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Assessment of Behavioral and Palynological Attributes of *Apis mellifera* L. Foragers in a Mustard Ecosystem

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Abstract

An investigation was carried out to assess different foraging activities of *Apis mellifera* L. in the mustard ecosystem by placing six strong *A. mellifera* colonies along the borders of mustard fields in the Terai agroecological region of West Bengal during 2019 and 2020. During this period, foragers of *A. mellifera* had a foraging duration of 9.41 ± 0.33 hours/day. Their activity was higher during mid-day than in the early morning and late evening hours. Temperature and bright sunshine hours strongly correlate with foraging duration and intensity. On the other hand, relative humidity and rainfall were negatively correlated with bee foraging. Foragers were found to spend maximum time on each flower at 11:00 – 13:00 hours, and their abundance was also high during this time interval. At initial hours of observation, i.e., at 07:00 – 09:00, foraging was minimal. The palynological assessment suggested that the foragers carry about 568,640 ± 15426.52 pollen on their bodies during peak foraging time. Apart from the pollen of *Brassica* sp., some other pollen types belonging to *Ageratum conyzoides*, *Amaranthus*/*Chenopodium* sp., *Cajanus cajan*, *Leucas aspera*, *Spilanthes* sp., and species of Polygonaceae and Solanaceae were also recovered from pollen load. However, these non-Brassica pollen frequency was very low in the pollen spectrum. This study provides information to assist local beekeepers in effectively managing their colonies by correlating foraging activity with weather parameters, and it will also advise mustard growers on establishing a prudent pesticide application schedule that will protect foraging bees from pesticide exposure.

Introduction

Pollinators are the key constructors of both natural and agroecosystems, providing highly valued pollination services to various crops and maintaining the natural balance of the ecosystems. Thus, pollinators form the fundamental pillar of crop production and link agriculture to the cycle of life (Khalifa et al., 2021). The pollination service builds a mutualistic relationship as it helps plant reproduction by transferring pollen among flowers, and as a reward, plants provide pollen as a source of nutrients to the pollinators (Campos et al., 2010). For cross-pollinated crops, this is the only way to ensure plant reproduction and food production. Pollination through bees accounts for about 30% of the world's food production (de Figueiredo-Mecca et al., 2013). Bees (Hymenoptera: Apiformes) are considered the most predominant pollinators in the world, pollinating about 80% of all flowering plants (Michener, 2007). Gallai et al. (2009) estimated that insect pollination services have attributed an annual valuation of approximately €153 billion in agricultural production. Among different bees, the European honey bee, *Apis mellifera* L. (Apidae: Hymenoptera), is well known for its precious pollination services (Calderone, 2012) and is the most frequent pollinator of various crops worldwide (Garibaldi et al., 2013).

Brassica spp. (Brassicaceae), the rapeseed-mustard is an important contributor to the world economy and the third major edible oilseed crop (Chand et al., 2021). After soybean, rapeseed mustard is the second highest contributor to India's total oilseed production (27%) and also covers 24% of India's total oilseed growing area (GOI, 2023). Many *Brassica* spp. require biotic cross-pollination for reproduction, depending on the great extent of insect visitors (Atmowidi et al., 2007). The bright yellow-colored mustard flowers, rich in pollen and nectar, attract bees and other pollinators a lot (Solovjovs, 2020). Insect pollination significantly increases crop yield and ensures the formation of relatively large, well-shaped, viable seeds (Singh & Singh, 1992; Khan & Chaudory, 1995; Vishwakarma & Chand, 2017; Subedi & Subedi, 2019). Whenever self-pollination is performed without pollinators, it reduces mustard seed yield and seed size in the current generation and the yield in subsequent generations (Delaplane & Mayer, 2000).

Brassica spp. have been reported to be pollinated by many insects around the world (Atmowidi et al., 2007; Stanley et al., 2013; Roy et al., 2014; Vishwakarma & Chand, 2017; Shakeel et al., 2019; Subedi & Subedi, 2019). Among the different pollinators of mustard, *A. mellifera* is considered one of the most important pollinators. The present study has been designed to assess different foraging attributes of *A. mellifera* foragers, such as their abundance, foraging duration, intensity, speed, and rate, as well as their palynological attributes in the mustard ecosystem under the Terai agroecological region of West Bengal.

Materials and Methods

Study area

The field observations were recorded from the mustard farm area of Uttar Banga Krishi Viswavidyalaya (UBKV), Pundibari, West Bengal, India, during the winter season (January – February) of 2019 and 2020. The observational fields were 43 m.a.s.l. The experiments were conducted in three different mustard fields (observational fields). Two *A. mellifera* colonies were placed at each mustard field border (alley) (Fig 1); thus, six colonies were used. These fields were surrounded by natural vegetation, including different weed flora. Apart from *A. mellifera*, other insect pollinators were also present in the mustard fields, and the field observations were carried out without hampering their activity. The laboratory experiment regarding the palynological assessment was conducted in the Department of Entomology and Molecular Biology Laboratory, UBKV, during the respective years of study.

This study region is characterized by a typical per humid climate with temperatures usually ranging between 10 and 38 ^{o}C and high rainfall above 3000 mm (annual average). During June – September, most rainfall occurs due to the southwest monsoon. Winter prevails from December to February, with reduced bright sunny hours and no snowfall. Mustard is cultivated in this region in this late period.

Condition of the honey bee colonies

All six colonies were maintained in the farm area of UBKV before initiating the study. These colonies were purchased from an established beekeeper of this region with a queen of about one year; the queen was fully fertile during the study period. All these colonies were healthy and strong, having eight frames each, and all the frames were entirely covered with bees on both sides. Seasonal and pest management strategies were performed to maintain the healthy condition of the colonies.

Observation recording

The observations were recorded on different foraging attributes of honey bees, such as foraging duration, intensity, speed, rate, abundance, and bee foraging on mustard. Data was recorded six times at weekly intervals, starting from the first week of January (right from the early flowering period of mustard) to mid-February, i.e., the winter season, when the crop was in the reproductive stage. For foraging duration and intensity, only one year of data was taken from 7th January to 11th February 2019 at weekly intervals. However, in the case of other parameters where two-year data were taken, the dates differed for both years.

The period for recording data varied depending on the foraging parameter. Observations on the temporal variation in foraging rate, speed, and bee abundance of *A. mellifera* foragers were carried out at five intervals in a day (i.e., 07:00 – 09:00, 09:00 – 11:00, 11:00 – 13:00, 13:00 – 15:00, and 15:00 – 17:00 hours, respectively) and foraging intensity was recorded at three periods in a day (i.e., 09:00, 12:00, and 15:00 hours, respectively).

Here, observation from each mustard field was treated as a separate replication for recording foraging speed, rate, and forager abundance. For recording foraging intensity, the average value of the two hives from each mustard field was treated as a single replication. Thus, three replications were performed to conduct the studies.

Foraging behavior

a. *Foraging duration*

Foraging duration was measured as the time gap between the first exit from the hive and the last entry into the hive, and it was estimated during the mustard flowering period at weekly intervals. The hive entrance gate was carefully monitored starting at 06:00h before sunrise (sunrise usually occurs after 06:00h in this region during the winter months) to ensure the exact time of the first exit from the hive. To confirm the last entry, we waited and monitored the hive entrance gates for an extended period until it became clear that no other bees remained to enter.

