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IMPACT OF PROTEINACEOUS DIETS ON FORAGING BEHAVIOR, BROOD PRODUCTION AND HONEY YIELD IN *APIS CERANA INDICA* F. COLONIES OF HIMACHAL PRADESH, INDIA

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ABSTRACT

Pollen and nectar supplies are essential for honey bee survival in the nature. Pollen substitute diets become vital to honeybee when they cannot collect nectar or pollen, while foraging. The present study was conducted to assess the effect of pollen substitutes and pollen supplement on colony performance in *Apis cerana* F. Five groups of 20 colonies were used for the experiment. These were prepared from locally available pulse/ cereals flour viz. defatted soy flour (DSF), wheat flour (WF), gram powder (GP), multi grain flour (MGF). UHF Solan Pollen substitute (T_1) was prepared by mixing DSF + WF+ deactivated yeast (*Saccharomyces cerevisiae*) (DY)+ locally available sugar (S)+ tap water (TW)+ rum (R); PAU Pollen substitute (T_2) by mixing yeast (Y)+ GP+ skimmed milk (SM)+S+TW; Pant Nagar Pollen substitute (T_3) by mixing MGF+ SM+ dried yeast (DrY)+honey (H). In addition to these, one pollen supplement was also provided as T_4 comprising of corbicular pollen (55-60%) (maize) + honey (40-45%). Sugar syrup (50%) was taken as control (T_5). Data analysis showed significant differences between pollen substitutes as compared to pollen supplement and control. All three pollen substitutes and pollen supplement were accepted by honeybee with 79.07 % to 98.30 % consumption over the time of observation which resulted in increased brood area, honey stores, pollen stores and bee strength. Positive correlation was observed among all studied parameters. Pant Nagar Pollen substitute resulted significant increase in pollen stores (1.63-fold), brood area (1.79-fold) and honey stores (1.64-fold) over control.

Keywords: Bee strength, colony parameters, diet consumption, Indian bees, pollen diets.

Introduction

Honeybee are an essential component of the natural heritage of the mountain community due to their ability to pollinate mountain crops such as apple, mustard, maize and support plant biodiversity through pollination service (Hung *et al*., 2018). Indian honey bee (*Apis cerana indica* Fabricius) is endemic to mountain regions of Himachal Pradesh state of India (Sharma *et al*., 2020) and is considered the third smallest honey bee species among other honey bees. The smallest honey bee measures 2.1 mm, while the largest measures 39 mm (Koeniger, 2010; Carr, 2011). Himachal Pradesh has a vast wild forest area with plentiful pollen and nectar plants from the natural endowment. *A. cerana* has been described as docile, mild, tolerant, and timid with a gentle temperament and relatively low stinging tendency however, it does sting when cornered /disturbed (Sharma *et al*., 2022). *Apis cerana* plays a vital role in beekeeping activities especially in collecting honey rich in antioxidants, serving as energy source and having healing power besides maintaining biodiversity as pollinating agent thereby increasing the income and life style of farmers (Mohammad *et al*., 2022). Honey bee keeping with *A. cerana* is a part of traditional knowledge and the farmers are well acquaint with this entrepreneur. Beekeeping with this honey bee is a small household activity as one to two wall hives can be seen with almost every farmer (Nuru *et al*., 2012; Singh, 2014; Sharma *et al*., 2022). Some farmers are using Indian standard institute (ISI) approved bee hives. *A. cerana* has been described as the exact equivalent of its European/African sister species *A. mellifera*, the European honeybee, showing as a wide range and capacity for variation and adaptation (Koetz, 2013). Indian bee tends to forage earlier and pollinate flowers for longer time as compared to European bees, hence *A. cerana* is considered as excellent crop pollinators and thus outperforming *A. mellifera* (Partap 2011). Bee population is affected by multiple factors like available flora (Aston, 2010; Topolska *et al*., 2010; Gray *et al*., 2020; Ahmad *et al*., 2021) climate change (Van *et al*., 2010), pesticide applications (Naggar *et al*., 2015), diseases and its parasites (Chandra and Mattu, 2017). Availability of sufficient food during the dearth period is essential for the growth and development of honeybee (Saboor *et al*., 2021) which may otherwise deplete bee hive's food stockpiles and nutritional reserves. Adequate honey and nectar sources are not available throughout the year, hence there is a need to provide artificial pollen supplements and pollen substitutes to maintain the strength of bee colony by increasing brood area and longevity of bees as per the reports of Safari (2006).Lack of protein diet results reduction/fall in queen egg laying frequency consequence of which the performance and strength of the colony declines (Irandoust and Ebadi, 2013). An estimated 40% of bee colonies are thought to perish due to starvation during migrating (Kumar and Aggarwal, 2014). Because of this, beekeepers are compelled to employ artificial seasonal bee feeding in order to maintain essential population activities, like as egg laying and brood development (Di *et al*., 2013; Pande *et al*., 2015; Shehata, 2016). Rashid *et al*. (2013) also reported that beekeepers frequently supplement honey bee colonies with defatted soybean, maize, and gram flour during dearth period when the natural pollen sources are insufficient to support the growth and development of the colonies.

