap et al., 2012). The assembly worth of fertilized reliant crops in Country was estimated approximately to be 1.59 billion US dollars. About one of our entire nutrition is at the mercy of insect-pollinated plants, either reliably or ultimately (Irshad and Stephen, 2013). Each year, honey bees donated around \$200 billion to the worldwide market, primarily through crop pollination. In Pakistan, the productivity value of pollination-dependent crops equals US\$ 590 million, with 61 basic pollinate crops declared foodstuff (26 fruits, 19 vegetables, 7 oil producing seeds, 4 grain leguminous plant, 2 additive crops, and 3 nut undergrowth) relying on honey bee pollination (Ahmad and Aziz, 2017).

Because of the floral abundance of oil seed crops during the flowering seasons of Brassica, Acacia, and Ber in January-March, April-May, and September-October, a significant number of beekeepers relocate from other regions of Pakistan (especially KPK) to the Pothwar region (Rawalpindi, Attock, Jhelum, Chakwal, and so on) of Province of Punjab. Bees are important because of their efficacy and wide availability. It is approximated that bees subsidized USD 11.68 billion to farming in the United States (Calderon, 2009). Honey bees are among the most rich pollinators, inducing efficiency and excellence (Meena et al., 2018), with their allogamy action aggregate the amount of germplasm and persuading over-all

profit, prominent to the suggestion that bee farmers localize bee collections close to cultivating lands to enhance pollination (Saboor et al., 2018). The cultivated honeybee *Apis mellifera* L. is essential for crop pollination as well as honey production. Many widely cultivated crops, such as *Citrus sinensis* (by 30%), watermelon (by 100%), and tomatoes (by 25%), have been reported to benefit from honeybee pollination services (Khalifa et al., 2021).

Cucurbits are an important vegetable that is widely consumed in Pakistan, among other places. Cucumber (*Cucumis sativus* L.) of the Cucurbitaceae family is a significant commercial crop grown in Pakistan. The plants are monoecious, meaning they produce two very different male and female flowers on the same plant. Cucumber is primarily a pollinated crop. Because pollen grains are heavy, large, and sticky, wind cannot transport pollen from one flower to another, and pollination is primarily done by insects, including the primary pollinator, honey bees (Tew and Caron, 1988). The effect of this pollination varies between 60 and 80% based on the environment and the presence of insect pollinators (Khalifa et al., 2021). In overall, any insect species' pollination efficiency is determined by its seeking rate (number of flowers visited per minute) and seeking speed (time spent per flower). (Saskia et al. 2019). Across all insect pollinators, the honeybee is the predominant and only reliable pollinator, accounting for 84 to 96% of total flower visitors. (2021, Khalifa et al.) Honeybees, primarily *A. mellifera*, are managed in hives to improve pollination. In cross-pollinated *Cucumis sativus* L., bee pollination is deemed significant (Chaurasia et al., 2008).

In Pakistan, with the help of honeybees pollinator to manage crop pollination is limited

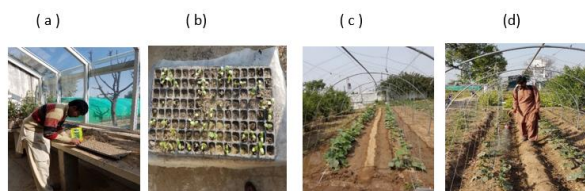
because the farming in the region is unaware of the pollination role of honeybees. As a result, the main goal of this research study is to design such a research work that aims to enhance the knowledge of the use of conserved honeybee pollination for cross pollinated cucurbits and to assess the colony level pollination effectiveness in *Apis mellifera* on cucurbit crops at Chakwal with regard to determining pollens transported by honeybees and foraging rate on cucurbit crops, discovering alternate pollen sources of honeybees on cucurbits.