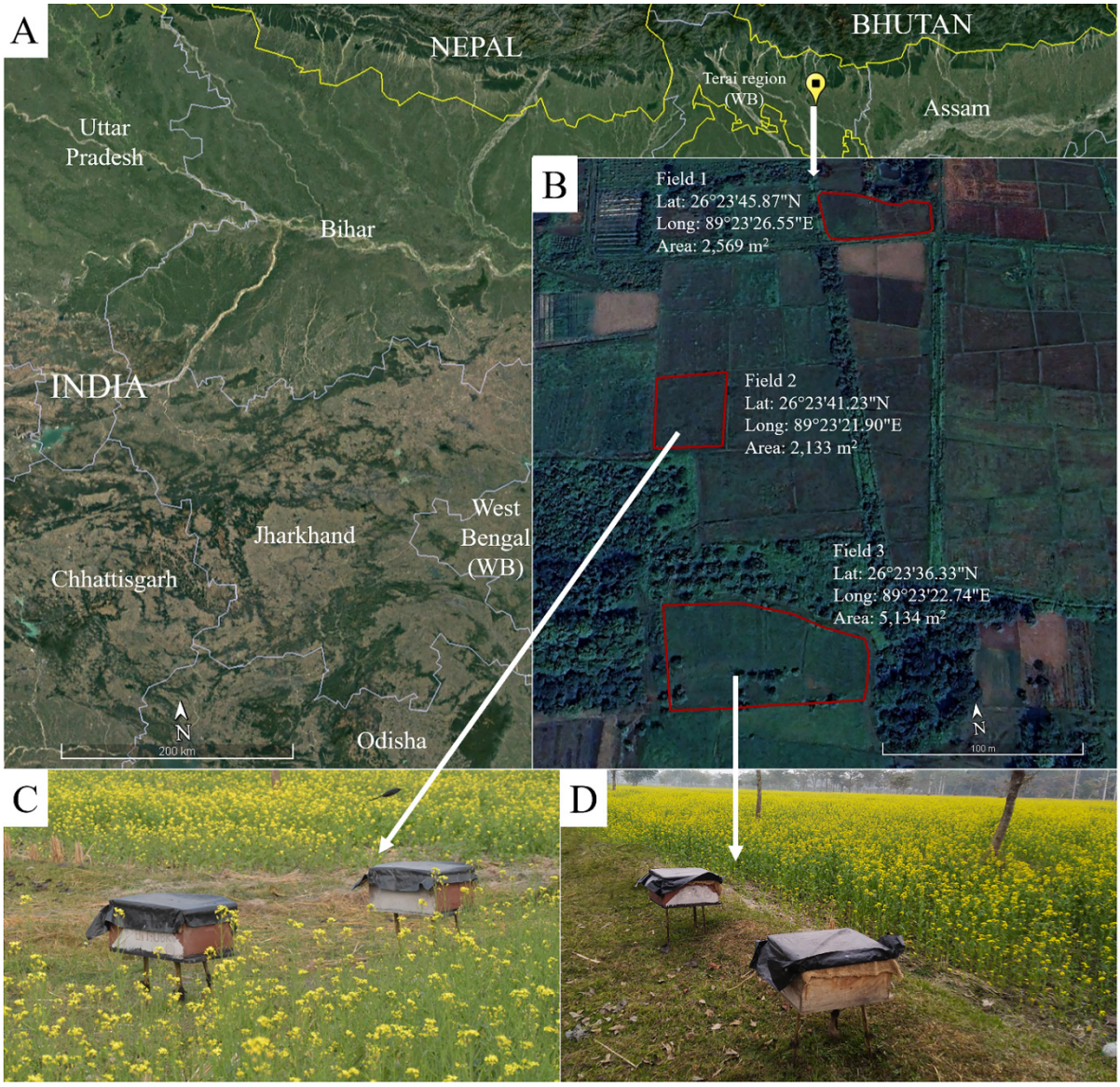

Fig 1. Satellite image of the study area. (A) Location of the study area. (B) Location of the observational fields. (C–D) Position of *Apis mellifera* hives at the border (allay) of mustard fields.

b. *Foraging intensity*

The foraging intensity was measured as the number of forager bees coming out (exit) and entering (entry) the hive within 5 minutes. Here, the foraging intensity (number of bees exiting and entering) of *A. mellifera* foragers at both the hives placed in each observational field was recorded, and their average was treated as a single replication (a total of three replications from three observational fields). Data was recorded at three different time intervals, i.e., 09:00, 12:00, and 15:00 hours, for six days at weekly intervals during the flowering period of mustard.

c. *Foraging speed*

Foraging speed was measured as the time spent (in seconds) by an individual worker honey bee per flower. It was recorded using eye estimation using a stopwatch. In each observational field, one forager bee was observed to record its foraging speed at the aforesaid date and time interval. Thus, three observations (replications) were considered for data recording from three observational fields.

d. *Foraging rate*

The foraging rate was measured as the number of flowers visited by an individual forager at one minute. This one-minute time included the time spent in flowers and the flight time they spent during trips from one flower to another. The number of flowers visited by a single *A. mellifera* forager was determined by eye estimation, and time was measured using a stopwatch. Here also, from each observational field, one *A. mellifera* forager was observed to record the number of flowers visited by that forager for one minute at the aforesaid date and time intervals. Thus, from three observational fields, a total of three replications were made.

e. *Forager abundance*

The abundance of *A. mellifera* foragers was recorded as the number of worker honey bees visits per m^2 of floral area per minute. For recording data, 1 m^2 areas were randomly selected and demarcated by pegging with bamboo pegs. Three 1 m^2 areas (three replications) were selected, one from each observational field, and the number of *A. mellifera* foragers in that 1 m^2 area for one minute was determined by eye estimation at the aforesaid date and time interval.

In the case of determining the abundance, foraging speed, and rate, the observed foragers were tagged by using bright color paint on the thorax (Betterbee, 2024) to avoid pseudoreplication. In order to note the accurate first exit and last entry time, and to count the foraging intensity of all the boxes almost simultaneously, three observers were assigned to take the data. A few glimpses of the foraging activity of *A. mellifera* foragers are shown in Fig 2.

Recording of weather data

Data about maximum temperature (T_{max}), minimum temperature (T_{min}), maximum relative humidity (RH_{max}), minimum relative humidity (RH_{min}), rainfall, and bright sunshine hours (BSH) were recorded from the Meteorological Unit, UBKV, located at the farm which is within 500 meters from the observational field.

Palynological assessment

The absolute pollen count (APC) or number of pollen carried by *A. mellifera* foragers was also estimated. Ten foragers with pollen loads were captured using an aerial net during their peak foraging time from the mustard field and kept in vials containing 70% ethanol. The vials were shaken vigorously to remove the pollen from the forager's body. After removing the bees from the vials, the dilution volume was made up to 1 ml and then vortexed for proper mixing. From there, a 10 µl aliquot was taken in a micropipette and placed slowly in the chambers of a hemocytometer, and pollen was counted in medium chambers containing 0.00625 µl of suspension (Balina et al., 2012).

To determine the presence of pollen types in the pollen load, 0.5 ml of the same dilution of pollen load was taken in a centrifuge tube and centrifuged at 1050 × g (c. 2500 rpm) (Pendleton, 2006) for 10 min. Centrifugation was performed