Artificial diets are frequently used by beekeepers to replenish lost protein, fat, vitamins, and minerals in situations where natural pollen is either unavailable or of inadequate quality. When these diets don't contain any real pollen, they're referred to as pollen substitutes; when they do, they're referred to as pollen supplements (Dalia and Kareem, 2016; Mortensen *et al*., 2019; Noordyke and Ellis, 2021). The most popular formula for a substitute is soy flour, dry brewers' yeast and dry skimmed milk with honey or sugar syrup added to form it into pellets or patties (Prakash *et al*., 2007; Brodschneider and Crailsheim, 2010). A large number of diet formulations have been developed by combining various protein rich ingredients including soybean flour, soya flour, parched gram, brewer's yeast, guar meal, egg yolk powder, pea powder, skimmed milk powder, protein hydrolysate powder, casein, fish meal, and rice bran (Abd *et al*., 2016; Kumari and Kumar, 2020). Soybean products are the most widely used ingredient of the diet of honeybees. Bees find pollen supplement diets with over 20 percent soybean flour to be highly papatable and very nutritious, meeting their needs for growth and reproduction (Mattila and Otis, 2006). In comparison to honeybee colonies without pollen substitute, those supplemented with flours such as soybean, maize, or wheat had larger brood areas (Castagmino *et al*., 2004). Similarly, yeast plays an important role in honey bee nutrition which could provide enzymes, amino acids, vitamins, and minerals to change pollen to bee bread biochemically (Gilliam, 1979; Somerville, 2000; Somerville, 2005). Despite the ample information available in the literature regarding pollen substitutes, the beekeepers of Chamba district were unaware about their use. Brood decline in honey bee colonies as a result of pollen scarcity is a major issue for the beekeepers in Himachal Pradesh. As a result, as part of effective beekeeping management practices, native alternative pollen sources need to be assessed in order to maintain the strength of honeybee colonies during dearth period. The development of a reliable pollen substitute holds the key to increase beekeeping productivity. Thus, an attempt was undertaken in the current study to assess the impact of various pollen diets on *Apis cerana indica* colony parameters, such as diet consumption rate, bee strength, brood area, pollen stores, and honey stores which in turn helped in conservation of honeybee and maintaining bee strength during a dearth period.

Materials and Methods

Selection of the bee colonies

The experiment was conducted at Kalsuien village of Bharmour block (district Chamba) (32.72°N and 76.08 $^{\circ}$E) on 20 randomly selected disease-free colonies comprising of four replicates per treatment. Colonies with equal bee populations, sealed and unsealed broods, honey stores, and pollen stores were selected for consistency and uniformity at the beginning of the trial. Pollen traps were placed in front of each colony to collect and weigh the fresh pollen collected by honeybee and also to encourage honeybee to consume the maximum quantity of pollen substitutes.

Preparation of pollen substitute diet

Following protein diets were prepared to assess the effect of pollen diets on colony parameters of *A. cerana*. The ingredients of these diets were procured from the local market.

$\mathbf{T_1}$ (UHF Solan pollen substitute): DSF = 150 g; WF = 150 g; DY= 100 g; S = 266 g; TW = 134 ml; Total = 800 g + 40 ml R

UHF- University of Horticulture and Forestry

$\mathbf{T_2}$ (PAU pollen substitute): Y (42%) = 336 g; GP (4%) = 32 g; SM (4%) = 32 g; Locally Available S = 200 g; TW = 200 ml; Total = 800 g

PAU- Punjab Agricultural University

$\mathbf{T_3}$ (Pant Nagar pollen substitute): MGF (3%) =150 g; SM (3%) =150 g; DrY (2%) =100 g; H (8%) = 400 g, Total = 800 g

$\mathbf{T_4}$ (Pollen supplement): Corbicular Pollen (55-60%) of maize crop collected during the honey flow season from pollen traps mounted in the entrance of strong honey bee colonies + honey (45-40%)

$\mathbf{T_5}$: Control (S solution) (50% sugar syrup)

The mixture of various pollen supplements and sugar syrup were prepared separately and were mixed thoroughly in a dough maker to make a smooth patty and were kept in this state for two hours. The diets were stuffed in patties, placed directly on brood frames, and covered with butter paper to avoid drying (Somerville, 2005). Patties were prepared freshly and each selected colony received 100 grams of each supplemental diet at 7 days intervals till the end of the experiment.

Pollen supplement diet was prepared by collecting pollen from the colonies by installing pollen traps at the entrance of bee colonies during kharif season. The stored pollen 55-60 % was mixed with honey. This mixture was covered with butter paper in patty form which was placed above the frames. Sugar syrup (50%) was given every week as Diet 5 to the control colonies to prevent starvation. Parameters on diet consumption, pollen area, honey stores, bee strength, and brood area were measured to assess the efficacy of pollen substitutes on colony health.

