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Effect of pollen substitutes on influencing the colony growth of Asiatic hive bee, *Apis cerana indica* F. (Hymenoptera : Apidae)

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ABSTRACT

Apiculture is a critical component in increasing agricultural yield across the world. Honey bees' high health and cleanliness are essential for apiculture's enrichment and advancement. To meet their dietary needs, honeybees require pollen and nectar-rich blooms. The experiments were done at multiple locations of Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore to assess the influence of pollen substitute on enhancing the colony growth of Asiatic hive bee, *Apis cerana indica*. The palatability, consumption and effects of protein-rich pollen substitutes diet were evaluated on honey bee colonies, *Apis cerana indica*. Supplementary pollen feeding include the combination of roasted Bengal gram powder, Parched red gram powder, soya bean powder, glucose, skimmed milk powder, multivitamins, brewer'yeast, honey and sugar at various proportions in eight different treatments and compared with control. For a month, data collection was conducted regularly on a weekly basis. From the observation, Roasted Bengal gram powder (30%) + Skimmed milk powder (25%) + Honey (34%) + Brewer's yeast (10%) + multivitamin (1%) substantially increased colony growth metrics such as brood area, nectar store area, pollen store area, and foraging activity when compared to the other treatments.

Key words : *Apis cerana indica, Colony growth, Pollen substitute.*

Introduction

Honey bees, also known as "Angels of Agriculture," are arguably the most vital insects on the planet. They are necessary for effective crop fertilisation and to advance the food creation process (Chantawannakul, 2018). Pollen is the primary supply of protein, vitamins, and minerals, whereas nectar is the primary source of carbohydrates for honey bees. The diversity and availability of blooming plants that provide nectar and pollen are also influenced by farming practices. Commercially manufactured bee diets can augment available pollen and provide a viable way for supporting brood raising in honey bee colonies when there isn't enough pollen available from plants (Matilla and Otis, 2006). The native bee flora begins to vanish in April, resulting in a lack of food (pollen and nectar) for bees. Periodic pollen shortages result in poor nutritional reserves, which have a negative impact on colony performance owing to a halt or reduction in egg laying and brood rearing. James *et al.*, (2008) revealed that the native bee flora begins to diminish in April, resulting in food shortage (pollen and nectar), causes reduced egg laying and brood rearing. Pollen scarcity cause insufficient nutritional reserves, resulting in shorter lifespans, poor hypopharyngeal gland growth, lower royal jelly output, and poor worker

larvae and young bee growth (Zaytoon *et al*., 1988). The implementation of effective pollen substitute to feed the colonies when pollen is sparse has the potential to improve beekeeping efficiency by optimising honey output, crop pollination, overcoming pesticide damage, and producing healthy colonies for package-bee production. The main objective of this research is to determine an acceptable alternative pollen substitute and its influence on the growth and development of Asiatic bee colonies.

Materials and Methods

The field research was conducted in selected Asian bee colonies located around the Tamil Nadu Agricultural University Campus in Coimbatore. Supplemental pollen feeding should be provided to Asiatic hive bee colonies to satisfy their energy requirements during the dearth season. In multiple colonies, nine different treatments were tested, including T_1-Bee pollen (65%) + Honey (34%) + multivitamin (1%), T_2-Roasted Bengal gram powder (49%) + Honey (40%) + Brewer's yeast (10%) + multivitamin (1%), T_3-Roasted Bengal Gram Powder (49%) + SMP (40%) + Brewer's yeast (10%) + multivitamin (1%), T_4 – Roasted Bengal gram powder (30%) + Skimmed milk powder (25%) + Honey (34%) + Brewer's yeast (10%) + multivitamin (1%), T_5- Soy bean powder (49%) + Skimmed milk powder (40%) + brewer's yeast (10%) + multivitamin (1%), T_6- Soy bean powder (49%), honey (40%), brewer's yeast (10%), multivitamin(1%), T_7- Soy bean powder (30%) + SMP (25%) + Honey (34%) + Brewer's yeast (10%) + multivitamin (1%), T_8 - Parched red gram powder (26%)+ SMP (24%) + Honey (35%) + sugar (5%)+ Glucose (10%) and T_9-Control. There were nine treatments over all, including the control, and two bee colonies were chosen for each treatment and given two more pollen feedings (20 gram per serving) at 14-day intervals. For feeding, a weighed amount of pollen substitutes (20 g) were placed on butter paper on top bars within the hives. For a month, the observations were made at weekly intervals. For each treatment colonies with four-frame strength will be chosen for the research purpose.

