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Influence of Pollen Trapping on Growth of *Apis mellifera* Linneaus, 1758 Colony Under Mustard Flowering Season

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ABSTRACT

Bee pollen has diverse applications beyond human consumption and bee supplement. Its economic advantages may encourage beekeepers to adopt frequent pollen trapping, yet its impacts on brood rearing remains poorly understood. This study aimed to standardize pollen trapping using front-mounted traps of 50% efficiency on *Apis mellifera* L. colonies. The effects of different trapping frequencies on pollen load collection, pollen foraging, and brood production were evaluated in 12-frame colonies across two apiaries in Hisar and Kaul, Haryana, India during the mustard flowering seasons of 2017 and 2018. Five experimental groups each with three colonies were established in both apiaries and mounted with traps based on pollen trapping frequencies *viz.* daily, alternate day, third day, weekly and control (no

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trapping) with respective duration of 42, 21, 14, 7 and 0 days. Daily trapping yielded maximum pollen load collection (0.56 Kg/colony at Hisar and 1.89 Kg/ colony at Kaul) but reduced brood area by 27.3% at Hisar and retarded brood expansion at Kaul. Conversely, weekly-trapped and control colonies, exhibited larger brood area, indicating a strong negative correlation between trapping frequency and brood area. Mustard season at Kaul exhibited greater pollen diversity (0.70-1.42) and higher protein content (24.3-30.2%). In both locations, pollen foraging peaked between 10:00 AM and 2:00 PM. Therefore, adopting intermittent pollen trapping or restricting daily trapping to peak foraging hours (10:00 AM- 2:00 PM) could be viable strategies to balance pollen collection with colony health.

Keywords: Bee nutrition, Foraging, Trap efficiency, Brood area, Honey flow season, Pollen load.

INTRODUCTION

Brood rearing in honeybee colonies depend upon the availability of pollen, which is a crucial source of protein, vitamins, minerals, free amino acids, and lipids (Mattila & Otis, 2005; Thakur & Nanda, 2020). Worker bees feed these pollen loads to nurse brood, develop pharyngeal glands, and secrete royal jelly (Keller, Fluri, & Imdorf, 2005; Bryś, Skowronek, & Strachecka, 2021). An *Apis mellifera* L. colony of nearly 15,000 bees requires approximately 13-18 kg of pollen annually to meet its needs (Avni, Hendriksma, Dag, Uni, & Shafir, 2014). Foraging bees groom and pack pollen grains into loads, which they deposit in hive cells to make bee bread (Anderson et al, 2014). Crude protein of pollen which was recorded 2.3% in *Cupressus arizonica* and 61.7% in *Dodecatheon clevelandii*, can significantly influence brood rearing (Roulston, Cane, & Buchmann, 2000). Protein-deficient pollen loads (less than 20%) can affect brood rearing activity, leading to poor development and potential colony loss (Naug, 2009). The crude protein range (23-30%) of pollen loads is considered optimum for successful brood rearing (Herbert, Shimanuki, & Caron, 1977; Corby-Harris, Snyder, Meador, & Ayote, 2018; Taha, Al-Kahtani, & Taha, 2019). Continuous brood rearing ensures a robust supply of in-hive and foraging worker bee force, essential for the colony's strength and various vital functions (Ismail, Owayss, Mohanny, & Salem, 2012). Previous study has confirmed that pollen diet can also improve bee lifespan, metabolism, and immunity (Li et al., 2019). Pollen loads collected with traps have multiple uses, including human consumption, cosmetics, skincare products and poultry feed (Haščík et al., 2017; Kurek-Górecka, Górecki, Rzepecka-Stojko, Balwierz, & Stojko, 2020; Paray et al., 2021; Topal et al., 2022). Bee-collected pollen is superior to pollen collected directly from plants due to its nutritive benefits and therapeutic effects, making it a rich proteinaceous diet consumed worldwide (Wright, Nicolson, & Shafir, 2018; Thakur & Nanda, 2020). Additionally, pollen loads are supplemented in bee diets to mitigate food shortages and maintain colony growth during extreme pollen dearth periods (Hoover, Ovinge, & Kearns, 2022). This practice is preferred over feeding pollen substitutes, which can have detrimental effects on the colony (Vaudo, Tooker, Grozinger, & Patch, 2015; Topal et al., 2022). However, excessive pollen trapping can cause shortages of stored pollen within the colony, adversely affecting colony growth (Ovinge & Hoover, 2018). Pollen trapped colonies produce less amount of royal jelly than trap-free colonies (Mohanny, Aslam, & Shahira, 2022). Shortages in stored pollen due to trapping and abrupt weather conditions also induce cannibalism of bee larvae, thus reducing the

overall brood area (Schmikl & Crailsheim, 2001). Furthermore, pollen-trapped colonies may experience relapses of chronic bee paralysis virus, leading to significant adult bee mortality (Dubois, Reis, Schurr, Cougoule, & Ribière-Chabert, 2018).

The pollen traps of varied designs are attached either inside or outside of the colony entrance (front mounted) depending on the climatic conditions of the surrounding area. The available flora also influences the efficiency of pollen traps (Levin & Loper, 1984; Goodwin & Perry, 1992). While bottom-mounted pollen traps are more efficient than front-mounted and plastic slide traps (Mohamed, Ali, & Ghazala, 2022), the use of front-mounted traps remains prevalent in India and other parts of Asia (Mahmood et al., 2017; Taha, Al-Kahtani, & Taha, 2019; Naveen, Yadav, & Singh, 2024; Rout, Srinivasan, Saminathan Suganthi, & Geetha, 2023). Pollen trapping efficiency varies from 3 to 70 percent across trap designs and flowering seasons (Levin & Loper, 1984; Goodwin & Perry, 1992; Keller et al., 2005; Taha, Al-Kahtani, & Taha, 2019). Numerous factors such as bee size, size and number of circular holes, pollen load size, and flowering seasons contribute to this variation (Levin & loper, 1984; Hoover & Ovinge, 2018). However, the efficiency of front-mounted traps remained in the range of 25-28 per cent (Ismail, Owayss, Mohanny, & Salem, 2013; Taha, Al-Kahtani, & Taha, 2019; Omar & Amro, 2023).

The maximum pollen foraging activity and pollen load collection in *A. mellifera* colonies during mustard flowering were observed between 10:00 AM and 2:00 PM, identifying this four-hour window as the most suitable time for pollen trapping (Mahmood et al., 2017). However, the impact of pollen trapping on brood production remains inconclusive, with studies reporting both negligible and adverse effects (McLellan, 1974; Waller, Caron, & loper, 1981; Webster, Thorp, Briggs, Skinner, & Parisian, 1985; Duff & Furgula, 1986; Nelson, McKenna, & Zumwalt, 1987; Pidek, 1988; Ismail, et al., 2012). Given the economic benefits of bee pollen, beekeepers might be inclined towards intensive pollen trapping, which is potentially detrimental to brood rearing. Therefore, it is crucial to optimize pollen trapping regimes to collect pollen loads without significantly impacting brood rearing. Considering these factors, this study was conducted to standardize pollen trapping methods with the dual objective of maximizing benefits for beekeepers while minimizing colony losses. The investigation specifically aimed to mitigate the negative effects of pollen traps on brood development.