MATERIALS AND METHODS

The research was conducted on Cucumber crop was grown at the BARI Institute, Chakwal located at Latitude 32.93183 N 32° 55'54.60209" and Longitude 72.71815 E 72° 43'5.32271 (GPS Coordinates of the experimental site) from March to July 2019. The cucumber genotype Waris F1 (purchased domestically) was utilized for the plantation and experimentations. The Institute's vegetable section's 2 tunnels, measuring 351 ft² (9 ft. x 39 ft) and 1590 ft² (26.5 ft. x 60 ft.), were used for trials. The area was ready for cucumber cultivation using farm machinery and hand equipment in each tunnel. The soil was cultured in the ratios of 20:20:20 with N, P, and K, and irrigation was suitably provided. Weeds were thoroughly eliminated during the study period. Bed rows of 30-40 centimeters in height, 1 m in width, and adaptable length were set up. Due to the hostile climate in area, seeds were first propagated in the nursery during February in artificial elastic boxes protected with clear polyethylene sheets and placed in a separate glass tunnel. The polyethylene sheets were removed just after plants began to grow after 3-5 days, and the plants were moved to their stable place, i.e. vegetal tunnels. The ranges between rows and plants in the vegetable tunnels demonstrated in were kept at 50 cm and

25 cm, respectively (Figure 1). Two *Apis mellifera* honeybee colonies were put in a cage-free environment near the tunnels. To allow honeybees' accessibility to Cucumber flowers, and the visitation was noted. The cucumber vines had been later backed with plastic mesh (size 3 x 3 inch) and side-sticks to assist the plants rise up and develop (when the plants had 8-10 leaves). Wood ash powder was used to control the problem of Red Pumpkin Beetle as it was affecting the plant health. For fruit collection, the tunnels were divided into four plots (each of 1m x 1m or 1m²) from where Cucumber fruits were collected on random days and analyzed at the Fruit & Disease Quality Diagnostics Laboratory, BARI, and Chakwal.

Fig.1 (a) (b) (c) (d) presenting proper seed sowing, and tunnel management.



In order to carry out the task of colony level pollination efficacy in *Apis mellifera* on cucurbits the three activities were carried out.

1. Determination of Pollens Transported By Honeybees and Foraging Rate on Cucurbit Crops.

Two bee colonies were selected in a 1 Acre field to conclude the total of honey bee outings and optimize flower-patterned means, and to access the calibration and Perseverance of pollen removed from bee body corbicula of ten Honeybees on a regular basis throughout the flowering season from a randomly selected ten flowers.

The observers sat near enough the honeybee colony to be able to see whether honeybees

were carrying pollen pellets on their legs and documented how many bees during 3-minutes period returning to the colonies carried pollen (visible) or not during the Cucumber blooming.

The data was collected on a weekly basis at three distinct periods of day: 10 a.m., 12 p.m., and 2 p.m. The Foraging rate was calculated by using the following formula:

$$\text{Foraging Rate} = \frac{\text{No. of Bees carrying Pollen pellets (in 3 minutes)} \times 100}{\text{Total no. of bees returning to hive (in 3 minutes)}}$$

Pollens collected on the bodies, legs, and pollen baskets of ten bees were quantified and determined on a weekly basis throughout the flowering period. The honeybees were captured using an aerial net and examined under a microscope in the Fruit & Disease Laboratory/ Pathology Laboratory at the Barani Agricultural Research Institute in Chakwal. SAWCRI, Chakwal, was used to collect metrological data.

All samples of honeybees were collected during the peak flowering (80-90%) and fruit setting phase of the Cucumber crop. Statistical analyses were later conducted to determine whether foraging activity differed significantly under different timings of the days for each of the two bee colonies.

2. Finding Out Alternate Pollen Sources of Honeybees on Cucurbit Crops Two Colonies of bees in 01 acre of field area have been selected to find out information regarding diversity and flowering of plants. Start regulating the quantity and consistency of pollen of flowering vegetation on a routine basis using 10 bees' bodies, legs, and pollen baskets. Florets acquired from the vegetation nearby the investigational plot, which also included equally wild and cultivated foliage. In order to categorize the native floras, the samples of ripe pollen particles were directly obtained from the field from mature flowers after the plant was identified as a bee plant by inspection of bees foraging on the plant for nectar, pollen, or both. Ripe pollen grains kept in Seventy % liquor for future research (Mailula et al., 2017). After

that, Pollen reference slides were created and stockpiled.

3. Impact of Weather Conditions on Foraging Rate of Bees on Cucurbits Crops.

Two colonies of bees in 1 Acre of field to determine the honey bees ability to forage at 10 am, 12 pm and 2 pm under different temperature, light intensity, weather condition etc. from 10 randomly no of selected flowers. According to the research by (Mommott et al., 2007), the ecological situations enforce a serious impact on association between plant life and the pollinating agents.