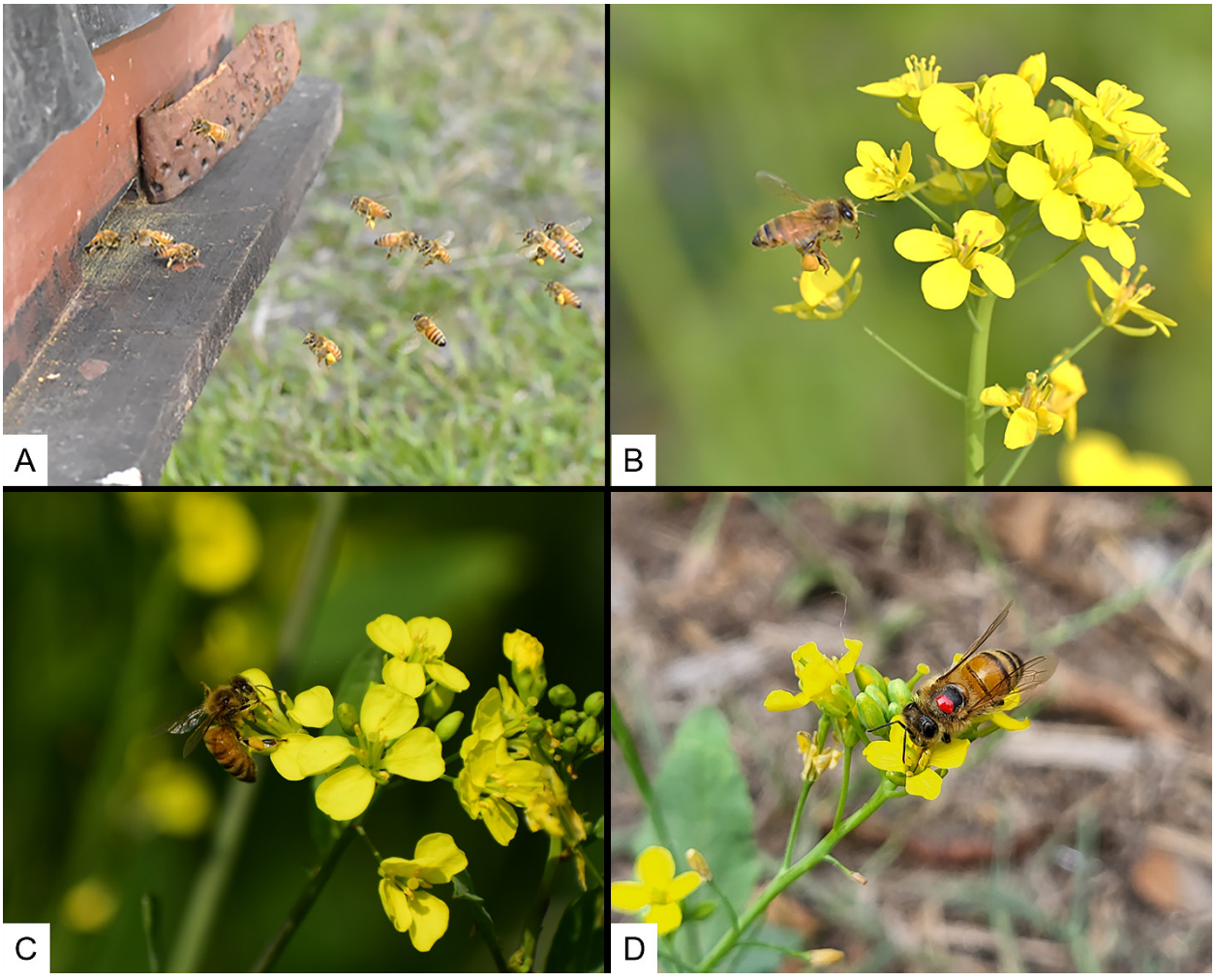

Fig 2. Glimpses of the foraging activity of *Apis mellifera* foragers during study. (A) Foragers coming out and entering into the hive. (B–C) *Apis mellifera* foragers with pollen load visiting mustard flowers. (D) An observed and tagged forager visiting mustard flower.

in two steps without acetolysis, first with 0.5 ml of glacial acetic acid and then with 0.5 ml of 50% aqueous glycerin (Saha et al., 2023a). Then, a small amount of supernatant was taken in a glass slide and observed under the ZEISS Axioscope 5 microscope, and photomicrographs of suitable magnification were captured using the Axiocam 503 color microscope camera. Visual identification was carried out by comparing the morphological features of that pollen with the pollen from locally available floral samples. For quantification, 500 number of pollen from each forager sample were counted. Frequency classes of different pollen types were expressed as 'predominant pollen' (> 45% of total pollen count), 'secondary pollen' (16–45%), 'important minor pollen' (3 – 15%), and 'minor pollen' (< 3%) (Louveaux et al., 1978). The presence of honeydew elements (HDEs) including fungal spores or hyphae was also determined as HDE/P ratio (P = total pollen count) and expressed as 'practically none' (HDE/P = 0.00–0.09), 'few' (HDE/P = 0.10 – 1.49), 'medium quantity' (HDE/P = 1.50 – 2.99), 'numerous' (HDE/P = 3.00 – 4.49), and 'very numerous' (HDE/P> 4.50) (Louveaux et al., 1978).

Statistical analysis

Data on foraging intensity, rate, and forager abundance were normalized using the square root [$\sqrt{(x+0.5)}$] transformation. Analyses were carried out in a two-factor factorial analysis of variance (ANOVA) to evaluate the effect of variables (date and time) on the parameters (foraging intensity, speed, rate, and forager abundance). Duncan's new multiple range test (DNMRT) was done to compare differences in the means of various observational units at $\alpha = 0.05$. Pearson's correlation between weather data and foraging duration and intensity was also performed to evaluate their impact on these foraging parameters. Statistical analyses were conducted using R statistical software, version 4.3.1 (R Core Team, 2023).

Results

Foraging duration

As presented in Table 1, the earliest exit of foragers from the hive and last entry into the hive were noticed at 6:54h and 17:32h, respectively, at the last observation date.

Table 1. Time of first exit and last entry and foraging duration of *Apis mellifera* during flowering period of mustard.

Dates	1st exit from the hive (at hour)	Last entry into the hive (at hour)	Foraging duration (in hours)
07/01/2019	7:22	16:58	9.600
14/01/2019	7:51	17:13	9.367
21/01/2019	7:56	17:16	9.333
28/01/2019	7:57	17:23	9.433
04/02/2019	9.03	17:10	8.117
11/04/2019	6:54	17:32	10.633

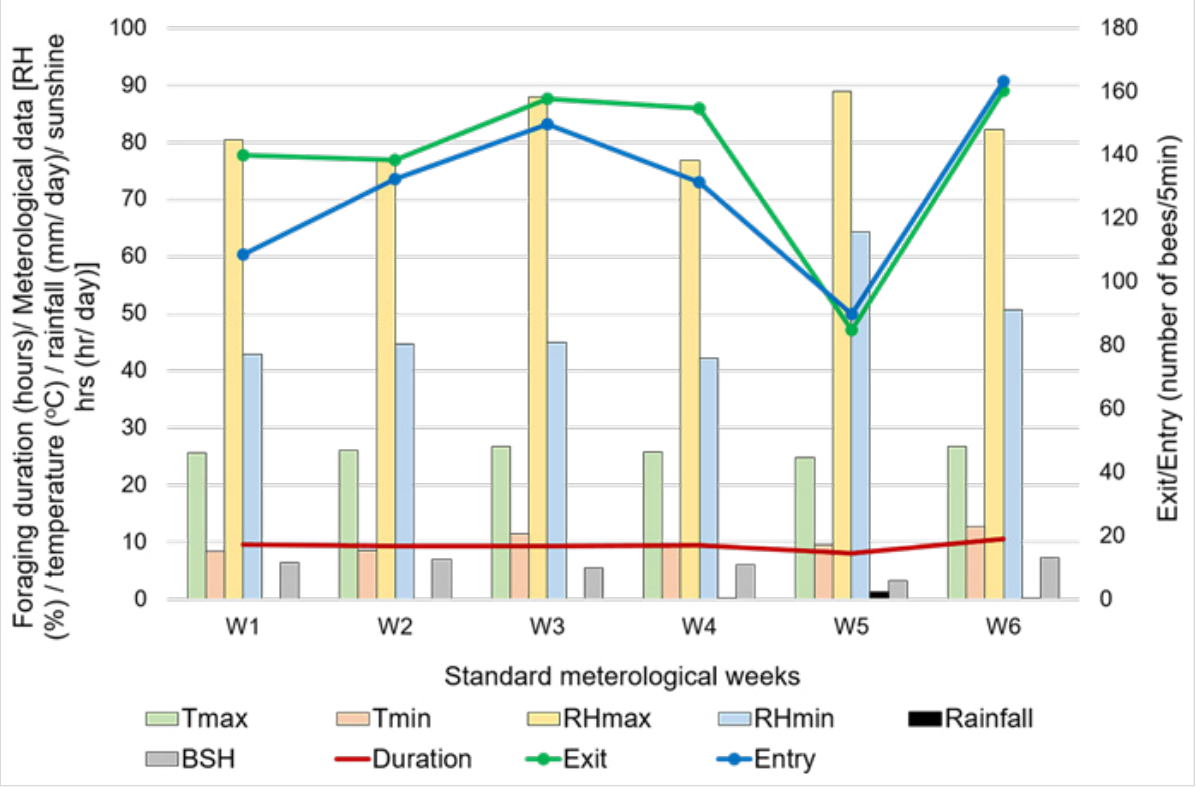

Fig 3. Graphical representation of weather parameters with the foraging duration and number of exit and entry/5 minute time period. Abbriviations stand for, Tmax: maximum temperature; Tmin: minimum temperature; RHmax: maximum relative humidity; RHmin: minimum relative humidity; and BSH: bright sunshine hours.

The mean time duration spent by the foragers during the mustard flowering period was 9.414 ± 0.327 hours/day (mean ± SEM).

Foraging intensity

From the results of ANOVA, we found a significant ($P \leq 0.05$) effect of date, time, and their interaction effect on the foraging intensity of *A. mellifera* (Table 2). Here, the activity of the foragers at the entrance of the hive was found to be higher (i.e., 160.22 bees exiting and 163.11 bees entering at 5 minutes) during the last date of observation, i.e., during mid-February (11/04/2019) and least on the date before. Coming to the time variable, the activity of the foragers was extremely low in the morning (i.e., 09:00h). However, their activity increased multiple times during the mid-day hour (i.e., 12:00h), and then again, their activity reduced in the late afternoon hours. Table 2 also presents that the number of entries was always less than the number of exits.

Relation of weather parameters on foraging duration and intensity

A graphical representation of weather parameters and foraging duration and intensity is shown in Fig 3. Correlation analysis (Fig 4) revealed a strong positive correlation between T_{max} and the foraging duration and the number of bees exiting and entering the hive. BSH showed a strong, significant, positive correlation with foraging duration ($r = 0.90$, $p < 0.05$). However, RH_{max}, RH_{min}, and rainfall negatively correlated with these foraging parameters. Rainfall had a strong negatively significant correlation with the number of bee exits ($r = -0.90$, $p<0.05$).

