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Comparative study on agricultural extracts for brood management in Indian bees (*Apis cerana indica*) in tropical ecosystems

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Abstract

The performance of worker bees fed with different agricultural extracts such as nectar and pollen substitutes compared to the naturally fed workers was determined on (*Apis cerana indica* Fab.) was evaluated using a randomized block design (RBD). The results revealed that the worker bee fed with nectar and pollen substitutes was significantly superior to the naturally fed workers. The maximum consumption of nectar substitutes was 199.7, and 199.5 ml out of 200 ml were observed in T_8 (black gram flour + sugar syrup) and T_2 (soybean flour + sugar syrup). The maximum consumption (84.5 g) of pollen substitutes, maximum population (35.66/5 cm^2), the maximum length of a worker bee (9.73 mm) and the maximum egg-laying capacity (103.5/5 cm^2) were observed in T_8 (black gram flour + sugar syrup). The minimum population of 29.08/5 cm^2 and the minimum egg-laying activity (83.33/5 cm^2) was observed in T_1 (naturally fed colony), and the minimum length of worker bee of 9.63 mm was observed in T_7 (soybean flour + tender coconut). These findings suggest that black gram flour and sugar syrup can significantly enhance colony productivity during dearth periods.

Keywords

agricultural extract; egg laying activity; nectar substitutes; pollen substitutes; population

Introduction

Apiculture involves managing and caring for honeybee colonies, primarily to produce honey, beeswax and other bee-related products. Honey, a natural substance produced from the nectar of flowers and processed by honeybees, has been esteemed for centuries for both its medicinal benefits and culinary uses (1). Bees are vital pollinators for both agricultural and forestry crops. Pollen supplies the colony with essential nutrients, including proteins, lipids, vitamins and minerals necessary for brood growth (2). Honeybees convert nectar into honey, which is their primary source of carbohydrates. Carbohydrates are crucial in a honeybees' diet, providing the energy needed for muscle activity, regulating body heat, and supporting vital functions of specific organs and glands, including those responsible for wax production (3).

Additionally, bees create bee bread by fermenting pollen. This process involves collecting pollen from flowers, mixing it with their saliva and a small amount of honey, and allowing it to undergo lactic fermentation (4). For instance, high-quality pollen enhances bees' capacity to resist stressors like pests and diseases (5). Bees require a balanced mix of carbohydrates, proteins,

lipids and micronutrients from nectar and pollen to survive, reproduce and withstand stress (6). However, seasonal and climatic variations, including extreme temperatures, heavy rainfall and hailstorms, greatly diminish floral resources throughout the year.

Tropical climates are typically found within the region spanning from approximately 23.5 degrees north to 23.5 degrees south of the equator, between the Tropic of Cancer and the Tropic of Capricorn. In these areas, temperatures are consistently warm year-round, with monthly averages always exceeding 64°F (18°C). For successful beekeeping in such climates, it is essential to understand local environmental conditions, adapt to specific challenges and engage in proactive hive management (7). Insights and support from local beekeeping associations and experienced beekeepers can be invaluable. When natural flora is inadequate, the queen bee's egg-laying rate declines, decreasing the colonys' population (8). Global honeybee colonies are experiencing significant losses due to parasitic infections and various stressors.

Key factors contributing to these losses include pathogen infections, pesticide exposure and nutritional stress (9). Beekeepers frequently employ artificial diets to substitute for the protein, fat, vitamins and minerals lacking or insufficient quality when natural pollen is unavailable (10). Various plant and animal protein alternatives have been examined as substitutes for pollen, encompassing items such as soybean flour, whole wheat flour, peanut meal, brewer's yeast, ground dry fish, powdered skim milk, corn flour, egg albumin and similar options. B blending bee-collected pollen with additional components, such as soybean flour and honey, creates supplements to achieve the desired patty texture (11). These artificial diets are termed "pollen substitutes" if they lack natural pollen and "pollen supplements" if they contain some natural pollen. Increased honey production is often expected when using pollen substitutes, as colonies with more honeybees and broods typically produce honey at a higher rate (12). The weight of worker honeybees increased linearly with the higher protein content in pollen substitutes (13).