Colony growth is influenced by a variety of parameters, including frame strength, bee population, honey store, pollen store, bee strength, brood rearing, swarming, and pest and disease occurrence. When the larvae transform into pupae, honey bees use wax to seal their brood. As a result, a colony's sealed brood is a predictor of adult population growth in the future. Pollen is collected from flowers and stored in the upper layer of cells in each comb for use by bees later. With the aid of a frame-sized wire grid, the brood raising area, honey, and pollen storage were measured in cm^2 and then multiplied by 6.45.

The number of worker bees going out and returning to the hive with and without pollen loads was counted using a manual tally counter and a stop watch to assess bee foraging activities. The pollen-carrying bees and empty foragers were identified independently. Non-pollen foragers/nectar gatherers were defined as bees that returned without pollen burdens. Foraging activity was recorded for a month during the year 2021 at weekly intervals, and observations were taken three times a day, at 1000, 1300, and 1600h for five minutes each, with the sum of the three intervals being used to calculate the total foragers of the hive per five minutes for that day. In completely randomized design and the observations were statistically analysed by one-way ANOVA using SPSS software.

Results and Discussion

Pollen replacements were used to test the consumption and effectiveness of formulated diets in experimental colonies. The colonies were fed nine different treatments, including eight different pollen substitutes and a control.

Sealed brood area

The observations on brood development in treated colonies recorded during the study period are presented in Table 1. Among all the nine treatments, the results revealed that the colonies fed with T_4 – Roasted Bengal gram powder (30%) + Skimmed milk powder (25%) + Honey (34%) + Brewer's yeast (10%) + multivitamin (1%) recorded maximum brood area development (193.25cm^2) followed by T_1-Bee pollen (65%) + Honey (34%) + multivitamin (1%) which recorded brood area of about(186.25 cm^2) . The sealed brood area of the other pollen substitute was lesser than those of T_4 and T_1. In comparison to all other treatments, the control colonies had a minimum growth in brood area (159.87cm^2).

The increase in brood area over control were ranged from 8.90 to 55.75 per cent in different treatments. The maximum increase of 55.75 per cent recorded in T_4 – Roasted Bengal gram powder (30%) + Skimmed milk powder (25%) + Honey (34%) +

Brewer's yeast (10%) + multivitamin (1%). The minimum increase of 8.90 per cent recorded in T_8 - Parched red gram powder (26%) + SMP (24%) + Honey (35%) + sugar (5%) + Glucose (10%). In T_4 – Roasted Bengal gram powder (30%) + Skimmed milk powder (25%) + Honey (34%) + Brewer's yeast (10%) + multivitamin (1%) had had more egg laying growth in worker population and few drone patches compared to other treatments including control colonies. The incidence of pest and disease was nil for this treatment. In the absence of pollen, honey bee colonies can be given substitutes that accelerate egg laying and support brood raising under less-than-ideal conditions, according to Standifer *et al*. (1960).

According to Herbert *et al.* (1977), the quantity of protein in a pollen supplement should be between 23 and 30 percent in order to achieve optimum brood growth. Brood growth is inhibited by protein concentrations of less than 10% and more than 50%.