Diet consumption

The data on the amount of diet consumed per colony was calculated every week by measuring the difference between the fresh weight of patty given to the colony and the weight of patty remaining after seven days interval (patty consumed = weight of patty at beginning–the weight of patty after seven days) (measured in g per colony). The total patty consumed by each colony was calculated at the end of the experiment.

Foraging activity

The foraging activity of *A. cerana* was studied in colonies fed on the pollen supplement and pollen substitute diets. At the hive entrance, the foraging activity was visually assessed by counting the number of bees returned to their respective hive with and without corbicular pollen loads over the 15 minutes between the hours of 8:00 AM, 11.00 AM, 02:00 PM, and 05:00 PM. Foraging activity was recorded at 15 days interval for a period of four months starting from 1st November till 29nd February.

Pollen weight

Pollen traps were mounted at the entry of colonies under assessment. The quantity of pollen collected by each colony was calculated by weighing the amount of pollen collected in each pollen trap at 15 days interval. Mean pollen weight per trap was recorded and the traps were emptied and again mounted in front of the colonies.

Sealed brood area

The mean sealed brood area was measured at 15-day intervals in all experimental colonies. The brood area was calculated with the help of modified grid system as per the method given by (Chhuneja *et al*., 1992). Brood area was measured with the help of a measuring grid having squares, each square measuring one square inch. The factor 6.45 was used to convert brood area from $inch^2$ to cm^2.Fully covered spaces between combs were counted as 1. Partially covered ones were counted proportionately in quarters of a comb (0.25, 0.5, and 0.75).

Honey bee strength

Honey bee strength was estimated by using the method given by DeGrandi *et al*., (2008). Honey bee strength was calculated by measuring the total number of frames covered with bees at 15-day interval. A fully covered frame with brood (sealed/unsealed) having a dense covering of bees on both sides of frames, was counted to be 1000 bees.

Honey storage

Honey stores were visually assessed at 15 days interval based on the assumption that each Langstroth frame sealed with honey holds 2 kg of honey. The area of the ripe (sealed) honey was measured using the wire-grid measuring frame to record the quantity of honey contained in combs. The total area of ripe honey was measured in square inches which were multiplied by 6.45 (to convert the area into cm^2) x 1.25 (to convert cm^2 into g) to get the honey quantity in grams.

Statistical analysis

Data on total amount of patty consumed, foraging activity, pollen weight, sealed worker brood area, honey bee population, and honey yield data were collected and compared across the treatments for exploring the trend in various honey bee activities over comprehensive time period. The results were analyzed by repeated measures of ANOVA using OPSTAT and MS-Excel. The correlation analysis was also performed to explore the relationship between different parameters of honey bee activities and significance of correlation coefficient was evaluated using t-test.

Results and Discussion

Diet consumption

Data in Table 2 represents mean amount of patty consumed after every week for four months (November to February). There was no significant variation in patty consumption over time, however there was a significant difference across all treatments at the $P<0.05$ level. Honeybee consumed a statistically higher amount of Pant Nagar Pollen substitutes (T_3) (98. 30 g) in comparison to all other treatments. This was followed by 94.22 g patty consumption in T_1 over the time of observation. Unexpectedly, minimum amount of patty (79.07 g) was consumed in pollen supplement (T_4) per week. From our patty feeding experiments made on the Indian honeybee colonies, we concluded that T_3, Pant Nagar pollen substitute with MGF was much more accepted by honeybee as compared to other pollen substitutes. The difference in acceptability among different pollen substitutes is not explained by nutritional value but presumable reflects palatability (Saffari *et al*., 2010) and their preference for the pollen substitutes as stated by Brodschneider and Crailsheim, 2010. Similar results on diet consumption by honeybee have been reported by Saboor *et al*. (2021); Islam *et al*. (2020) who also reported a significant difference in protein supplement diet consumption over control. However, our study is in contrary to the findings of Mahfouz (2016) who had reported maximum consumption rate of defatted soybean flour by *A. mellifera* in winter season. In the present study, 100% pollen supplement consumption was recorded which get support from the findings of Dodologlu and Emsen (2007) who similarly observed complete feed consumption of diet consisting of honey and pollen cake when given as supplementary feed for *A. mellifera*.