Similarly, colonies with diets containing 35% protein produced worker bees with longer lifespans than those with diets with 15% protein (14). Supplying pollen and nectar substitutes to bee colonies during dearth periods can significantly enhance hives' overall health and development in tropical climatic conditions (15). The present study aimed at rearing Indian bee colonies fed with nectar and pollen substitutes through specific methods and evaluation of its performances with a naturally fed bee colony.

Materials and Methods

The investigation on morphometric analysis of *A. cerana indica* Fab by feeding of nectar and pollen substitutes was carried out in 2024 at Post Graduate Laboratory, Department of Entomology, ADAC & RI, Tamil Nadu Agricultural University, Tiruchirappalli (Latitude 10°45'N and Longitude 78°36'E). The materials utilized and methodologies employed are outlined below. Worker Bees of *A. cerana indica* Fab (adult), glass jars (100 ml), Ethanol, Glass slides, Coverslips, Compound microscope, Digital Vernier calliper, dissecting box, Knife and Weighing balance.

Date of experiment

The period of insufficient floral resources is commonly called a dearth period (16). In the tropical regions, dearth periods can occur during dry or rainy seasons. During these times, bee colonies may migrate to find better conditions for survival. This experiment commenced in the second week of March (11/3) and continued until the end of April (29/4), with readings taken at seven-day intervals during 2024.

Treatment details

T_1 - Control

T_2 - Soybean flour + Sugar syrup

T_3 - Soybean flour + Jaggery syrup

T_4 - Soybean flour + Sugarcane juice

T_5 - Soybean flour + Banana syrup

T_6 - Soybean flour + Date syrup

T_7 - Soybean flour + Tender Coconut

T_8 - Black gram flour + Sugar syrup

T_9 - Black gram flour + Jaggery syrup

T_{10} - Black gram flour + Banana syrup

T_{11} - Black gram flour + Sugarcane juice

T_{12} - Black gram flour + Date syrup

T_{13} - Black gram flour + Tender Coconut

Components of diets

Pollen substitutes:

a) Soy bean flour
b) Black gram flour
c) Sugar powder
d) Hot water

Nectar substitutes:

a) Sugar syrup
b) Jaggery syrup
c) Cane juice
d) Banana syrup
e) Date syrup
f) Tender coconut

Diet preparation

Protein-rich black gram and soybean flour, along with low-cost sucrose-rich ingredients like banana syrup, jaggery syrup, sugarcane juice, date syrup and tender coconut, are incorporated into the formulation of nectar and pollen substitutes.

Pollen substitute diet preparation:

A pollen substitute diet was prepared by mixing wet and dry components. The wet element of the pollen substitute contains hot water (75 ml) and honey (10 ml). The dry component of the pollen substitute contains sugar powder (20 g), soybean flour and black gram flour (100 g). After mixing wet and dry

components, patties were created to feed the Indian bee (*A. cerana indica*).

Nectar substitute diet preparation:

Nectar substitutes were given as syrup to the bees. In the case of sugar and jaggery, equal parts of the dry ingredients and water were combined to create the syrup. However, the fruits were soaked in water for approximately two hours to make banana and date syrup. Following this, the fruits were mashed and the resulting liquid was filtered. Subsequently, the liquid underwent boiling to produce the syrup.

Place of the diets in beehives

Weekly feeding can reduce the tendency of bees to forage outside during the dearth period, while the control colonies were not provided with any feeding. All twelve treatments were administered and the diets were as previously described at seven-day intervals. The substitutes were placed directly inside the hives on the brood combs' top bars throughout the experiment.

Bee sampling

Morphologically, identified bees of *A. cerana indica* were collected from Bee Garden, Department of Entomology, ADAC & RI, Tiruchirappalli. One hundred thirty adult workers were collected from 26 colonies during every observation.

Characters studied

The collected worker bees (larva, pupa, adult) were dissected to study various characters before and after feeding nectar and pollen substitutes with the help of a Dissecting box, compound microscope (40X) and Digital Vernier calliper. Multiple characteristics of the bees were studied: length of worker adult, population, egg-laying activity and consumption rate of nectar and pollen substitutes.

