Journal of Experimental Agriculture International

Volume 46, Issue 9, Page 796-803, 2024; Article no.JEAI.121970
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Evaluation of Some Pollen Substitutes on Productivity of Honey bee Colonies (*Apis cerana* F.)

Udeshna Borgohain [a], M.K. Deka [a], Abhigyan Rajkhowa [b*], Dipankar Brahma [c], and Karthik R [a]

[a] *Department of Entomology, Assam Agricultural University, Jorhat -785013, India.*
[b] *Department of Sericulture, Assam Agricultural University, Jorhat -785013, India.*
[c] *Department of Sericulture, Forest College and Research Institute, Mettupalayam, Coimbatore – 641301, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jeai/2024/v46i92876

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/121970

Original Research Article

Received: 26/06/2024
Accepted: 28/08/2024
Published: 13/09/2024

ABSTRACT

This study examined the effects of different pollen substitutes on the performance of honey bee colonies in Assam, India. Natural Corbicula pollen (CPF) and six artificial diets were compared with a sugar syrup control for 15 days. CPF consistently outperformed other treatments in all parameters. Regarding the honey area, the CPF reached 22.63 cm^2 and 22.65 cm^2 in 2021 and 2022, respectively, while in the control group it was 15.61 cm^2 and 15.72 cm^2, respectively. CPF also had the highest pollen area (15.59 cm^2 and 15.68 cm^2) and brood area (20.19 cm^2 and 20.35 cm^2) in both years. Colony strength was maximized in the CPF (7.55 and 7.59 for

**Corresponding author: E-mail: abhirajkhowa13@gmail.com;*

Cite as: *Borgohain, Udeshna, M.K. Deka, Abhigyan Rajkhowa, Dipankar Brahma, and Karthik R. 2024. "Evaluation of Some Pollen Substitutes on Productivity of Honey Bee Colonies (Apis Cerana F.)". Journal of Experimental Agriculture International 46 (9):796-803. https://doi.org/10.9734/jeai/2024/v46i92876.*

frames 2021 and 2022). Among artificial foods, OUAT-PS (soy flour, honey, yeast, multivitamin) generally performed best, with no significant difference from CPF in colony strength. All pollen substitutes stimulated honey production and brood development with foods rich in proteins, vitamins and amino acids, and significantly improved colony performance compared to controls. These results can help beekeepers select appropriate additional nutrients in times of pollen shortage.

Keywords: Pollen substitutes; honey bee; honey area; pollen area; brood area; colony strength.

1. INTRODUCTION

Honey bees are regarded as most important eusocial insects, providing honey and various products such as beeswax, royal jelly, pollen, bee venom and propolis for the welfare of human beings [1]. Additionally, bees play a significant role in increasing agricultural and horticultural productivity through their pollination services, accounting for 80% of pollinating insects worldwide [2]. Beekeeping, also known as apiculture, involves the cultivation and management of domesticated honey bee species. In India, the most important domesticated bee species are *Apis cerana* and *Apis mellifera*. Among these two bee species, *A. cerana* is distributed across the tropical regions. In India, it is a prominent domesticated species, particularly in southern and north-eastern regions, including Assam [3,4].

Honey bees primarily visit flowers for their food sources, which consists of nectar and pollen [5]. Nectar is an important carbohydrate source, while pollen is the main source of protein [6]. Pollen also contains lipids, minerals and macro and micronutrients that are essential for the overall colony development [7]. In Assam, the peak seasons for floral availability, providing ample pollen resources for honey bees, are spring and winter, coinciding with honey harvesting. However, intense rainfall during the monsoon season hampers beekeepers' ability to harvest honey. During this period, particularly in June and July, honey bees face challenges such as scarcity of natural food sources, the presence of natural adversaries, and frequent absconding. These challenges lead to decreased colony development and egg-laying capacity [8]. To address this issue, bee keepers can relocate their colonies to areas with sufficient food sources. However, this approach is time-consuming and expensive, and it also has its own set of risks. The colony's movement during the dearth period demonstrates that there was an estimated 40% loss of colonies annually [9]. Therefore, artificial feeding consists of pollen substitutes is necessary to sustain *A. cerana* beekeeping in Assam.

Limited information exists regarding the effects of pollen alternatives on the growth and development of the Indian honey bee, *Apis cerana*. Several agricultural universities in India have developed different pollen substitutes for managing honey bee colonies during periods of pollen dearth. Therefore, the prime aim of this study was to examine the impacts of pollen substitutes on colony performance of *Apis cerana* by comparing the various pollen substitutes developed by agricultural universities in India and determining the best pollen substitute suitable for conditions in Assam.

