

Assessment of consumption and digestibility of artificial diets and their effects on few life study parameters of *Apis mellifera* L.

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Abstract

Artificial diets are necessary for managed honeybees (*Apis mellifera* L.) in arid regions when naturally occurring flora becomes scarce. The current study was planned to evaluate different artificial diets in terms of consumability and digestibility of *A. mellifera* and their effects on weight of queen bee, worker bee longevity and number of broods for colony survival and management in dearth period. Different artificial diets were administered separately to 33 colonies in three replications. The best combination was 25 g, each of soya flour, dry apricot powder, date paste and powder sugar, mixed in 8 ml of vegetable oil. Honeybees consumed most of diet-T₈ in all weeks (average 49.53 g), followed by diet-T₇ (average 38.81 g) and diet-T₆ (average 34.55 g); while the minimum consumption was of diet-T₁ (average 6.67 g). In diet digestibility experiment, diet-T₂ showed the highest digestibility (73.9%) followed by diet-T₁ (71.3%) and diet-T₄ (66.7%) with the lowest digestibility in diet-T₉ (59%). The protein present in hemolymph was highest in diet-T₈ (26.9 ug/ul) followed by diet-T₇ (24.1 ug/ul) and diet-T₆ (22.9 ug/ul) with the lowest in diet-T₁ (17.7 ug/ul). As a response to the feeding on the prepared diets, the queen's bee weight was highest in diet-T₈ (198.65 ± 0.36 mg) followed by diet-T₇ and diet-T₆ while the lowest in diet-T₀ (control group, 182.28 ± 0.55 mg). Similarly, the worker bee longevity was highest in diet-T₈ (53 ± 2.14 days) followed by diet-T₇ and diet-T₆ and lowest in diet-T₀ (control group, 34 ± 1.73 days). In the brood experiment, the number of eggs, larvae and pupae was highest in diet-T₈ (85 ± 2.03 eggs, 66 ± 1.15 larvae and 78 ± 1.15 pupae) while lowest in diet-T₀ (51 ± 1.5 eggs, 26 ± 1.5 larvae, 23 ± 1.5 pupae) after 28 days of diet feeding. Therefore, diet-T₈ can be used as substitute diet for the honeybees during dearth period for the survival and colony management of *A. mellifera*.

Keywords: *Apis mellifera* diet, Substitute diet, Dearth period management, Diet digestibility, Weight of queen, Worker bee longevity, Number of broods

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Introduction

Honeybees require highly nutritive, diets as being eusocial, they have to perform different tasks in their life (Seeley, 2019; Free and Williams, 1972) e.g., they feed their larvae and build comb for about the first half of their life (Seeley, 2019). They require proteins and royal jelly for the larval growth (Helm et al., 2017). During a certain period of the year, floral scarcity occurs due to seasonal changes and environmental stressors in various parts of the world (Paray et al., 2021). In this period when natural flora is insufficient or unavailable for the colony's need, beekeepers feed artificial diets to ensure survival, strengthen colonies and reduce their susceptibility to diseases (Fleming et al., 2015). In Pakistan, during the dearth eras (June-July), beekeepers frequently feed honeybee colonies with sugar syrup and fruit supplements (Al-Ghamdi et al., 2011; Mahmood et al., 2013). The majority of beekeepers also use powder sugar syrup as an additional nutrition to promote brood growth and fertilization of queen (Usha et al., 2014). But only sugar syrup is not enough as a diet of honeybees for better survival and growth. Low nutrition adversely affects colony performance and health (Paray et al., 2021). It also leads to insect pest and disease attacks, which may result in colony collapse disorder.

Honeybees require a balanced diet of protein, carbohydrates, lipids, vitamins, and minerals to maintain their health and productivity (Brodschneider and Crailsheim, 2010). Black (2006) proposed the specifications for an efficient pollen substitute diet: approximately 25–30% protein, 5% lipids, 10–20% fiber, 40–60% sugar, 1–1.5% minerals and vitamins, along with the appropriate amount of essential amino acids, linoleic acid, and cholesterol. Antioxidants can be included to ensure the long-term preservation of the diet (Weirich et al., 2002). High quality dietary sources are necessary to enhance bee production. Diets containing artificial pollen can be used for honeybee colonies' survival and reproduction in the absence of naturally occurring pollen sources (Mattila and Otis, 2006) rich protein feedings are essential for the reproduction and care of bee colonies, bee brood rearing, and younger bee development (Manning, 2001) and also for honey production (Saffari et al.,

2004). Beans and pulses are the important source of proteins and increase the longevity and overall health of the bees. Carbohydrates are most important for the development of honeybee colonies and are found in the plant nectar. However natural sources of nectar are not available throughout the year (Pawlikowski, 2010). The most important energy source for many birds and animals is fruits as they mainly provide sugar, vitamins, and minerals (Howe, 1986). Therefore, the fruits function similarly to nectar. Many insect species including honeybees forage on fruits (Evison and Ratnieks, 2007; Helanterä and Ratnieks, 2008).

Significant research work on substitute diets has been conducted worldwide, utilizing various ingredients to prepare artificial diets (Gemeda, 2014; Usha et al., 2014; Aly et al., 2014; Amro et al., 2020). However, these ingredients or artificial diets cannot be used and implemented everywhere due to vast differences in climatic and environmental conditions, floral variety, presence of raw ingredients, specific needs and economic aspects. Scientists have also explored the influence of pollen and nectar substitute diet formulations on honeybee colonies. A large number of diet formulations have been developed by the scientists combining different ingredients (Paray et al., 2021) but no substitute diet has been formulated so far that can completely replace the nutrition provided by the pollen (Manning, 2018).

The goal of this study was to discover alternative diets which would be most palatable and easily digested by honeybees and how these diets affect life study parameters during food scarcity, in order to enhance colony survival. The results of this study may help in providing guidelines to beekeepers on how to run their businesses and come up with creative solutions to deal with the issue of food scarcity during the dearth period.

Material and Methods

Study site

The study was conducted in the Apiculture research area in the Department of Entomology, The Islamia University of Bahawalpur (29.372800 longitudes, 71.764988 latitudes). Bahawalpur is located in the Punjab province of Pakistan. Bahawalpur has a desert climate characterized by extremely hot summers (41-



43°C average maximum temperature) and mild winters (9.4-22.8°C) and recorded rainfall in summer is 31 mm to 58 mm, while in winter it is 5 mm to 14 mm. During the winter season, beekeepers from various regions come to Bahawalpur to take advantage of the flowering mustard fields. Mustard blooms provide an abundant source of nectar and pollen for bees, which is essential for the growth and maintenance of bee colonies during the colder months.