For pollen identification transported by the honeybees, ten bees with pollens attached on their body were collected from each of the two hives on weekly intervals. Acetolysis protocol was used for pollen identification (Dafni et al., 2005 and Louveaux et al., 1978).

For cytology, pollen interruption was prepared by washing bee body in seventy percent ethanol. Then 5ml of pollen suspension was added in 2ml glacial acetic acid for 10-15 minutes. After that diverging it for 2-3 minutes at 2400 rpm and afloat was discarded. The product obtained was unruffled for few minutes and repeat the procedure again to obtain the clear required material of pollen substance that was well-preserved for archival reference slides according to the proposed protocol, then a cover slip was put on, wrapped with nail coating. Then pollens were acknowledged using Credentials keys and online image catalogs (Kearns and Inouye, 1993).

RESULTS

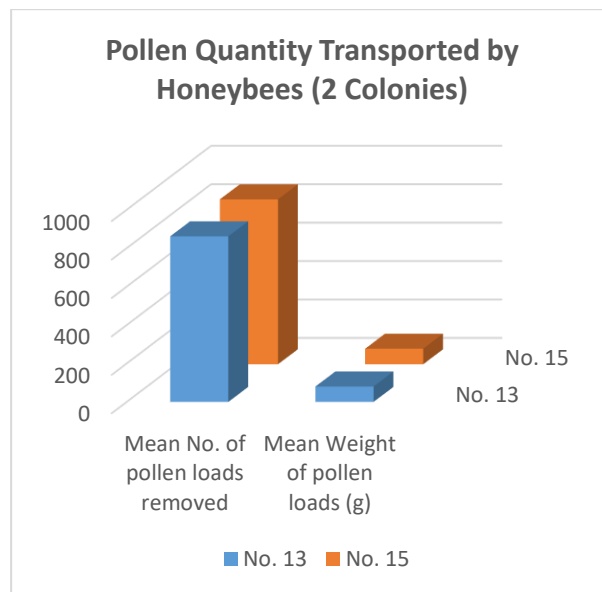
All samples of honeybees were collected during the peak flowering and fruit setting phase of the Cucumber crop, the mode of carrying the pollen grains by the body parts of bees presented in (Figure 3 A, 3 B, 3 C).

Statistical analyses showed that maximum no. of pollen load collected by forager bee was 861.79 ± 6.43 on colony no 13 with weight 80.18 ± 0.62 g followed by colony no.15 with average no. of pollen load 858.25 ± 6.58 having mean weight of this pollen load 80.87 ± 0.63 respectively presented in (Table.1 and Figure 2 graph) showed that foraging activity of bees was differed significantly under different timings of the days for each of the two bee colonies. The Amount of honeybees pollinator in Colony No. 13 and Colony No. 15 with and without pollen were detected when came back to their hives after foraging the flowering crop plants and observed for the period of 3 minutes time span during March – July 2019 were presented in Fig 4.

Table 1 shows the weekly mean number and weight of pollen loads excluded from *Apis mellifera* bodies from March 27th to July 9th, 2019.

Bee Colony	Mean No. of pollen loads removed \pm SE	Mean Weight of pollen loads (g) \pm SE
No. 13	861.79 ± 6.43	80.18 ± 0.62
No. 15	858.25 ± 6.58	80.87 ± 0.63

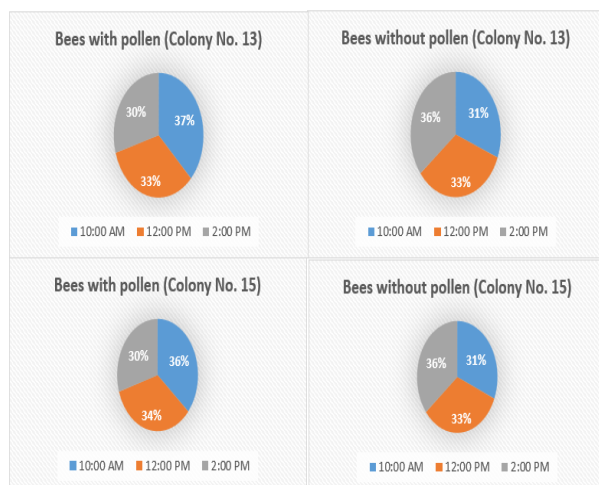
Figure 2: Weekly graph of the average number and bulk of pollen left out from *Apis mellifera* bodies from March 27th to July 9th, 2019.