2. MATERIALS AND METHODS

2.1 Study Site and Bee Colony Maintenance

The study was conducted from June 2021 to July 2022 at the Instructional Cum Research (ICR) Farm, Horticultural Orchard, and Departmental Apiary and Apiculture Laboratory of Assam Agricultural University, Jorhat, India (26°45'N, 94°12'E, 87m above MSL).

Bee colonies were located away from high-traffic areas, with access to various forages and a water source, sheltered from strong winds and exposed to morning sunlight to remain healthy and productive. Hives were inspected every 7-10 days during the active season to monitor colony health, queen presence, brood patterns, disease, and pest presence. Bee hives needed to be cleaned and supplemented with sugar syrup during nectar dearths. It was ensured that hive space and ventilation were maintained, and queen cells were monitored. Regular cleaning was done, protective gear was used and detailed documentation of inspections and treatments was carried out during the study period for effective colony management.

2.2 Experimental Design and Treatment Details

The present experiment employed a Randomized Block Design (RBD). Seven treatments were followed with 3 replications. The treatments comprised: Punjab Agricultural University pollen substitute (PAU-PS), Govind Ballabh Pant University of Agriculture and Technology pollen substitute (GBPUAT-PS), Dr. Yashwant Singh Parmar University of Horticulture and Forestry (YSPUHF-PS), Corbicular Pollen Feeding (CPF), Odisha University of Agriculture and Technology (OUAT-PS), Assam Agricultural University pollen substitute (AAU- PS) and control. PAU-PS, GBPUAT-PS, YSPUHF-PS, CPF, OUAT-PS, AAU- PS and control. The pollen diet components of different treatments were as follows:

1. PAU-PS - Brewer's yeast (41.8 g) + Skimmed milk powder (4 g) + Dehusked parched gram (4.2 g) and mix the gram and sugar solution in a 13:12 ratio
2. GBPUAT-PS- Soya bari (18.75 g) + Honey (50 g) + Amul (12.5 g) + Yeast (12.5 g)
3. YSPUHF- PS- Soya (150 g) + wheat (150 g) + Yeast (100 g) + Sugar solution (400 ml) + Dark Rum (20 ml)
4. CPF- Pre-collected natural corbicular pollen (3g with a pollen was collected from natural floral sources and 750 grams of sugar were dissolved as a sugar solution by adding it to 1 litre of water.)
5. OUAT- PS- Soya (60 g) + Honey (35 g) + Yeast (5 g) + multi-vitamin (1 g)
6. AAU-PS - Soybean flour + Sugar + Honey + Water + Brewer's yeast + Skimmed milk powder + multi-vitamin. It was developed by Assam Agricultural University, Jorhat. It contained 100 g soy flour, 100 g sugar, 10 g honey and 100 ml water Brewer's yeast 50 g, skimmed milk powder 5 g, multivitamins 5 g Mix well in a container and feed the bees in a Petri dish soaked in water Cotton.
7. Control- sugar solution feeding (SSF) i.e., sugar solution prepared by dissolving 750 g of sugar in 1L of water.

These pollen substitutes were obtained from the respective institutions.

2.3 Feeding of Pollen Diets and Observation

Diets were prepared by combining the respective components and providing them to the colonies in petri dishes with cotton soaked in syrup, and placed on the top bars of the brood chamber. After the diet was established, three measurements were taken for each treatment, before treatment, 7 and 15 days after treatment, for growth and development of brood (unsealed/sealed) and stored contents (nectar/pollen). A preliminary calculation was made before the provision of the regime.

2.4 Procedure for Honey Area

The paper grid method was used to record the honey area. The grid was created by cutting out a 10-centimeter square area and placing it in the bee's honeycomb. From this area we can obtain several parameters. The paper grid method is a low-technology and low-cost way to convert data from a smaller area to a larger area, as well as to reproduce and/or enlarge an image. It was measured in cm^2.

2.5 Procedure for Pollen Area

The pollen area was also measured using the paper grid method. To obtain the desired parameters, a grid was installed in the pollen area of the honeycomb. Pollen is mostly found between the honey and brood areas. It was measured in cm^2.

2.6 Procedure for Brood Area

The brood area was also evaluated using the paper grid method. To obtain the required parameter, a grid was installed in the brood region of the bee comb. It was also measured in cm^2.