MATERIALS AND METHODS

Experimental sites and period of study

Apiaries were established at two different locations in Haryana, India during the mustard (*Brassica* spp.) flowering seasons, involving a total of 30 high-strength Langstroth *A. mellifera* colonies (15 colonies per location). Each colony contained 12 bee-covered frames. 15 colonies at each location were divided into 5 treatment groups, each consisting of 3 colonies (5 groups × 3 colonies = 15 colonies per location). Each colony within a group acted as a replication. Five distinct treatment groups were pollen trapping frequencies *viz*., daily, alternate days, third day, weekly and control (No trapping). Three replicate colonies in the daily group, labelled as 'D,' were fitted with three-piece plastic pollen traps at the colony

entrances on a daily basis (Fig. 2). Similarly, colonies in the alternate-day, third-day, weekly, and control (no pollen trapping) groups were labelled as 'AD,' 'TD,' 'W,' and 'C,' respectively, and were subjected to pollen trapping according to their specific trapping schedules. The trapping durations for the daily, alternate-day, third-day, weekly, and control groups were 42, 21, 14, 7, and 0 days, respectively, during the mustard flowering season. This study was performed for 47 days during mustard flowering season at both locations, from January 10 to February 25, in the years 2017 (location 1) and 2018 (location 2). Location 1 was situated at Ram Dhan Singh Seed Farm, CCS Haryana Agricultural University (CCSHAU), Hisar (29.2447°N, 75.7209°E; 215 m above mean sea level), while location 2 was located at the College of Agriculture, CCSHAU, Kaul, Haryana, India (29.8498°N, 76.6615°E; 237 m above mean sea level). The two locations were 150 km apart. For clarity, the mustard flowering seasons at the two locations are referred to as location-1 (Hisar) and location-2 (Kaul), respectively. The mustard crop was cultivated approximately 200 m away from the apiary sites at both locations. The colonies at both locations were placed according to randomized block design. During both years, the recommended agricultural practices from CCS Haryana Agricultural University were followed for mustard crop production (Anonymous, 2022), excluding the application of pesticides. Fig. 1 illustrates the experimental setup and the geographical locations of the study sites.

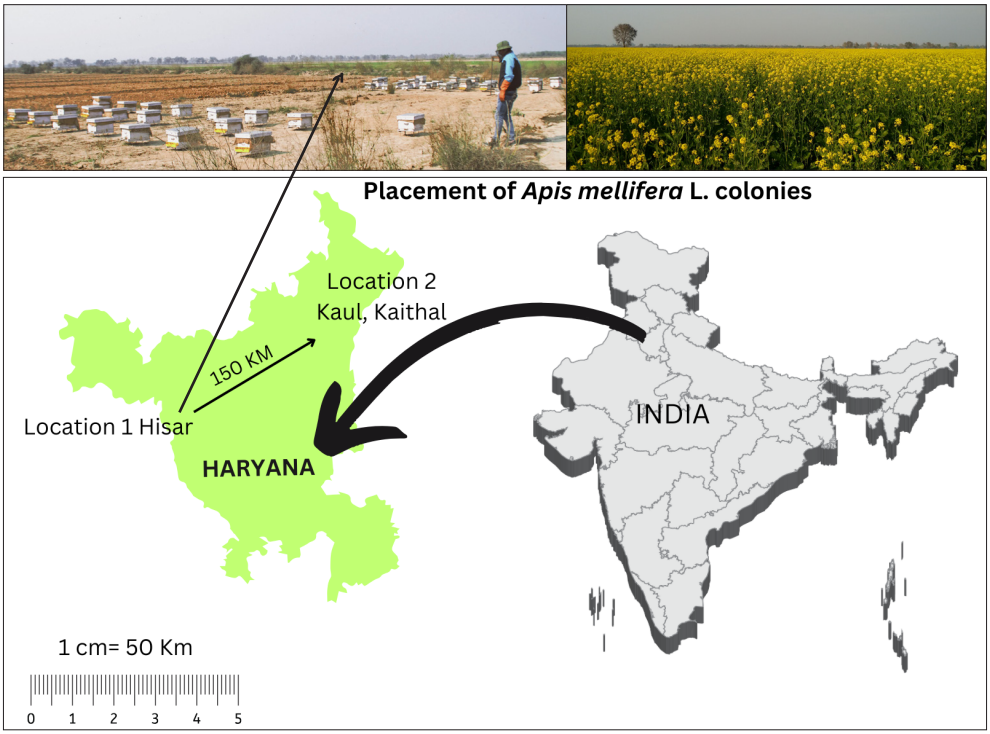

Figure 1. Mapped view of placement of *A. mellifera* colonies.

Preparation of *A. mellifera* colonies for the experiment

To prepare the *A. mellifera* colonies for the experiment, 10 frames were placed in the brood chamber of each high-strength colony, while the remaining two frames were kept inside the super, positioned above the brood chamber. Prior to the start of the experiment, all colonies were standardized for queen age, worker population, stored honey and nectar area, stored pollen area, and brood area (both capped and open brood, including eggs) at the onset of the flowering seasons, following the standard protocol by Delaplane, Van Der Steen, & Guzman-Novoa (2013).

Pollen trap design, its efficiency and analysis of pollen loads

The three piece front-mounted plastic pollen trap exhibited a total of 225 holes on grid for passage of foraging bees and tray for collection of pollen loads. The length and breadth of pollen trap was 39.0 and 3.7 cm, respectively. Each hole had the diameter of 5 mm. All pollen traps used in the entire study at both locations were identical in size, design, and other specifications (Fig. 2). The efficiency of pollen traps were determined by counting the total number of pollen loads falling in the tray of traps from 100 pollen loads carrying bees (with 200 pollen loads) entering the colony through front mounted pollen traps. The pollen trap efficiency was calculated according to equation described by Ismail et al. (2013) and Omar & Amro (2023).

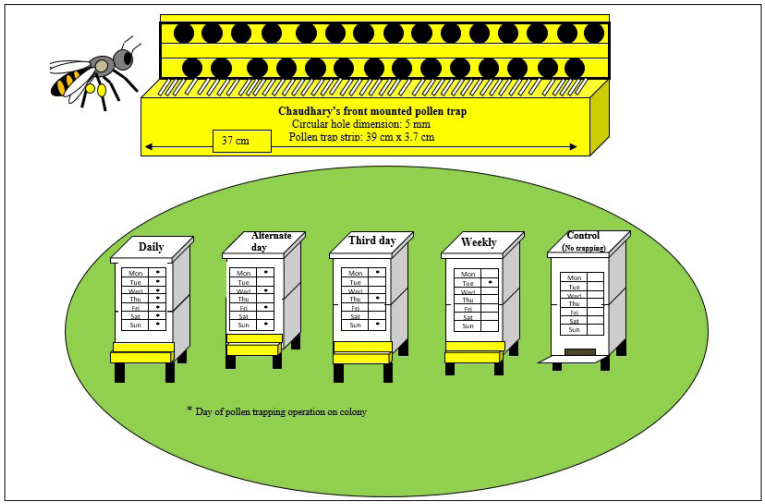

Figure 2. Graphical illustration of pollen trap design and colonies with different frequencies of pollen trapping.

$$\text{Pollen trap efficiency}\,(\%) = \frac{\text{Number of pollen pellets in the tray of trap}}{200} \text{X}100$$

Applying the above equation, the efficiency of the trap was calculated as 50 per cent. Pollen trapping was suspended on days with precipitation. From 8 a.m. to 6 p.m.(10 hours), traps were affixed to their respective colonies according to the trapping frequency. The pollen reference slides from collected and segregated pollen loads were prepared at the Post Graduate Lab, Department of Entomology, CCSHAU, Hisar. The pollen load samples

were also submitted to the Central Bee Research and Training Institute, KVIC (CBRTI), Pune for confirmation of their botanical origin. Estimation of crude protein content (%) of collected pollen loads was performed at Avon Food Labs Pvt. Ltd., Delhi, India.