Figs 3. (A) Dorsal view of a worker bee showing pollen grains attached to its legs and thorax (B) Ventral view depicting large size pollen loads attached to its hind legs (C) Lateral view showing pollen load on its hind leg



Figs 4. Represented that Proportion of honeybees in Colony No. 13 and Colony No. 15 with and without pollen that were observed returning to their hives observed for a 3-minute period during March – July 2019.


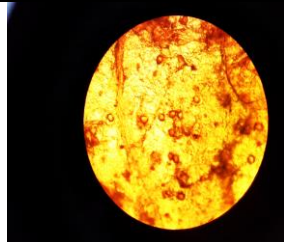


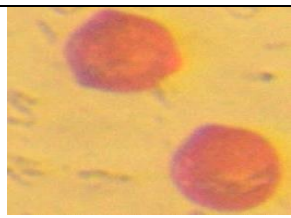

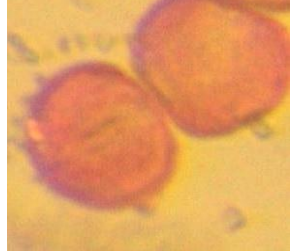

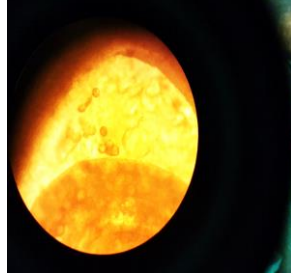

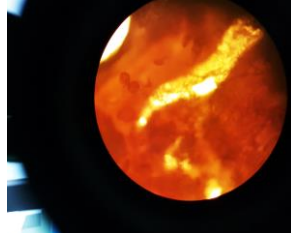
Two bee colonies were chosen in one acre of field to collect information about plant biodiversity and flowering, as well as to regulate the quantity and determination of pollen from flowering plants composed on the bodies, legs, and pollen baskets of ten bees on a weekly basis. Pollens obtained by *A. mellifera* foragers from various floral sources during the flowering period of *C. sativus* were distinguished in laboratory and the results were shown in the Table 2. Ten unlike plant species (with *C. sativus*) were recognized as *A. mellifera* forage sources.

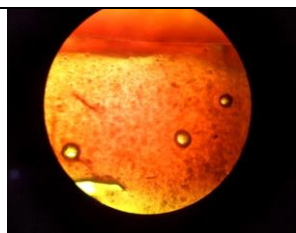
For pollen classification, an acetolysis protocol was used (Dafni et al., 2005). The pollens were recognized using identifying keys and online image databases after the slides were organized (Kearns and Inouye, 1993).

Finding Out Alternate Pollen Sources of Honeybees on Cucurbit Crops.

Table 2 shows the various floral resources explore by the *A. mellifera* honeybee during the Blossom of *Cucumis sativus* at BARI, Chakwal.

Sr.	Pollen Picture	Plant's Common Name	Family Name	Morphology	Flowering Period	Forage Source
1		Sarsoon	<i>Brassica napus</i>	Monoporate, Spherical shape, thick layer covering the structure	January-March	Crop
2		Cucumber	<i>Cucumis sativus</i>	Monoporate, pointed at its one end and shows bilateral symmetry	February-June/July	Crop

3		Black night shad	<i>Solanum nigrum</i>	Colporate, prolate, oblate spheroid, radially symmetrical	March – June	Weed
4		Red chick weed	<i>Anagallis arvensis</i>	Radial symmetry, exine	March – July	Weed
5		Onion weed	<i>Asphodelus tenuifolius</i>	Spheroid, two-sided proportion, with dense outer covering.	February-March	Weed
6		Sundial lupine	<i>Lupinus perennis</i>	Spheroid shaped pollens, bilateral symmetry, prolate	March-July	Weed
7		Chilli	<i>Capsicum sp</i>	Spheroid pollens, bilateral symmetry,	April-July	Crop
8		Bhakra		Monoporate, Spherical shape, thick layer covering the structure	May-July	Weed
9		Bittergourd	<i>Momordica charantia</i>	Spheroid pollens, bilateral symmetry,	May-September	Crop

10		Toria	<i>Brassica sp</i>	Spheroid shaped pollens, bilateral symmetry, prolate	May-September	Crop
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Impact of Weather Conditions on Foraging Rate of Bees on Cucurbits Crops.

