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Development of pollen substitute diets from medicinal plants for *Apis mellifera* colonies and their impact on colony development

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Abstract

The study aimed to develop pollen substitute diets using medicinal plants for *Apis mellifera* colonies to satisfy their pollen and nectar requirements during drought conditions. A full-fledged pollen substitute diet plays a vital role during the floral dearth period and helps maintain the honey bee's health and strength from April to June 2023. Eight different pollen substitute diets were fed to 8 treatment groups of honeybee colonies. Diet-1, containing only sugar syrup (50 %) was used as a control. Pollen Diet-2 to 8 containing leaf and fruit powders of locally available medicinal plants and other contents with sugar syrup (50 %). The study parameters such as feed consumption rate, foraging activity, pollen storage, capped brood area, and population strength of bees were studied. The honeybee colonies fed with Diet-8, with mixed contents, showed the highest consumption rate, foraging activity, pollen storage, capped brood area and total strength of bees as compared to other treatment groups of colonies. The result showed high potential of a pollen substitute diet to improve the overall health of honeybee colonies under adverse conditions of nectar and pollen sources.

Key words: Pollen substitute diets, medicinal plants, consumption rate, foraging activity, population strength, brood development, *Apis mellifera* L.

Introduction

Honeybees are crucial for crop pollination. Beekeeping has numerous benefits that help farmer beekeepers better their livelihood. It is significant for honey production, food security, poverty reduction, employment creation, income generation, environmental protection, and human health. Honeybees can also be raised to generate honey, beeswax, propolis, royal jelly, and bee venom, as well as for apitherapy purposes. They also help to preserve biodiversity, pollinate crops, and create income.

In Maharashtra (India), the dearth period is longer, artificial feeding is essential to maintain honeybee colonies. It was observed that food resources were found to be drastically reduced during dearth periods of the year (Kumar and Agrawal, 2014). Due to lesser natural food availability, there would be a reduction in the number of worker bees in the hive. This feed decline also causes a decreased rate of the queen's egg-laying capacity, diminishes the survival rate of individuals, makes the colony susceptible to diseases, and increases the rate of absconding or abandonment (Morais *et al.*, 2013; Paiva *et al.*, 2016). Infection of pathogens, poor nutrition and their interactions are the reasons for colony losses such as the deaths of bees due to colony collapse disorder, *i.e.*, CCD (Brodschneider and Crailsheim, 2010). Lack of nutrition and starvation lead to colony losses in various parts of the world (De Grandi-Hoffman *et al.*, 2010; Khalil, 2017).

To mitigate the effects of the dearth period, artificial pollen diets complete colony needs. These supplements contain balanced formulae that suit the dietary needs of bees, ensuring the colony's longevity and performance. However, it should be highlighted that artificial food resources cannot successfully replace actual natural food (bee bread) because this natural protein source is more palatable and nutritionally complete, meeting all the necessities of the bees (Saffari *et al.*, 2010; Morais *et al.*, 2013).

Bees depend on the pollen and nectar of angiosperm plants as their primary food source (Stephen *et al.*, 2024; Vignesh, 2024). Nectar is primarily a carbohydrate source (Najarpoor, 2025; Liu *et al.*, 2024). Pollen contains carbohydrates, fats, vitamins, and minerals (Anjum *et al.*, 2024; El Ghouizi *et al.*, 2023). Honeybees require carbohydrates, protein, vitamins, minerals, and water (Di Pasquale *et al.*, 2013; Tsuruda *et al.*, 2021).