Pollen store area

The observations made during the research period on pollen storage development in treated colonies shown in Table 2. The colonies fed with T_4 – Roasted Bengal gram powder (30%) + Skimmed milk powder (25%) + Honey (34%) + Brewer's yeast (10%) + multivitamin (1%) had the greatest pollen storage (68.62 cm^2), followed by T_1 – Bee pollen (65%) + Honey (34%) + multivitamin (1%) of about (61.62 cm^2) . The other pollen substitute had a smaller pollen storage than T_4 and T_1. The control colonies were observed the least amount of pollen storage area compared to the other treatments (44.62cm^2).

In different treatments, the increase in pollen storage area above control ranged from 9.25 to 37.27 percent. In T_4- Roasted bengal gram powder (30%) + Skimmed milk powder (25%) + Honey (34%) + Brewer's yeast (10%) + multivitamin (1%) shows the maximum increase of 37.27 percent. In T_8 - Parched red gram powder (26%) + Skimmed milk powder (24%) + Honey (35%) + sugar (5 %) + Glucose (10%) showed the least improvement of 9.25 percent. Honey bees compensate for the lack of pollen by lowering brood production, reducing oviposition, or cannibalism of young larvae. In conditions where pollen is scarce, older larvae survive (Schmickl and Crailsheim, 2001).

Table 1. Effect of pollen substitutes feeding on brood area development in Apis cerana colonies

Treatments	Brood area (cm^2) W 1	W 2	W 3	W 4	MEAN	% increase over control
T_1 – BP(65%) + H(34%) + M(1%) (12.41)ab	153.50 (13.30)^b	176.50 (13.89)^{ab}	192.50 (14.92)^b	222.50	186.25	44.06
T_2 – RBGP(49%) + H(40%) + BY(10%) + M(1%)	153.50 (12.36)^{bc}	174.00 (13.21)^{bc}	187.00 (13.69)^{bc}	215.50 (14.69)^b	182.25	37.38
T_3 - RBGP(49%) + SMP(40%) + BY(10%) + M (1%)	156.00 (12.51)^a	177.00 (13.32)^b	187.00 (13.69)^{bc}	210.00 (14.50)^b	182.50	37.79
T_4- RBGP(30%) + SMP(25%) + H(34%) + BY(10%) +M (1%)	153.50 (12.41)^{ab}	181.50 (13.49)^a	203.00 (14.25)^a	235.00 (15.34)^a	193.25	55.75
T_5- SBP(49%) + SMP (40%) + BY(10%) + M(1%)	155.00 (12.47)^{ab}	166.50 (12.90)^c	177.50 (13.34)^c	185.00 (13.58)^c	171.00	18.59
T_6- SBP(49%) + H (40%) + BY(10%) + M(1%)	150.00 (12.26)^c	162.50 (12.72)^c	176.50 (13.30)^c	182.50 (13.50)^c	167.87	13.36
T_7- SBP(30%) + SMP(25%) + H(34%) + BY(10%) + M(1%)	154.00 (12.43)^{ab}	160.00 (12.66)^{cd}	175.00 (13.24)^d	180.00 (13.43)^{cd}	167.25	12.44
T_8- PRGP(26%) + SMP(24%) + H(35%) + S(5%) + G(10%)	152.00 (12.36)^{bc}	159.00 (12.62)^{cd}	174.00 (13.21)^d	176.00 (13.28)^d	165.25	8.90
T_9- Control (12.30)^{bc}	151.00 (12.53)^d	156.50 (12.84)^e	164.50 (12.95)^e	167.50	159.87	
SED	0.07	0.52	0.33	0.69		
CD(0.05)	0.16	1.21	0.76	1.59		

BP- Bee pollen, RBGP- roasted bengal gram powder, PRGP- parched red gram powder, SMP- skimmed milk powder, SBP- soyabean powder, BY- brewer's yeast, S -sugar, G- glucose, H- honey, M-multivitamin, W- week

Figures:* In a column means followed by a common alphabet are significantly different at five per cent

* Mean of two replications.