The cumulative mean temperature increase from April (22.45° C) to July (30.25° C) (Table. 5 and 6) found to be significantly higher the only concern was that the differences were not marked between the temperature in June and July 2019 (30.65° C and 30.25° C respectively). There have been significantly more *A. mellifera* visits to the Cucumber flowers in April and May 2019 (foraging rate 44.1 ± 0.92 and 46.61 ± 0.39 respectively) as compared to the months of June and July (foraging rate 30.43 ± 1.31 and 20.14 ± 1.6 respectively) 2019.

Table 3

Colony Number	Time Interval	Bees with pollen	Bees without pollen
13	10:00 AM	42.1	57.9
	12:00 PM	37.67	62.33
	2:00 PM	33.44	66.56

Table 4

Colony Number	Time Interval	Bees with pollen	Bees without pollen
15	10:00 AM	43.97	56.03
	12:00 PM	41.22	58.78
	2:00 PM	36.1	63.9

Table 3 & 4 representing the proportion of honeybees in Colony No. 13 and Colony No. 15 with and without pollen that were observed returning to their hives observed for a 3-minute period during 3-time intervals from March till July.

The pollen collection was measured by counting the total of bees that entered with pollen. The quantity of honeybees accessing with pollen differed to a certain extent between the two bee colonies (37.74% and 40.43% for Colonies 13 and 15, respectively). Conversely, pollen accumulation diverse depending on the time of day (in first colony. 42.1, 37.67, and 33.44; in 2nd colony, 43.97%, 41.22%, and 36.1% at 10 a.m., 12 p.m., and 2 p.m., respectively) presented in Table 3 and 4 correspondingly.

Table. 5. Average MET Data from March till July 2019 (courtesy by Soil and Water Conservation Research Institute, Chakwal)

Month	Rainfall (mm)/day	Min. Temp. (°C)	Max. Temp. (°C)	Humidity Mean (%)	Sunshine (Hours)/day	Run of wind (km/day)	Evaporation (mm/day)
Mar-19	1.3	8.20	22.10	66.8	5.30	42.7	1.9
Apr-19	2.7	14.6	30.3	57.7	7.50	51.80	4.5

May-19	1	18.3	35.4	44.6	9.70	59.0	7.0
Jun-19	0.30	21.90	39.40	41.50	8.80	67.70	9.40
Jul-19	2.5	24.4	36.1	69.6	6.50	95.5	6.8

Table 6

Sr. No.	Date	Bee Colony No.	Foraging Rate of Bees	Weather Conditions					
				Temp. (°C)		Rel. Hum.	Sunshine	Wind	Rainfall
				Min	Max	Mean %	Hours/Day	Km/Day	mm/Day
1	27/03/2019	13	32.77	11.0	30.0	52.0	5.50	24.8	0.0
		15	33.75						
2	02/04/2019	13	39.89	11.0	33.0	34.0	10.10	28.71	0.0
		15	41.99						
3	08/04/2019	13	42.45	14.0	31.5	53.5	7.40	27.22	0.0
		15	45.89						
4	14/04/2019	13	40.72	16.5	27.5	67.5	2.50	123.71	0.0
		15	46.19						
5	22/04/2019	13	46.39	19.0	36.5	60.5	7.20	116.30	0.0
		15	49.49						
6	29/04/2019	13	43.13	16.0	35.0	65.0	9.30	71.12	0.0
		15	44.91						
7	07/05/2019	13	44.48	18.0	38.0	37.5	9.10	44.6	0.0
		15	45.38						
8	13/05/2019	13	47.11	21.5	35.0	56.0	4.00	89.3	Traces
		15	47.88						
9	20/05/2019	13	48.5	16.5	34.0	43.0	11.30	51.1	0.0
		15	46.42						
10	27/05/2019	13	46.43	19.0	36.5	42.0	10.30	51.3	0.0
		15	46.66						
11	03/06/2019	13	30.39	22.5	41.0	31.0	10.00	92.97	0.0
		15	36.89						
12	10/06/2019	13	28.69	23.0	43.0	31.0	9.25	99.45	0.0
		15	33.81						

13	17/06/2019	13	28.14	22.5	40.5	40.0	7.55	102.60	Traces
		15	34.38						
14	24/06/2019	13	22.77	24.5	36.0	52.0	5.20	70.75	0.0
		15	28.33						
15	02/07/2019	13	19.44	27.0	41.0	43.0	8.35	87.2	8.9
		15	22.7						
16	08/07/2019	13	18.23	27.0	40.0	51.0	6.05	106.3	7.6
		15	20.19						

Average MET data from one weather station (SAWCRI, Chakwal) starting from late March through early July, 2019 represented in (Table. 6 & Fig.5).