Materials used for observations

Colony growth parameters (CGP) measuring grid frame was constructed from a typical deep Langstroth wooden frame with holes drilled every inch along all four bars. A coloured plastic wire was crisscrossed through these holes to create a square cell measuring one inch². This counting frame contained a total of 112 square cells arranged in 16 columns and seven rows. During colony inspection, the CGP measuring frame was superimposed over frames of treatment colonies to record the area covered under brood (including open brood, capped brood, and eggs). A Magnus MLX microscope with 10x magnification was used to observe the prepared glass slides from collected pollen loads. To weigh the pollen loads collected from the experimental colonies, a battery-powered, pocket-sized (10x10x2 cm), high-sensitivity (0.01-200 g) digital weighing balance was used.

Experimental Details

Recording of Observations

To investigate the effect of varied pollen trapping frequencies on brood area of colony, the following observations were recorded from the experimental group colonies.

Quantity of Collected Pollen Loads and Their Segregation

Pollen traps were removed from all experimental group colonies at dusk, and the trapped pollen loads were collected in plastic beakers (85 x 65 mm) and weighed using the digital balance. Weekly average of quantity of trapped pollen loads from specific pollen trapping frequency (treatment) in addition to overall quantity of pollen loads trapped for the whole season were recorded. The following day, pollen loads were initially dried under morning sunlight (before 10:00 a.m.) and stored in plastic zip pouches (20x15 cm) in a refrigerator (4°C). Monthly stocks of collected pollen loads were maintained. Five replicates of 10 g samples of pollen loads (n=5) were taken from these monthly lots and segregated based on their colour (Dimou & Thrasyvoulou, 2007). The segregated pollen loads were then weighed to determine the proportion of each coloured pollen type and subjected to three procedures: preparation of reference slides (Louveaux, Maurizio, & Vorwohl, 1978), evaluation of the Shannon-Weiner diversity index, and estimation of crude protein content (Shannon & Weiner, 1949; Liolios et al, 2015).

Melissopalynological analysis of pollen loads

Pollen reference slides were prepared from different coloured pollen loads and bee foraging flowering plants in the vicinity of the apiary setup, which were viewed under the microscope at 10x magnification. Both slides were matched to confirm the botanical origin of these pollen loads.

Determination of Diversity of Collected Pollen Loads

The segregated pollen loads based on colour were weighed, and the diversity of pollen load types was determined for each month of flowering seasons of both locations using the Shannon-Weiner diversity index equation 1 (Shannon & Weiner, 1949). High value of H' indicates greater pollen diversity.

$$H' = -\sum_{i}^{n} p_i \, ln_i \, p_i \quad (1)$$

Where H' is Shannon-Weiner diversity index, p_i is the proportion of each pollen type i found in the 10 g sample, ln is natural logarithm, n is the number of pollen species.

Crude protein content of pollen loads

Pollen loads from each month of the mustard flowering seasons at both locations were collected. 1 g sample of bee pollen loads was digested with 20 ml of H_2SO_4 (95-97%) for 4 hours until the solution turned dark blue. The digested sample was then mixed with 90 ml of NaOH (30%) and distilled for 2 minutes using 30 ml of H_3BO_4 solution (4%), followed by titration with HCl solution (0.1M). The amount of HCl (0.1M) required for titration determined the nitrogen content. Crude protein content (%) was then estimated using the equation 2 described by Rabie, Wellis, & Dent (1983):

Crude Protein(%)=Nitrogen content × 5.60 (2)

Colony Inspections to Record Growth Parameters

During both mustard locations, four colony inspections were performed using a CGP measurement frame. Brood area (sum of areas of eggs, uncapped brood, and capped brood), were recorded from all treatment group colonies in $inch^2$ units. Then, these areas were converted into cm^2 by multiplying with a factor of 6.45 (1 $inch^2$= 6.45 cm^2). The first inspection began prior to mounting pollen traps on day 0 and continued at fortnight intervals (15 days) after mounting traps until the 45th day (fourth inspection). The net area of brood produced or lost in a colony (cm^2/colony) under different pollen trapping frequencies at different locations was evaluated in cm^2 using equation 3:

Net brood area in a colony = Brood area on 0 day - Brood area on 45 day (3)

Statistical analysis

All the data analyses were performed using SPSS version 23.0 software. The effects of pollen trapping frequency, weeks, and their interaction on quantity of pollen loads and number of pollen foragers were evaluated using two-way ANOVA with Fisher's LSD post hoc test ($p<0.05$). Similarly, brood area variation due to trapping frequency, 15-day colony inspection intervals, and their interaction was also assessed using two-way ANOVA with Fisher's LSD test ($p<0.05$). Differences in the pollen load diversity, measured via the Shannon-Weiner index and crude protein content of pollen loads between mustard flowering months were tested using one-way ANOVA with

Tukey's HSD ($p<0.05$). Additionally, overall pollen load collection and net brood area per trapping frequency was measured, along with correlation analysis to examine relationships among pollen trapping frequency, pollen loads collection, number of pollen foragers, net brood area, and weather parameters.

RESULTS

Pollen load collections from *A. mellifera* colonies subjected to different pollen trapping frequencies

Quantity of pollen load collection per colony per day was evaluated across trapping frequencies and weeks at both locations. In Hisar (Location 1), the highest pollen collection (13.3 g/colony/day) was observed during the fourth week of the mustard flowering season (Table 1; $F_{6,68}$= 5.54, P<0.001). In Kaul (Location 2), the maximum yield (30.2 g/colony/day) was recorded in the sixth week (Table 2; $F_{6,68}$ = 3.071, P = 0.01). Across pollen trapping frequencies in location 1, daily, alternate-day, and third-day trapping trapped statistically similar quantity of pollen loads, all significantly higher than weekly trapping ($F_{4,68}$ = 13.19, P<0.001). Notably, daily trapping at location 2 resulted in the maximum pollen load yield, averaging 37.1 g/colony/day which was significantly higher than alternate day, third day and weekly trapping ($F_{4,68}$ = 18.09, P = 0.001). In addition, a non-significant interaction effect was recorded between pollen trapping frequencies and weeks in both locations, which indicate similar variation in pollen load collection in colonies subjected to different pollen trapping frequencies in different weeks of mustard flowering season, which is likely to be influenced by different meteorological parametres.

Table 1. Effect of pollen trapping frequency on pollen load collection (mean±standard error) from 12-frame *A. mellifera* colony during mustard flowering season at location 1 (Hisar, Haryana) in 2017.

Pollen load collection (g/colony/day) (n= 3 bee colonies per PTF)						
Pollen Trapping Frequency (PTF)→ Weeks↓	**Daily (42)***	**Alternate Day (21)**	**Third Day (14)**	**Weekly (7)**	**Control (0)**	**Week Mean**
Week 1 (11-17 Jan)	1.3±0.7	2.9±0.7	0.5±0.2	0.0±0.0	0.0±0.0	0.9±0.2c
Week 2(18-24 Jan)	14.9±5.8	17.4±4.7	8.7±4.0	5.7±3.6	0.0±0.0	9.3±2.9a
Week 3(25-30 Jan)	19.5±7.6	19.7±7.9	15.5±8.3	8.4±7.0	0.0±0.0	12.6±4.5a
Week 4(31 Jan-06 Feb)	22.4±5.1	19.0±5.3	14.6±9.8	10.4±8.8	0.0±0.0	13.3±4.5a
Week 5 (07-13 Feb)	19.9±3.3	17.8±6.4	13.5±6.9	2.5±1.3	0.0±0.0	10.7±1.1a
Week 6 (14-20 Feb)	11.1±3.9	14.5±4.2	14.4±7.2	3.2±1.8	0.0±0.0	8.6±1.6ab
Week 7 (21 Feb-28 Feb)	4.8±1.9	4.0±1.7	7.7±4.5	2.5±1.6	0.0±0.0	3.8±0.9bc
PTF Mean	13.4±3.1A	13.6±3.3A	10.7±5.5A	4.7±3.3B	0.0±0.0C	
ANOVA						
	PTF		Week		Interaction (PTF×Week)	
F-Value	13.19		5.54		0.73	
DF	4,68		6,68		24,68	
p	0.000		0.000		0.804	
C.D.	4.63		5.48		N.S.	