Foraging activity

The observations on foraging activity include pollen foragers, outgoing bees and nectar gatherers is recorded for about five minutes thrice a day at weekly interval for a month and the mean value is calculated. The effect of pollen substitutes on foraging activity is illustrated in the graph 1. Although honeybee foraging is altruistic and socially controlled, individual nutritional physiology may also have an influence in foraging behaviour (Toth *et al*., 2005). In T_4 – Roasted Bengal gram powder (30%) + Skimmed milk powder (25%) + Honey (34%) + Brewer's yeast (10%) + multivitamin (1%) had the highest pollen foragers with a mean value of (40.5)bees/5min , followed by T_1 – Bee pollen (65%) + Honey (34%) + multivitamin (1%) with a mean value of (38.75) bees/ 5 min. Pollen foragers were less effective than T_4 and T_1 when compared to other pollen substitutes. Pollen foragers were significantly more abundant in the pollen substitute colonies than in the control colonies. The control colonies had the least pollen foragers (22.25/ 5min) compared to the other treatments.

Under different treatments, the increase in pollen foragers compared to the control ranged from 38.74 to 82.02 percent. The peak elevation rise of pollen foragers about 82.02 percent is shown in T_4- Roasted Bengal gram powder (30%) + Skimmed milk powder (25%) + Honey (34%) + Brewer's yeast (10%) + multivitamin (1%) over the control colonies. In T_8 - PRGP(26%) + SMP(24%) + H(35%) + S(5%) + G(10%) had the smallest improvement of 38.74 percent over the control colonies. Sammataro and Weiss (2013) reported that the supplemental feedings can also help with brood development, which results in more pollen foraging.

Incase of nectar gatherers T_4 – Roasted Bengal gram powder (30%) + Skimmed milk powder (25%) + Honey (34%) + Brewer's yeast (10%) + multivitamin (1%) had the maximum nectar gatherers with a mean value of (42.5) bees/5min , followed by T_1 – Bee pollen (65%) + Honey (34%) + multivitamin (1%) with a mean value of (37.87) bees/ 5 min. The control colonies had the least nectar gatherers (27.12/ 5min) compared to the other treatments. Among different treatments, the rise in nectar gatherers over control l ranged from 23.52 to 56.71 percent. The peak elevation of nectar gatherers about

Table 2. Effect of pollen substitutes feeding on pollen store area development in *Apis cerana* colonies

Treatments	Pollen store area (cm^2) W 1	W 2	W 3	W 4	MEAN	% increase over control
T_1 – BP(65%) + H(34%) + M(1%)	41.00 (6.44)^c	55.00 (7.44)^b	70.00 (8.39)^b	80.50 (8.99)^b	61.62	37.27
T_2 – RBGP(49%) + H(40%) + BY(10%) + M(1%)	42.50 (6.55)^b	55.50 (7.48)^b	67.50 (8.24)^b	76.00 (8.74)^b	60.37	35.29
T_3 - RBGP(49%) + SMP(40%) + BY(10%) + M (1%)	42.50 (6.55)^b	54.50 (7.41)^b	68.50 (8.30)^b	79.00 (8.91)^b	61.12	36.97
T_4- RBGP(30%) + SMP(25%) + H(34%) + BY(10%) + M (1%)	43.50 (6.63)^a	59.50 (7.71)^a	77.50 (8.80)^a	94.00 (9.72)^a	68.62	53.78
T_5- SBP(49%) + SMP (40%) + BY(10%) + M(1%)	43.00 (6.59)^b	47.50 (6.92)^c	59.00 (7.71)^c	72.50 (8.54)^c	55.50	24.38
T_6- SBP(49%) + H (40%) + BY(10%) + M(1%)	41.50 (6.48)^c	44.50 (6.70)^{cd}	58.00 (7.64)^c	67.50 (8.23)^c	52.87	18.48
T_7- SBP(30%) + SMP(25%) + H(34%) + BY(10%) + M(1%)	42.50 (6.55)^b	46.50 (6.80)^{cd}	59.00 (7.70)^c	56.50 (7.51)^d	51.12	14.56
T_8- PRGP(26%) + SMP(24%) + H(35%) + S(5%) + G(10%)	43.50 (6.63)^a	44.00 (6.60)^d	53.00 (7.31)^c	54.50 (7.35)^d	48.75	9.25
T_9- Control	40.50 (6.40)^c	42.50 (6.55)^d	46.50 (6.85)^d	49.00 (7.03)^e	44.62	
SED	0.10	0.23	0.42	0.67		
CD(0.05)	0.23	0.53	0.98	1.56		