Honeybees need about ten different amino acids such as threonine-3.0 g, valine- 4.0 g, methionine- 1.5 g, leucine- 4.5 g, iso-leucine-4.0 g, phenylalanine- 2.5 g, lysine- 3.0 g, histidine- 1.5 g, arginine- 3.0 g and tryptophan- 1.0 g (/100 g). The development of a single larva requires approximately 25-37.5 mg of protein (De Groot, 1953). Carbohydrates contribute the required energy for metabolic processes to support cellular immune reactions. An adult worker bee needs approximately 04 mg of sugar in a day for its survival (Brodschneider and Crailsheim, 2010; Erler *et al.*, 2014). Lipids are the most essential and pioneer source for biosynthesis and metabolism during the brood stage; 02- 04 % more extraction of lipids from pollen, affects high brood rearing. An artificial diet with fat-soluble vitamins in combination with A, D, E and K results in increased brood production (Herbert *et al.*, 1980; Cantrill *et al.*, 1981). Water is important to make food for larvae as well as to supply minerals and it is collected as per demand as it is not stored as pollen and honey in the colony (Nicolson, 2009; Lindauer, 1954). Phytosterol of 24-methylene cholesterol was essential for the production of the moulting hormone ecdysone, brood rearing and bee longevity (longer survival) (Herbert *et al.*, 1980).

Medicinal plants have almost all phytochemicals that fulfill the requirement of nutrition and disease resistance capacity. Caffeic acid, gallic acid, p-coumaric acids and kaempferol prolonged the honeybee's lifespan most effectively. It is necessary to boost growth, improve immunity and to increase the resistance of bees against diseases. It also helps to enhance consumption rate, brood production, foraging activity, body weight, longevity and population strength in honeybees (Bernklau *et al.*, 2019).

Existing pollen substitute diets did not fulfill the nutrient requirement of bees. Nutritionally, almost all diets lack phytochemicals and other natural ingredients. Beekeepers often feed honeybee colonies with pollen supplements such as defatted soybean, maize and gram flour, when natural pollen supplies are not sufficient to promote colony development and health. Low nutritional reserves adversely affect colony performance. Various pests and diseases affect the colonies badly. Due to the reason that these factors cause honeybee colonies to weaken and even die, so additional care must be taken while managing bee colonies (Saffari *et al.*, 2010; Rashid *et al.*, 2013; Islam *et al.*, 2020).

The present study aimed to develop and test a diet formulation from locally available protein sources from medicinal plants and other ingredients that would be preferably palatable for bees and to determine their nutritional effects on the consumption rate, capped brood, foraging activity, pollen storage and population strength in *A. mellifera* colonies.

Materials and methods

The study was conducted at Dr. Babasaheb Ambedkar Marathwada University, Campus, Chhatrapati Sambhajinagar. Feeding experiments were carried out on honey bee colonies (*Apis mellifera* L.). Pollen substitute diets were composed and fed to honeybee colonies during the period from April 1, 2023 to June 19, 2023. Temperature ranged between 24.3 ºC to 40.7 ºC; 33.5±3.8 ºC and humidity between 16.9 % to 69.5 %; 37.1±12.0 %.

A total of sixteen (16) honeybee colonies were chosen to test pollen substitute diet treatments. Each colony had eight frames, out of eight frames, five frames were filled with almost the same area of brood, pollen and population strength of honeybees, two frames are empty but well-built-in wax cells and one frame was empty (with only a comb foundation sheet).

Selection and preparation of plant leaves and fruit powders for pollen feed diets: Plant leaves/ fruits based on high nutritional values and phytochemical profiles as per honeybee's dietary requirements were selected. Plant leaves material of coriander (*Coriandrum sativum*), moringa (*Moringa oleifera*), tulsi (*Ocimum sanctum*), lemongrass (*Cymbopogon citratus*) and fruit powders of amla (*Embilica officinalis*) and guava (*Psidium guava*) were selected.

All plant leaves were washed with clean water and then shade-dried completely. The fruit pulp of amla and guava was also shade-dried completely. Dried leaves and fruit pulp were then ground up separately by using a mixer and sieved through a fine mesh to get fine powders and stored separately in air-tight containers.

Preparation of sugar syrup (Diet-1/ Control) using white crystal sugar, it was prepared in a 1:1 ratio (*i.e.*, 50 %), boiled for 4 to 5 minutes and then filtered. Every time freshly prepared sugar syrup was used.

Method of pollen substitute diet feed treatments: Sixteen colonies were allotted as eight groups; each group contained two colonies. Colonies were then labeled as Diet-1 (Control), Diet-2 (Coriander), Diet-3 (Moringa), Diet-4 (Tulsi), Diet-5 (Lemongrass), Diet-6 (Amla), Diet-7 (Guava) and Diet-8 (Mixed diet). The feeders of all the colonies were washed with water and dried properly. The feed treatments to all the above eight groups of colonies were given the respective pollen substitute diets in the feeders, as per the labeled marking of colonies. All colonies have been given pollen substitute feed as per the allotment of colonies, except the control colonies. The control colonies were fed with 50 % sugar syrup only.