DISCUSSION

Determination of Pollens Transported By Honeybees and Foraging Rate on Cucurbit Crops.

Data revealed that based on entire samples of honeybees composed during the top flowering and fruit setting stage of the Cucumber crop, Honeybees of both colonies were observed carrying more pollen during early morning i.e. 10 AM while this pollen collection (Foraging Rate) gradually decreased after 12 PM and 2 PM from (37 % to 31 %) respectively. The findings were also consistent with (Tew and Caron, 1988,) who stated that honeybees obtain pollen on crop plants mostly in the initial hours of the day because pollen scavenging is greatest between 10 and 12 a.m. and declines in the later hours.

Finding Out Alternate Pollen Sources of Honeybees on Cucurbit Crops.

On the basis of facts and figures regarding plant biodiversity and flowering, with respect to bee colonies in order to quantify the pollen collected from the bodies of honeybees and

pollen baskets from the hindlegs the quantification results indicated that Pollens obtained by *A. mellifera* foragers from various floral sources during the flowering period of *C. sativus* were indicated after laboratory study that Ten unlike plant species (with *C. sativus*) were recognized as *A. mellifera* forage sources and out of these ten 5 are recognized as crops plants and remaining 5 as weeds. The results also specified that Bee foraging activities were discovered to be slow at the start of the flowering period. Moreover, because most plants flowered from late March to early April, foraging activities have risen. *C. sativus* was deemed to be the primary pollen and nectar source for bees throughout this time period.

Impact of Weather Conditions on Foraging Rate of Bees on Cucurbits Crops.

The pollen assortment was measured by counting the total of bees that entered with pollen during the study time. In all significant pair-wise comparisons for March to July 2019, where *A. mellifera* visits to Cucumber flowers were greatest during the month of April and May 2019 and lowest in June and July 2019. The results also indicated that during rainy season the activity of foragers, visitation and pollen collection in accordance with (Poulson, 1996) decreases during rainy periods and causes low visitation rate of flowers as

precipitation conditions become more severe and the abundance of flying insects decreases as the duration of precipitation increases. Bad weather conditions which combine with higher wind speeds and decreases in temperature, also prevent pollen foraging bees from returning to their colonies when compared to returns during good weather (Tuell and Isaacs, 2010).

The results indicated clearly that the seasonal effects shows a vital role as significant driver of ecological conditions. Biotic pollination rest on the positive transferal of pollens from one flower to the other, allowing sexual reproduction in plants. Bad meteorological conditions such as rains, can blow up the whole process and therefore prevent the generative efforts of flowering plants through several mechanisms.

CONCLUSION

Pollen collection varied across three different times of the day, revealing that the most pollen was collected during the early hours of the day. Bee foraging activities were quite low at early stage of flowering but increased during the flowers bloomed time. *A. mellifera* visited cucumber flowers far more oftenly in April and May than in June and July. The percentage of honeybees accessing pollen slightly differ between both the two colonies (37.74% and 40.43%, respectively).

RECOMMENDATION

It is recommended for the local farmer that encourages the presence of honeybee apiaries to locate near the fields of their crop, orchard, and farm area etc. where the bee activities remain maximum during early hours of the day and play a significant role in pollination ultimately may help to improve fruit quality, quantity and finally yield. The bees carrying the pollen load, equally from crops as well as from

natural weeds etc. with respect to optimal weather conditions

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DATA AVAILABILITY STATEMENT

All data and materials are available from the corresponding author. Therefore, at a reasonable request, the corresponding author shared it via email.

COMPETING INTERESTS

Authors have declared that no competing interests exists

ETHICAL APPROVAL: The ethical issues is not applicable

CONSENT OF PARTICIPATE: Not applicable

CONSENT OF PUBLICATION: Not applicable

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