Analysis of length of worker adult

Before feeding nectar and pollen substitutes and every seven days after feeding, the length of the worker adult was measured using a digital Vernier calliper.

Analysis of the population of worker bees and egg-laying activity of queen

Before feeding off nectar and pollen substitutes and every seven days after feeding, observations of colony performance, like the population of worker bees and the queen's egg-laying activity, were measured using a 5 cm^2 transparent polythene sheet above the brood frames. The observations were recorded by counting within the 5 cm^2 transparent polythene sheet.

Statistical analysis

The experiment was set up in a Randomized Block design (RBD) with 2 replications. A one-way analysis of variance (ANOVA) was performed. The significant result is indicated when means were compared using LSD (least significance difference) and the resulting P-value was < 0.05. Statistical analysis was performed with the AGRESS Software.

Results and Discussion

At the apiary of ADAC & RI in Tiruchirappalli, Indian bee colonies were supplied with artificial pollen and nectar substitutes. The pollen substitutes included soybean flour and black gram flour. For nectar, six different substitutes were utilized: sugar syrup, jaggery syrup, sugarcane juice, banana syrup, date syrup and tender coconut.

Consumption rate of pollen and nectar substitutes and the effect of those substitutes on bee population

Pollen and nectar substitutes were offered to *A. cerana indica* during off season to check their preference and consumption. 100g pollen substitute and 200 ml of nectar substitute were given to each colony. The net weight of pollen substitutes and the net quantity of nectar substitutes consumed within seven days for 5 weeks was recorded for each treatment. For nectar substitutes among the treatments, T_8& T_2,T_4& T_6,T_7& T_{13} are on par. Indian bees prefer maximum to T_8(black gram flour + sugar syrup) and T_2 (soybean flour + sugar syrup) at the mean rate of 199.7 ml and 199.5 out of 200 ml. The lowest preference (Fig. 1) was recorded at T_{13}(black gram flour + Tender Coconut) and T_7 (soybean flour + tender coconut) at the mean rate of 113.2 ml and 113.9 ml out of 200 ml. In the case of pollen substitutes, bees prefer maximum (Table 1.) to T_8(black gram flour + sugar syrup) at the mean rate of 84.5 g out of 100 g. The lowest preference (Fig. 2) was recorded at T_7 (soybean flour + tender coconut) and T_5 (soybean flour + banana syrup)at the mean rate of 56.9 g and 57.1 g out of 100 g. Diets rich in protein sources such as brewers' yeast, chickpea flour and a moderate level of pollen, combined with sugar syrup, were highly palatable and beneficial for honeybee colonies (17). The commonality between these studies indicates that protein-rich substitutes, especially those with leguminous components like black gram and chickpea flour, fulfil the nutritional needs of bees by closely mimicking natural pollen. The worker bee population was measured using a 5 cm^2 transparent polythene sheet (Fig. 3.). For a population of worker bee adults, among the treatments, T_2 and T_8 are on par with each other. The maximum population (Table 3.) of worker bees were recorded at T_8 (black gram flour + sugar syrup) and T_2 (Soybean flour + sugar syrup) with mean values of 35.66 and 34.75. The lowest population was recorded at T_1 (Control) at the mean value 29.08. Colonies with enriched pollen substitutes exhibited higher worker bee populations and brood-rearing rates than those with fewer comprehensive substitutes (18). A significant correlation was found between the quantity of diet consumed and variations in both brood area and adult bee population (19). Similarly, studies showed increased brood production with pollen substitute feeding compared to those not receiving

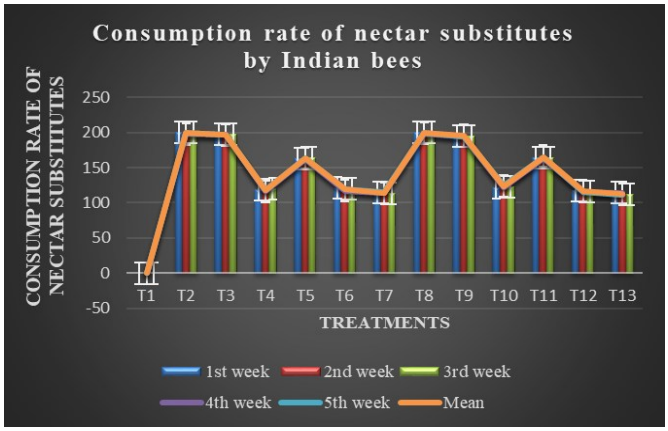

Fig. 1. Consumption rate of nectar substitutes by Indian bees.