2.7 Procedure for Colony Strength

To determine the strength of the colony/bee, we counted the total number of covered bee frames contained in the hive.

2.8 Statistical Analysis

The data on colony performance of *A. cerana* was subjected to a two-way analysis of variance (ANOVA) with the RBD. In the present experiment, the level of significance (α) was fixed at 5%. Hence all conclusions were made at a 95% confidence level. The normality of the data was examined by Shapiro Wilk's test Shapiro and Wilk, [10] and the homogeneity of variance assumption was examined using Bertlett's Test [11]. All the statistical analyses were done by using the R Software R core Team [12] with the R packages "agricolae" [13].

3. RESULTS

3.1 Influence of Pollen Substitute Dietary Treatments on Honey Area

In both 2021 and 2022, the pre-treatment data no significant differences among the treatments. Seven Days After Feeding (DAF), CPF treatment produced the highest honey area, measuring 15.68 cm^2 and 15.73 cm^2 in 2021 and 2022, respectively, compared to other treatments. In contrast, the control had the lowest honey area measuring 10.67 cm^2 and 10.78 cm^2 during the corresponding years. The other pollen substitute treatments also demonstrated significantly higher honey areas compared to the control.

Fifteen days after feeding, the CPF treatment continued to yield the maximum honey area of 22.63 cm^2 and 22.65 cm^2 in 2021 and 2022, respectively, while the control had the minimum honey area of 15.61 cm^2 and 15.72 cm^2. The other treatments also showed a significant increase in honey storage area compared to the control. In both years, PAU-PS and OUAT-PS treatments, among the artificial pollen diets, exhibited the maximum honey area (Table 1).

3.2 Influence of Pollen Substitute Dietary Treatments on Pollen Area

In the case of pollen area, no significant difference was observed in the pre- treatment data. However, after feeding with pollen substitutes, all treatments showed significant difference compared to the control. Among all treatments tested, CPF exhibited the highest pollen area after 7 DAF, with 10.83 cm^2 and 10.9 cm^2 in 2021 and 2022, respectively. This was followed by PAU-PS, OUAT-PS and YSPUHF-PS, while the control showed the lowest pollen area of 5.90 cm^2 and 5.98 cm^2 during the corresponding years. After 15 DAF, CPF yielded the maximum pollen area of 15.59 cm^2 and 15.68 cm^2 in 2021 and 2022, respectively, while the control had the minimum pollen area of 8.54 cm^2 and 8.73 cm^2 (Table 2). The other artificial pollen substitute treatments also exhibited a significant increase in pollen storage area relative to the control.

Table 1. Effect of different pollen substitutes on the honey area

Treatments	Honey area (cm^2) during 2021			Honey area (cm^2) during 2022		
Treatments	Pre-treatment	Post-treatment		Pre-treatment	Post-treatment	
		7 DAF	15 DAF		7 DAF	15 DAF
PAU-PS	4.66	14.70^b	21.74^b	4.63	15.13^b	21.82^b
GBPUAT-PS	4.70	13.53^d	20.64^d	4.65	13.78^d	20.82^d
YSPUHF-PS	4.68	14.55^c	21.65^c	4.71	14.57^{bc}	21.22^c
CPF	4.76	15.68^a	22.63^a	4.78	15.73^a	22.65^a
OUAT-PS	4.69	14.71^b	21.82^b	4.65	14.84^b	21.86^b
AAU-PS	4.72	13.48^d	20.52^e	4.70	13.76^d	20.72^d
Control	4.68	10.67^e	15.61^f	4.71	10.78^e	15.72^e

Data are mean of 10 observations from 3 replications
Lower case letters indicate significant difference at $p \le 0.05$ following two-way ANOVA

Table 2. Effect of different pollen substitutes on the pollen area

Treatments	Pollen area (cm^2) during 2021			Pollen area (cm^2) during 2022		
	Pre-treatment	Post treatment		Pre-treatment	Post treatment	
		7 DAF	15 DAF		7 DAF	15 DAF
PAU-PS	5.68	9.93^b	14.75^b	5.74	9.93^b	14.83^b
GBPUAT-PS	5.70	8.65^c	14.68^b	5.72	8.76^c	14.74^b
YSPUHF-PS	5.68	9.78^b	14.74^b	5.69	9.87^b	14.78^b
CPF	5.66	10.83^a	15.59^a	5.74	10.90^a	15.68^a
OUAT-PS	5.71	9.89^b	14.81^b	5.71	9.99^b	14.83^b
AAU-PS	5.68	8.69^c	14.53^{bc}	5.73	8.83^c	14.64^b
Control	5.67	5.90^d	8.54^d	5.74	5.98^d	8.73^c