Foraging speed

Here, the results of the ANOVA suggested a significant ($P \leq 0.05$) effect of both date and time on the foraging speed of *A. mellifera*. However, date-time interaction showed no significant effect on the foraging speed (Table 3). Foragers

Table 2. Assessment of *Apis mellifera* foraging intensity [foragers going out (exit) and coming into (entry) the hive within 5 minutes] at different times and dates during the mustard flowering period using two-factor factorial ANOVA and DNMRT *post-hoc* test (α = 0.05).

Date	Number of bees exit/5 minute (N = 7522)				Number of bees entering/5 minute (N = 6977)			
	Time			Mean	Time			Mean
	09:00	12:00	15:00		09:00	12:00	15:00	
07/01/2019	37.67 (6.17)	248.00 (15.76)	134.00 (11.58)	139.89 (11.17)^{a}	30.33 (5.54)	223.00 (14.93)	72.33 (8.51)	108.56 (9.66)^{c}
14/01/2019	29.00 (5.25)	265.00 (16.26)	121.33 (10.98)	138.44 (10.83)^{a}	28.00 (5.26)	262.67 (16.16)	106.33 (10.29)	132.33 (10.57)^{bc}
21/01/2019	28.00 (5.27)	307.33 (17.55)	137.33 (11.67)	157.56 (11.49)^{a}	25.33 (5.04)	309.00 (17.59)	115.00 (10.74)	149.78 (11.12)^{ab}
28/01/2019	13.33 (3.68)	309.00 (17.59)	141.67 (11.87)	154.67 (11.05)^{a}	15.33 (3.97)	294.33 (17.17)	84.67 (9.22)	131.44 (10.12)^{c}
04/02/2019	5.00 (2.34)	149.00 (12.23)	101.00 (9.98)	85.00 (8.18)^{b}	8.33 (2.97)	152.33 (12.36)	109.33 (10.30)	90.00 (8.54)^{d}
11/02/2019	20.67 (4.59)	290.67 (17.05)	169.33 (13.03)	160.22 (11.56)^{a}	24.00 (4.90)	304.00 (17.45)	161.33 (12.71)	163.11 (11.69)^{a}
Mean	22.28 (4.55)^{c}	261.50 (16.07)^{a}	134.11 (11.52)^{b}	—	21.89 (4.61)^{c}	257.56 (15.94)^{a}	108.17 (10.29)^{b}	—
Parameters	Factors and interaction							
	Date	Time	Date × Time		Date	Time	Date × Time	
SEM ±	0.333	0.236	0.577		0.316	0.224	0.548	
CD	0.956	0.676	1.656		0.907	0.641	1.571	
CV	9.334				9.226			
	ANOVA							
Source	df	Sum of square	Mean square	F value	P	df	Sum of square	Mean square	F value	P
Date	5	72.600	14.500	15.254	<0.001	5	55.800	11.200	12.157	<0.001
Time	2	1212.400	606.200	637.119	<0.001	2	1155.100	577.500	628.680	<0.001
Date × Time	10	31.600	3.200	3.323	0.004	10	50.400	5.000	5.481	<0.001
Error	36	34.300	1.000			36	33.100	0.900		

Note: Means suffixed with different letters are significantly different. Figures in the parenthesis are square root transformed values. N= total number of forager bees encountered at the entrance of the hive either exiting or entering into the hive; SEM= Standard error of the mean; CD = Critical difference; CV = Coefficient of variation.

Table 3. Assessment of foraging speed [time spent per flower (in seconds)] of *Apis mellifera* at different times and dates during the study period using two-factor factorial ANOVA and DNMRT *post-hoc* test (α = 0.05).

Date	2019						2020						Pooled					
	Time					Mean	Time					Mean	Time					Mean
	07:00-09:00	09:00-11:00	11:00-13:00	13:00-15:00	15:00-17:00		07:00-09:00	09:00-11:00	11:00-13:00	13:00-15:00	15:00-17:00		07:00-09:00	09:00-11:00	11:00-13:00	13:00-15:00	15:00-17:00	
1st Week	0.92	1.54	2.94	2.47	0.78	1.73^{d}	1.12	1.90	3.45	2.66	0.73	1.97^{c}	1.02	1.72	3.19	2.57	0.75	1.85^{b}
2nd Week	1.21	2.44	3.54	2.80	1.10	2.22^{b}	0.92	1.11	2.91	2.34	0.67	1.59^{d}	1.07	1.78	3.22	2.57	0.89	1.91^{b}
3rd Week	1.06	2.40	3.51	2.64	1.06	2.13^{bc}	0.95	1.66	3.35	2.42	0.71	1.82^{cd}	1.01	2.03	3.43	2.53	0.89	1.98^{b}
4th Week	0.96	1.89	3.35	2.57	1.02	1.96^{cd}	0.94	1.80	3.44	2.36	0.70	1.85^{cd}	0.95	1.85	3.39	2.47	0.86	1.91^{b}
5th Week	0.93	1.70	3.11	2.56	1.00	1.86^{d}	1.47	2.86	4.10	3.58	1.29	2.66^{a}	1.20	2.28	3.60	3.07	1.15	2.26^{a}
6th Week	1.59	2.89	4.03	3.13	1.11	2.55^{a}	1.46	2.54	3.56	2.85	1.25	2.33^{b}	1.53	2.71	3.80	2.99	1.18	2.44^{a}
Mean	1.11^{d}	2.14^{c}	3.41^{a}	2.70^{b}	1.01^{d}	—	1.14^{d}	1.98^{c}	3.47^{a}	2.70^{b}	0.89^{e}	—	1.13^{d}	2.06^{c}	3.44^{a}	2.70^{b}	0.95^{e}	—

Parameters	Factors and interaction								
	Date	Time	Date × Time	Date	Time	Date × Time	Date	Time	Date × Time
SEM ±	0.079	0.072	0.177	0.097	0.089	0.217	0.073	0.066	0.162
CD	0.224	0.204	NA	0.274	0.250	NA	0.205	0.187	NA
CV	14.782			18.432			13.651		

ANOVA

Source	df	Sum of square	Mean square	F value	P	df	Sum of square	Mean square	F value	P	df	Sum of square	Mean square	F value	P
Date	5	6.430	1.285	13.682	<0.001	5	11.410	2.283	16.194	<0.001	5	4.280	0.856	10.810	<0.001
Time	4	76.210	19.051	202.830	<0.001	4	82.850	20.713	146.932	<0.001	4	79.370	19.842	250.76	<0.001
Date × Time	20	1.850	0.093	0.985	0.492	20	2.380	0.119	0.843	0.654	20	0.790	0.040	0.500	0.956
Error	60	5.640	0.094			60	8.460	0.141			60	4.750	0.079		

Note: Means suffixed with different letters are significantly different. SEM = Standard error of mean; CD = Critical difference; CV = Coefficient of variation; NA = determination of CD is not applicable or not required as the effect is non-significant.