Each feeder of the experimental colony (*i.e.*, Diet-2 to Diet-8) was filled up with 10 gm of pollen diet powder + 100 mL sugar syrup (50 %) and the control colony was fed with 100 mL of Diet-1 (50 % sugar syrup). All diets were provided at 07.00 P.M. every day. Feeding was started from April 1, 2023 to April 10, 2023 for 10 days. After 10 days of feeding, feeding was stopped from April 11, 2023 to April 20, 2023 for 10 days. Feeding treatments were continued up to the date of June 19, 2023. A total of five feeding cycles were completed during this study period. Various parameters were studied during feeding treatment.

Preferred amount of the nutrient components, pH and particle size of the pollen diets (Cohen *et al.*, 2011): Preferred ranges of components, protein (15-45 gm), carbohydrate (35-42 gm), lipid (1-4 gm), ash (1-3 gm), ascorbic acid/ vitamin C (0.25-1.0 gm), pH (3.7-5.5). Particle size was less than (< 27). The preferred pH range was 3.7-5.5.

Diet consumption rate: All the experimental colonies (*i.e.*, Diet-2 to Diet-8) were given 100 mL of pollen substitute diets

Table 1. Composition of pollen substitute diets (100 g)

Diets	DSF	SMP	BY	PS	TP	Vit.	CA	GGP	E202	E281	CLP	MLP	TLP	LLP	AFP	GFP
Diet 1	1 Liter of 50 % Sugar syrup (Control)															
Diet 2	45 g	15 g	6.4 g	20 g	0.7 g	0.8 g	5.0 g	0.5 g	0.3 g	0.3 g	6.0 g	-	-	-	-	-
Diet 3	45 g	15 g	6.4 g	20 g	0.7 g	0.8 g	5.0 g	0.5 g	0.3 g	0.3 g	-	6.0 g	-	-	-	-
Diet 4	45 g	15 g	6.4 g	20 g	0.7 g	0.8 g	5.0 g	0.5 g	0.3 g	0.3 g	-	-	6.0 g	-	-	-
Diet 5	45 g	15 g	6.4 g	20 g	0.7 g	0.8 g	5.0 g	0.5 g	0.3 g	0.3 g	-	-	-	6.0 g	-	-
Diet 6	45 g	15 g	6.4 g	20 g	0.7 g	0.8 g	5.0 g	0.5 g	0.3 g	0.3 g	-	-	-	-	6.0 g	-
Diet 7	45 g	15 g	6.4 g	20 g	0.7 g	0.8 g	5.0 g	0.5 g	0.3 g	0.3 g	-	-	-	-	-	6.0 g
Diet 8	45 g	15 g	6.4 g	20 g	0.7 g	0.8 g	5.0 g	0.5 g	0.3 g	0.3 g	1.0 g	1.0 g	1.0 g	1.0 g	1.0 g	1.0 g

Abbreviations: DSF: Defatted Soya flour, SMP: Skimmed milk powder, BY: Brewer's yeast, PS: Powdered sugar, TP: Turmeric powder, Vit.: Vitamin A, D, E & K, CA: Citric acid, GGP: Guar gum powder, E202: Potassium sorbate, E281: Sodium propionate, CLE: Coriander leaves powder, MLE: Moringa leaves powder, TLE: Tulsi leaves powder, LLE: Lemongrass leaves powder, AFP: Amla fruit powder, GFP: Guava fruit powder.

and sugar syrup (Diet-1) was provided for control colony at 7 P.M. The amount of feed consumed by bees during the night was recorded the next day morning at 8.00 AM. The amount of feed consumed by the bees was determined using the earlier assessment (Amro *et al.*, 2016; Khalil, 2017; Ahmad *et al.*, 2021).