Table 1. Consumption rate of pollen substitutes by Indian bees during dearth period

Consumption rate of pollen substitute (g)						
Treatments	**Week 1***	**Week 2***	**Week 3***	**Week 4***	**Week 5***	**Mean**
T_1	0.0	0.0	0.0	0.0	0.0	0.0
T_2	66.0	69.5	69.5	69.0	67.5	68.3^{e}
T_3	66.0	68.5	66.5	67.5	65.5	66.8^{f}
T_4	61.5	63.5	61.0	62.0	60.0	61.6^{g}
T_5	57.0	54.0	61.0	56.0	57.5	57.1^{h}
T_6	62.0	64.5	64.0	62.0	61.5	62.8^{g}
T_7	55.5	58.0	60.0	56.5	54.5	56.9^{h}
T_8	85.0	83.5	84.5	85.5	84.0	84.5^{a}
T_9	82.0	84.5	82.0	82.5	82.0	82.6^{b}
T_{10}	79.0	76.0	76.0	79.0	77.0	77.4^{d}
T_{11}	81.0	79.0	79.0	78.5	78.5	79.2^{c}
T_{12}	78.0	80.0	81.5	81.0	80.0	80.1^{c}
T_{13}	81.5	83.0	80.5	82.5	80.0	81.5^{b}
Mean	65.73	66.46	66.57	66.30	65.23	66.06
SE d	0.98	0.96	1.02	0.95	0.84	0.57
C.D. (0.05)	2.13	2.10	2.22	2.07	1.84	1.25

*Mean of two replications

Table 3. Population of worker bees before and after feeding of nectar and pollen substitutes

Population of worker bees / 5cm^2 brood area							
Treatments	**Before feeding***	**Week 1***	**Week 2***	**Week 3***	**Week 4***	**Week 5***	**Mean**
T_1	30.0	30.0	28.5	27.5	29.5	29.0	29.08^{f}
T_2	28.0	32.0	34.5	36.0	38.0	40.0	34.75^{a}
T_3	31.5	33.5	34.5	34.5	36.0	37.0	34.50^{b}
T_4	30.5	30.5	32.0	31.0	32.0	32.5	31.41^{c}
T_5	33.0	31.5	33.0	34.0	35.0	34.0	33.41^{b}
T_6	28.0	29.0	31.0	30.5	32.5	32.5	30.58^{d}
T_7	29.0	30.5	31.5	30.0	32.0	33.0	31.00^{d}
T_8	29.5	33.5	34.5	36.0	39.0	41.5	35.66^{a}
T_9	29.5	32.0	33.5	34.5	37.5	38.5	34.25^{b}
T_{10}	30.0	31.0	31.0	31.5	32.5	34.5	31.75^{c}
T_{11}	29.0	29.5	30.5	30.0	31.0	32.0	30.33^{d}
T_{12}	31.0	32.0	33.0	30.5	32.5	33.5	32.08^{c}
T_{13}	29.5	30.5	32.0	29.5.0	31.0	32.0	30.75^{d}
Mean	29.88	31.19	32.26	31.96	33.73	34.61	32.27
SE d	0.94	0.49	0.68	0.87	0.99	0.98	0.53
C.D. (0.05)	2.05	1.06	1.49	1.89	2.16	2.13	1.15

*Mean of two replications

Table 2. Length of worker bee adult before and after feeding of nectar and pollen substitutes