Foraging activity

Figure 1 presents the foraging activity of honeybee based on a number of bees returning to their respective hives at the time of observation with corbicular pollen. The results revealed that foraging efforts differed significantly between the treatments and different time intervals at the $P<0.05$ level. A significant difference between the treatments at different time intervals was found (Table 3). Among all treatments, Pant Nagar Pollen substitutes (Treatment 3) fed colonies showed significant higher foraging activity at 8:00 AM, 11.00 AM, 02:00 PM, and 05:00 PM. The mean foragers recorded over the time of observations also revealed that foraging activity was significantly higher in Pant Nagar Pollen substitutes fed colonies (60.51 bees/15 minutes) compared to other treatments. Foraging activity recorded at different time intervals revealed maximum number of foragers with corbicular pollen (55.74 bees/15 minutes) at 11.00 am. However, mean number of foragers (39.03 bees/15 minutes and 34.48 bees/15 minutes) at 2.00 and 8.00 am, respectively, were significantly at par with each other. The minimum foraging activity (23.14 bees/15 minutes) was observed during evening hours (5.00 pm). Our study revealed that different pollen substitutes and pollen supplement have a significant impact on the foraging effort and pollen collection by honeybee (Table 5). Foraging success was assessed by directly measuring the mean weight of pollen that bees collected and trapped at the colony entrance. Artificial pollen supplements and pollen substitutes have been provided as adequate flora for honeybee was not available all year round. The study gets the support from the findings of Safari (2006) who also reported that the artificial pollen supplements and pollen substitutes have increased the brood area and longevity of the bees, maintaining the strength of the bee colony. Our study revealed that the foraging effort of *A. cerana* was maximum (55.74 bees/15 minutes) at 11.00 am while minimum foraging activity (23.14 bees/15 minutes) was observed during evening hours (5.00 pm). The results support the previous findings of Painkra *et al*. (2021); Mohanty *et al*. (2023) who also reported that the foraging activity of *A. cerana* was highest in morning hours (9.00 am) and lowest at evening hours (5.00 pm).

Pollen stores

The mean pollen weight collected from pollen traps positioned at the entrances of bee colonies differed significantly between pollen substitutes and pollen supplements over the time of observation (Table 4). The results indicate that irrespective of the days, statistically higher pollen weight was recorded for Treatment T_3, Pant Nagar pollen substitute (126.38 g) followed by pollen weight (116.99 g) in T_1 (UHF Solan pollen substitute). However, statistically lowest pollen weight was observed in T_5, control condition (77.64 g) followed by pollen weight in pollen supplement (97.43 g). Irrespective of the treatments, statistically highest pollen weight was recorded in Day 120 (137.95 g) followed by pollen weight in Day 105 (126.89 g). Whereas, statistically lowest pollen weight was recorded in Day 15 (75.64 g) followed by pollen stores in Day 30 (83.56 g). Increase in pollen weight (g) over control was calculated for each treatment which indicated 1.51, 1.38, 1.63, 1.25-fold increase for T_1, T_2, T_3 and T_4, respectively, over control. Thus, time fold increase over control was highest for Pant Nagar pollen substitute (1.63) and lowest in pollen supplement (1.25). Interaction effect of treatment and months of feeding, indicated that highest average pollen store was recorded for Pant Nagar pollen substitute (T_3) in day 120 (168.62 g) and day 105 (159.12 g) which was statistically at par with pollen stores of UHF Solan pollen substitute in day 120 (157.34 g). Whereas, lowest average pollen store was recorded for control condition in the day 15 to day 75 (66.83 g to 76.32 g, respectively) which was statistically at par with pollen store of T_4 (Pollen supplement) in day 15 (70.44 g) and day 30 (77.52 g) and PAU pollen substitute in day 15 (76.40 g). Statistically higher pollen weight (126.38 g) was recorded for T_3(Pant Nagar pollen substitute) followed by pollen weight (116.99 g) in T_1 (UHF Solan pollen substitute). However, statistically lowest pollen weight was observed in T_5 (77.64 g). The results indicated that the pollen storage in pollen supplement-fed colonies was higher as compared to those were not given pollen supplements. Rachna *et al*. (2011) also reported that pollen storage in pollen substitute fed colonies increased significantly as compared to the control. Similar results were reported by Mitta and Srinivasan (2016) who registered 57 % increase in pollen storage compared to colonies where no pollen supplements were provided.

Worker sealed brood area

A significant difference in worker sealed brood area was observed between the consumption of different diets (Table 5). The maximum sealed brood area (3199.40 cm^2) was observed in the consumption of Pant Nagar pollen substitute (T_3) which was statistically at par with consumption of UHF Solan pollen substitute (T_1) (3125.01 cm^2). Whereas, lowest average brood area was recorded in T_5, control condition (1789.85 cm^2) followed by 2868.11 cm^2 and 2828.74 cm^2 with the consumption of T_4 (Pollen supplement) and T_2 (PAU pollen substitute), respectively, which were statistically at par with each other. The data further showed that irrespective of treatment, highest average brood was recorded in day 90 (2892.24 cm^2) which was though statistically at par with brood area in day 75 (2846.79 cm^2). Whereas, brood area was statistically at par at different day intervals, irrespective of the treatments. Increase in brood area (cm^2) over control was calculated for each treatment which indicated 1.75, 1.58, 1.79, 1.60-fold increase respectively for T_1, T_2, T_3 and T_4 over control. Thus, increase over control was highest for Pant Nagar pollen substitute (1.79) closely followed by UHF Solan pollen substitute (1.75) and lowest in T_2 (PAU pollen substitute) (1.58). However, the interaction between treatments and days was non-significant. The consumption of pollen supplement and pollen substitutes increased the worker sealed brood area (Table 7), bee strength (Table 9) and honey yield compared to control (Table 11). The findings of the studies are in consonance with the results of Abd *et al*. (2016) who reported that sealed brood area increased in colonies fed with supplementary pollen diets in relation to un-fed bee colonies. Sihag and Gupta (2011), Lamontagne *et al*. (2019), Islam *et al*. (2020) and Samson *et al*. (2020) also revealed that the surface of sealed brood area increased following the consumption of several supplements and pollen substitutes by honey bees. Musa *et al*. (1989); Chhuneja *et al*. (1993); Nabors (2000); Rajesh *et al*. (2013) also reported that protein supplement diets increased brood rearing by bees fed in pollen supplements in comparison to control.