BP- Bee pollen, RBGP-roasted bengal gram powder, PRGP- parched red gram powder, SMP- skimmed milk powder, SBP- soyabean powder, BY- brewer's yeast, S -sugar, G- glucose, H- honey, M-multivitamin, W- week
Figures:* In a column means followed by a common alphabet are significantly different at five per cent
* Mean of two replications.

56.71 percent is shown in T_4- Roasted Bengal gram powder (30%) + Skimmed milk powder (25%) + Honey (34%) + Brewer's yeast (10%) + multivitamin (1%) over the control colonies. According to nectar gatherers, T_2 – RBGP (49%) + H(40%) + BY(10%) + M(1%) received the smallest improvement of 23.52 percent over the control colonies.

In T_4 – Roasted Bengal gram powder (30%) + Skimmed milk powder (25%) + Honey (34%) + Brewer's yeast (10%) + multivitamin (1%) had the highest outgoing foragers with a mean value of (55.00) bees/5min , followed by T_1 – Bee pollen (65%) + Honey (34%) + multivitamin (1%) with a mean value of (53.12) bees/ 5 min.. The control colonies had the least outgoing foragers (35.37/ 5min) compared to the other treatments. Among different treatments, the elevation of outgoing foragers over control ranged from 18.74 to 55.49 percent. The peak elevation of nectar gatherers about 55.49 percent is shown in T_4- Roasted Bengal gram powder (30%) + Skimmed milk powder (25%) + Honey (34%) + Brewer's yeast (10%) + multivitamin (1%) over the control colonies. According to outgoing foragers , T_6- SBP(49%) + H (40%) + BY(10%) + M(1%) received the smallest improvement of 18.74 percent over the control colonies. Padmashree *et al*.(2021) revealed that probiotic supplementation can enhance honeybee colonies' foraging behaviour.

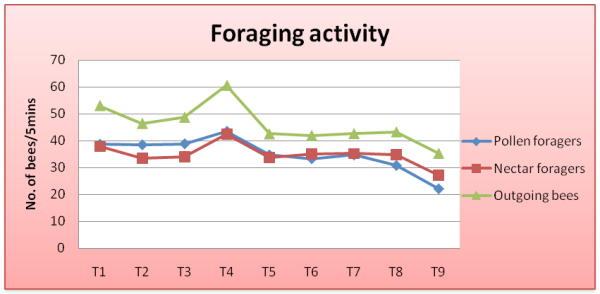

Fig. 1. Effect of pollen substitutes on the foraging activity of Asian honey bee, *Apis cerana indica.*

Conclusion

Pollen substitute feeding improved the overall colony growth parameters of Asian honey bee colonies. Pollen substitute with Roasted Bengal gram powder (49%) + Honey (40%) + Brewer's yeast (10%) + multivitamin (1%) significantly increases the brood area (193.25 cm^2), pollen store area (68.62 cm^2), pollen foragers (40.50/ 5min), nectar gatherers (42.50/5 min) and outgoing bees(55.00/ 5min) . Pollen substitute feeding during the off season period will help the colonies to develop faster and perform better than control colonies. Pollen substitute feeding is one of the finest options for keeping the colony healthy particularly in brood area development throughout the dearth period.

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Conflict of interest

The authors declare that there is no conflict of interest.

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