Experimental colonies

Thirty three colonies were selected for diet consumability and digestibility experiments and 12 colonies were selected for the life study parameters. Each colony had nine frames, a single fertile queen and approximately twenty thousand bee population. The colonies were homogeneous in population, ensuring consistent and reliable results across the study. Experiments were done during summer from 5th June to 5th October 2023.

Preparation of different combinations of diet ingredients

Economically cheaper ingredients were selected for diet preparation. The main components included soya flour (main source of protein), date paste (Source of protein and carbohydrate), dry apricot powder (source of carbohydrate vitamins A & C and minerals), skimmed milk (source of protein carbohydrate and vitamins) and the powdered sugar.

In the mixing process, the soya flour and dry apricot powder were combined in a large bowl, while the date paste was gradually added and thoroughly mixed to achieve an even distribution. Powdered sugar was then incorporated slowly to achieve a consistent texture. Skimmed milk was also added and thoroughly mixed in treatments where required. Finally, vegetable oil was added as to our specific composition ratio. The detailed ingredients are outlined in Table 1.

Table-1. Different ingredients selected for diet preparation

Sr. No.	Ingredients
1	Soya Flour
2	Date Paste
3	Dry Apricot Powder
4	Skimmed Milk
5	Powder Sugar
6	Vegetable Oil

The specific composition ratio of ingredients is described below:

Diet 1 (T1) = 33.33 g soya flour + 33.33 g skimmed milk + 33.33 g powder sugar

Diet 2 (T2) = 33.33 g soya flour + 33.33 g skimmed milk + 33.33 g powder sugar + 8 ml vegetable oil

Diet 3 (T3) = 33.33 g soya flour + 33.33 g dry apricot powder + 33.33 g powder sugar

Diet 4 (T4) = 33.33 g soya flour + 33.33 g dry apricot powder + 33.33 g powder sugar + 8 ml vegetable oil

Diet 5 (T5) = 33.33 g soya flour + 33.33 g date paste + 33.33 g powder sugar

Diet 6 (T6) = 33.33 g soya flour + 33.33 g date paste + 33.33 g powder sugar + 8 ml vegetable oil

Diet 7 (T7) = 25 g soya flour + 25 g dry apricot powder + 25 g date paste + 25 g powder sugar

Diet 8 (T8) = 25 g soya flour + 25 g dry apricot powder + 25 g date paste + 25 g powder sugar + 8 ml vegetable oil

Diet 9 (T9) = 20 g soya flour + 20 g skimmed milk + 20 g dry apricot powder + 20 g date paste + 20 g powder sugar

Diet 10 (T10) = 20 g soya flour + 20 g skimmed milk + 20 g dry apricot powder + 20 g date paste + 20 g powder sugar + 8 ml vegetable oil

Diet 11 (T₀) = Control (50% sugar solution)

Petty feeding diet

The diet was prepared as to the composition ratio and were filled equally in the petri dishes and were wrapped with polythene paper leaving the space for bees to consume the diet as proposed in the study of Amro et al. (2016). Petri dishes were placed under the bee frames inside the bee colony.

Consumability of diet

The data were recorded after at one week interval up to 8th week. At first, fresh diet was weighed with electronic weight balance (OHAUS Diamond MCT 500) and then weighed it again after 1 week and the data were recorded up to 8th week. The diet was replaced with fresh diet after every week (Islam et al., 2020).

Digestibility of diet

This experiment was conducted during the summer, from August 7th to August 21st, 2023. The diets were fed for two weeks to determine the digestibility of diet, only the nurse bees were selected from the hives. For this purpose, concentration of the protein



present in contents of the hindgut of a nurse bee were studied and compared with the concentration present in the provided diet. 3 random nurse bees were collected from the hives of all the colonies (from each treatment and replication) and were dissected, to extract and weighted their hindgut. The hindgut content from of 1 µl was mixed with 99 µl Phosphate-Buffered Saline (PBS) and 1% EDTA (ethylene diamine tetra acetic acid) without HPIC (High-Performance Ion Chromatography) reagents. Then, mixed solutions were added to 900 µl of PBS containing 1% EDTA-free HPIC and kept at -80°C until we used a spectrophotometer (UV-Visible spectrophotometer) to measure the total soluble proteins at 595 nm (Cremonz et al., 1998). Then protein concentrations of samples of diets were to find out. Three random samples were collected from the diets. 1 mg from the diet sample were added to the 999 µl of PBS containing 1 EDTA-free HPIC. The same procedure was adopted to find out the soluble proteins present in the diet. Diet digestibility was determined as to the following formula.

$$\text{Approximate Digestibility} = \frac{\text{Protein conc. in diet} - \text{Protein conc. in hindgut}}{\text{Protein conc. in diet}} \times 100$$

Then, the amount of total protein present in nurse bee hemolymph was measured. For this purpose, three honeybees were randomly selected from each colony from all the treatments and hemolymph was harvested following Borsuk et al. (2017) technique. Briefly, after separating the bee's antenna, the abdomen of the bee was carefully pressed. A drop of hemolymph appeared at the excised antenna's base as a result, and it was scooped up using a pipette. Hemolymph samples were stored at -80°C. Total protein contents were measured with the same procedure as adopted for protein contents in hindgut and diet using 1 µl of hemolymph samples and mixing it with 99 µl of PBS and 1% EDTA-free HPIC.

Weight of queen

This trial consisted of 12 colonies including 3 replications. Colonies were fed on the screened diet (i.e., T₆, T₇, T₈ and T₀) for 4 weeks from 7th August to 4th September 2023. After being fed on the screened diet, the frames in the colonies were observed to find the queen cells. When the queen emerged, it was weighed on the electronic balance (Akyol et al., 2008). The data were calculated based on the weight

of the emerged queens (treatments) and the weight of the queen in the unfed colony (control) and were analyzed according to statistical standard procedures.

Worker bee longevity

For this experiment, 12 honeybee colonies were selected along with three replications. Experiments were carried out from 7th August to 5th October 2023. As the adult honeybees emerged from the cells, they were marked with a permanent color marker (Shurjeel et al., 2020). The date and time of the bee emergence was noted. In this manner, 15 honeybees were marked daily from each colony for three days. Longevity was assessed by daily inspection of the bee colony and identification of bees (Martin et al., 2021). The colony was inspected daily in the morning at 10 am. Worker bee longevity data were collected and analyzed according to statistical standard procedures.

Number of broods

For this experiment, 12 honeybee colonies were selected along with three replications. The worker bee broods (eggs, larvae and pupae) were counted after 14 days and 28 days of feeding diet. For this purpose, a region in the brood area of the honeybee hive was selected and counted the newly emerging broods (Amir and Peveling, 2004). The data were compared with the controlled colony (with no fed screened diets) and statistically analyzed.