Data are mean of 10 observations from 3 replications
Lower case letters indicate significant difference at $p \le 0.05$ following two-way ANOVA

Table 3. Effect of different pollen substitutes on the brood area

Treatments	Brood area (cm^2) during 2021			Brood area (cm^2) during 2022		
	Pre-treatment	Post treatment		Pre-treatment	Post treatment	
		7 DAF	15 DAF		7 DAF	15 DAF
PAU-PS	7.87	17.54^{c}	19.58^{b}	7.88	17.54^{b}	19.56^{b}
GBPUAT-PS	7.85	16.76^{e}	18.48^{c}	7.85	16.76^{d}	18.57^{c}
YSPUHF-PS	7.79	17.34^{d}	19.33^{bc}	7.82	17.33^{c}	19.36^{b}
CPF	7.78	17.97^{a}	20.19^{a}	7.83	17.96^{a}	20.35^{a}
OUAT-PS	7.76	17.58^{b}	19.74^{b}	7.81	17.55^{b}	19.70^{b}
AAU-PS	7.68	16.68^{f}	18.23^{c}	7.88	16.66^{e}	18.21^{c}
Control	7.85	11.16^{g}	12.89^{d}	7.85	11.19^{f}	12.96^{d}

Data are mean of 10 observations from 3 replications
Lower case letters indicate significant difference at p ≤ 0.05 following two-way ANOVA

Table 4. Effect of different pollen substitutes on the colony strength

Treatments	Colony strength (nos.) during 2021			Colony strength (nos.) during 2022		
	Pre-treatment	Post treatment		Pre-treatment	Post treatment	
		7 DAF	15 DAF		7 DAF	15 DAF
PAU-PS	5.58	6.54^{ab}	7.32^{b}	5.59	6.59^{a}	7.35^{b}
GBPUAT-PS	5.59	6.36^{c}	7.25^{b}	5.92	6.43^{ab}	7.25^{b}
YSPUHF-PS	5.60	6.53^{b}	7.28^{b}	5.62	6.53^{a}	7.31^{b}
CPF	5.57	6.62^{a}	7.55^{a}	5.70	6.64^{a}	7.59^{a}
OUAT-PS	5.60	6.52^{b}	7.35^{ab}	5.60	6.50^{a}	7.39^{ab}
AAU-PS	5.56	6.24^{d}	7.03^{c}	5.58	6.46^{a}	7.05^{bc}
Control	5.58	5.64^{e}	6.38^{d}	5.60	5.57^{b}	6.42^{d}

Data are mean of 10 observations from 3 replications
Lower case letters indicate significant difference at p ≤ 0.05 following two-way ANOVA

3.3 Influence of Pollen Substitute Dietary Treatments on Brood Area

The pre-treatment data did not reveal any significant difference in brood area. However, during both 2021 and 2022, the CPF treatment consistently exhibited the highest brood area at both 7 and 15 DAF (Table 3). Specifically, the brood area for CPF was 17.97 cm^2 and 17.96 cm^2 in 2021 and 2022, respectively, while at 15 DAF, it measured 20.19 $cm^2$and 20.35 cm^2in 2021 and 2022, respectively. Following CPF, the OUAT-PS and PAU-PS showed the next highest brood area. In contrast, control displayed the lowest brood area in both years. For the control, the brood area at 7 DAF was 11.16 cm^2 and 11.19 cm^2 in 2021 and 2022, respectively and at 15 DAF, it was 12.89 cm^2 and 12.96 cm^2.

3.4 Influence of Pollen Substitute Dietary Treatments on Colony Strength

Before feeding with pollen substitutes, there were no significant differences in colony strength between the treatments. In 2021, at 7 DAF, the highest colony strength (6.63 nos.) was observed in CPF which was statistically at par with PAU-PS (6.54 nos.). A similar pattern was observed in 2022. In both the years, at 7 DAF, the lowest colony strength was observed in control. At 15 DAF, the results showed that CPF had the maximum colony strength in CPF (7.55 and 7.59 nos. in 2021 and 2022, respectively), while the minimum colony strength was recorded in control (6.38 and 6.42 nos. in 2021 and 2022, respectively). At 15 DAF, in both the years, OUAT-PS showed no significant difference in colony strength between CPF (Table 4).