Length of worker adult (mm)							
Treatments	**Before feeding***	**Week 1***	**Week 2***	**Week 3***	**Week 4***	**Week 5***	**Mean**
T_1	9.64	9.66	9.66	9.65	9.66	9.65	9.65^{b}
T_2	9.68	9.71	9.71	9.72	9.73	9.74	9.71^{a}
T_3	9.64	9.67	9.66	9.67	9.67	9.68	9.66^{b}
T_4	9.63	9.63	9.65	9.65	9.66	9.66	9.64^{b}
T_5	9.68	9.69	9.71	9.71	9.71	9.71	9.70^{a}
T_6	9.68	9.69	9.70	9.70	9.71	9.71	9.70^{a}
T_7	9.62	9.63	9.64	9.63	9.64	9.64	9.63^{b}
T_8	9.68	9.74	9.75	9.74	9.75	9.76	9.73^{a}
T_9	9.63	9.68	9.68	9.68	9.69	9.69	9.67^{b}
T_{10}	9.66	9.64	9.65	9.65	9.66	9.67	9.65^{b}
T_{11}	9.69	9.69	9.67	9.69	9.7	9.71	9.69^{a}
T_{12}	9.69	9.71	9.69	9.70	9.69	9.71	9.70^{a}
T_{13}	9.66	9.65	9.64	9.67	9.68	9.70	9.66^{b}
Mean	9.66	9.67	9.67	9.68	9.68	9.69	9.68
SE d	0.04	0.03	0.03	0.03	0.03	0.03	0.03
C.D. (0.05)	0.08	0.08	0.06	0.06	0.06	0.06	0.06

*Mean of two replications

Fig. 2. Pollen substitute consumption by Indian bees.

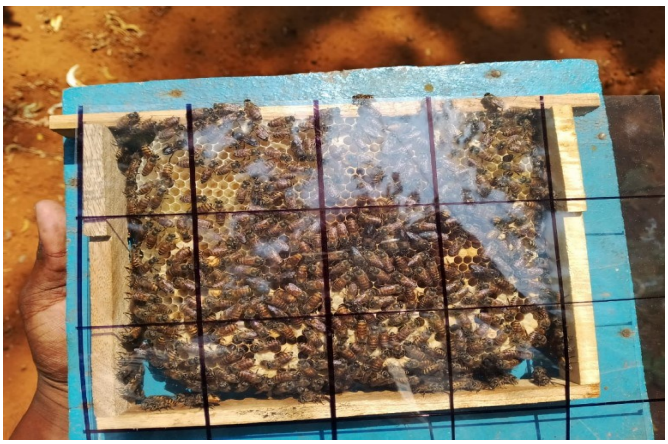

Fig. 3. Measurement of bee population by using a transparent polythene sheet.

any diet, almost usually during pollen scarcity or unfavourable weather conditions (20-22). So, feeding nectar and pollen substitutes during the dearth period can improve the population of worker bees under the dearth period.

Effect of nectar and pollen substitutes on the length of worker bee adult during dearth period

The length of worker adult was measured using a vernier calliper (Fig. 4) after feeding nectar and pollen substitutes at seven days for 5 weeks. For the length of worker bee adult, among the treatments, T_2, T_5, T_6, T_8, T_{11} & T_{12} are on par with each other. Similarly, T_1, T_3, T_4, T_7, T_9, T_{10}& T_{13} are on par. The worker adult's maximum length was recorded at T_8(Black gram flour + sugar syrup) at the mean value of 9.73 mm (Table 2.). The lowest length was recorded at T_7(soybean flour + tender coconut) at the mean value of 9.63 mm.

Similarly, in an experiment, worker bees in colonies fed sugar syrups and high-protein pollen substitutes had more considerable body lengths than those fed sugar syrups alone (23, 24). Due to its high protein content, soybean flour effectively promotes brood development, which in turn impacts the size of the bees. Similarly, black gram flour, known for its high levels of protein and amino acids, provides essential nutrients that support physiological growth (25). The addition of sugar syrup in both treatments served as a vital carbohydrate source, supplying the energy needed for development while allowing the protein from black gram and soybean flour to be efficiently utilized. This emphasizes the importance of a balanced diet that resembles natural forage. So, nectar and pollen substitutes can improve the time worker bee adults are under a dearth period.