Bee Strength

The impact of various treatments on bee strength was described in Table 6. The number of frames covered with honeybee showed significant difference among all treatments. Irrespective of days interval, statistically highest honey bee strength was recorded in T_3, Pant Nagar pollen substitute (9.10 bee-frame) followed by honey bee strength in T_1, UHF Solan pollen substitute (8.64 bee-frame). Whereas, statistically lowest bee strength was recorded in T_5, control (5.05 bee-frame) condition followed by honey bee strength in T_4, Pollen supplement (7.75 bee-frame). Irrespective of treatments, statistically highest honey bee strength was recorded in day 120 (8.29 bee frame) and day 60 (8.11 bee-frame). Whereas, lowest bee strength was recorded in day 30 (7.49 bee-frame) which was statistically at par with honey bee strength in the day 105 (7.53 bee-frame), day 45 (7.54 bee-frame), day 15 (7.56 bee-frame), day 75 (7.60 bee-frame) and day 90 (7.85bee-frame). Interaction effect of treatment and days of feeding was found to be non-significant. Our results indicated a significant increase in bee strength is all colonies fed on pollen supplement and pollen substitutes over control. Statistically highest honey bee strength was recorded in Pant Nagar pollen substitute (T_3) (9.10 bee-frame) while lowest bee strength was recorded in T_5(control) (5.05 bee-frame). The results are in concurrence with the findings of Sharma (2002); Akyol *et al*. (2006); De-Grandi *et al*. (2008); Sihag and Gupta (2013); Kumar and Agarwal (2014); Mitta and Srinivasan (2016); Islam *et al*. (2020) who also reported increased bee strength in pollen substitute fed colonies in both Indian bees and European bees.

Honey stores

The amount of honey yield per colony after the consumption of various diet was presented in Table 7. The results revealed that irrespective of days, statistically highest honey store was recorded in T_3, Pant Nagar pollen substitute (135.29 g) followed by honey stores in T_1, UHF Solan pollen substitute (124.58 g). Whereas lowest honey store was recorded in T_5, control condition (82.27 g) followed by honey stores in Pollen supplement (104.99 g). Irrespective of treatments, highest honey stores were recorded in day 120 (149.55 g) which was followed by honey stores recorded in day 105 (137.39 g). However, statistically lowest honey stores were recorded in day 15 (79.79 g) followed by honey stores in day 30 (88.26 g). The interaction effect of treatment and days of feeding indicated that highest honey stores were recorded for the treatment T_3, Pant Nagar pollen substitute (183.12 g on day 15 and 171.87 g on day 105). Whereas statistically lowest honey stores were recorded in control in the day 15 (70.58 g), day 30 (75.96 g), day 45 (79.28 g) and day 60 (81.68 g) which was though statistically at par with honey stores for T_4 (Pollen supplement) on day 15 (74.19 g) and day 30 (82.27 g). Increase in honey stores (g) over control was calculated for each treatment which indicated 1.51, 1.40, 1.64, 1.28-fold increase respectively for T_1, T_2, T_3 and T_4 over control. Thus, time fold increase over control was highest for Pant Nagar pollen substitute (1.64) and lowest in T_4 (pollen supplement) (1.28). Significant Increase in honey yield in colonies fed with pollen supplements has also been reported by Shehata (2016); Abd *et al*. (2016); Islam *et al*. (2020) than the control colonies fed only on sugar syrup. In unfavourable foraging conditions, these pollen substitutes may represent a temporary solution to keep bee losses under control, but they are not likely to be a long-term solution during pollen shortage.

Correlation studies

Correlation studies of various colony parameters among themselves showed that all the parameters were positively correlated to each other. The correlation studies revealed that pollen store was found positively correlated bee strength (r=0.94), brood area (r=0.94) and honey store (r=0.99), which was statistically significant (p=0.05).A significant positive correlation of honey store with diet consumption (r=0.98), brood area (r=0.94) and bee strength(r=0.95) (p=0.05).