*Values in the parenthesis indicates number of days pollen traps mounted on colonies; Means followed by different uppercase letters along row (PTF means) are significantly different as per Fisher' LSD post hoc test ($p<0.05$); Means followed by different lowercase letters along column (week mean) are significantly different as per Fisher' LSD post hoc test ($p<0.05$).

Table 2. Effect of pollen trapping frequency on pollen load collection (mean±standard error) from 12-frame *A. mellifera* colony during mustard flowering season at location 2 (Kaul, Haryana) in 2018.

Pollen load collection (g/colony/day) (n= 3 bee colonies per PTF)						
Pollen Trapping Frequency (PTF)→ Weeks↓	**Daily (42)***	**Alternate Day (21)**	**Third Day (14)**	**Weekly (7)**	**Control (0)**	**Week Mean**
Week 1 (11-17 Jan)	22.3±13.5	8.4±0.4	12.6±3.6	12.4±11.4	0.0±0.0	11.2±3.1c
Week 2(18-24 Jan)	39.6±16.6	27.0±0.8	32.8±4.9	21.0±11.6	0.0±0.0	24.1±5.6ab
Week 3(25-30 Jan)	34.2±8.1	27.5±0.8	22.0±8.4	10.2±4.0	0.0±0.0	18.8±1.5bc
Week 4(31 Jan-06 Feb)	31.0±3.8	23.4±2.4	11.3±3.6	24.5±9.8	0.0±0.0	18.1±1.8bc
Week 5 (07-13 Feb)	44.2±7.8	32.2±0.9	17.6±6.9	46.3±18.3	0.0±0.0	28.1±4.0ab
Week 6 (14-20 Feb)	62.0±19.1	26.3±0.6	30.6±12.4	32.1±12.2	0.0±0.0	30.2±3.3a
Week 7 (21 Feb-28 Feb)	26.7±7.1	17.4±1.1	37.1±16.0	16.4±9.1	0.0±0.0	19.5±0.8bc
PTF Mean	37.1±8.5A	23.2±0.7B	23.4±7.0B	23.3±9.3B	0.0±0.0C	
ANOVA						
	PTF		Week		Interaction (PTF×Week)	
F-Value	18.092		3.071		1.037	
DF	4,68		6,68		24,68	
p	0.001		0.01		0.436	
C.D.	8.89		10.53		N.S.	

*Values in the parenthesis indicates number of days pollen traps mounted on colonies; Means followed by different uppercase letters along row (PTF means) are significantly different as per Fisher' LSD post hoc test ($p<0.05$); Means followed by different lowercase letters along column (week mean) are significantly different as per Fisher' LSD post hoc test ($p<0.05$).

In terms of overall quantity of pollen loads collected during the mustard flowering season, in location 1 (Fig. 3), maximum quantity were collected from colonies under daily trapping (0.56 Kg/colony) followed by alternate day trapping (0.31Kg/colony). Similarly in location 2, the overall pollen collection (Fig. 3), were substantially higher in daily trapping (1.89±0.34 Kg/colony) followed by alternate day trapping (0.39±0.08 Kg/colony). The segregation analysis of trapped pollen loads revealed *Brassica campestris* as the most predominant pollen load type under both mustard location 1 (69.0-88.4%) and location 2 (46.43-79.73%) (Table 3). However, most diverse (Shannon-Weiner diversity index-1.42±0.3) pollen loads (6-7 species of pollen loads) were collected during February 2018 at location 2 (Table 3), and least diverse (0.42±0.1) during January 2017 at location 1 ($F_{3,16}$=31.3; $p<0.05$). Pollen loads of location 1 exhibited 22.4-23.0% (Table 3) crude protein, which was significantly less than the corresponding values (24.3-30.2%) recorded under location 2 ($F_{3,16}$=107.6; $p<0.05$).

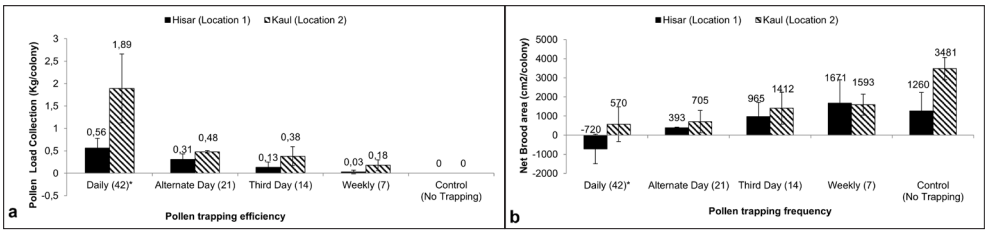

Figure 3. Effect of pollen trapping frequency on A) overall pollen loads collected and B) net brood area developed during mustard flowering seasons at two locations in Haryana, India.

*Values in the parenthesis indicates number of days pollen traps mounted on colonies.

Table 3. Diversity and crude protein content of collected pollen loads under different mustard flowering months at different locations.

Mustard location	Month and year	Pollen types	Proportion of pollen loads (%)	Shannon-Weiner diversity index (H')[Mean±SD]	Crude protein% [Mean±SD] of collected pollen loads
Mustard location 1	Jan-2017	*Brassica campestris*	88.40	0.42±0.1a	22.4±0.5a
		Asphodelus tenuifoleus	10.00		
		Pisum sativum	0.75		
		Eucalyptus sp.	0.85		
	Feb-2017	*Brassica campestris*	69.00	0.91±0.2b	23.0±0.5a
		Asphodelus tenuifoleus	14.78		
		Pisum sativum	13.32		
		Eucalyptus sp.	2.90		
Mustard location 2	Jan-2018	*Brassica campestris*	79.73	0.70±0.1b	24.3±0.5b
		Eucalyptus sp.	10.8		
		Ageratum conyzoides	7.98		
		Brassica campestris and *Eucalyptus* sp. (Bifloral load)	0.00		
		Cicer arietinum and *Eucalyptus* sp.(Bifloral Load)	2.18		
		Apiaceace	0.00		
		Sida acuta	0.00		
	Feb-2018	*Brassica campestris*	46.43	1.42±0.3c	30.2±1.3c
		Eucalyptus sp.	24.68		
		Ageratum conyzoides	16.50		
		Brassica campestris and *Eucalyptus* sp. (Bifloral load)	4.8		
		Cicer arietinum and *Eucalyptus* sp. (Bifloral Load)	3.65		
		Apiaceace	1.65		
		Sida acuta	3.00		
F-value				31.3	107.6
DF				3,16	3,16
p				0.000	0.000

Means within columns followed by different letters are significantly different as per Tukey's HSD post hoc test ($p<0.05$).