Fig. 4. Measurement of worker adult length by vernier calliper.

Effect of nectar and pollen substitutes on the egg-laying activity of queen during dearth period

The queen's egg-laying activity was measured using a 5 cm^2 transparent polythene sheet. The maximum egg-laying activity (Table 4.) of worker bees was recorded at T_8(black gram flour + sugar syrup) at the mean value of 103.5. The minimum egg-laying activity was recorded at T_1(control) at the mean value of 83.33. So, feeding nectar and pollen substitutes during the dearth period can improve the egg-laying activity of the queen. Compared to control colonies, colonies that received superior pollen substitutes had noticeably larger brood areas and egg-laying rates (26). This field study emphasizes how high-quality alternatives might support queen production in a useful way. The colony's nutritional state affects the queen's ability to lay eggs. Egg-laying activity is increased in colonies given balanced nectar and pollen substitutes, indicating sustained colony development and productivity (18). Using pollen substitutes boosts egg laying activity and, as a result, improves colony development. Pollen substitutes can significantly increase egg laying and support overall colony development (27).

Table 4. Egg-laying activity of the queen before and after the feeding of nectar and pollen substitutes

	Egg laying activity of queen / 5cm^2 brood area						
Treatments	**Before feeding***	**Week 1***	**Week 2***	**Week 3***	**Week 4***	**Week 5***	**Mean**
T_1	84.00	82.50	83.50	83.00	83.50	83.50	83.33^h
T_2	87.50	99.50	103.50	104.50	106.00	108.00	101.50^b
T_3	86.50	98.50	102.00	103.50	105.00	106.00	100.25^b
T_4	87.50	94.50	96.50	96.50	97.50	100.00	95.41^c
T_5	85.00	92.50	93.50	94.50	94.50	95.50	92.58^d
T_6	85.50	87.50	89.50	88.50	89.50	91.50	88.66^f
T_7	88.50	91.00	91.50	91.50	92.50	94.50	91.58^d
T_8	88.50	100.0	106.00	107.00	109.50	110.00	103.50^a
T_9	86.50	98.50	103.50	104.50	105.50	106.50	100.83^b
T_{10}	89.00	90.00	91.50	91.00	92.50	94.50	91.41^d
T_{11}	83.50	85.50	87.50	88.50	89.50	91.50	87.66^f
T_{12}	82.50	87.00	87.00	86.00	88.00	90.50	86.83^g
T_{13}	86.50	89.00	91.00	91.50	91.50	93.00	90.41^e
Mean	86.23	92.00	94.34	94.65	95.76	97.30	93.38
SE d	0.66	0.98	0.82	0.92	0.76	0.88	0.56
C.D. (0.05)	1.43	2.14	1.80	2.00	1.66	1.93	1.22

*Mean of two replications

Conclusion

The present findings were consistent with previous research, which found that colonies typically show an increase in brood production with agricultural extracts such as pollen and nectar substitute feeding compared to those not receiving any diet, particularly during pollen scarcity or unfavourable weather conditions. Based on these results, Black gram flour and sugar syrup performed better than other nectar and pollen substitutes in tropical climatic conditions. However, variability in the responses across treatments was observed. Therefore, providing Black gram flour and sugar syrup to Indian bee colonies during the dearth period appears to be a promising strategy to enhance colony productivity. Planting a variety of natural forage remains the most effective long-term solution for alleviating nutritional stress in honeybees (22). In summary, supplementing the *A. cerana indica* diet with nectar and pollen substitutes in tropical environments can improve bee health and colony viability by mitigating challenges from erratic flower supplies. Future research should optimise these substitutes' nutritional profiles and assess their long-term impacts on bee populations and biodiversity.

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Authors' contributions

Each author has made an active contribution. SK conducted experiments in both the lab and the field. AK and VA conceptualized and managed the experiment. SK and AK prepared and revised the manuscript. SJ provided an interpretation and critique of the outcome.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Did you use generative AI to write this manuscript?

Yes

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used AI ChatGPT to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the publication's content.

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