Statistical analysis

The total amount of diet consumed, and diet digested were compared across the treatments. Analysis of variance (ANOVA) following the Completely Randomized Design (CRD) was performed to test the statistically significant differences among the treatments. Further, the Least Significant Difference (LSD) test was performed for multiple comparisons among the groups at 5% level of significance for the diet consumability, digestibility, weight of queen and worker bee longevity experiments. While two-way ANOVA (factorial design) was used for the number of broods to test the statistically significant differences among the treatments. Further, the Tukey's HSD post-hoc test was performed for multiple comparisons among the groups at the 5% level of significance. Analyses of variance were performed by Statistix (8.1) software, and the graphs were developed in MS Excel (2016).



Results

Diet consumption

In week 1, there was a statistically significant difference among ten diets in terms of mass consumed ($p < 0.000$). Honeybees consumed maximum diet-T₈ (41.29 ± 0.66 g) followed by diet-T₇ and T₆; while minimum diet-T₁ (4.13 ± 0.50 g). In week-2, there were also significant differences among all the treatments ($p < 0.001$). Maximum diet-T₈ (44.40 ± 1.25 g) was consumed by honeybees followed by diet-T₇ and T₆, while the diet-T₁ was consumed minimum (4.59 ± 0.59 g). In week-3, there were also significant differences among the treatments ($p < 0.001$). Honeybees consumed maximum diet-T₈ (45.64 ± 0.85 g) followed by diet-T₇ and T₆; while minimum diet-T₁ (8.4 ± 0.55 g). In week-3, there was also significant difference among all the diets ($p < 0.001$). Maximum diet consumption was recorded in diet-T₈ (45.10 ± 1.06 g) followed by diet-T₇ and T₆; while minimum in diet-T₁ (8.4 ± 0.55 g). In week-4, there were statistically significant

differences among all the treatments ($p < 0.001$). Honeybees consumed maximum diet-T₈ (45.10 ± 1.06 g) followed by diet-T₇ and T₆; while minimum diet-T₁ (8.73 ± 0.68 g). In week 5, there were also significant differences among all the treatments ($p < 0.001$). Maximum diet-T₈ (52.02 ± 1.21 g) was consumed by honeybees followed by diet-T₇ and T₆, while the diet-T₁ was consumed minimum (7.39 ± 1.27 g). In week-6, there were also significant differences among the treatments ($p < 0.001$). Honeybees consumed maximum diet-T₈ (52.57 ± 0.36 g) followed by diet diet-T₇ and T₆; while minimum diet-T₁ (7.50 ± 0.47). In week 7, there were also significant differences among all diets ($p < 0.001$). The maximum diet consumption was recorded in diet-T₈ (58.53 ± 1.41 g) followed by diet-T₇ and T₆; while minimum diet-T₁ (6.38 ± 1.17 g). In week-8, there were also statistically significant differences among all the treatments ($p < 0.001$). Honeybees consumed maximum diet-T₈ (56.74 ± 0.58 g) followed by diet-T₇ and T₆; while minimum diet-T₁ (6.28 ± 0.17 g).

Table-2. Diet consumed by honeybees (*Apis mellifera*) in 8 weeks

Diet	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Diet 1	4.13 ± 0.50 h	4.59 ± 0.59 i	8.4 ± 0.55 i	8.73 ± 0.68 h	7.39 ± 1.27 h	7.50 ± 0.47 i	6.38 ± 1.17 i	6.28 ± 0.17 j
Diet 2	7.95 ± 0.41 g	7.51 ± 0.35 h	11.56 ± 1.20 h	11.7 ± 0.66 g	14.42 ± 0.63 g	11.5 ± 0.51 h	12.8 ± 0.64 h	12.51 ± 0.50 t
Diet 3	13.69 ± 0.38 f	13.07 ± 0.67 g	12.96 ± 0.78 gh	16.38 ± 0.94 f	15.48 ± 0.93 g	16.07 ± 0.52 g	17.98 ± 0.71 g	17.19 ± 0.37 f
Diet 4	20.83 ± 0.92 d	23.04 ± 0.82 e	22.77 ± 0.96 e	24.96 ± 0.97 de	25.21 ± 1.14 e	27.04 ± 1.47 d	27.73 ± 0.82 e	26.84 ± 0.41 e
Diet 5	24.01 ± 1.31 c	26.29 ± 0.42 d	27.02 ± 0.46 d	27.83 ± 0.46 d	29.30 ± 0.32 d	29.86 ± 2.24 d	33.74 ± 0.70 d	32.56 ± 0.37 d
Diet 6	30.44 ± 0.89 b	30.94 ± 1.20 c	32.56 ± 1.22 c	35.37 ± 1.19 c	35.11 ± 1.31 c	33.71 ± 0.52 c	39.7 ± 1.00 c	38.62 ± 0.77 c
Diet 7	32.94 ± 1.37 b	36.93 ± 1.13 b	36.20 ± 0.82 b	38.57 ± 1.36 b	40.67 ± 0.60 b	39.59 ± 0.78 b	43.44 ± 0.58 b	42.19 ± 0.73 b
Diet 8	41.29 ± 0.66 a	44.40 ± 1.25 a	45.64 ± 0.85 a	45.10 ± 1.06 a	52.02 ± 1.21 a	52.57 ± 0.36 a	58.53 ± 1.41 a	56.74 ± 0.58 a
Diet 9	16.77 ± 0.93 e	18.10 ± 0.43 f	15.38 ± 1.08 g	19.05 ± 1.37 f	19.36 ± 1.04 f	20.35 ± 0.94 f	20.18 ± 1.41 g	19.52 ± 0.71 g
Diet 10	19.08 ± 0.77 de	21.33 ± 1.07 e	19.82 ± 1.56 f	22.37 ± 0.72 e	21.6 ± 1.36 f	23.71 ± 0.68 e	23.63 ± 0.39 f	23.07 ± 0.78 f
ANOVA	d.f	9	9	9	9	9	9	9
Results	f	170	215	146	144	170	178	300
	p	0	0	0	0	0	0	0