Effect of pollen trapping frequencies on brood area of *A. mellifera* colonies under different mustard locations

The variation in brood area affected by different pollen trapping frequencies at different fortnight intervals in two locations is presented in tables 4-5. Mean brood area at both locations expanded gradually along the mustard flowering season ($F_{3,38}$=21.287, P=0.000 location 1; $F_{3,38}$=10.981, P=0.000 Location 2). However, wide variation in expansion of brood area was recorded in colonies subjected to different pollen trapping frequencies. In location 1, all the colonies irrespective of pollen trapping frequencies showed expansion of brood area till first fortnight (15 day; Table 4) however, thereafter considerable reduction in brood area was recorded in daily trapped colonies causing it to fall even below its initial levels (-720 cm^2/colony; Fig. 3; Table 4). In contrast, weekly trapped colonies showed consistent expansion of brood area resulting in maximum gain in net brood area (1671 cm^2/colony) over a mustard flowering season ($F_{4,48}$=6.058, P=0.001). Similarly at location 2 (Table 5), gradual increase in brood area was recorded from all the colonies subjected to different pollen trapping frequencies however net brood area (Fig. 3) occupied by daily trapped colonies

was fairly less (570 cm^2/colony) in comparison to weekly (1593 cm^2/colony) and control colonies (3481 cm^2/colony). Secondly, a significant interaction effect between pollen trapping frequency and colony inspection on brood area indicate that brood area expansion under different trapping frequency varies with colony inspection whereas, non-significant interaction effect was recorded for corresponding values at location 2. Thus, it become evident that irrespective of location, colonies under daily pollen trapping suffered significant reduction in brood area whereas, colonies subjected to intermittent trapping exhibited larger brood area. A strong inverse correlation (r = -0.950) was also established between net brood area and trapping frequency at location 1 (Table 11). Daily trapped colonies at location 2 exhibited a gain in brood area (570±768 cm^2/colony), while those at location 1 experienced net losses.

Table 4. Effect of pollen trapping frequency on brood area (mean±standard error) of the 12-Frame *A. mellifera* colony during mustard flowering season at location 1 (Hisar, Haryana) in 2017.

Brood area (cm^2/colony) (n= 3 bee colonies per PTF)						
Pollen Trapping Frequency (PTF)→ Inspection↓	**Daily (42)***	**Alternate Day (21)**	**Third Day (14)**	**Weekly (7)**	**Control (0)**	**Inspection Mean**
0 day (Pre-trapping)	2,632±93	2,326±30	2,047±34	2,083±212	1,959±245	2,209±70a
15 days	2,791±93	2,928±91	2,485±59	3,040±117	2,589±59	2,767±34b
30 days	2,408±35	3,178±35	2,874±56	3,053±360	3,208±229	2,944±110b
45 days	1,911±29	2,720±48	3,012±72	3,754±420	3,219±153	2,923±108b
PTF Mean	2,435±48C	2,788±29AB	2,605±41BC	2,983±248B	2,743±98B	
ANOVA						
	PTF		Inspection		Interaction (PTF×Inspection)	
F-Value	6.058		21.287		6.361	
DF	4,38		3,38		12,38	
p	0.001		0.000		0.000	
C.D.	239.3		214.0		478.6	

*Values in the parenthesis indicates number of days pollen traps mounted on colonies; Means followed by different uppercase letters along row (PTF means) are significantly different as per Fisher' LSD post hoc test ($p<0.05$); Means followed by different lowercase letters along column (Inspection mean) are significantly different as per Fisher' LSD post hoc test ($p<0.05$).

Table 5. Effect of pollen trapping frequency on brood area (mean±standard error) of the 12-Frame *A. mellifera* colony during mustard flowering season at location 2 (Kaul, Haryana) in 2018.

Brood area (cm^2/colony) (n= 3 bee colonies per PTF)						
Pollen Trapping Frequency (PTF)→ Inspection↓	**Daily (42)***	**Alternate Day (21)**	**Third Day(14)**	**Weekly (7)**	**Control (0)**	**Inspection Mean**
0 day (Pre-trapping)	2,533±1437	2,546±155	1,954±1036	2,767±1846	1,810±701	2,322±278c
15 days	2,090±1448	2,774±40	2,739±1045	3,272±1352	2,814±341	2,738±230bc
30 days	2,812±346	3,064±133	3,038±946	3,668±1308	3,399±259	3,196±79b
45 days	3,102±673	3,251±156	3,367±322	4,360±1023	5,291±1622	3,874±273a
PTF Mean	2,634±251C	2,908±46ABC	2,775±251BC	3,517±774A	3,329±415AB	
ANOVA						
	PTF		Inspection		Interaction (PTF×Inspection)	
F-Value	2.795		10.981		1.278	
DF	4,38		3,38		12,38	
p	0.04		0.000		0.271	
C.D.	644.9		576.8		N.S.	

*Values in the parenthesis indicates number of days pollen traps mounted on colonies; Means followed by different uppercase letters along row (PTF means) are significantly different as per Fisher' LSD post hoc test ($p<0.05$); Means followed by different lowercase letters along column (inspection mean) are significantly different as per Fisher' LSD post hoc test ($p<0.05$).

Effect of pollen trapping frequencies on number of pollen foragers entering the *A. mellifera* colonies

The present study has also studied the role of pollen trapping on number of pollen foragers entering the *A. mellifera* colonies for both locations during different dates of the mustard season and time of the day. Higher number of pollen foragers entering trap equipped colonies implies increased preference of foragers towards pollen foraging driven by less stored pollen in the colony, which in turn raises the quantity of pollen load collection in the tray of the trap. The results revealed significant variations caused by pollen trapping on the number of pollen foragers entering the colonies ($F_{4,78}$=6.611; P=0.000). In location 1, significantly less number of pollen foragers was recorded in weekly trapped colonies (5.4±0.8 pollen foragers/ 2 min) in relation to daily (9.8±1.28 pollen foragers/ 2 min) and alternate day trapped colonies (9.5±1.4 pollen foragers/ 2 min). Similarly, in location 2, maximum number of pollen foragers entered the daily trapped colonies (24.9±6.5 pollen foragers/ 2 min) followed by alternate day, which was considerably higher than the corresponding numbers recorded from control colonies (13.4±1.1 pollen foragers/ 2 min). Thus it can be inferred from the above results of both locations that the frequent placement of traps either daily or alternate day on colony entrance increases the pollen foraging impulse in the worker bees. With reference to the time of day during mustard flowering season at location 1, higher number of pollen foragers (16.4-10.3 pollen foragers/ 2 min) were observed entering colonies during 10:00 am to 2:00 pm whereas in location 2, high number of pollen foragers entered colonies during 10:00 am to 4:00 pm with peak numbers recorded during 12:00 pm (35.9±5.1 pollen foragers/ 2 min). In addition, during the mustard flowering season in location 1, number of pollen foragers entering colonies increased significantly from 5.1±0.8 pollen foragers/ 2 min on 11 Jan 2017 to 11.0 pollen foragers/ 2 min on 7 Feb-2017 whereas, in location 2, higher number of pollen foragers was witnessed entering the colonies from 25 Jan 2018 onwards with maximum numbers recorded on 14 Feb 2018 (32.7±4.1 pollen foragers/ 2 min).

Table 6. Effect of pollen trapping frequency on number of pollen foragers (mean±standard error) entering 12-Frame *A. mellifera* colonies during a day of mustard flowering season at location 1 (Hisar, Haryana) in 2017.

Number of pollen foragers/2min (n= 3 bee colonies per PTF)						
Pollen Trapping Frequency (PTF) → Time↓	**Daily (42)****	**Alternate Day (21)**	**Third Day (14)**	**Weekly (7)**	**Control (0)**	**Time Mean**
8:00 AM	1.3±0.2 (1.5)*	0.8±0.1 (1.3)	1.3±0.5 (1.5)	1.0±0.4 (1.4)	1.1±0.2 (1.5)	1.1±0.3d (1.4)
10:00 AM	18.8±3.1 (4.4)	17.8±4.3 (4.3)	10.8±5.2 (3.2)	10.7±1.5 (3.4)	23.7±4.0 (4.9)	16.4±2.7a (4.0)
12:00 AM	22.2±2.6 (4.8)	21.2±2.8 (4.7)	15.9±6.7 (3.9)	11.2±2.3 (3.5)	22.3±4.0 (4.8)	18.6±2.3a (4.3)
2:00 PM	11.5±1.8 (3.5)	11.4±1.3 (3.5)	11.6±3.4 (3.5)	5.8±0.9 (2.6)	11.3±0.2 (3.5)	10.3±1.2b (3.3)
4:00 PM	3.3±0.5 (2.1)	4.4±0.8 (2.3)	6.1±2.4 (2.6)	2.6±0.8 (1.9)	4.1±0.6 (2.2)	4.1±0.4c (2.2)
6:00 PM	1.6±0.4 (1.6)	1.4±0.2 (1.6)	2.7±1.4 (1.8)	1.2±0.3 (1.5)	0.8±0.2 (1.3)	1.6±0.3cd (1.6)
PTF Mean	9.8±1.2A (3.0)	9.5±1.4A (2.9)	8.1±3.2A (2.7)	5.4±0.8B (2.4)	10.6±1.3A (3.0)	

table continued	ANOVA		
	PTF	Time	Interaction (PTF×Time)
F-Value	4.51	78.03	1.46
DF	4,58	5,58	20,58
p	0.003	0.000	0.133
C.D.	(0.367)	(0.402)	N.S.