4. DISCUSSION

4.1 Influence of Pollen Substitute Dietary Treatments on Honey Area

In the present investigation, the maximum honey area was observed in the treatment CPF. Generally, bees consume more natural pollen than they consume pollen substitutes [14]. It could be attributed to the nutritional composition of natural pollen which is rich in protein, vitamin B complex, amino acids, and folic acid, which are essential for enhancing adult bee activity [15]. It is also to be noted that the previous studies demonstrated that the maximum preference of pollen by bees over substitute diets and increased the honey area [16]. In the present research, among the artificial pollen substitute treatments, OUAT-PS (soybean flour, honey, yeast, and multivitamin) exhibited the maximum honey area of 21.83±0.11 cm^2, likely due to its balanced nutritional profile. These findings are consistent with the results reported by Sabir et al. [17], who observed the highest honey area (195.79 square inches) in colonies supplemented with maize flour, four vitamin B complexes, and methionine (T8), outperforming other treatment groups. The inclusion of essential nutrients, such as proteins, vitamins, and amino acids, in pollen substitute diets appears to stimulate honey production in honey bee colonies.

4.2 Influence of Pollen Substitute Dietary Treatments on Pollen Area

In case of pollen area, CPF demonstrated maximum pollen area compared to other treatments. This could be attributed to the nutritional composition of pollen, which is rich in proteins (about 40%), vitamins like B-complex, free amino acids, folic acid and ascorbic acid. Among artificial diets tested in the study, the treatment OUAT-PS (soya + honey + yeast + multi-vitamin) was found to be the most suitable, showing a maximum pollen area of 14.81cm^2 and 14.81cm^2 in 2021 and 2022, respectively. Similar findings were reported by Sihug and Gupta [18], who found that a diet consisting of soybean flour + yeast + vitamins + minerals was the most effective in terms of pollen area, with an increase from 79.0cm^2 to 239cm^2 , which was statistically significant compared to all other treatments.

4.3 Influence of Pollen Substitute Dietary Treatments on Brood Area

In the current study, both CPF and other pollen substitutes were found to significantly enhance the brood area of *A. cerana*. It is known that pollen diet increases number and weight of ovaries of honey bees [19]. Based on these findings, it can be suggested that the observed increase in brood area after feeding with the pollen substitutes may be attributed to the enhancement in ovaries. A congruent finding was also reported by Kumar et al. [20]. who observed a substantial augmentation in the brood area (2053.0 cm^2 per colony) when colonies were provided with CPF- Pre-collected natural corbicular pollen, wherein natural pollen constituted a significant component. This phenomenon could potentially be attributed to the presence of ascorbic acid within the pollen, which appears to play a crucial role in brood development Sihug and Gupta [18].

4.4 Influence of Pollen Substitute Dietary Treatments on Colony Strength

In this study, it was found that colony strength was significantly enhanced with the CPF diet. Interestingly, the treatment OUAT-PS, which mainly consisted of soybean flour, showed no significant difference compared to CPF. This could be attributed to the fact that soybean soybean flour is readily consumed by the adult bees. Soybean flour is known for its richness in protein and carbohydrates, with a small amount of fat and fiber, which may contribute to increased adult bee activity in the hive during dearth periods [21]. A similar result was also observed by Sabir et al. [17], who reported the highest colony strength of 6.24nos [22,23]. covering 84 frames on average when colonies were fed with soybean flour along with vitamin B-complex and methionine [24,25].

