

## Comparative analysis of various pollen substitute diets on the colony performance of Honey bee, *Apis*

*cerana* F.

### Abstract

The present investigation aims to study the impact of various pollen substitutes on different parameters of the colony of the Indian honey bee (*Apis cerana*) such as honey area, pollen area, brood area, and colony strength during the dearth period. The experiment was conducted at Assam Agricultural University (AAU), Jorhat with a Randomized Block Design (RBD). Seven pollen dietary treatments viz., 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 i.e., Sugar Solution Feeding (SSF) were evaluated. The results indicate that the treatment CPF significantly increased the colony parameters of *A. cerana* compared to all other treatments. Furthermore, the other pollen substitute treatments also significantly enhanced colony performance compared to the control. This study highlights the importance of supplementing *A. cerana* with pollen diets during periods of pollen dearth.

**Keywords:** Pollen substitutes, honey bee, honey area, pollen area, brood area, colony strength

### 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 (Requier et al. 2019). Additionally, bees play a significant role in increasing agricultural and horticultural productivity through their pollination services, accounting for 80% of pollinating insects worldwide (Breeze et al., 2011). 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 (Bhagawati et al. 2016; Koetz, 2013).

Honey bees primarily visit flowers for their food sources, which consists of nectar and pollen (Wright et al. 2018). Nectar is an important carbohydrate source, while pollen is the main source of protein (Abou-Shaara, 2014). Pollen also contains lipids, minerals and macro and micronutrients that are essential for the

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**Comment [i2]:** Pollen sources and various pollen substitute methods are repeated in detail in the methods. It is preferable to express them a little and mention more results in the abstract.

**Comment [i3]:** Writing references in the text from old to new

overall colony development (Loidl and Crailsheim, 2001). 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 (Awad et al. 2017). 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 (Kumar and Agrawal, 2014). 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.

## Materials and Methods

### 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 ~~was~~relocated away from ~~high-traffic~~high-traffic areas, with access to ~~a variety of forages and a water source, sheltered from strong winds and exposed to morning sunlight in order to~~various forages and a water source, sheltered from strong winds and exposed to morning sunlight to remain healthy and productive.~~Inspections of hives was conducted~~Hives were inspected every 7-10 days during the active season to monitor colony health, queen presence, brood patterns, disease, and pest presence.~~It was necessary that bee hives was~~Bee hives needed to be cleaned and supplemented with sugar syrup during nectardearths.It was ensured that hive space and ventilation~~was 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.

### 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 + Skimmed milk powder + Dehusked parched gram + Sugar solution
2. GBPUAT-PS- Soya-baribean + Honey + Amul + Yeast
3. YSPUHF- PS- Soya + wheat + Yeast + Sugar solution + Dark Rum
4. CPF- Pre-collected natural corbicular pollen
5. OUAT- PS- Soya + Honey + Yeast + Multi-vitamin
6. AAU-PS - Soybean flour + Sugar + Honey + Water + Brewer's yeast + Skimmed milk powder + Multi-vitamin. It was developed by Assam Agricultural University Assam Agricultural University developed it, Jorhat contained 100 g soy flour, 100 g sugar, 10 g honey, 100 ml water, 50 g brewer's yeast, 5 g skimmed milk powder and 5 g multivitamins Mixed well in a container and fed to the bees in a soaked petri dish Cotton.
7. Control- sugar solution feeding (SSF) i.e., sugar solution prepared by dissolving 750g of sugar in 1L of water.

These pollen substitutes were obtained from the respective institutions.

### 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

**Comment [i4]:** The number of honey bee colonies and the components of each colony must be added to the number of combs, as they must be of the same strength and the same ages of queens, in order to judge the progress of the colonies under the influence of the nutritional alternative.

**Comment [i5]:** The protein content of each alternative had to be measured.

**Comment [i6]:** The weight of each component of the food substitute must be stated.

and development of brood (unsealed/sealed) and stored contents (nectar/pollen). A preliminary calculation was made before the provision of the regime.

#### 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<sup>2</sup>.

**Comment [i7]:** It is best to weigh the honeycombs before and after extracting the honey from the colonies.

#### 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<sup>2</sup>.

**Comment [i8]:** How is the effect of pollen substitutes known in the presence of a natural pollen source measured?

#### Procedure for brood area

The brood area was also evaluated using the paper grid method. In order to obtain the required parameter, a grid was installed in the brood region of the bee comb. It was also measured in cm<sup>2</sup>.

**Comment [i9]:** How long is the time between each worker brood measurement?

**Comment [i10]:** When measuring the sealed brood every 13 days, but when measuring the sealed and unsealed brood every 20 or 21 days.

#### 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.

**Comment [i11]:** How strong was the bee colony at the start of the experiment?

#### Statistical Analysis

The data on colony performance of *A. ceranawas* was subjected to a two-way analysis of variance (ANOVA) with the RBD. In the present experiment, the level of significance ( $\alpha$ ) was fixed as 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, 1965) and the homogeneity of variance assumption was examined using Bertlett's Test (Chao and Glaser, 1978). All the statistical analyses were done by using the R Software (R core Team 2022) with the R packages "agricolae" (Mendiburu and Yaseen 2020).