*Values in parentheses are square root transformed values; **Values in the parenthesis indicates number of days pollen traps mounted on colonies; Means followed by different uppercase letters along row (PTF means) are significantly different as per Fisher' LSD post hoc test (p<0.05); Means followed by different lowercase letters along column (Time mean) are significantly different as per Fisher' LSD post hoc test (p<0.05).

Table 7. Effect of pollen trapping frequency on number of pollen foragers (mean±standard error) entering 12-Frame *A. mellifera* colonies during mustard flowering season at location 1 (Hisar, Haryana) in 2017.

Number of pollen foragers/ 2min (n= 3 bee colonies per PTF)						
Pollen Trapping Frequency (PTF)→ Dates↓	**Daily (42)****	**Alternate Day (21)**	**Third Day (14)**	**Weekly (7)**	**Control (0)**	**Date Mean**
11-Jan	3.5±0.6 (2.1)*	8.1±1.9 (3.0)	2.9±0.3 (2.0)	1.1±1.0 (1.4)	9.9±1.2 (3.3)	5.1±0.9c (2.3)
18-Jan	11.1±5.6 (3.2)	14.7±6.3 (3.8)	9.3±3.0 (3.1)	0.5±0.2 (1.2)	9.5±1.8 (3.2)	9.0±1.9ab (2.9)
25-Jan	14.3±0.8 (3.9)	7.8±1.3 (3.0)	10.1±2.5 (3.3)	2.6±1.7 (1.8)	15.2±3.9 (4.0)	10.0±1.5ab (3.2)
1-Feb	7.2±0.8 (2.9)	7.0±2.2 (2.8)	6.1±3.6 (2.4)	3.7±2.2 (2.0)	12.5±2.0 (3.7)	7.3±2.1bc (2.8)
7-Feb	14.3±3.3 (3.9)	13.2±2.5 (3.7)	7.4±4.0 (2.7)	6.4±2.6 (2.6)	13.5±1.9 (3.8)	11.0±2.0a (3.3)
14-Feb	8.1±1.3 (3.0)	13.3±2.9 (3.7)	12.9±6.2 (3.4)	9.2±6.3 (2.9)	8.3±0.7 (3.0)	10.4±0.7ab (3.2)
21-Feb	8.0±1.9 (3.0)	15.4±2.2 (2.5)	8.7±4.0 (2.9)	11.8±5.8 (3.2)	7.3±0.6 (2.9)	8.2±2.5abc (2.9)
28-Feb	11.8±0.3 (3.6)	6.3±2.7 (2.6)	7.1±3.8 (2.6)	8.1±3.9 (2.9)	8.3±1.4 (3.0)	8.3±0.5abc (2.9)
PTF Mean	9.8±1.2A (3.2)	9.5±1.4A (3.1)	8.1±3.2AB (2.8)	5.4±0.8B (2.3)	10.6±1.3A (3.4)	
ANOVA						
	PTF		Dates		Interaction (PTF×Date)	
F-Value	6.611		2.141		1.237	
DF	4,78		7,78		28,78	
p	0.000		0.049		0.23	
C.D.	(0.477)		(0.603)		N.S.	

*Values in parenthesis are square root transformed values; **Values in the parenthesis indicates number of days pollen traps mounted on colonies; Means followed by different uppercase letters along row (PTF means) are significantly different as per Fisher' LSD post hoc test (p<0.05); Means followed by different lowercase letters along column (week mean) are significantly different as per Fisher' LSD post hoc test (p<0.05); N.S. means Non-significant.

Table 8. Effect of pollen trapping frequency on number of pollen foragers (mean±standard error) entering 12-Frame *A. mellifera* colonies during a day of mustard flowering season at location 2 (Kaul, Haryana) in 2018.

Number of pollen foragers/2 min (n= 3 bee colonies per PTF)						
Pollen Trapping Frequency (PTF) → Time↓	**Daily (42)****	**Alternate Day (21)**	**Third Day (14)**	**Weekly (7)**	**Control (0)**	**Time Mean**
8:00 AM	4.2±1.5 (2.2)*	4.4±0.5 (2.3)	1.8±0.6 (1.6)	5.4±2.2 (2.5)	1.9±0.2 (1.7)	3.6±0.5d (2.1)
10:00 AM	28.9±7.7 (5.4)	21.8±2.6 (4.8)	20.0±6.1 (4.5)	27.6±9.5 (5.2)	18.2±1.8 (4.4)	23.3±3.7bc (4.8)
12:00 AM	44.0±13.8 (6.5)	36.7±2.7 (6.1)	33.3±7.3 (5.8)	35.5±12.5 (5.9)	30.1±2.6 (5.6)	35.9±5.1a (6.0)
2:00 PM	40.7±13.4 (6.3)	35.2±2.0 (6.0)	30.3±7.5 (5.5)	22.0±7.7 (4.7)	17.0±1.3 (4.2)	29.0±3.2ab (5.3)
4:00 PM	24.8±9.5 (4.9)	19.3±1.3 (4.5)	18.0±5.6 (4.2)	14.6±4.6 (3.9)	10.0±0.6 (3.3)	17.3±1.8c (4.2)
6:00 PM	6.7±3.7 (2.6)	5.0±0.7 (2.4)	5.6±1.8 (2.5)	4.1±1.1 (2.2)	3.3±0.6 (2.1) (2.1)	4.9±0.7d (2.4)
PTF Mean	24.9±8.1A (4.7)	20.4±1.5AB (4.4)	18.2±4.5BC (4.0)	18.2±6.2BC (4.0)	13.4±1.1C (3.5)	
ANOVA						
	PTF		Time		Interaction (PTF×Time)	
F-Value	3.85		46.82		0.45	
DF	4,58		5,58		20,58	
p	0.008		0.000		0.974	
C.D.	(0.603)		(0.661)		N.S.	

*Values in parenthesis are square root transformed values; **Values in the parenthesis indicates number of days pollen traps mounted on colonies; Means followed by different uppercase letters along row (PTF means) are significantly different as per Fisher' LSD post hoc test (p<0.05); Means followed by different lowercase letters along column (time mean) are significantly different as per Fisher' LSD post hoc test (p<0.05); N.S. means Non-significant.

Table 9. Effect of pollen trapping frequency on number of pollen foragers (mean±standard error) entering 12-Frame *A. mellifera* colonies during mustard flowering season at location 2 (Kaul, Haryana) in 2018.