Foraging activity and pollen storage area: Foraging activity was determined from April to June 2023 at intervals of 21 days. The foraging activity of every honeybee colony was monitored visually for one minute, to record the number of honeybees coming back to the hive. The records of the foraging activity of bees were made six times a day from 08.00 A. M. to 06.00 P. M. (Ullah *et al.*, 2021).

The area of pollen storage was measured at four times; such as Initial (before feeding), after 15 days, after 30 days and after 45 days. Measurements of pollen areas were recorded with the help of transparent plastic grid paper placed on the frame, then pollen occupied areas in inch^2 and it was then multiplied by 6.45 factor to convert into cm^2 (Abd EI-Wahab *et al.*, 2016).

Measurement of capped brood area: The capped brood area of worker bees was measured at 12-day intervals utilizing a computing frame with a plastic transparent paper grid with scaling of one square inch (inch^2) and it was converted into cm^2 by multiplying factor 6.45, as described by Abd EI-Wahab *et al.* (2016). The sealed brood area was referred to as a standard to predict the growth of experimental bee colonies.

Population strength of worker bees: The honeybee population was measured by the number of frames covered with bees. The worker honeybee population was estimated after every 12 days by calculating the total number of frames entirely covered by bees (Ahmad *et al.*, 2021). The area occupied by worker bees was measured in square inches (inch^2). Single worker bees occupied an area of 1.25 cm^2/ bee (Imdorf and Gerig, 2001).

Statistical analysis: Data were analyzed using one-way ANOVA and the Post Hoc Duncan Multiple Range Test (DMRT) by SPSS 29.0.2.0.. Results are presented as mean ± standard error, with homogeneous subsets of means indicated by small letters. Means sharing the same letter indicate no significant difference, while P<0.05 signifies significant differences among subsets.

Results and discussion

The pollen substitute diet contained the following amounts per 100 g: protein (31.23 g), carbohydrate (51.92 g), lipid (1.05 g), ash (5.05 g), moisture (10.75 g), vitamin C (0.03068 g) and zinc (0.00486 g). The pH of the pollen substitute diet was 3.9, which is favorable because bees are attracted to acidic feed and consume more pollen diets. The particle size of the pollen substitute diet was maintained at 1.7 µm. This particle size is essential in the consumption of diets and the process of digestion.

Proteinaceous diets in the present study also significantly performed and co-related with the results of Ullah *et al.* (2021) studies. These findings are consistent with those of Kumar and Agrawal (2014), Kumar *et al.* (2013), Saffari *et al.* (2004), and Saffari *et al.* (2010). We found that in some cases, natural protein sources combined with other ingredients in diet formulations performed better than existing pollen supplement formulations.

Consumption rate: The consumption rate of pollen substitute diet was found to be highest in colonies consumed Diet-8 (74.66±0.49 mL), containing all ingredients such as defatted soya flour, skimmed milk powder, brewers' yeast, powdered sugar, turmeric powder, vitamin A, D, E & K, citric acid, guar gum powder, potassium sorbate, sodium propionate, leaves powder of coriander, moringa, tulsi, lemongrass and fruit powders of amla & guava. Other diets with consumption rates in the treatment group of colonies were, Diet-2 (49.66±1.06 mL), Diet-3 (44.86±0.83 mL), Diet-4 (51.46±0.91 mL), Diet-5 (43.96±0.55 mL), Diet-6 (43.76±0.97 mL) and Diet-7 (58.5±0.93 mL) showing almost same responses for consumption rate. Diet-1 *i.e.*, the control diet containing only 50% sugar syrup, showed the lowest consumption rate (41.62±0.54 mL) as compared to other diets, as shown in Table 2. Bees were attracted to citrus-flavored diets and well-aromatic plants such as tulsi and lemongrass. Diet-8 (mixed with all ingredients) a balanced aroma, so the feeding rate was highest in Diet-8. Bees were easily attracted and consumed more feed.

Results revealed that the pollen diet was easy to uptake as well as easy to digest. Similar results have been reported by Ullah *et al.* (2021), Kumar *et al.* (2013), Saffari *et al.* (2004), and Saffari *et al.* (2010), who noted protein substitute diet consumption rates higher than pollen/traditional artificial diets (*i.e.*, sugar syrup).