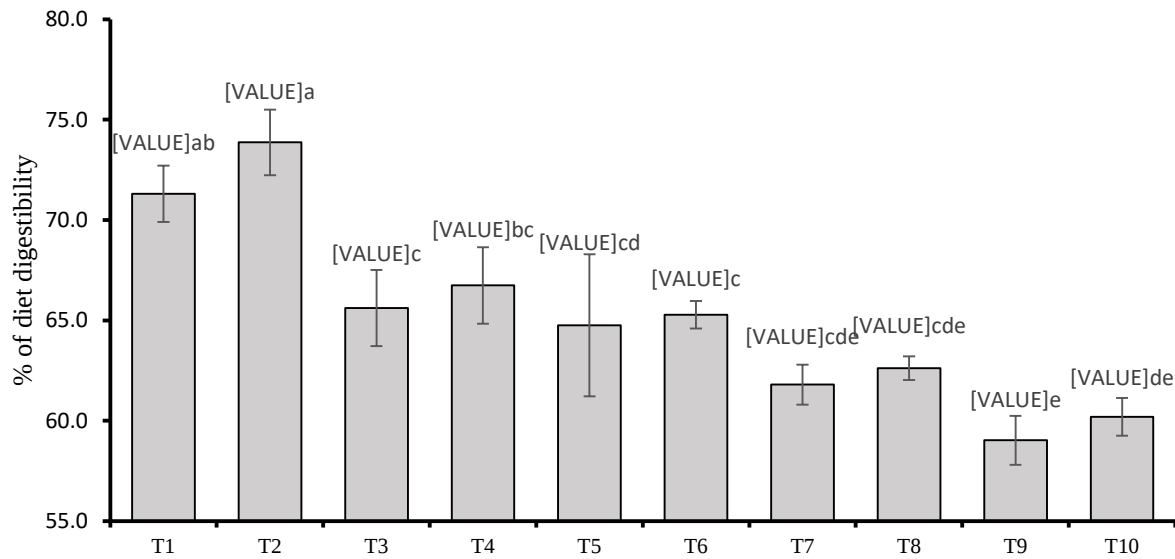


Figure-1. Percentage diet digestibility of all the treatments

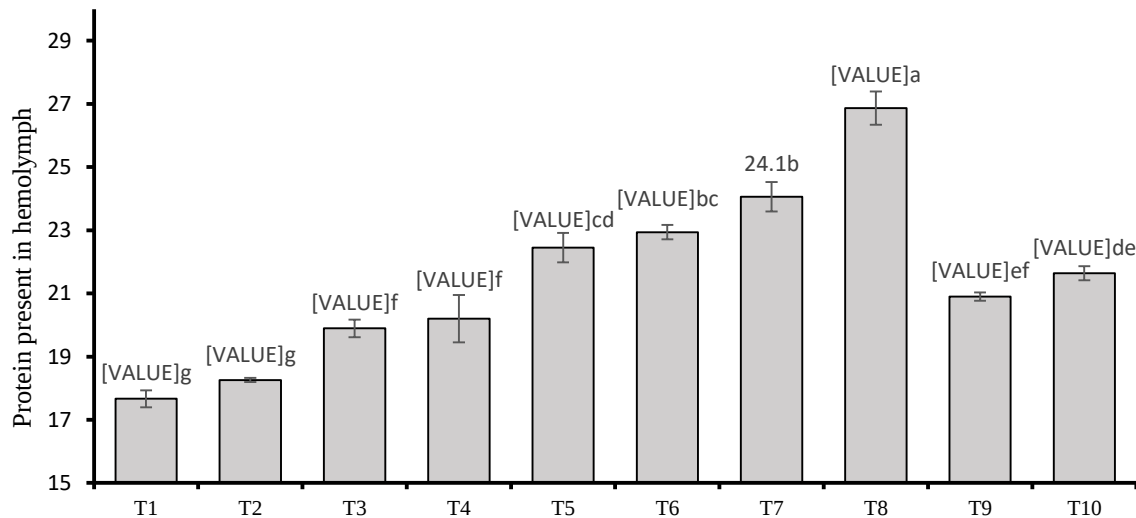


Figure-2. Protein present in the honeybee hemolymph in ug/ul

Diet digestibility

Treatments 6, 7 and 8 were statistically similar, while maximum diet digestibility (%) was recorded in T₂ (73.9 ± 1.3 %) followed by T₁ and T₄, while minimum was observed in T₉ (59.0 ± 1.22 %).

Protein present in hemolymph

Statistically significant differences were observed among all the treatments in terms of protein availability in hemolymph ($df= 9$, $F= 49.0$, $p < 0.001$). However, maximum presence of protein in hemolymph was recorded in T₈ (26.9 ± 0.52 ug/ul) followed by T₇ and T₆, while minimum was observed in T₁ (17.7 ± 0.27 ug/ul).

Correlation matrix

Correlation between variables i.e. Diet digestibility, Protein present in hemolymph with respect to diet consumption is explained in Table (3).

Table-3. Correlation of diet digestibility, protein levels in hemolymph, and diet consumption

Variables	Diet Digestibility	Protein hemolymph µg/µl
Protein hemolymph µg/µl	-0.640 (0.046)	
Consumption	-0.525 (0.119)	0.974 (0.0001)



There is a moderate negative correlation between diet digestibility and protein levels in hemolymph, indicating that as diet digestibility increases, the protein concentration in hemolymph tends to decrease. This relationship is statistically significant, with a p-value of 0.046. Similarly, there is a moderate negative correlation between diet digestibility and consumption; however, this relationship is not statistically significant, as indicated by a p-value of 0.119. On the other hand, a very strong positive correlation exists between protein levels in hemolymph and consumption, suggesting that higher consumption is associated with increased protein levels in hemolymph. This correlation is highly significant, with a p-value of 0.0001.

Effect of artificial diets on weight of queen bee

After feeding on the artificial diets, there were observed statistically significant differences among all the diets in terms of weight of queen ($p < 0.001$). The queen emerged after feeding on T_8 weight was highest (198.65 ± 0.36 mg) followed by T_6 and T_7 ; while minimum performance was observed in control group, T_0 (182.25 ± 0.55 mg).

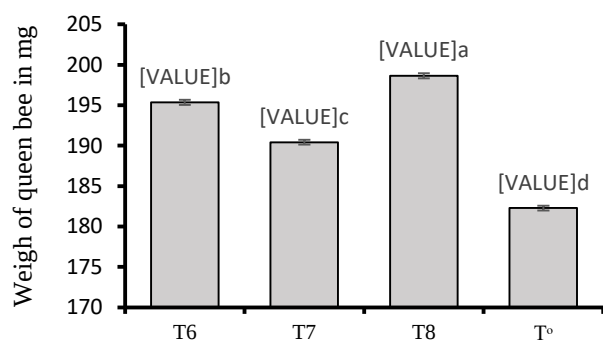


Figure-4. Comparison of bee's longevity in colonies offered selected diets with respect to control treatment

Effect of artificial diets on brood

Effect of artificial diets on number of eggs of honeybees

At the time of diet placement the diet on 1st day, there was no-statistically significant difference between all the treatments. After 14 days of diet

feeding the position was different. All the treatments had statistically significant difference ($p < 0.001$). T_8 had the maximum number of eggs (62 ± 2.03 eggs) followed by T_6 and T_7 , while the minimum eggs were recorded in T_0 control group (43 ± 1.5 eggs). After 14 days, there was also a significant difference between all the treatments ($p < 0.001$). Maximum number of eggs were recorded in T_8 (85 ± 2.03 eggs) followed by T_6 and T_7 , while the minimum was observed in control group T_0 (51 ± 1.5 eggs).