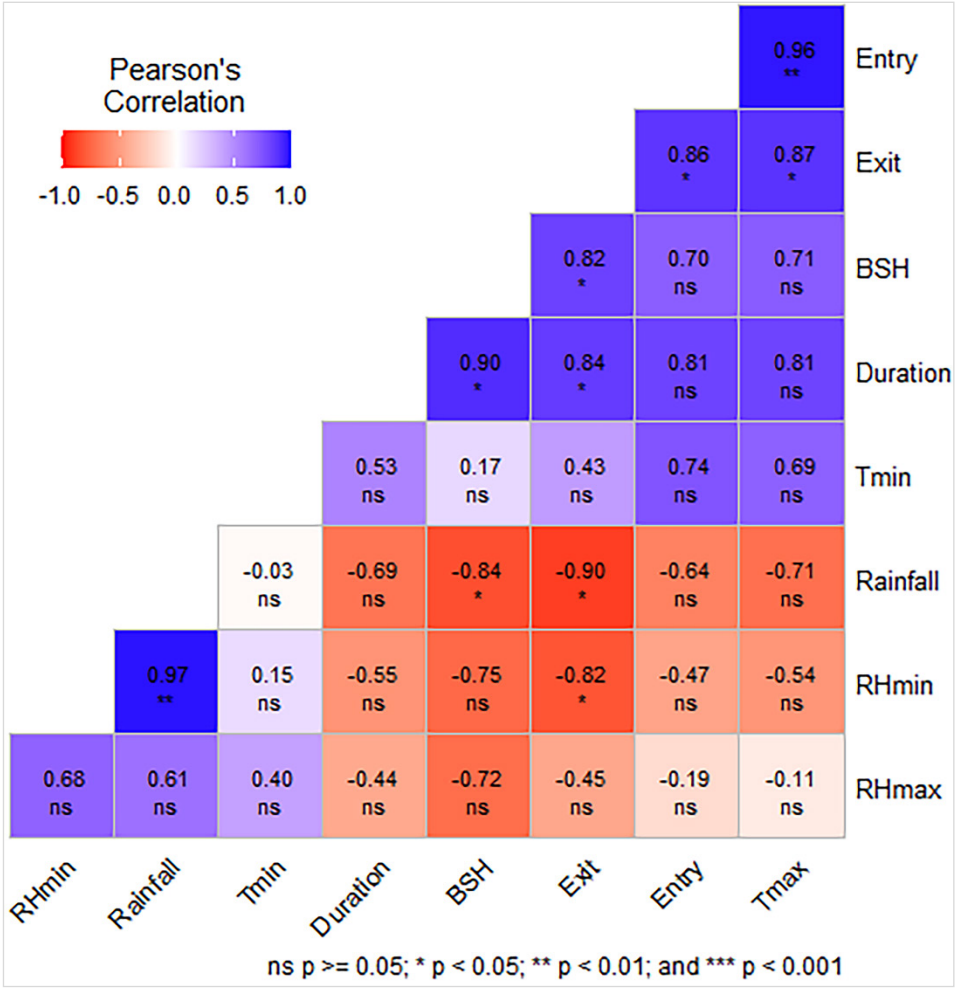

Fig 4. Pearson correlation matrix plot of the foraging duration and number of exit and entry/5 minute time period with weather parameters. Abbriviations stand for, Tmax: maximum temperature; Tmin: minimum temperature; RHmax: maximum relative humidity; RHmin: minimum relative humidity; and BSH: bright sunshine hours.

spent less time on each flower in the initial weeks than in the later weeks. In the case of the time variable, 11:00–13:00 hours was when the foragers spent the maximum time on each flower, i.e., 3.41 and 3.47 seconds/flower during 2019 and 2020, respectively. Their foraging speed was very low in the early morning and late evening hours.

Foraging rate

Here, the results of the ANOVA suggested a significant ($P \leq 0.05$) effect of only time on the foraging rate of *A. mellifera*. Date and date-time interaction did not significantly affect (Table 4). So, there was no significant variation in the mean foraging rate in different weeks. However, the foraging rate was highly variable with time. During the initial hour, the foraging rate was very low (i.e., 5.06 and 5.89 number of flowers visited/forager/minute during 2019 and 2020, respectively). After that, the foraging rate increased significantly, peaking at 13:00–15:00 hours (i.e., 17.72 and 18.06 flowers visited/forager/minute during 2019 and 2020, respectively). However, the foraging rate of *A. mellifera* foragers at 11:00–13:00 hours was slightly less than that of 09:00–11:00 and 13:00–15:00 hours.

Forager abundance

In the case of forager abundance, the ANOVA table suggested a significant ($P \leq 0.05$) effect of both date and time on the *A. mellifera* abundance, with a non-significant effect of date-time interaction on this parameter (Table 5). *A. mellifera* was found to be more abundant in the later weeks as compared to the initial weeks of observation. Fig 5 suggests that the total abundance of *A. mellifera* foragers was almost similar in both years. Their abundance was higher in the mid-hours (i.e., 11:00–13:00 hours), which was 10.28 and 10.22 foragers/m^2/minute during 2019 and 2020, respectively. Abundance was very low in the initial hours and evening hours of the day.

Palynological assessment

Foragers of *A. mellifera* were found to carry about 568,640 ± 15426.52 (mean ± SEM) numbers of pollen grains (APC) on their bodies during their peak foraging period on mustard fields. Analysis of pollen load suggested the presence of seven different pollen types (other than mustard) belonging to six different plant families. This pollen spectrum included pollen of *Ageratum conyzoides* L., *Amaranthus*/*Chenopodium* sp., *Brassica* sp., *Cajanus cajan* (L.), *Leucas aspera* (Willd.),

Table 4. Assessment of foraging rate (number of flowers visited/forager/minute) of *Apis mellifera* at different times and dates during the study period using two-factor factorial ANOVA and DNMRT *post-hoc* test (α = 0.05).

Date	2019 (N = 1280)						2020 (N = 1284)						Pooled (N = 2564)					
	Time					Mean	Time					Mean	Time					Mean
	07:00-09:00	09:00-11:00	11:00-13:00	13:00-15:00	15:00-17:00		07:00-09:00	09:00-11:00	11:00-13:00	13:00-15:00	15:00-17:00		07:00-09:00	09:00-11:00	11:00-13:00	13:00-15:00	15:00-17:00	
1st Week	2.67 (1.74)	17.00 (4.18)	15.33 (3.98)	18.00 (4.30)	14.67 (3.89)	13.53 (3.62)^{a}	3.67 (1.60)	19.33 (4.45)	16.00 (4.05)	19.33 (4.45)	14.33 (3.85)	14.53 (3.68)^{a}	3.17 (1.67)	18.17 (4.31)	15.67 (4.01)	18.67 (4.37)	14.50 (3.87)	14.03 (3.65)^{a}
2nd Week	5.33 (2.23)	17.00 (4.18)	15.67 (4.02)	18.00 (4.30)	15.67 (4.02)	14.33 (3.75)^{a}	3.33 (1.88)	16.33 (4.10)	14.00 (3.81)	16.67 (4.14)	17.00 (4.18)	13.47 (3.62)^{a}	4.33 (2.06)	16.67 (4.14)	14.83 (3.91)	17.33 (4.22)	16.33 (4.10)	13.90 (3.69)^{a}
3rd Week	7.33 (2.68)	17.00 (4.18)	13.00 (3.67)	17.00 (4.18)	15.33 (3.97)	13.93 (3.74)^{a}	4.67 (2.18)	16.33 (4.10)	13.33 (3.72)	16.00 (4.05)	16.33 (4.10)	13.33 (3.63)^{a}	6.00 (2.43)	16.67 (4.14)	13.17 (3.69)	16.50 (4.11)	15.83 (4.04)	13.63 (3.68)^{a}
4th Week	3.67 (2.00)	15.33 (3.97)	16.00 (4.06)	19.33 (4.45)	15.67 (4.01)	14.00 (3.70)^{a}	6.00 (2.41)	17.33 (4.22)	15.67 (4.02)	18.33 (4.34)	15.33 (3.97)	14.53 (3.79)^{a}	4.83 (2.21)	16.33 (4.09)	15.83 (4.04)	18.83 (4.40)	15.50 (3.99)	14.27 (3.74)^{a}
5th Week	4.67 (2.18)	18.67 (4.38)	16.67 (4.14)	15.67 (4.01)	16.67 (4.14)	14.47 (3.77)^{a}	7.00 (2.60)	15.67 (4.02)	13.67 (3.75)	19.33 (4.45)	16.67 (4.14)	14.47 (3.79)^{a}	5.83 (2.39)	17.17 (4.20)	15.17 (3.95)	17.50 (4.23)	16.67 (4.14)	14.47 (3.78)^{a}
6th Week	6.67 (2.53)	18.00 (4.30)	16.67 (4.14)	18.33 (4.34)	15.67 (4.02)	15.07 (3.87)^{a}	10.67 (3.15)	16.67 (4.14)	16.00 (4.06)	18.67 (4.37)	14.33 (3.85)	15.27 (3.91)^{a}	8.67 (2.84)	17.33 (4.22)	16.33 (4.10)	18.50 (4.35)	15.00 (3.93)	15.17 (3.89)^{a}
Mean	5.06 (2.23)^{b}	17.17 (4.20)^{ab}	15.56 (4.00)^{a}	17.72 (4.26)^{a}	15.61 (4.01)^{ab}	—	5.89 (2.30)^{c}	16.94 (4.17)^{ab}	14.78 (3.90)^{b}	18.06 (4.30)^{a}	15.67 (4.01)^{ab}	—	5.47 (2.27)^{c}	17.06 (4.18)^{ab}	15.17 (3.95)^{b}	17.89 (4.28)^{a}	15.64 (4.01)^{ab}	—

Parameters	Factors and interaction								
	Date	Time	Date × Time	Date	Time	Date × Time	Date	Time	Date × Time
SEM ±	0.110	0.101	0.247	0.140	0.127	0.312	0.111	0.101	0.248
CD	NA	0.285	NA	NA	0.360	NA	NA	0.286	NA
CV	11.440			14.456			11.474		

ANOVA

Source	df	Sum of square	Mean square	F value	P	df	Sum of square	Mean square	F value	P	df	Sum of square	Mean square	F value	P
Date	5	0.510	0.102	0.557	0.732	5	0.990	0.198	0.677	0.642	5	0.580	0.116	0.632	0.676
Time	4	52.400	13.099	71.656	<0.001	4	47.900	11.974	40.980	<0.001	4	50.000	12.499	67.980	<0.001
Date × Time	20	2.480	0.124	0.677	0.832	20	5.00	0.250	0.855	0.640	20	2.490	0.125	0.678	0.832
Error	60	10.970	0.183			60	17.530	0.292			60	11.030	0.184		

Note: Means suffixed with different letters are significantly different. Figures in the parenthesis are square root transformed values. N = total number of flowers encountered to be visited by the foragers observed during the respective year of study; SEM = Standard error of the mean; CD = Critical difference; CV = Coefficient of variation; NA = determination of CD is not applicable or not required as the effect is non-significant.