Foraging activity and pollen storage area: Foraging activity was recorded as the number of bees returned per minute per colony to their respective colonies. The average values of the records after every 21 days interval is given in (Table 3). The highest foraging activity was observed between (08:00 to 12:00 AM). The major foraging difference was recorded in the control group of colonies *i.e.*, Diet-1 and the treatment group of colonies *i.e.*, Diet-8 (Mixed diet), the highest foraging activity was found in Diet-8 (30±1.15) as compared to the lowest foraging activity in Diet-1 (12.33±1.45) at 10:00 AM. Similarly, Diet-3 (25.33±1.45), Diet-4 (25.67±1.45)

Table 2. The effect of pollen substitute diets on consumption rate [mL day/ colony] (Mean ± S.E)

Date	Diet-1 (Control)	Diet-2	Diet-3	Diet-4	Diet-5	Diet-6	Diet-7	Diet-8
01/04/2023 to 10/04/2023	44.30± 1.63a	53.00± 0.93b	43.00± 1.07a	54.80± 0.89b	42.40± 0.76a	45.30± 1.73a	59.20± 1.02c	73.10± 1.16d
11/04/2023 to 20/04/2023	No feedings							
21/04/2023 to 30/04/2023	43.30± 1.66a	51.30± 1.09b	44.10± 1.43a	51.40± 1.21b	43.50± 1.10a	43.10± 0.91a	57.20± 1.22c	75.60± 1.13d
01/05/2023 to 10/05/2023	No feedings							
11/05/2023 to 20/05/2023	44.50± 1.55b	47.40± 1.38bc	46.60± 1.65bc	49.50± 1.67c	45.80± 1.72bc	40.40± 0.64a	60.90± 0.57d	75.60± 1.10e
21/05/2023 to 30/05/2023	No feedings							
31/05/2023 to 09/06/2023	45.20± 1.63ab	48.50± 1.75bc	47.10± 1.34ab	51.50± 1.15c	44.00± 0.91a	44.00± 0.80a	59.60± 0.62d	75.10± 0.62e
10/06/2023 to 20/06/2023	No feedings							
21/06/2023 to 30/06/2023	44.80± 2.27a	48.10± 2.16ab	43.50± 1.18a	50.10± 1.45b	44.10± 1.22a	46.00± 0.79ab	55.60± 1.29c	73.90± 0.94d
Average	41.62±0.54a	49.66±1.06c	44.86±0.83b	51.46±0.91d	43.96±0.55b	43.76±0.97b	58.5±0.93e	74.66±0.49f

Different small letters within every column represent statistically significant differences (P<0.05).

and Diet-6 (25.67±1.45) had almost the same response at 10:00 AM. Other diets Diet-2 (22.67±1.76), Diet-5 (21.67±0.88) and Diet-7 (23.33±0.88) showed average performance. The lowest foraging activity was recorded between 04:00 to 06:00 PM.

Pollen storage area: The effect of different diets on pollen storage area, measured in square centimeters (cm²), showed a gradual increase after 15 days. Diet-8 demonstrated the highest storage rate compared to the control (Diet-1) and other diets, which showed moderate responses (Table 4). After 45 days, Diet-8 achieved the largest storage area (367.6 ± 3.72 cm²), followed by Diet-2 and Diet-6 with better responses than other diets, excluding Diet-8. Diet-3, Diet-4, Diet-5 and Diet-7 showed similar storage levels, while the control had the smallest area (225.7 ± 3.72 cm²).

Similar results were observed by Ullah *et al.* (2021), Kumar *et al.* (2013), Saffari *et al.* (2004), Saffari *et al.* (2010), and Abd EI-Wahab *et al.* (2016), who noted that feeding protein substitute diets had a significant impact on the daily foraging activity of bees and increased pollen storage capacity.