Effect of artificial diets on number of larvae of honeybees

At the time of diet placement (i.e. day-1), there were no significant differences between the treatments. After 14 days of diet feeding; the position was different. All the treatments were statistically significant difference ($p < 0.001$). Diet- T_8 had the maximum number of larvae (47 ± 1.15 larvae) followed by diet- T_6 and T_7 , while the minimum larvae were recorded in control group- T_0 (23 ± 1.5 larvae). After 14 days, there was also a significant difference among all the treatments ($p < 0.001$). Maximum number of larvae were recorded in diet- T_8 (66 ± 1.15 larvae) followed by diet- T_6 and T_7 , while the minimum were in control group- T_0 (26 ± 1.5 larvae).

Effect of artificial diets on number of pupae of honeybees

At the time of diet placement (i.e. day-1), there were no significant differences among all the treatments. While after 14 days of diet feeding the position was different. All the treatments were having statistically significant differences ($p < 0.001$). Diet- T_8 had the maximum number of larvae (29 ± 1.15 pupae) followed by diet- T_6 and T_7 , while the minimum larvae were recorded in control group- T_0 (19 ± 1.5 pupae). After 14 days, there was also a significant difference between all the treatments ($p < 0.001$). The maximum number of larvae were recorded in diet- T_8 (78 ± 1.15 pupae) followed by diet- T_6 and T_7 while the minimum was in control group- T_0 (23 ± 1.5 pupae).



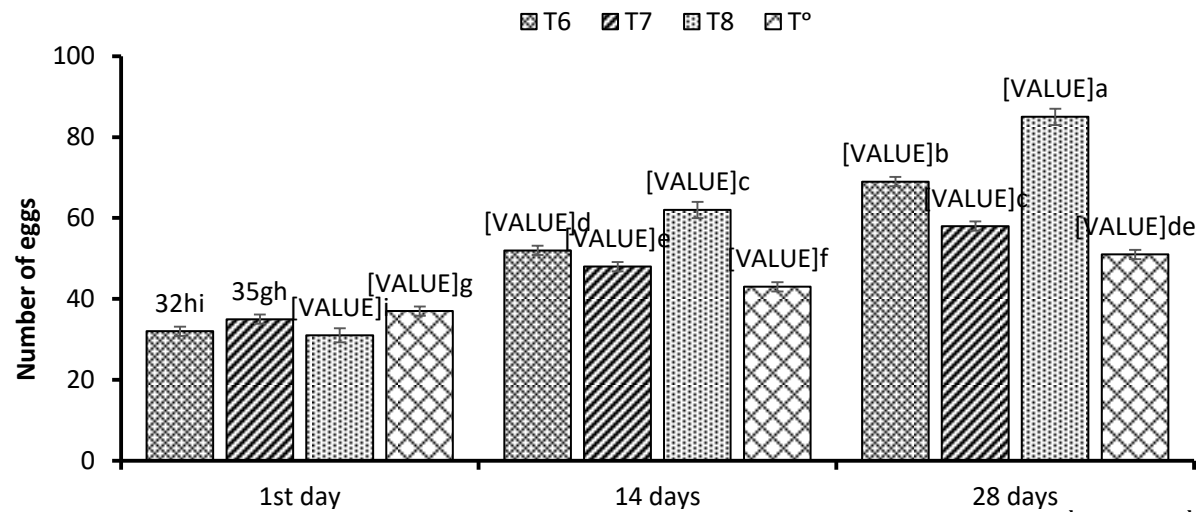


Figure-5. Comparison of effect of all the diets on number of eggs of honeybees in 1st, 14th, and 28th days, all the diets with respect to control group.

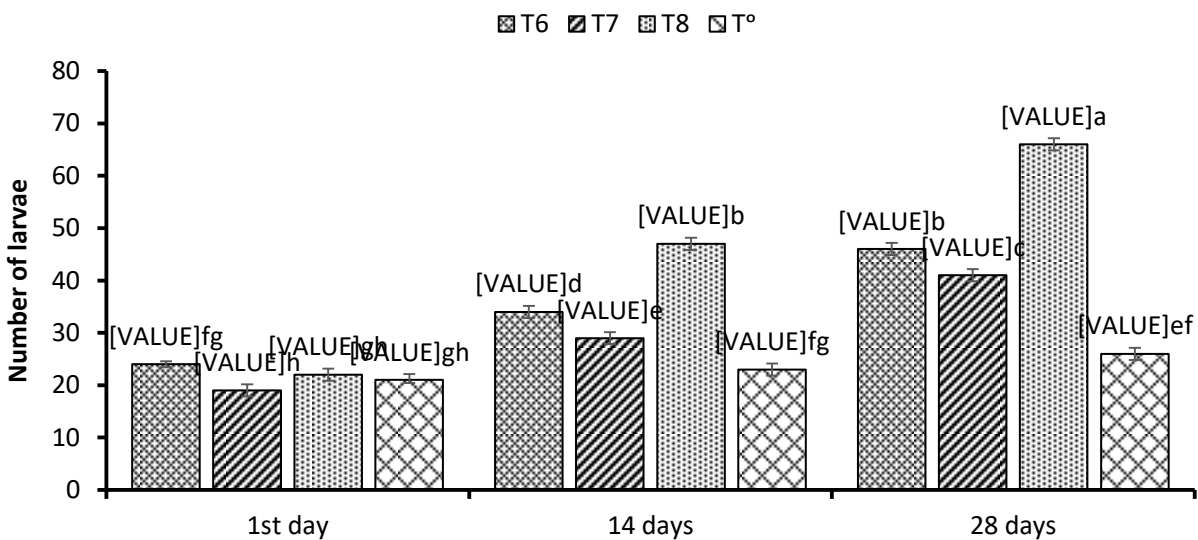


Figure-6. Comparison of effect of all the diets on number of larvae of honeybees in 1st, 14th, and 28th days, all the diets with respect to control group.