Conclusion

The results of our studies indicate that pollen substitutes and pollen supplements have significant effect on honey bee strength, honey and pollen storage. Among different pollen supplements, Pant Nagar pollen substitute was found significantly superior compared to other substitutes and pollen supplement. However, our studies are limited because most of the bee colonies used various amount of diet quantity per week. As a result, we are unable to determine the precise quantity of supplement diet that each bee colony consumes on a daily basis. Further, we are unsure of how bees use different supplemental protein diets. Additionally, more field research is required to ascertain how these additional diets affect the health and productivity of honey bee colonies. However, this research could potentially assist beekeepers in creating more suitable feeding materials that would reduce waste and boost their bee colonies' nutritional intake.

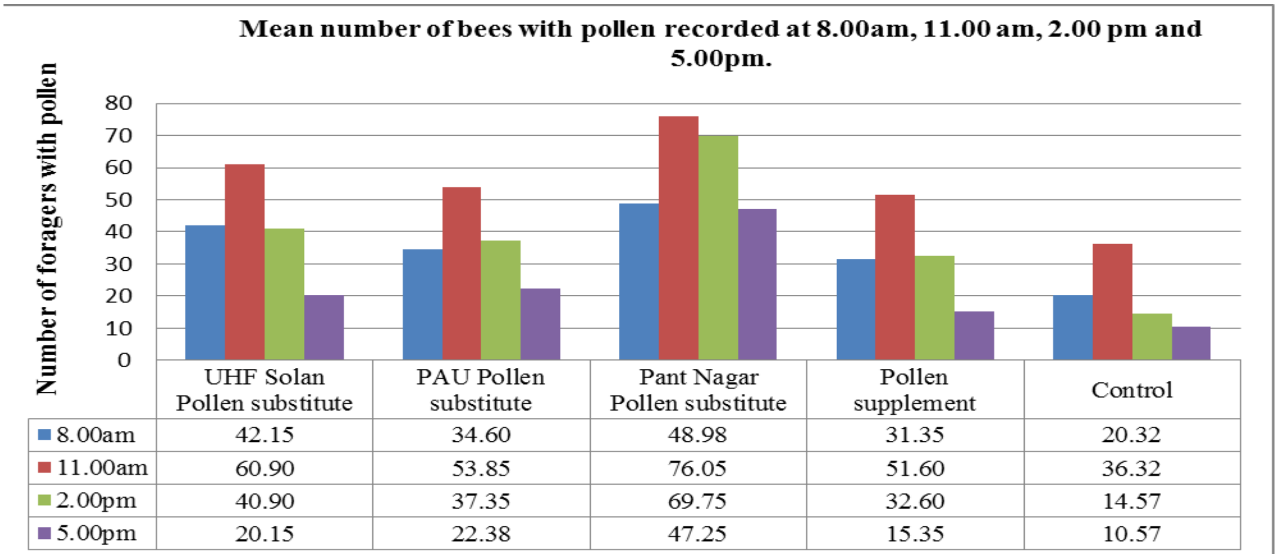

Fig. 1: Mean number of bees with pollen recorded at 8.00am, 11.00 am, 2.00 pm and 5.00 pm.

Table 1 : Composition of pollen supplements and substitutes

Treatments	Name of treatment	Ingredients
T_1	UHF Solan Pollen substitute	Defatted soya flour = 150 g; Wheat flour = 150 g; Deactivated yeast = 100 g; Locally Available Sugar = 266 g; Tap Water = 134 ml Total = 800 g + 40 ml Rum
T_2	PAU Pollen substitute	Yeast (42%) = 336 g; Gram Powder (4%) = 32 g; Skimmed milk (4%) = 32 g; Locally Available Sugar = 200 g; Tap Water = 200 ml Total = 800 g
T_3	Pant Nagar Pollen substitute	Multi grain flour (3%) =150 g; Skimmed milk (3%) =150 g; Dried yeast (2%) =100 g; Honey (8%) = 400 g Total = 800 g
T_4	Pollen supplement	Pollen 55-60% + Honey (45-40%)
T_5	Control	50% sugar syrup

Table 2: Impact of proteinaceous diets on patty consumption (in gram) over a week interval