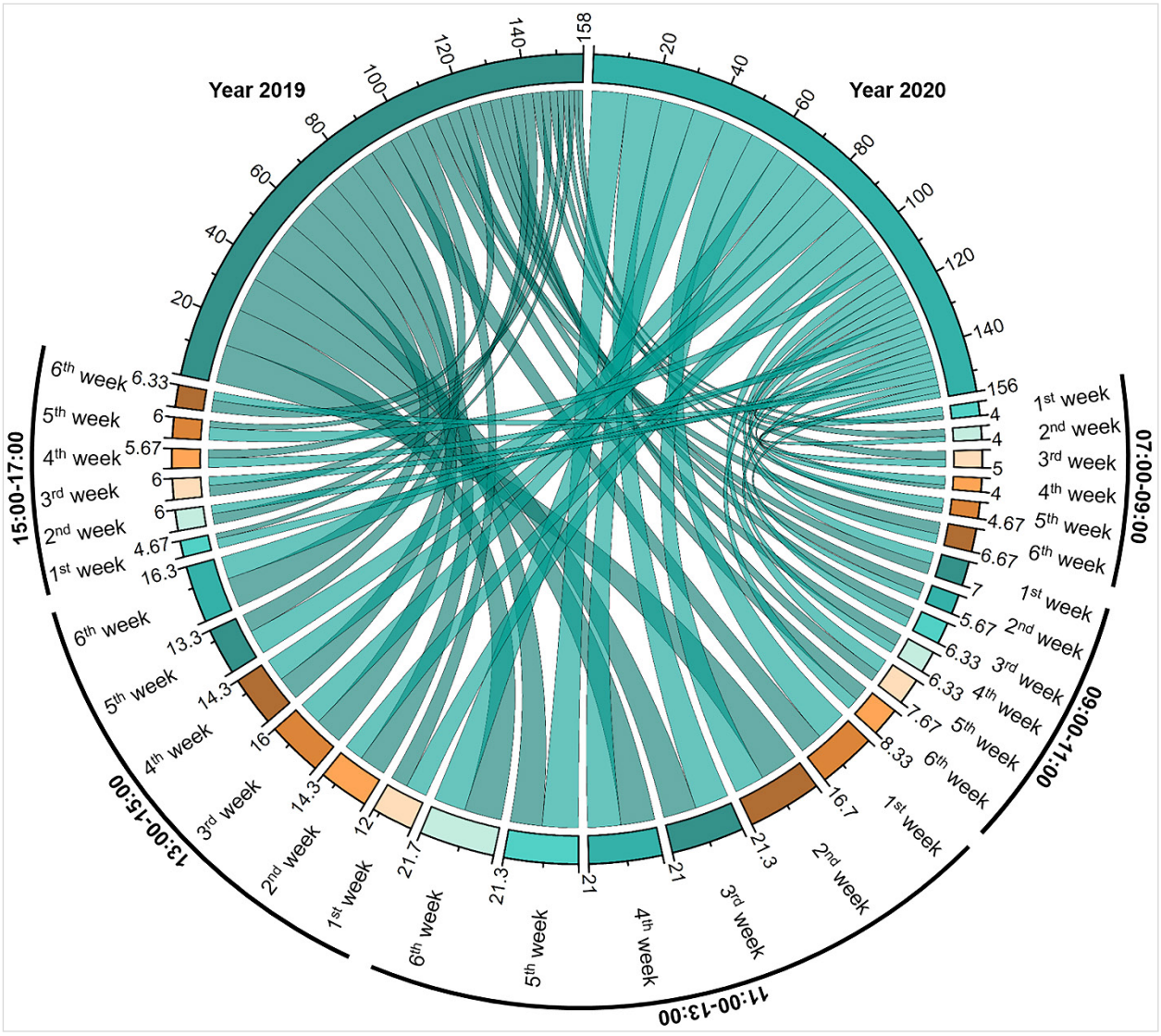

Fig 5. Chord diagram representing the abundance of *Apis mellifera* in the mustard ecosystem at different dates at different time intervals during 2019 and 2020.

Polygonaceae type, Solanaceae type, and *Spilanthes* sp. (Table 6). However, the fraction of this associated pollen taxa in the pollen load was found to be extremely low compared to the pollen of *Brassica* sp. In most cases, their frequency of occurrence falls into the minor group. The presence of honeydew elements in the pollen load was also very low, and the HDE/P ratio ranged between 0.000 and 0.008, indicating their presence as practically none. Photomicrographs of different pollen taxa with suitable magnification are presented in Fig 6.

Discussion

Foraging behavior

Various factors influence the foraging activity of honey bees, of which the need for pollen (Weidenmüller & Tautz, 2002) and the impact of the weather parameters (Riessberger & Crailsheim, 1997) are two important ones. That is why there are spatial and temporal variations in the foraging activity of honey bees (Abou-Shaara, 2014). In our present observation, we have recorded a comparatively short foraging duration of *A. mellifera* foragers during the mustard flowering season (January – mid-February) than the mean annual foraging duration [11.85 ± 1.79 hours/day (mean ± SD)] of *A. mellifera* in the region under consideration (Nath et al., 2023a). Usually, this region's blooming season of mustard is characterized by the prevalence of cool temperatures with short day lengths (Roy et al., 2018). Our correlation indicates a positive significant relation between BSH and foraging duration. Temperature was also found to have a positive relation with foraging duration. The low temperature might be the reason for honey bees to delay the initiation of their foraging activity and return to the hive much earlier during the mustard blooming season than the rest of the year, significantly reducing the foraging duration. Moreover, the last observation date is characterized by higher temperature and BSH, as shown in Fig 3. So, the combined effect of increased environmental temperature and BSH might be responsible for the increased foraging duration on the last observation date compared to the earlier dates.

Table 5. Assessment of *Apis mellifera* abundance (number of foragers/m²/minute) at different times and dates during the study period using two-factor factorial ANOVA and DNMRT *post-hoc* test (α = 0.05).