Capped brood area: Capped brood area in colonies fed with pollen substitute Diet-8 after 48 days was found to be the highest, *i.e.*, (2386.5±3.72 cm^2) as compared to control Diet-1 (1161±3.72 cm^2). Capped brood area in another treatment group of colonies fed with pollen substitute diets after 48 days were, Diet-2 (1354.5±3.72 cm^2), Diet-3 (1386.75±3.72 cm^2), Diet-4 (1470.6±3.72 cm^2), Diet-5 (1406.1±3.72 cm^2), Diet-6 (1380.3±3.72 cm^2) and Diet-7 (1367.4±3.72 cm^2), showing almost the same responses for capped brood areas. The control diet (Diet-1) containing only 50% sugar syrup, shows the lowest capped brood area, while the mixed diet (Diet-8) showed the highest capped brood area with all ingredients (Table 1), as compared to other Diets-2, 3, 4, 5, 6 and 7 as shown in (Table 5).

Similar results were observed by Saffari *et al.* (2004), Saffari *et al.* (2010), Khalil (2017), and Islam *et al.* (2020), who noted an increased capped brood area after feeding pollen substitute diets.

Table 5. The effect of pollen substitute diet treatments on capped brood area (cm^2) (Mean ± S.E) (CBA- capped brood area)

DWIC	Initial CBA	After 12 days	After 24 days	After 36 days	After 48 days
Diet-1	806.25±3.72d	877.2±3.72a	954.6±3.72a	1064.25±3.72a	1161±3.72a
Diet-2	799.8±3.72cd	903±3.72bc	1032±3.72b	1161±3.72c	1354.5±3.72b
Diet-3	786.9±3.72b	935.25±3.72d	1070.7±3.72e	1180.35±3.72d	1386.75±3.72d
Diet-4	774±3.72a	954.6±3.72e	1077.15±3.72e	1212.6±3.72f	1470.6±3.72f
Diet-5	806.25±3.72d	941.7±3.72d	1057.8±3.72d	1193.25±3.72e	1406.1±3.72e
Diet-6	786.9±3.72b	896.55±3.72b	1044.9±3.72c	1148.1±3.72b	1380.3±3.72d
Diet-7	793.35±3.72bc	909.45±3.72c	1038.45±3.72bc	1141.65±3.72b	1367.4±3.72c
Diet-8	786.9±3.72b	1096.5±3.72f	1341.6±3.72f	1728.6±3.72g	2386.5±3.72g

DWIC: Day-wise increased CBA. Small letters within every column represents statistically significant differences (P<0.05).

Population strength: The mean area of frames (inch^2) covered with bees varies significantly after consuming different diets (Table 6). The area of frames covered with bees after 48 days of feeding treatment was found to be highest in Diet-8 (1165±0.577 inch^2) as compared to other diets. The lowest response to population strength was found in control Diet-1 (1060±0.577 inch^2). Almost similar diet response to population strength was recorded in Diet-2 (1103±0.577 inch^2), Diet-3 (1100±0.577 inch^2), Diet-4 (1108±0.577 inch^2), Diet-5 (1105±0.577 inch^2), Diet-6 (1101±0.577 inch^2) and Diet-7 (1106±0.577 inch^2). Similar results were observed by Herbert (1980), Imdorf and Gerig (2001), Islam *et al.* (2020), and Ahmad *et al.* (2021), who noted increased population strength with the feeding of various types of pollen substitute diets.

The study has shown that honey bee colonies' performance can be improved by feeding them on artificial pollen supplemental diets. The honeybees preferred to consume liquid type diets. The research also showed that a well-prepared pollen liquid feed enhances diet consumption, foraging activity, pollen storage area, brood and population growth. Diet-8 (*i.e.*, a combination of medicinal plants and fruits) was found to be the best for the improvement in colony performance. Medicinal plant powders and essential nutrients provided the phytochemicals for growth and reproduction as required. Hence, Diet-8 is recommended for further improving bee development and productivity in commercial beekeeping.