**Comment [i12]:** RCBD is more accurate in analyzing results than RBD.

#### Results

### 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<sup>2</sup> and 15.73 cm<sup>2</sup> in 2021 and 2022, respectively, compared to other treatments. In contrast, the control had the lowest honey area measuring 10.67 cm<sup>2</sup> and 10.78 cm<sup>2</sup> 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<sup>2</sup> and 22.65 cm<sup>2</sup> in 2021 and 2022, respectively, while the control had the minimum honey area of 15.61 cm<sup>2</sup> and 15.72 cm<sup>2</sup>. The other treatments also showed a significant increase in honey storage area compared to the control. In both the years, PAU-PS and OUAT-PS treatments, among the artificial pollen diets, exhibited the maximum honey area (Table 1).

**Comment [113]:** Do pollen substitutes affect the quality of honey produced?

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

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

Data are mean of 10 observations from 3 replications

Lower case letters indicate significant difference at  $p \leq 0.05$  following two-way ANOVA

### 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<sup>2</sup> and 10.9 cm<sup>2</sup> 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<sup>2</sup> and 5.98 cm<sup>2</sup> during the corresponding years. After 15 DAF, CPF yielded the maximum pollen area of 15.59 cm<sup>2</sup> and 15.68 cm<sup>2</sup> in 2021 and 2022, respectively, while the control had the minimum pollen area of 8.54 cm<sup>2</sup> and 8.73 cm<sup>2</sup> (Table 2). The other artificial pollen substitute treatments also exhibited a significant increase in pollen storage area relative to the control.

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

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

Data are mean of 10 observations from 3 replications

Lower case letters indicate significant difference at  $p \leq 0.05$  following two-way ANOVA

#### **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<sup>2</sup> and 17.96 cm<sup>2</sup> in 2021 and 2022, respectively, while at 15 DAF, it measured 20.19 cm<sup>2</sup> and 20.35 cm<sup>2</sup> in 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<sup>2</sup> and 11.19 cm<sup>2</sup> in 2021 and 2022, respectively and at 15 DAF, it was 12.89 cm<sup>2</sup> and 12.96 cm<sup>2</sup>.

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

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

Data are mean of 10 observations from 3 replications

Lower case letters indicate significant difference at  $p \leq 0.05$  following two-way ANOVA

#### 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).

**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
<b>PAU-PS</b>	5.58	6.54 <sup>ab</sup>	7.32 <sup>b</sup>	5.59	6.59 <sup>a</sup>	7.35 <sup>b</sup>
<b>GBPUAT-PS</b>	5.59	6.36 <sup>c</sup>	7.25 <sup>b</sup>	5.92	6.43 <sup>ab</sup>	7.25 <sup>b</sup>
<b>YSPUHF-PS</b>	5.60	6.53 <sup>b</sup>	7.28 <sup>b</sup>	5.62	6.53 <sup>a</sup>	7.31 <sup>b</sup>
<b>CPF</b>	5.57	6.62 <sup>a</sup>	7.55 <sup>a</sup>	5.70	6.64 <sup>a</sup>	7.59 <sup>a</sup>
<b>OUAT-PS</b>	5.60	6.52 <sup>b</sup>	7.35 <sup>ab</sup>	5.60	6.50 <sup>a</sup>	7.39 <sup>ab</sup>
<b>AAU-PS</b>	5.56	6.24 <sup>d</sup>	7.03 <sup>c</sup>	5.58	6.46 <sup>a</sup>	7.05 <sup>bc</sup>
<b>Control</b>	5.58	5.64 <sup>e</sup>	6.38 <sup>d</sup>	5.60	5.57 <sup>b</sup>	6.42 <sup>d</sup>

Data are mean of 10 observations from 3 replications

Lower case letters indicate significant difference at  $p \leq 0.05$  following two-way ANOVA

## Discussion

### 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 (Ricigliano and Simone- Finstrom, 2020). 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 (Lan *et al.* 2021). 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 (Saffari *et al.* 2010). In the present research, among the artificial pollen substitute treatments, OUAT-PS (soybean flour, honey, yeast, and multivitamin) exhibited the maximum honey area of  $21.83 \pm 0.11 \text{ cm}^2$ , likely due to its balanced nutritional profile. These findings are consistent with the results reported by Sabir *et al.* (2000), 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.

### 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<sup>2</sup> and 14.81cm<sup>2</sup> in 2021 and 2022, respectively. Similar findings were reported by Sihug and Gupta (2013), 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<sup>2</sup> to 239cm<sup>2</sup>, which was statistically significant compared to all other treatments.

#### **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 (Eldin *et al.* 2018). 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.* (2013), who observed a substantial augmentation in the brood area (2053.0 cm<sup>2</sup> 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 (2013).

#### **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 ~~soyabean~~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 (Ahmad *et al.* 2021). A similar result was also observed by Sabir *et al.* (2000), who reported the highest colony strength of 6.24nos. covering 84 frames on average when colonies were fed with soybean flour along with vitamin B-complex and methionine.

#### **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.

## Reference

**Comment [i14]:** All references must be accompanied by a DOI number or website.

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