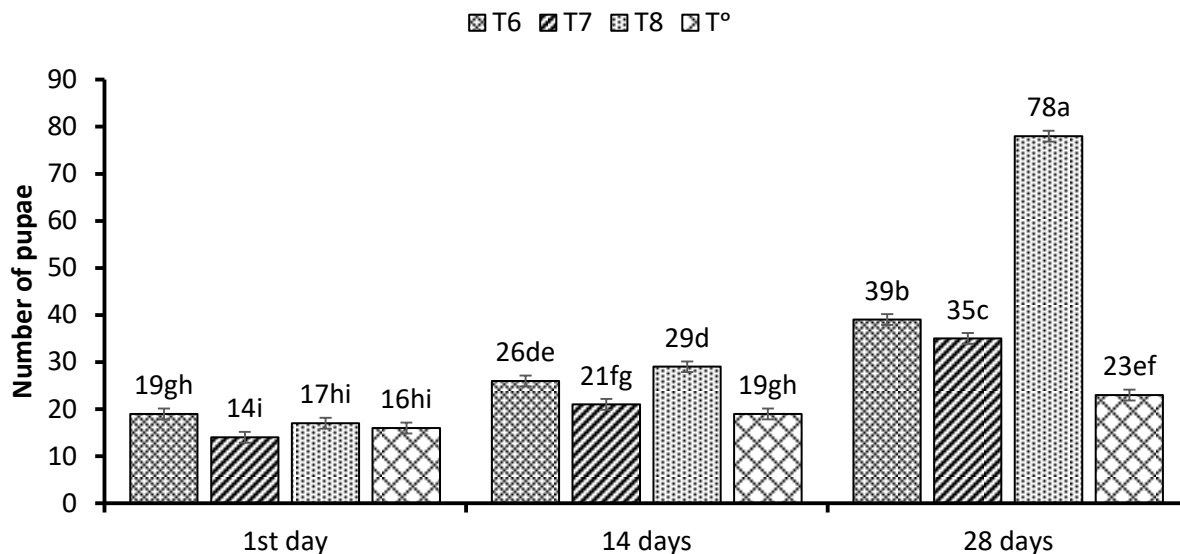


Figure-7. Comparison of effect of all the diets on number of pupae of honeybees in 1st, 14th, and 28th days, all the diets with respect to control group.

Discussion

Best artificial diet (among different diet combinations), the honeybee responses to artificial diet consumability and diet's digestibility in honeybee were evaluated during the current study. Results revealed that T₈ (i.e. 25 g soya flour + 25 g dry apricot powder + 25 g date paste + 25 g powder sugar + 8 ml vegetable oil) had a significant impact. Honeybees consumed maximum diet-T₈ (41.29 ± 0.66 g) followed by diet-T₇ and diet-T₆; while minimum diet-T₁ (4.13 ± 0.50 g). The same trend was observed in the 2nd, 3rd, 4th, 5th, 7th and 8th week of diet consumption. The reason behind this honeybee preferred to eat candy diet in comparison to dry diet and also preferred to eat protein enriched diet; diet-T₈ was high in protein diet rather than other diets (Saffari et al., 2004; Mattila and Otis, 2006) so it was consumed maximum. The mean food consumption throughout the whole sample of honeybee colonies showed notable variability ($P < 0.05$). Similar findings from prior studies have shown that bees choose synthetic protein-rich foods over naturally occurring pollen (Saffari et al., 2004; Mattila and Otis, 2006).

Diet-T₇ also showed a good consumability after diet-T₈, in all eight weeks of feeding diet. The diet was in dry form not in the form of candy but showed good results after T₈ rather than other treatments. The reason behind this consumption may be the

ingredients were the same as diet-T₈, but the only difference was the addition of vegetable oil. Diet-T₆ was consumed mostly by bees after diets T₇ and T₈. It was a combination of the ingredients without dry apricot powder but in the form of candy. While the Diet-T₅ was the same as Diet-T₆ but not in the form of candy so its consumption was also less than diet-T₆. The same reason was with diet-T₃ and diet-T₄ as this diet combination was without date paste.

In the treatments where we had used skimmed milk as a source of protein and vitamins, the bees didn't prefer to feed on the diet having skimmed milk ever in the powder form or in candy. This contrasts with the findings of Paray et al. (2021). The lack of preference in our study may be due to the combination of skimmed milk with other ingredients, such as soy flour and powdered sugar, which the bees found less palatable, particularly when compared to other diets, especially diet T₈, which was the most consumed.

In diet digestibility experiment, current outcomes resemble the findings of Omar et al. (2022), as they compared the digestibility of crushed and non-crushed pollens of the maize, we also used the crushed form of diet ingredients and found that our diet-T₈ (that was consumed maximum) showed the 62.6 percent diet digestibility that is lower as comparison to the other treatments. The maximum diet digestibility was recorded in diet-T₂ (73.9%) followed by diet-T₁ (71.3%) and T₄ (66.7%). Diet



digestibility was good in diet-T₂ but it was not consumed even in a good quantity for the survival of the honeybee colony, so it was not recommended. The reason might be as the diet-T₈ had higher concentration of protein (13.2%) in comparison to the diet-T₆ and T₇ (i.e. 12.6 and 12.8 percent, respectively) as to Du Rand et al. (2020) high protein diets are difficult to digest.

The effectiveness of protein diets is often evaluated based on the hemolymph's protein content (Kunc et al., 2019; Noordyke and Ellis, 2021). High protein contents and nutrients increase bee lives and boost their defense against disease (Skowronek et al., 2021). Currently, hemolymph contents were found higher in diet-T₈ sample of hemolymph i.e. 26.9 µg/µl followed by diet-T₆ and T₇, as they had less protein contents in the diet so it proved the statement of Basualdo et al. (2014) the more protein available in the diet, the more protein contents should be available in the hemolymph.

The results showed that the artificial diets had a significant impact on the weight of queen bees. The queens that emerged after feeding on diet-T₈ had the highest weight (198.65 ± 0.36 mg), followed by diet-T₆ and T₇, while the control group (T₀) had the lowest weight (182.25 ± 0.55 mg). These findings were consistent with previous studies that had reported the influence of diet on queen bee weight and development (Keller et al., 2005; DeGrandi-Hoffman et al., 2010).

The longevity of worker bees was also significantly affected by artificial diets. Workers fed on diet-T₈ had the longest lifespan (53 ± 2.1 days), followed by diet-T₇ and T₆, while the control group (T₀) had the shortest lifespan (34 ± 1.7 days). This aligns with previous research highlighting the importance of diet composition on worker bee longevity and colony health. On the first day of diet placement, the control group (T₀) had the highest number of eggs (37 ± 1.5 eggs), followed by diet-T₇ and T₆, while diet-T₃ had the lowest (31 ± 1.5 eggs). However, after 14 and 28 days, the trend reversed, with diet-T₈ having the highest number of eggs (62 ± 2.03 and 85 ± 2.03 eggs, respectively), followed by diet-T₆ and T₇, while diet-T₀ had the lowest (43 ± 1.5 and 51 ± 1.5 eggs, respectively). These results suggest that the artificial diets, particularly diet-T₈, had a positive impact on egg production over time, which is consistent with previous studies on the effect of diet on brood production (Ullah et al., 2021; kumari and kumar,

2020).