Number of pollen foragers/ 2min (n= 3 bee colonies per PTF)						
Pollen Trapping Frequency (PTF)→ Date↓	**Daily (42)****	**Alternate Day (21)**	**Third Day (14)**	**Weekly (7)**	**Control (0)**	**Date Mean**
11-Jan	8.8±2.0 (3.1)*	8.2±1.2 (3.0)	15.2±10.4 (3.5)	6.1±5.2 (2.3)	6.2±0.4 (2.7)	8.9±3.4d (2.9)
18-Jan	15.7±2.0 (4.1)	11.7±0.3 (3.6)	9.3±0.8 (3.2)	10.3±7.0 (3.0)	9.4±0.6 (3.2)	11.3±1.8cd (3.4)
25-Jan	35.6±6.3 (6.0)	25.9±2.2 (5.2)	18.7±5.4 (4.3)	21.2±11.8 (4.3)	16.1±1.8 (4.1)	23.5±3.1b (4.8)
1-Feb	20.3±3.5 (4.6)	21.0±0.7 (4.7)	18.8±7.3 (4.2)	18.1±6.0 (4.2)	12.7±1.3 (3.7)	18.2±0.6bc (4.3)
7-Feb	20.0±5.4 (4.5)	13.6±3.0 (3.8)	14.2±5.3 (3.7)	18.0±6.4 (4.2)	14.3±1.7 (3.9)	16.0±1.5bcd (4.0)
14-Feb	52.3±22.2 (7.0)	36.4±1.7 (6.1)	26.7±9.0 (5.1)	31.0±5.0 (5.6)	17.0±1.2 (4.2)	32.7±4.1a (5.6)
21-Feb	22.8±3.0 (4.9)	27.5±1.9 (5.3)	20.0±10.8 (4.2)	21.0±5.0 (4.6)	13.1±1.4 (3.7)	20.9±1.4b (4.5)
28-Feb	23.5±9.9 (4.8)	18.5±3.5 (4.4)	22.1±11.7 (4.4)	19.7±7.0 (4.4)	18.4±3.3 (4.4)	20.5±2.2b (4.5)
PTF Mean	24.9±6.5A (4.9)	20.4±1.5A (4.5)	18.1±4.5AB (4.1)	18.2±6.2AB (4.1)	13.4±1.1B (3.7)	
ANOVA						
	PTF		Date		Interaction (PTF×Date)	
F-Value	3.233		7.457		0.4	
DF	4,78		7,78		28,78	
p	0.017		0.000		0.996	
C.D.	(0.677)		(0.856)		N.S.	

*Values in parenthesis are square root transformed values; **Values in the parenthesis indicates number of days pollen traps mounted on colonies; Means followed by different uppercase letters along row (PTF means) are significantly different as per Fisher' LSD post hoc test (p<0.05); Means followed by different lowercase letters along column (date mean) are significantly different as per Fisher' LSD post hoc test (p<0.05); N.S. means Non-significant.

Relationships between pollen trapping, pollen collection, brood area, pollen foragers and weather parameters

Pollen trapping in daily, alternate day, third day, weekly and control was implemented for 42, 21, 14, 7 and 0 days, respectively. This in turn caused variation in the tested colony growth parameters in this study. Thus, a need was felt to understand the relationship between these parameters along with meteorological data through correlation analysis (Table 11 and 12). It was revealed that pollen trapping frequency at location 1, exhibit a significantly positive correlation with quantity of pollen loads collected (r=0.987; p<0.01) and negative correlation with net brood area (r=-0.950; p<0.05). Even pollen load collection showed significantly negative correlation with net brood area (r=-0.978; p<0.05). Similarly, in location 2, pollen trapping frequency demonstrated significantly positive relationship with pollen loads collected (r=0.966; p<0.01) and number of pollen foragers (r=0.964; p<0.01) whereas, non-significant negative correlation with net brood area (r=-0.809). Furthermore, net brood area demonstrated significantly negative correlation with number of pollen foragers (r=-0.912; p<0.05). Therefore, it can be inferred from the above results that frequent pollen trapping for long duration that collect large quantity of pollen loads from entry of high number of pollen foragers can cause significant reduction in brood area of *A. mellifera* colonies. On the contrary, no definite correlation was established between meteorological parameters and pollen load collection except with minimum temperature which showed moderate correlation in location 1 (r=0.616) whereas, with morning relative humidity it demonstrated moderate correlation in both location (Table 12).

Table 10. Meteorological parameters during mustard flowering season at location 1 and 2 in Haryana 2017.

Meteorological parameters during mustard flowering season at location 1 (Hisar) in 2017						
Flowering Period	**Max Temp ºC**	**Min Temp ºC**	**RH (%) Morning**	**RH (%) Evening**	**Bright Sunshine hours**	**Wind speed Km/hr**
Week 1 (10-17 Jan)	17.1	2.6	95.1	61.4	6.1	2.9
Week 2(18-24 Jan)	18.5	5.4	100.0	68.0	5.1	2.2
Week 3(25-31 Jan)	18.7	10.3	98.5	82.0	2.1	4.6
Week 4(01-07 Feb)	22.3	9.1	95.9	60.3	5.6	3.7
Week 5 (08-15 Feb)	21.6	4.8	92.7	48.6	8.7	2.0
Week 6 (16-22 Feb)	26.5	10.2	91.1	43.9	7.2	2.1
Week 7 (23 Feb-01 Mar)	26.3	7.3	89.3	35.1	9.3	2.8
Meteorological parameters during Mustard flowering season at location 2 (Kaul) in 2018						
Flowering Period	**Max Temp ºC**	**Min Temp ºC**	**RH (%) Morning**	**RH (%) Evening**	**Bright Sunshine hours**	**Wind speed Km/hr**
Week 1 (10-17 Jan)	24.1	5.0	95.3	51.7	7.0	5.0
Week 2(18-24 Jan)	21.6	3.8	97.1	60.6	7.3	3.0
Week 3(25-31 Jan)	16.7	6.6	95.7	74.7	3.6	6.0
Week 4(01-07 Feb)	22.1	6.7	91.6	52.6	7.7	3.8
Week 5 (08-15 Feb)	20.9	4.5	97.9	54.6	6.7	3.6
Week 6 (16-22 Feb)	21.2	7.0	96.4	60.4	8.1	5.4
Week 7 (23 Feb-01 Mar)	25.4	10.1	95.0	60.4	6.8	3.2

Table 11. Correlation analysis of pollen trapping frequencies with pollen load collection and colony growth parameters in two locations.

Correlation coefficient (r) at location 1 (Hisar, 2017) (n= 5)				
	Pollen Trapping Frequency	**Pollen load collection**	**Net Brood area**	**Number of pollen foragers**
Pollen Trapping Frequency	1	0.987**	-0.950*	0.252
Pollen load collection		1	-0.978**	0.373
Net Brood area			1	-0.542
Number of pollen foragers				1
Correlation coefficient (r) at location 1 (Kaul, 2018) (n= 5)				
Pollen Trapping Frequency	1	0.966**	-0.809	0.964**
Pollen load collection		1	-0.659	0.903*
Net Brood area			1	-0.912*
Number of pollen foragers				1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Table 12. Correlation analysis of pollen load collection and number of pollen foragers with meteorological parameters in two locations.