5. CONCLUSION

Based on the findings of this study, it can be concluded that the colony performance of *A. cerana* was significantly improved with the use of CPF compared to other pollen substitutes. Moreover, other pollen substitutes also demonstrated effectiveness when compared to control. One of the factors contributing decline of honey bee population is an unavailability and unbalanced diet. Therefore, future research should focus on investigating the impact of these pollen substitutes on the physiology of *A. cerana*, with a particular emphasis on their dietary requirements.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Requier F, Garnery L, Kohl PL, Njovu HK, Pirk CW, Crewe RM, Steffan-Dewenter I. The conservation of native honey bees is crucial. Trends in Ecology and Evolution. 2019;34(9):789-798.
2. Breeze TD, Bailey AP, Balcombe KG, Potts SG.(2011). Pollination services in the UK: How important are honeybees? Agriculture, Ecosystems and Environment. 2011;142(3-4):137-143.
3. Bhagawati S, Rahman A, Deka MK. Diversity of insect foragers with reference to foraging behaviour of Asian honey bee, Apis cerana F. on sesamum, Sesamum indicum L. Journal of Entomological Research. 2016;40(3):213-216.
4. Koetz AH. Ecology, behaviour and control of Apis cerana with a focus on relevance to the Australian incursion. Insects. 2013;4(4):558-592.
5. Wright GA, Nicolson SW, Shafir S. Nutritional physiology and ecology of honey bees. Annual Review of Entomology. 2018;63:327-344.
6. Abou-Shaara HF. The foraging behaviour of honey bees, *Apis mellifera*: A review. *Veterinarni medicina.* 2014;59(1).
7. Loidl A, Crailsheim K. Free fatty acids digested from pollen and triolein in the honeybee (*Apis mellifera* carnica Pollmann) midgut. Journal of Comparative Physiology B. 2011;171:313-319.
8. Awad AM, Owayss AA, Alqarni AS. Performance of two honey bee subspecies during harsh weather and Acacia gerrardii nectar-rich flow. *Scientia Agricola*. 2017;*74*:474-480.
9. Kumar R, Agrawal OP. Comparative performance of honey bee colonies fed with artificial diets in Gwalior and Panchkula region. Journal of Entomology and Zoology Studies. 2014;2(4):104-107.
10. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). *Biometrika.* 1965;52(3):591–611
11. Chao MT, Glaser RE. The exact distribution of Bartlett's test statistic for homogeneity of variances with unequal sample sizes. Journal of the American Statistical Association. 1978;73 (362):422-426.
12. Core Team R. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria; 2022.
13. Mendiburu FD, Yaseen M. agricolae: Statistical Procedures for Agricultural Research. R package version; 2020 1. 4..0
14. Ricigliano VA, Simone-Finstrom M. Nutritional and prebiotic efficacy of the microalga *Arthrospira platensis* (spirulina) in honey bees. Apidologie. 2020;51(5):898-910.
15. Lan J, Ding G, Ma W, Jiang Y, Huang J. Pollen source affects development and behavioral preferences in honey bees. Insects. 2021;12(2):130.
16. Saffari A, Kevan PG, Atkinson JL. Palatability and consumption of patty-formulated pollen and pollen substitutes and their effects on honeybee colony performance. Journal of Apicultural Science. 2010;54(2):63-71.
17. Sabir AM, Suhail A, Akram W, Sarwar G, Saleem M. Effect of some pollen substitute diets on the development of Apis mellifera L. colonies. Pakistan Journal of Biological Sciences. 2000;3(5):890-891.
18. Sihag RC, Gupta M. Testing the Effects of Some Pollen Substitute Diets on Colony Build up and Economics of Beekeeping vvith *Apis mellifera* L. Journal of Entomology. 2013;10(3):120-35.
19. Eldin GNK, Ebeid AA, Sallam AM, Basuny NK. Effect of pollen supplements and substitutes on honey bee queen ovaries and worker hypopharyngeal glands. Journal of Plant Protection and Pathology. 2018;9(2):83-91.
20. Kumar R, Mishra RC, Agrawal OP. Effect of feeding artificial diets to honey bees during dearth period under Panchkula (Haryana) conditions. Journal of Entomological Research. 2013;37(1):41-46.
21. Ahmad S, Khan KA, Khan SA, Ghramh HA, Gul A. 2021). Comparative assessment of various supplementary diets on commercial honey bee (Apis mellifera) health and colony performance. PLoS One. 2021;16(10):e0258430. Available:https://doi.org/10.1371/journal.pone.0258430
22. Al-Ghamdi AA, Al-Khaibari AM, Omar MO. 2011). Consumption rate of some proteinic diets affecting hypopharyngeal glands development in honeybee workers. Saudi Journal of Biological Sciences. 2011;18(1): 73-77.
23. Haydak MH. Brood rearing by honey bees confined to a pure carbohydrate diet. Journal of Economic Entomology. 1935;28: 657-660.

24. Islam N, Mahmood R, Sarwar G, Ahmad S, Abid S. Development of pollen substitute diets for Apis mellifera ligustica colonies and their impact on brood development and honey production. Pakistan Journal of Agricultural Research. 2020;33(2):381-388.

25. Sihag RC, Gupta M. Development of an artificial pollen substitute/supplement diet to help tide the colonies of honeybee (*Apis mellifera* L.) over the dearth season. Journal of Apicultural Science. 2011;55(2): 15-28.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/121970