Similar to egg production, artificial diets had a significant effect on the number of larvae produced. On the first day, diet-T₆ had the highest number of larvae (24 ± 0.58 larvae), followed by diet-T₈ and T₀, while diet-T₇ had the lowest (19 ± 1.5 larvae). However, after 14 and 28 days, diet-T₈ had the highest number of larvae (47 ± 1.15 and 66 ± 1.15 larvae, respectively), followed by diet-T₆ and T₇, while diet-T₀ had the lowest (23 ± 1.5 and 26 ± 1.5 larvae, respectively). These findings are consistent with the egg production results and further highlighted the positive impacts of the artificial diets, particularly diet-T₈, on brood production over time, which is consistent with previous studies on the effect of diet on brood production (Shurjeel et al., 2023; DeGrandi-Hoffman et al., 2010).

Overall, the results of this study demonstrated the potential of artificial diets, specifically T₈, in enhancing various aspects of honeybee colony performance, including queen bee weight, worker bee longevity, and brood production (eggs, larvae, and pupae). These findings contributed to the ongoing research efforts aimed at developing sustainable and efficient dietary solutions for improving honeybee health and colony productivity.

Conclusion

Diet-T₈ (i.e. 25 g soya flour + 25 g dry apricot powder + 25 g date paste + 25 g powder sugar + 8 ml vegetable oil) can be used as a suitable substitute in the dearth period for honeybee colony management and survival. It had higher consumption and good digestibility in comparison to other diets; study also demonstrated the potentials of diet T₈, in enhancing various aspects of honeybee colony performance, including queen bee weight, worker bee longevity, and brood production (eggs, larvae, and pupae). These findings contributed to the ongoing research efforts aimed at developing sustainable and efficient dietary solutions for improving honeybee health and colony productivity. The current study emphasized the value of providing honeybee colonies with additional foods when there is insufficient pollen supply. To learn how these artificial diets affect social behavior, the quality and quantity of honey under diverse climatic situations, more research work is required.



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Contribution of Authors

All the authors contributed equally for idea conception, designing research methodology, data collection, analysis and interpretation of results, manuscript write up and editing.

Moreover, all authors read and approved the final draft of the manuscript as well.

References

- Akyol E, Yeninar H and Kaftanoglu O, 2008. Live weight of queen honey bees (*Apis mellifera* L.) predicts reproductive characteristics. J. Kansas Entomol. Soc. 81(2): 92-100. <https://doi.org/10.2317/JKES-705.13.1>
- Al-Ghamdi AA, Al-Khaibari AM and Omar MO, 2011. Effect of honeybee race and worker age on development and histological structure of hypopharyngeal glands of honeybee. Saudi J. Biol. Sci. 18(2): 113-116. <https://doi.org/10.1016/j.sjbs.2011.01.001>
- Aly MZ, Osman KS, Mohanny K and Elsayeh WA, 2014. New formula of pollen supplemental diets to study honey bee (*Apis mellifera carnica*) attractiveness. Egypt. Acad. J. Biol. Sci. A, Entomol. 7(2): 47-55. <https://10.21608/EAJBSA.2014.12944>
- Amir OG and Peveling R, 2004. Effect of triflumuron on brood development and colony survival of free-flying honeybee, *Apis mellifera* L. J. Appl. Entomol. 128(4): 242-249. <https://doi.org/10.1111/j.1439-0418.2004.00782.x>
- Amro A, Omar M and Al-Ghamdi A, 2016. Influence of different proteinaceous diets on consumption, brood rearing, and honey bee quality parameters under isolation conditions. Turk. J. Vet. Animal Sci. 40(4): 468-475. <https://doi.org/10.3906/vet-1507-28>
- Amro A, Younis M and Ghania A, 2020. Physiological effects of some pollen substitutes diets on caged honey bee workers (*Apis mellifera* L.). Int. J. Environ. 9(1): 87-99. <http://dx.doi.org/10.3126/ije.v9i1.27589>
- Basualdo M, Barragán S and Antúnez K, 2014. Bee bread increases honeybee haemolymph protein and promote better survival despite of causing higher *Nosema ceranae* abundance in honeybees. Environ. Microbiol. Rep. 6(4): 396-400. <https://doi.org/10.1111/1758-2229.12169>
- Black JL, 2006. Honeybee nutrition: review of research and practices. Publication No. 06/052. Rural Industries Research and Development Corporation: Canberra.
- Borsuk G, Ptaszyńska AA, Olszewski K, Domaciuk M, Krutmuang P and Paleolog J, 2017. A new method for quick and easy hemolymph collection from *apidae* adults. PloS One 12(1): e0170487. <https://doi.org/10.1371/journal.pone.0170487>
- Brodschneider R and Crailsheim K, 2010. Nutrition and health in honey bees. Apidologie 41(3): 278-294. <https://doi.org/10.1051/apido/2010012>
- Cremonz TM, De Jong D and Bitondi MM, 1998. Quantification of hemolymph proteins as a fast method for testing protein diets for honey bees (Hymenoptera: Apidae). J. Econom. Entomol. 91(6): 1284-1289. <https://doi.org/10.1093/jee/91.6.1284>
- DeGrandi-Hoffman G, Chen Y, Huang E and Huang MH, 2010. The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (*Apis mellifera* L.). J. Insect Physiol. 56(9): 1184-1191. <https://doi.org/10.1016/j.jinsphys.2010.03.017>
- Du Rand EE, Stutzer C, Human H, Pirk CW and Nicolson SW, 2020. Antibiotic treatment impairs protein digestion in the honeybee, *Apis mellifera*. Apidologie 51: 94-106. <https://doi.org/10.1007/s13592-019-00718-4>
- Evison SE and Ratnieks FL, 2007. New role for majors in *Atta* leafcutter ants. Ecol. Entomol. 32(5): 451-454. <https://doi.org/10.1111/j.1365-2311.2007.00877.x>
- Fleming JC, Schmehl DR and Ellis JD, 2015. Characterizing the impact of commercial pollen substitute diets on the level of *Nosema* spp. in