Days Treat-ment	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56	Day 63	Day 70	Day 77	Day 84	Day 91	Day 98	Day 105	Day 112	Day 119	Mean	Rank-ing order
T_1 (UHF Solan pollen subst-itute)	92.58 (9.67)	93.03 (9.69)	93.34 (9.71)	94.51 (9.77)	95.51 (9.82)	95.31 (9.81)	95.61 (9.83)	95.71 (9.83)	93.82 (9.74)	92.03 (9.64)	95.61 (9.83)	93.97 (9.74)	93.81 (9.74)	92.24 (9.66)	96.07 (9.85)	95.41 (9.82)	93.23 (9.71)	94.22 (9.76)	2nd
T_2 (PAU pollen bsti-tute)	82.60 (9.33)	86.36 (9.34)	86.15 (9.33)	86.30 (9.33)	91.29 (9.60)	88.97 (9.48)	91.13 (9.60)	92.03 (9.64)	89.27 (9.50)	88.90 (9.48)	91.13 (9.60)	89.42 (9.51)	89.27 (9.50)	88.62 (9.47)	92.22 (9.65)	90.31 (9.55)	89.23 (9.50)	89.22 (9.49)	3rd
T_3 (Pant Nagar pollen bsti-tute)	96.43 (9.87)	96.57 (9.88)	96.71 (9.88)	97.63 (9.93)	98.19 (9.96)	98.06 (9.95)	97.95 (9.95)	98.22 (9.96)	99.44 (10.02)	99.07 (10.00)	97.95 (9.95)	99.58 (10.03)	99.10 (10.01)	98.92 (10.00)	98.70 (9.99)	99.52 (10.03)	99.07 (10.00)	98.30 (9.96)	1st
T_4 (Pollen supple-ment)	78.85 (8.93)	79.32 (8.96)	79.36 (8.96)	82.35 (9.13)	79.56 (8.96)	79.65 (8.96)	79.06 (8.93)	79.91 (8.98)	77.81 (8.87)	77.44 (8.85)	79.06 (8.93)	77.96 (8.88)	77.81 (8.87)	73.30 (8.62)	85.39 (9.29)	78.38 (8.90)	78.88 (8.93)	79.07 (8.94)	4th
Mean	88.51 (9.45)	88.89 (9.47)	88.89 (9.47)	90.20 (9.54)	91.14 (9.59)	90.50 (9.55)	90.94 (9.57)	91.47 (9.60)	90.09 (9.53)	89.36 (9.49)	90.94 (9.57)	90.23 (9.54)	90.00 (9.53)	88.27 (9.43)	93.10 (9.69)	90.90 (9.58)	90.10 (9.53)		

Figures in parenthesis are square root transformation values

C.D. $_{(0.05)}$ Treatments =0.105
S.E (d) Treatments =0.053
S.E (m) Treatments =0.038

C.D. $_{(0.05)}$ Days = NA
S.E (d) Days =0.11
S.E (m) Days =0.078

C.D. $_{(0.05)}$ Treatments x Days = NA
S.E (d) Treatments x Days =0.22
S.E (m) Treatments x Days =0.156

Table 3: Table of SEM, SED and C.D. for foraging activity of honeybee

Factors	C.D.	SE(d)	SE(m)
Treatments	5.12	2.55	1.80
Time	4.58	2.28	1.61
Treatments x Time	10.25	5.10	3.61

Table 4: Effect of different treatments on pollen storage by Indian honeybee

Treatment \ Days	Pollen weight (g/colony)									Increase over control
	Day 15	**Day 30**	**Day 45**	**Day 60**	**Day 75**	**Day 90**	**Day 105**	**Day 120**	**Mean**	
T_1 (UHF Solan pollen substitute)	81.02	90.90	98.10	111.08	120.83	134.12	142.50	157.34	116.99	**1.51**
T_2 (PAU pollen substitute)	76.40	84.50	92.49	102.93	110.74	119.22	130.58	143.11	107.50	**1.38**
T_3 (Pant Nagar pollen substitute)	83.52	93.40	102.82	124.43	131.46	147.70	159.12	168.62	126.38	**1.63**
T_4 (Pollen supplement)	70.44	77.52	83.05	92.74	100.13	108.46	116.74	130.37	97.43	**1.25**
T_5 (Control)	66.83	71.46	75.28	73.93	76.32	81.50	85.52	90.32	77.64	
Mean	75.64	83.56	90.35	101.02	107.90	118.20	126.89	137.95		

C.D. $_{(0.05)}$ Treatments =4.19
S.E (d) Treatments =2.11
S.E (m) Treatments =1.49

C.D. $_{(0.05)}$ Days = 5.30
S.E (d) Days =2.67
S.E (m) Days =5.98

C.D. $_{(0.05)}$ Treatments x Days = 11.85
S.E (d) Treatments x Days =5.98
S.E (m) Treatments x Days =4.23

Table 5 : Effect of different treatments on worker sealed brood area of Indian honeybee

Treatment \ Days	Brood area (cm^2)									Increase over control
	Day 15	**Day 30**	**Day 45**	**Day 60**	**Day 75**	**Day 90**	**Day 105**	**Day 120**	**Mean**	
T_1 (UHF Solan pollen substitute)	3011.03	3019.60	3042.06	3041.41	3251.92	3278.06	3174.43	3181.57	3125.01	**1.75**
T_2 (PAU pollen substitute)	2709.06	2748.17	2833.02	2845.32	2904.02	2967.28	2800.02	2823.05	2828.74	**1.58**
T_3 (Pant Nagar pollen substitute)	3123.04	3173.76	3135.78	3144.33	3264.07	3298.32	3221.00	3234.95	3199.40	**1.79**
T_4 (Pollen supplement)	2705.44	2772.62	2812.40	2911.75	2932.92	3040.07	2881.25	2888.40	2868.11	**1.60**
T_5 (Control)	1822.30	1811.76	1754.71	1752.61	1881.01	1877.49	1704.74	1714.17	1789.85	
Mean	2674.17	2705.18	2715.59	2739.08	2846.79	2892.24	2756.29	2768.43		