Correlation coefficient (r) at location 1 (Hisar, 2017) (n= 7)								
	Pollen load collection	**Number of Pollen Foragers**	**Max Temp (ºC)**	**Min Temp (ºC)**	**Morning RH (%)**	**Evening RH (%)**	**Wind Speed (Km/hr)**	**Bright Sunshine Hours**
Pollen load collection (g/colony/day)	1	0.595	0.015	0.616	0.406	0.408	0.331	-0.433
Number of Pollen Foragers/2 min		1	0.368	0.468	-0.108	-0.090	0.058	-0.214
Correlation coefficient (r) at location 2 (Kaul, 2018) (n= 7)								
Pollen load collection (g/colony/day)	1	0.548	0.059	-0.106	0.551	0.128	-0.138	0.268
Number of Pollen Foragers/2 min		1	-0.088	0.539	-0.018	0.461	0.473	-0.042

Table 13. An overview of different pollen trap designs, their efficiencies and their effect on brood of *A. mellifera.*

Sr. No.	Type of pollen trap	Flowering season	Efficiency of pollen trap	Country	Quantity of pollen loads collected	Effect on brood development	References
1.	Bottom drawer OAC pollen trap	Saguaro cacti and *Cereus giganteus* at two different locations	Location 1-33.6% Location 2- 60.1%	USA	Location 1-32.3 g/ colony/day Location 2-126.1g/colony/day	=*	Levin & Loper (1984)
2.	Bottom drawer OAC pollen trap	NA	NA	USA	Full time trapping 1st year-40.8 g/colony/day Full time trapping 2nd year- 52.5 g/colony/day	-ve	Duff & Furgula (1986)
3.	Bottom drawer OAC pollen trap	NA	NA	Canada	9.4 Kg/colony/season	=	Nelson, McKenna, & Zumwalt (1987)
4.	Front mounted pollen trap	Kiwi fruit	0-25%	New Zealand	NA	NA	Goodwin & Perry (1992)
5.	Front mounted pollen trap	Clover and Cotton	28.0%	Egypt	NA	-ve	Ismail, Owayss, Mohanny, & Salem (2012)
8.	Front Drawer pollen trap	Maize	NA	Egypt	1076.2 g/colony/season	NA	Mohamed, Ali, & Ghazala (2022)
9.	Bottom pollen traps	Maize	NA	Egypt	2303.0 g/colony/season	NA	Mohamed, Ali, & Ghazala (2022)
10.	Plastic slide traps	Maize	NA	Egypt	742.8 g/colony/season	NA	Mohamed, Ali, & Ghazala (2022)
11.	Front mounted pollen traps	Coconut (*A. cerana indica*)	NA	India	Daily trapping 42.33 g/ colony/day Once in three days trapping 44.58 g/colony/ day		Rout, Srinivasan, Saminathan, Suganthi, & Geetha (2023)
12.	Front mounted pollen traps	Mustard	NA	India	Daily trapping 1st year 167.45 g/colony/season Daily trapping 2nd year 157.34 g/colony/season	NA	Naveen, Yadav, & Singh (2024)
13.	Front mounted pollen trap	*Brassica* spp.	50%	India	Daily trapping Location 1-0.56 Kg/colony/ season Daily trapping Location 2-1.89 Kg/colony/ season	-ve	Present study

*-ve means reduction in brood area; = means little or no effect on brood area; NA means information not available.

CONCLUSIONS AND DISCUSSION

Our study provided key insights into the impact of pollen trapping frequencies on pollen load collection and brood rearing. The results from both locations showed a progressive increase in pollen collection with higher trapping frequency, following the order: daily > alternate day > third day > weekly > control. These findings align with previous studies (Table 13) on *A. mellifera* during mustard flowering (Naveen, et al., 2024) and *A. cerana indica* during coconut flowering (Rout et al., 2023). Since the duration of trap placement varied across treatments i.e. 42, 21, 14, 7, and 0 days for daily, alternate-day, third-day, weekly, and control groups, respectively therefore, it lead to corresponding differences in pollen load collection. In addition, frequent pollen trapping also enhanced the number of pollen foragers entering the trapped colonies as evident from our results (Table 6- 9), which possibly caused further increase in the quantity of pollen loads collected. Previously, Webster et al. (1985), Gemeda

et al, (2018) and Dalal et al, (2024) also recorded higher number and proportion of pollen foragers in colonies equipped with traps. In addition, the variations in pollen load collection within the location and between the locations can be substantiated with differences in the meteorological parameters (Table 10) along with prevalence of different species of flowering plants during different times of the season (Table 3). Previous studies (Table 13) have also reported variations in the efficiency of traps between different locations (Levin & Loper, 1984) and within the flowering season (Goodwin & Perry, 1992) due to spatial and temporal variations in floral sources of honey bees, which depends upon landscape characteristics (Lau et al, 2019). Furthermore, efficiency of traps can also vary depending on factors such as pollen load size, bee size, and diameter of circular hole (Keller et al., 2005; Hoover & Ovinge, 2018).

Our study demonstrates that daily pollen trapping significantly reduces brood area in 12-frame *Apis mellifera* colonies. At location 1, a 27.3% reduction in brood area was observed, while at location 2, brood expansion was notably slower in daily-trapped colonies compared to weekly-trapped and control colonies (Table 5; Fig 3). These findings align with previous studies reporting adverse effects of pollen trapping on brood development. Ibrahim & Salim (1974) recorded a 39.5% reduction in brood area during peak pollen availability, while Waller et al. (1981) and Webster et al. (1985) reported declines in brood area and colony population following prolonged pollen trapping. Similarly, Ismail et al. (2012) used pollen traps of 28% efficiency and observed brood area reductions of 25.16% and 50.72% in two consecutive years. However, contrasting evidence still exists regarding the impact of pollen trapping efficiency on brood development. While some studies reported minimal effects (Levin & Loper, 1984; Nelson et al., 1987), others demonstrated significant brood reduction (Waller et al. 1981; Webster et al., 1985; Duff & Furgula, 1986 Ismail et al., 2012). High-efficiency pollen traps (50%) used in our study likely exacerbated pollen shortages, negatively affecting brood development in both seasons. These findings underscore the necessity of regulating pollen trap efficiency and frequency to mitigate brood losses. We observed peak *A. mellifera* pollen foraging between 10:00 AM and 2:00 PM in both locations (Tables 6 and 8), consistent with findings from Pakistan during the mustard season (Mahmood et al, 2017).Thus, intermittent trapping or restricting trapping to peak foraging hours (10:00 AM- 2:00 PM) could be viable strategies to balance pollen collection with colony health. Weekly pollen trapping proved effective in maintaining robust brood areas, as confirmed by the negative correlation between trapping frequency and brood area in our study (Table 11). Similarly, a high pollen load collection was negatively correlated with brood area, reinforcing the idea that excessive trapping can hinder brood rearing. Pollen is a critical protein source for larval development and nurse bee sustenance, and frequent trapping can lead to pollen shortages within the colony (Nelson et al.,1987; Capela et al, 2023). Reduced pollen stores not only suppress brood production but may also affect the physiology and foraging efficiency of emerging worker bees (Ismail et al., 2013).Our findings highlight that mustard (*Brassica* spp.) flowering during January and February in northern India

serves as a major pollen source, making it an optimal period for pollen trapping (Noor, Khan, & Camphor, 2009). Brood development is closely linked to pollen diversity and crude protein content (Melin et al, 2020; Dufour, Fournier, & Giovenazzo, 2020). A minimum crude protein content of >20% is required for optimal brood rearing (Herbert et al, 1977; Thakur & Nada, 2020). In our study, pollen from location 2 exhibited higher diversity and >24% crude protein content, explaining the relatively better brood expansion observed under daily trapping at this site than location 1. While occasional trapping (twice or thrice per week) allowed partial brood expansion, it remains unclear whether such intermittent trapping can sustain colonies during floral dearth periods. In contrast, weekly trapping, which yielded lower quantity of pollen loads, resulted in higher brood areas comparable to control colonies. These results suggest that an intermittent trapping regime (once or twice a week) may be preferable for maintaining colony health while ensuring sufficient pollen collection. Additionally, an economic analysis of pollen trapping could provide further insights into optimizing trapping frequency. Overall, our findings suggest that pollen trapping must be carefully managed to prevent negative impacts on brood development. Regulating trapping frequency, optimizing trap efficiency, and considering seasonal pollen availability are crucial for sustainable pollen harvesting while maintaining colony health and productivity.

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