Date	2019 (N = 473)						2020 (N = 468)						Pooled (N = 941)					
	Time					Mean	Time					Mean	Time					Mean
	07:00-09:00	09:00-11:00	11:00-13:00	13:00-15:00	15:00-17:00		07:00-09:00	09:00-11:00	11:00-13:00	13:00-15:00	15:00-17:00		07:00-09:00	09:00-11:00	11:00-13:00	13:00-15:00	15:00-17:00	
1st Week	1.33 (1.34)	3.33 (1.94)	7.00 (2.74)	5.33 (2.42)	1.67 (1.46)	3.73 (1.98)^{d}	2.67 (1.74)	3.67 (2.02)	9.67 (3.18)	6.67 (2.67)	3.00 (1.86)	5.13 (2.29)^{bc}	2.00 (1.54)	3.50 (1.98)	8.33 (2.96)	6.00 (2.54)	2.33 (1.66)	4.43 (2.14)^{c}
2nd Week	2.33 (1.64)	3.33 (1.95)	12.00 (3.53)	8.33 (2.97)	3.33 (1.93)	5.87 (2.41)^{ab}	1.67 (1.46)	2.33 (1.68)	9.33 (3.12)	6.00 (2.55)	2.67 (1.76)	4.40 (2.11)^{c}	2.00 (1.55)	2.83 (1.82)	10.67 (3.32)	7.17 (2.76)	3.00 (1.85)	5.14 (2.26)^{bc}
3rd Week	2.67 (1.77)	3.33 (1.95)	10.33 (3.29)	8.67 (3.02)	3.33 (1.94)	5.67 (2.39)^{ab}	2.33 (1.64)	3.00 (1.86)	10.67 (3.34)	7.33 (2.80)	2.67 (1.77)	5.20 (2.28)^{bc}	2.50 (1.71)	3.17 (1.91)	10.50 (3.32)	8.00 (2.91)	3.00 (1.86)	5.44 (2.34)^{ab}
4th Week	1.33 (1.29)	3.67 (2.03)	10.67 (3.34)	7.33 (2.79)	2.67 (1.77)	5.13 (2.24)^{bc}	2.67 (1.77)	2.67 (1.77)	10.33 (3.28)	7.00 (2.73)	3.00 (1.86)	5.13 (2.28)^{bc}	2.00 (1.53)	3.17 (1.90)	10.50 (3.31)	7.17 (2.76)	2.83 (1.82)	5.13 (2.26)^{bc}
5th Week	1.67 (1.46)	3.33 (1.95)	10.00 (3.24)	5.67 (2.48)	2.00 (1.56)	4.53 (2.14)^{cd}	3.00 (1.86)	4.33 (2.20)	11.33 (3.44)	7.67 (2.86)	4.00 (2.11)	6.07 (2.49)^{a}	2.33 (1.66)	3.83 (2.08)	10.67 (3.34)	6.67 (2.67)	3.00 (1.84)	5.30 (2.32)^{ab}
6th Week	3.67 (2.04)	4.67 (2.27)	11.67 (3.48)	9.33 (3.11)	3.67 (2.02)	6.60 (2.58)^{a}	3.00 (1.86)	3.67 (2.04)	10.00 (3.24)	7.00 (2.74)	2.67 (1.77)	5.27 (2.33)^{ab}	3.33 (1.95)	4.17 (2.15)	10.83 (3.36)	8.17 (2.92)	3.17 (1.90)	5.94 (2.46)^{a}
Mean	2.17 (1.59)^{e}	3.61 (2.02)^{c}	10.28 (3.27)^{a}	7.44 (2.80)^{b}	2.78 (1.78)^{d}	—	2.56 (1.72)^{d}	3.28 (1.93)^{c}	10.22 (3.27)^{a}	6.94 (2.72)^{b}	3.00 (1.86)^{cd}	—	2.37 (1.66)^{e}	3.45 (1.97)^{c}	10.25 (3.27)^{a}	7.19 (2.76)^{b}	2.89 (1.82)^{d}	—

Parameters	Factors and interaction								
	Date	Time	Date × Time	Date	Time	Date × Time	Date	Time	Date × Time
SEM±	0.069	0.063	0.154	0.066	0.060	0.147	0.049	0.045	0.110
CD	0.195	0.178	NA	0.186	0.170	NA	0.139	0.127	NA
CV	11.631			11.091			8.267		

Source	ANOVA														
	df	Sum of square	Mean square	F value	P	df	Sum of square	Mean square	F value	P	df	Sum of square	Mean square	F value	P
Date	5	3.480	0.697	9.856	<0.001	5	1.110	0.222	3.410	0.009	5	0.840	0.167	4.605	0.001
Time	4	36.740	9.185	129.904	<0.001	4	32.200	8.049	123.752	<0.001	4	34.160	8.539	235.341	<0.001
Date × Time	20	1.220	0.061	0.866	0.628	20	0.430	0.021	0.327	0.996	20	0.540	0.027	0.742	0.767
Error	60	4.240	0.071			60	3.900	0.065			60	2.180	0.036		

Note: Means suffixed with different letters are significantly different. Figures in the parenthesis are square root transformed values. N = total number of foragers encountered while recording their abundance during the respective year of study; SEM = Standard error of mean; CD = Critical difference; CV = Coefficient of variation; NA = determination of CD is not applicable or not required as the effect is non-significant.

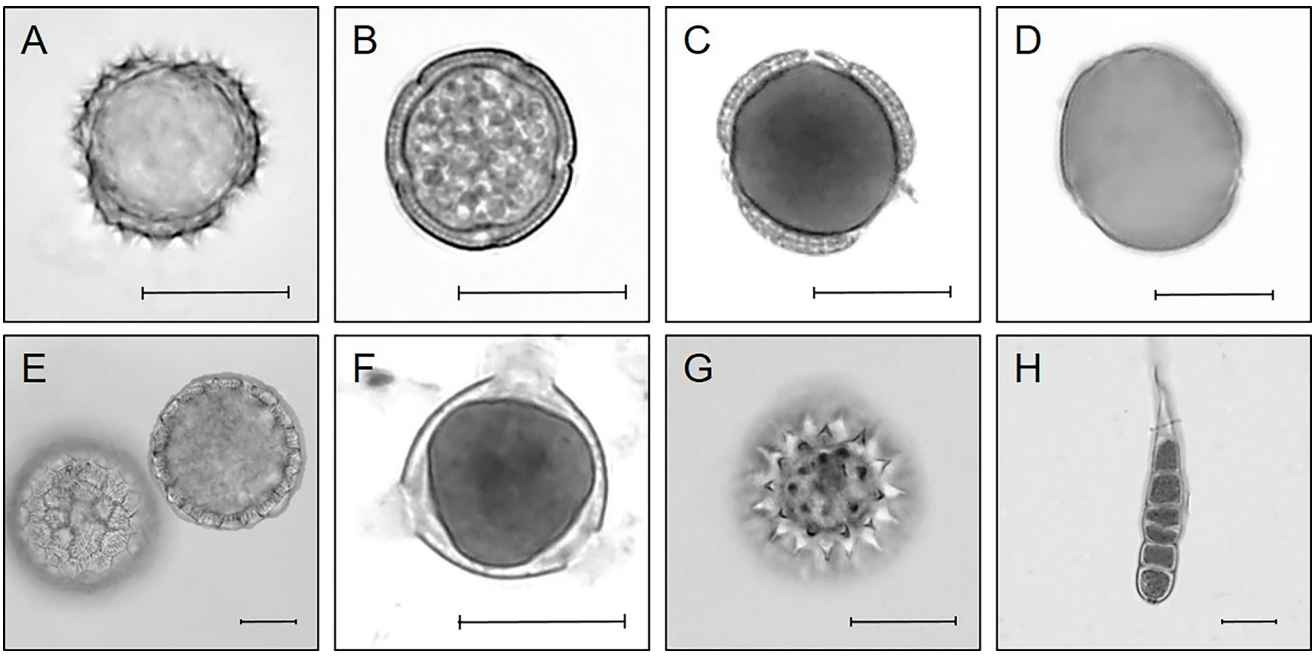

Fig 6. Photomicrographs of pollen types recovered from pollen load. **A:** *Ageratum conyzoides*. **B:** *Amaranthus*/*Chenopodium* sp. **C:** *Brassica* sp. **D:** *Leucas aspera*. **E:** Polygonaceae type. **F:** Solanaceae type. **G:** *Spilanthes* sp. **H:** Fungal pathogen. Scale bars – 10 µm.

Similar to foraging duration, foraging intensity is also influenced by weather parameters. This reason might be responsible for the highest foraging intensity in the last observation date. Rainfall and relative humidity were the factors harming foraging duration and intensity. Fig 3 depicts the occurrence of rainfall during the fifth observational date coupled with high relative humidity, low temperature, and BSH. These abiotic factors might be the reason for the least foraging duration and lesser activity of the foragers during this observational date, indicating that foragers prefer to stay within the hive instead of going out for foraging. So, weather factors significantly influence honey bees' foraging behavior despite their capacity to adapt to temperature changes through thermoregulation (Becsi et al., 2021; Barreiro et al., 2024). While comparing our data with different previously published literature (Abou-Shaara et al., 2013; Gebremedhn et al., 2014; Maity et al., 2014; Polatto et al., 2014), we found it was almost following and sometimes slightly varying from those. This variation might be due to the difference in the region, prevailing weather conditions, foraging sources, etc.

Regarding the temporal variation, the foragers were more active at mid-day hour (i.e., 12:00h) than the remaining times. An increase in environmental temperature during the mid-day hours might be the reason for this phenomenon. Apart from the environmental temperature, the time of anthesis might be another factor that also influences the foraging activity of the bee foragers (Kamaraj & Rasappan, 2024). Usually, during January-February, maximum anthesis of the mustard flower takes place between 10:00 to 12:00h (Devi et al., 2017), and honey bee foragers can remember the particular period of the day when the food resources are more abundant (Silva et al., 2013), which may correlate with their higher foraging activity. That could be another potential reason for the maximum foraging activity of *A. mellifera* foragers during mid-day hours, representing more food resource availability. These findings were corroborated by the results of Khan et al. (2021), as they also found the maximum exit and entry of honey bees from and into the hive at noon on *Brassica campestris* L.

Another thing that our result depicts is that the number of entries was always less than the number of exits, a clear indication that all the foragers were not able to return to the hive either due to the death of the foragers while foraging or their inability to find the route back to the hive because of different biotic and abiotic factors (Tan et al., 2013; Beck et al., 2023).