C.D. $_{(0.05)}$ Treatments =89.55
S.E (d) Treatments =45.16
S.E (m) Treatments =31.93

C.D. $_{(0.05)}$ Days = 113.27
S.E (d) Days =57.12
S.E (m) Days =40.39

C.D. $_{(0.05)}$ Treatments x Days = NS
S.E (d) Treatments x Days =127.73
S.E (m) Treatments x Days =90.32

Table 6 : Effect of different treatments on honey bee strength at different times of observation

Treatment \ Days	Bee Strength (bee frames)								
	Day 15	**Day 30**	**Day 45**	**Day 60**	**Day 75**	**Day 90**	**Day 105**	**Day 120**	**Mean**
T_1 (UHF Solan pollen substitute)	8.39	8.31	8.36	8.88	8.59	8.90	8.66	9.00	8.64
T_2 (PAU pollen substitute)	8.00	7.93	8.10	8.66	8.00	8.18	8.08	8.68	8.20
T_3 (Pant Nagar pollen substitute)	8.89	8.81	8.89	9.46	8.98	9.21	9.00	9.60	9.10
T_4 (Pollen supplement)	7.60	7.53	7.49	8.09	7.38	7.87	7.71	8.35	7.75
T_5 (Control)	4.94	4.87	4.86	5.45	5.14	5.09	4.23	5.83	5.05
Mean	7.56	7.49	7.54	8.11	7.62	7.85	7.53	8.29	

C.D. $_{(0.05)}$ Treatments =0.29
S.E (d) Treatments =0.15
S.E (m) Treatments =0.10

C.D. $_{(0.05)}$ Days = 0.37
S.E (d) Days =0.19
S.E (m) Days =0.13

C.D. $_{(0.05)}$ Treatments x Days = NS
S.E (d) Treatments x Days =0.42
S.E (m) Treatments x Days =0.30

Table 7 : Effect of different treatments on honey bee strength at different times of observation

Days Treatment	Honey stores (g/colony)									Increase over control
	Day 15	**Day 30**	**Day 45**	**Day 60**	**Day 75**	**Day 90**	**Day 105**	**Day 120**	**Mean**	
T_1 (UHF Solan pollen substitute)	85.02	95.65	102.85	116.58	126.08	143.12	156.50	170.84	124.58	**1.51**
T_2 (PAU pollen substitute)	80.15	89.00	96.99	108.68	117.49	127.72	142.08	156.36	114.81	**1.40**
T_3 (Pant Nagar pollen substitute)	89.02	98.40	109.82	132.43	141.21	156.45	171.87	183.12	135.29	**1.64**
T_4 (Pollen supplement)	74.19	82.27	88.55	99.24	106.13	117.46	127.99	144.12	104.99	**1.28**
T_5 (Control)	70.58	75.96	79.28	81.68	83.57	85.25	88.52	93.32	82.27	
Mean	79.79	88.26	95.50	107.72	114.90	126.00	137.39	149.55		

C.D. $_{(0.05)}$ Treatments =4.15 | C.D. $_{(0.05)}$ Days = 5.25 | C.D. $_{(0.05)}$ Treatments x Days = 11.75
S.E (d) Treatments =2.09 | S.E (d) Days =2.65 | S.E (d) Treatments x Days =5.92
S.E (m) Treatments =1.48 | S.E (m) Days =1.87 | S.E (m) Treatments x Days =4.19

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Author Contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication. AS carried out the experiment, took observations. VS visited the apiaries and collected the data. JC supervised the experiments and provided the important guidance to conduct the experiment. RSC, contributed by developing the ideas, guided the research by formulating the research concept. ID edited the research article, guided the research by formulating the research concept. MD edited the manuscript. AS supervised the experiments. RK editing the manuscript. B helped in analyzing the data. RR helped in securing research funds and providing technical support.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Scope Statement

Our study revealed that the all three pollen substitutes and pollen supplement were accepted by honeybee with 79.07 % to 98.30 % consumption over the time of observation which resulted in increased brood area, honey stores, pollen stores and bee strength. Moreover, these artificial diets are cost effective and nutritionally balanced and thus can be used for feeding honeybee during dearth period. This study highlights the importance of artificial pollen substitutes and emphasizes the significance of providing artificial diets for the honey bee colonies when the pollens are not readily available in sufficient amount. However, more research work is required to find out the effect of pollen supplements on different physiological parameters of honey bee races in diverse environments.

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