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Table 3. The effect of pollen diet treatment on the foraging activity of bees (*i.e.*, bees return/ min./ colony) (Mean ± S.E)

Time		Diet-1 (Control)	Diet-2	Diet-3	Diet-4	Diet-5	Diet-6	Diet-7	Diet-8
Morning	At 08 am	3.00 ± 0.58a	8.00 ± 1.15bc	9.67 ± 0.88cd	5.33 ± 0.88a	5.67 ± 1.20ab	5.00 ± 0.58a	4.00 ± 0.58a	12.00 ± 0.58d
	At 10 am	12.33 ± 1.45a	22.67 ± 1.76b	25.33 ± 1.45b	25.67 ± 1.45b	21.67 ± 0.88b	25.67 ± 1.45b	23.33 ± 0.88b	30.00 ± 1.15c
After noon	At 12 pm	12.33 ± 0.88a	16.67 ± 0.88bc	18.00 ± 0.58c	16.33 ± 0.88bc	12.67 ± 0.88a	14.67 ± 0.88ab	16.33 ± 0.88bc	22.67 ± 1.20d
	At 02 pm	7.67 ± 0.88a	14.33 ± 1.76bc	12.33 ± 1.85bc	14.33 ± 0.88bc	11.33 ± 0.88b	16.00 ± 0.58c	16.00 ± 0.58c	20.67 ± 0.88d
Evening	At 04 pm	5.33 ± 0.88a	8.33 ± 0.88abc	6.67 ± 0.88ab	8.67 ± 0.88bc	9.67 ± 0.88bc	13.00 ± 1.53d	10.67 ± 0.88cd	20.00 ± 0.58e
	At 06 pm	4.67 ± 0.88ab	5.67 ± 0.88ab	6.33 ± 0.88ab	9.00 ± 0.58c	7.00 ± 0.58bc	6.00 ± 1.15ab	4.00 ± 0.58a	14.00 ± 0.58d

Different small letters within every column represent statistically significant differences (P<0.05).

Table 4 The effect of pollen substitute diets on storage of pollen area (cm^2) (Mean ± S.E)

Days	Diet-1 (Control)	Diet-2	Diet-3	Diet-4	Diet-5	Diet-6	Diet-7	Diet-8
Initial pollen store area	70.95 ± 3.72b	64.50 ± 3.72ab	70.95 ± 3.72b	64.50 ± 3.72ab	58.0 ± 3.72a	64.50 ± 3.72ab	58.0 ± 3.72a	64.50 ± 3.72ab
After 15 days	103.2 ± 3.72a	116.1 ± 3.72b	122.5 ± 3.72bc	129.0 ± 3.72c	129.0 ± 3.72c	116.1 ± 3.72b	122.5 ± 3.72bc	154.8 ± 3.72d
After 30 days	154.8 ± 3.72a	180.6 ± 3.72bc	174.1 ± 3.72b	206.4 ± 3.72d	187.0 ± 3.72c	161.2 ± 3.72a	180.6 ± 3.72bc	245.1 ± 3.72e
After 45 days	225.7 ± 3.72a	296.7 ± 3.72d	283.8 ± 3.72bc	283.8 ± 3.72bc	277.3 ± 3.72b	290.2 ± 3.72cd	283.8 ± 3.72bc	367.6 ± 3.72e

Different small letters within every column represents statistically significant differences (P<0.05).

Table 6. The effect of pollen diet treatments on the population strength of bees (population of bees/ colony) inch^2 area. (Mean ± S.E)

Diet	Initial area	After 12 days	After 24 days	After 36 days	After 48 days
Diet-1	1013±0.577ab	1020±0.577a	1033±0.577a	1045±0.577a	1060±0.577a
Diet-2	1012±0.577a	1030±0.577c	1048±0.577d	1063±0.577cd	1103±0.577c
Diet-3	1014±0.577bc	1034±0.577e	1045±0.577c	1060±0.577b	1100±0.577b
Diet-4	1013±0.577ab	1036±0.577f	1058±0.577e	1067±0.577f	1108±0.577e
Diet-5	1015±0.577c	1032±0.577d	1048±0.577d	1065±0.577e	1105±0.577d
Diet-6	1014±0.577bc	1029±0.577c	1043±0.577b	1064±0.577de	1101±0.577b
Diet-7	1013±0.577ab	1025±0.577b	1046±0.577c	1062±0.577c	1106±0.577d
Diet-8	1015±0.577c	1045±0.577g	1080±0.577f	1120±0.577g	1165±0.577f

Different small letters within every column represents statistically significant differences (P<0.05).

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