Here, we found the low foraging speed of *A. mellifera* foragers in the early morning hours, then a gradual increase, peaking at 11:00 – 13:00 hours, and again a decrease after that. This pattern of variation in the foraging speed of *A. mellifera* foragers on mustard was strongly supported by other researchers who have worked in the same field (Kunjwal et al., 2014; Nagpal et al., 2020). Environmental factors might be vital in this temporal variation in foraging speed. This foraging speed also varies depending on the plant species (Abou-Shaara, 2014). There might be a relation between foraging speed and foraging rate. Quite a lower foraging rate at 11:00–13:00 hours might be due to the higher time spent by the foragers on each flower during this time interval. The minimum foraging speed and rate at the initial hours can be explained as, during this period, the foragers did not visit more flowers. Instead, they used to hover in the air for more time. Here, our findings were similar to those of Singh et al. (2005), Kunjwal et al. (2014), Devi et al. (2016), and Poonam et al. (2022).

Table 6. Palynological assessment of pollen loads from *Apis mellifera* foragers.

Sample	APC	Average APC (Mean ± SEM)	Total pollen count/sample	HDE/P	Pollen taxa recovered	Number of pollen grains and their frequency (%)
S-1	643,200	568,640 ± 15,426.52	500	1/500 = 0.002	*Ageratum conyzoides* (Asteraceae)	9 (1.8) M
					Brassica sp. (Brassicaceae)	488 (97.6) P
					Cajanus cajan (Fabaceae)	3 (0.6) M
S-2	608,000			0/500 = 0.000	*Ageratum conyzoides* (Asteraceae)	4 (0.8) M
					Amaranthus/*Chenopodium* sp. (Amaranthaceae/ Chenopodiaceae)	2 (0.4) M
					Brassica sp. (Brassicaceae)	490 (98.0) P
					Leucas aspera (Lamiaceae)	4 (0.8) M
S-3	582,400			0/500 = 0.000	*Brassica* sp. (Brassicaceae)	500 (100.0) P
S-4	558,400			2/500 = 0.004	*Ageratum conyzoides* (Asteraceae)	7 (1.4) M
					Brassica sp. (Brassicaceae)	476 (95.2) P
					Solanaceae type	16 (3.2) I
					Spilanthes sp. (Asteraceae)	1 (0.2) M
S-5	576,000			1/500 = 0.002	*Brassica* sp. (Brassicaceae)	492 (98.4) P
					Cajanus cajan (Fabaceae)	2 (0.04) M
					Leucas aspera (Lamiaceae)	6 (1.2) M
S-6	494,400			1/500 = 0.002	*Brassica* sp. (Brassicaceae)	485 (97.0) P
					Polygonaceae type	15 (3.0) I
					Brassica sp. (Brassicaceae)	488 (97.6) P
S-7	628,800			0/500 = 0.000	Polygonaceae type	8 (1.6) M
					Spilanthes sp. (Asteraceae)	4 (0.8) M
S-8	552,000			4/500 = 0.008	*Brassica* sp. (Brassicaceae)	500 (100.0) P
S-9	513,600			0/500 = 0.000	*Brassica* sp. (Brassicaceae)	496 (99.2) P
					Leucas aspera (Lamiaceae)	4 (0.8) M
S-10	529,600			2/500 = 0.004	*Ageratum conyzoides* (Asteraceae)	4 (0.8) M
					Brassica sp. (Brassicaceae)	483 (96.6) P
					Polygonaceae type	13 (2.6) M

Note: P = Predominant pollen. I = Important minor pollen. M = Minor pollen. S = Sample. APC = Absolute pollen count. HDE = Honey dew element. P = total pollen count/sample.

The lower abundance of *A. mellifera* in the early morning and late evening hours might also be due to the prevalence of cool temperature, which increases after that (maximum at 11:00 – 13:00 hours) with increasing temperature at mid-day hours. Moreover, as mentioned earlier, the foraging intensity of *A. mellifera* foragers was also high during the middle hours of the day. That means more foragers came out to forage at this time, increasing the abundance. Our previous study found a higher abundance of *A. mellifera* on litchi in this region at 09:00 – 11:00 hours (Nath et al., 2023b). This variation with the present study might be due to the differences in the prevailing weather conditions, where litchi blooms in the March – April months (spring season) when the atmospheric temperature is relatively higher than the mustard blooming months. That also might be the reason for their abundance to be increased with passing dates. These findings of the present study follow the findings of other researchers, who also have reported the highest abundance of *A. mellifera* on rapeseed mustard within 10:00 – 13:00 hours (Goswami & Khan, 2014; Devi et al., 2016; Vishwakarma & Chand, 2017; Singh et al., 2018; Poonam et al., 2022).

Palynological assessment

We found the *A. mellifera* foragers to carry about 568,640 ± 15426.52 pollen grains (APC) on the body during their peak foraging period, i.e., at the mid-day hours on mustard fields. Usually, the heaviest pollen loads are collected by *A. mellifera* foragers at mid-day (García-García et al., 2004). However, the amount of pollen the foragers carry also varies according to the plant species they visit (García-García et al., 2004). Usually, pollen size and stickiness govern the amount of pollen to be carried, whereas the smaller pollen grains can be more easily compacted and thus cause a heavier pollen load (Villalobos et al., n.d.). The size of *Brassica* pollen is moderately smaller (Amina et al., 2020; Saha & Begum, 2020), which might be attributed to more pollen grains carried by the *A. mellifera* foragers.

Our palynological study showed the occurrence of other pollen types in the pollen load, indicating the presence of these plants in the vicinity of the mustard field during the study period, and the *A. mellifera* foragers might also visit these plants. Moreover, the presence of these pollen in the pollen load might be due to contamination. Different authors have also reported such contamination (Silici & Gökceoglu, 2007; Forcone et al., 2009, 2011; Durán-Escalante et al., 2023; Saha et al., 2023a). Actually, at the time of foraging, pollen from these plants might get adhered to the body of the foragers due to the electrostatic charge of the body hairs (Vaknin et al., 2000; Zakon, 2016) and hence be detected in the pollen load. The presence of different associated pollen taxa in the pollen load at extremely low quantities as compared to the pollen of *Brassica* sp. might be due to the floral fidelity of the honey bees (Sanderson & Wells, 2005; Roman & Kulik, 2006) and in most cases, these other pollen categories fall into the minor group. Most of these associated pollen types were represented by weed pollen. The importance of weed flora in bee foraging has already been well documented (Bretagnolle & Gaba, 2015; Rollin et al., 2016; Saha et al., 2023b). Moreover, different weeds have already been reported as important bee forages in this region (Saha et al., 2023a).

A low HDE/P ratio indicates that the incidence of mustard crop diseases might be low in this region, or the mustard plantations might face a good amount of fungicide application. Previously, Mukhopadhyay et al. (2007) also found such a low HDE/P ratio in the honey samples of Sub-Himalayan West Bengal. Similarly, a low HDE/P ratio was also noted in the mustard honey samples from this region (Saha et al., 2023a).

Conclusion

Mustard highly depends on insect pollinators for pollination and fruit setting, and *A. mellifera* is one of the most important pollinators. From the study, it is clear that the foraging activity of *A. mellifera* is strongly correlated with weather factors. Among different weather parameters, rainfall is highly detrimental to the forager's activity, whereas the temperature positively influences their activity. So, from this aspect, we can conclude that during the rainy season, special care of the colonies (by providing supplementary food) needs to be taken to maintain colony strength. During the summer and spring seasons, when ample to high temperatures prevail, in addition to diverse bee forages, supering needs to be done to harvest a surplus amount of honey.

Moreover, the foraging behavior of the *A. mellifera* foragers varies during different periods of the day. It is the mid-hours of the day when the activity of the foragers is at its maximum in the mustard fields. So, the adoption of any pesticidal chemical measure during this active foraging period will result in their mortality, which will indirectly reduce the crop yield. Instead, chemicals with very short residual toxicity may be applied in extreme need in the early morning or late evening hours when their activity is relatively low. That will reduce the chance of chemical exposure to the foragers and ensure efficient pollination of mustard, giving a higher yield. Palynological assessment of pollen load suggests that even after foraging on mustard fields, foragers may visit other flowering plants, or pollen from other plants may adhere to the body of the foragers. This diverse pollen source will serve as an additional source of nutrition for the bees, as pollen from different plants differs in its nutritional composition. Moreover, different weed pollen also contributed to that pollen spectrum, indicating the importance of weeds in honey bee foraging.

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Declaration of Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Data Availability Statement

The raw data are available with the corresponding author and can be available upon request.

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