- honey bees (*Apis mellifera* L.). PLoS One 10(7): e0132014.
<https://doi.org/10.1371/journal.pone.0132014>
- Free JB and Williams IH, 1972. The influence of a honeybee (*Apis mellifera*) colony on egg-laying by its queen. Entomologia Experimentalis et Applicata 15(2): 224-228.
<https://doi.org/10.1111/j.1570-7458.1972.tb00200.x>
- Gemeda TK, 2014. Testing the effect of dearth period supplementary feeding of honeybee (*Apis mellifera*) on brood development and honey production. Int. J. Adv. Res. 2(11): 319-324.
- Helanterä H and Ratnieks FL, 2008. Geometry explains the benefits of division of labour in a leafcutter ant. Proceed. Royal Soc. B: Biol. Sci. 275(1640): 1255-1260.
<https://doi.org/10.1098/rspb.2008.0024>
- Helm BR, Slater GP, Rajamohan A, Yocum GD, Greenlee KJ and Bowsher JH, 2017. The geometric framework for nutrition reveals interactions between protein and carbohydrate during larval growth in honey bees. Biol. Open 6(6): 872-880.
<https://doi.org/10.1242/bio.022582>
- Howe HF, 1986. Seed dispersal by fruit-eating birds and mammals. Seed Dispersal 123: 189.
- Islam N, Mahmood R, Sarwar G, Ahmad S and Abid S, 2020. Development of pollen substitute diets for *Apis mellifera* ligustica colonies and their impact on brood development and honey production. Pak. J. Agric. Res. 33(2): 381-388.
<https://doi.org/10.17582/journal.pjar/2020/33.2.381.388>
- Keller I, Fluri P and Imdorf A, 2005. Pollen nutrition and colony development in honey bees: part 1. Bee world 86(1): 3-10.
<https://doi.org/10.1080/0005772X.2005.11099641>
- Kumari I and Kumar R, 2020. Pollen substitute diet for *Apis mellifera*: Consumption and effects on colony parameters in sub-tropical Himalaya. Indian J. Agric. Res. 54(2): 147-153.
<https://doi.org/10.18805/IJARE.5369>
- Kunc M, Dobeš P, Hurychová J, Vojtek L, Poiani SB, Danihlík J, Havlík J, Titěra D and Hyršl P, 2019. The year of the honey bee (*Apis mellifera* L.) with respect to its physiology and immunity: A search for biochemical markers of longevity. Insects 10(8): 244.
<https://doi.org/10.3390/insects10080244>
- Mahmood R, Wagchoure ES and Sarwar G, 2013. Influence of supplemental diets on *Apis mellifera* L. colonies for honey production. Pak. J. Agric. Res. 26(4): 290-294.
- Manning R, 2001. Fatty acids in pollen: a review of their importance for honey bees. Bee World 82(2): 60-75.
<https://doi.org/10.1080/0005772X.2001.11099504>
- Manning R, 2018. Chemical residues in beebread, honey, pollen and wax samples collected from bee hives placed on canola crops in Western Australia. J. Apicultural Res. 57(5): 696-708.
<https://doi.org/10.1080/00218839.2018.1494889>
- Martin N, Hulbert AJ, Bicudo JEPW, Mitchell TW and Else PL, 2021. The adult lifespan of the female honey bee (*Apis mellifera*): Metabolic rate, AGE pigment and the effect of dietary fatty acids. Mech. Ageing Develop. 199: 111562.
<https://doi.org/10.1016/j.mad.2021.111562>
- Mattila HR and Otis GW, 2006. Influence of pollen diet in spring on development of honey bee (Hymenoptera: Apidae) colonies. J. Econom. Entomol. 99(3): 604-613.
<https://doi.org/10.1093/jee/99.3.604>
- Noordyke ER and Ellis JD, 2021. Reviewing the efficacy of pollen substitutes as a management tool for improving the health and productivity of western honey bee (*Apis mellifera*) colonies. Front. Sustain. Food Syst. 5: 772897.
<https://doi.org/10.3389/fsufs.2021.772897>
- Omar EM, Darwish HY, Othman AA, El-Seedi HR and Al Naggar Y, 2022. Crushing corn pollen grains increased diet digestibility and hemolymph protein content while decreasing honey bee consumption. Apidologie 53(5): 52.
<https://doi.org/10.1007/s13592-022-00963-0>
- Paray BA, Kumari I, Hajam YA, Sharma B, Kumar R, Albeshr MF, Farah MA and Khan JM, 2021. Honeybee nutrition and pollen substitutes: A review. Saudi J. Biol. Sci. 28(1): 1167-1176.
<https://doi.org/10.1016/j.sjbs.2020.11.053>
- Pawlikowski T, 2010. Pollination activity of bees (Apoidea: Apiformes) visiting the flowers of *Tilia cordata* Mill. and *Tilia tomentosa* Moench in an urban environment. J. Apic. Sci. 54(2): 73-79.
- Saffari AM, Kevan PG and Atkinson JL, 2004. A promising pollen substitute for honey bees. American Bee J. 144(3): 230-231.
- Seeley TD, 2019. The history of honey bees in North America. Phylogenetics of Bees (eds. R. Ilyasov and HW Kwon): 222-232.



- Shurjeel HK, Aqueel MA, Ashraf E, Ali A and Rubab A, 2020. Effect of insecticides on the longevity of *Apis mellifera* L. (Hymenoptera: Apidae). Sarhad J. Agric. 36(3): 768-776. <http://dx.doi.org/10.17582/journal.sja/2017/36.3.768.776>
- Shurjeel HK, Aqueel MA, Rubab A, Iqbal S, Akram A and Saeed N, 2023. Impact of Yeast Diet on the Number of Eggs and Larvae Produced in Honey Bee Colonies (*Apis Mellifera* L.) Apidae: Hymenoptera. Proceed. Pak. Acad. Sci.: B. Life Environ. Sci. 60(4): 627-634. [https://doi.org/10.53560/PPASB\(60-4\)772](https://doi.org/10.53560/PPASB(60-4)772)
- Skowronek P, Wójcik Ł and Strachecka A, 2021. Cannabis extract has a positive-immunostimulating effect through proteolytic system and metabolic compounds of honey bee (*Apis mellifera*) workers. Animals 11(8): 2190. <https://doi.org/10.3390/ani11082190>
- Ullah A, Shahzad MF, Iqbal J and Baloch MS, 2021. Nutritional effects of supplementary diets on brood development, biological activities and honey production of *Apis mellifera* L. Saudi J. Biol. Sci. 28(12): 6861-6868. <https://doi.org/10.1016/j.sjbs.2021.07.067>
- Usha U, Srivastava P, Goswami V and Khan MS, 2014. Exploration of various flours as pollen substitutes for *Apis mellifera* L. during Dearth period at Tarai region of Uttarakhand, India. J. Appl. Nat. Sci. 6(2): 812-815. <https://doi.org/10.31018/jans.v6i2.541>
- Weirich GF, Collins AM and Williams VP, 2002. Antioxidant enzymes in the honey bee, *Apis mellifera*. Apidologie 33(1): 3-14. <https://doi.org/10.1051/apido:2001001>

