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Assessment of various pollen substitutes for sustaining brood development in *Apis cerana himalaya* Fabricius during the dearth period in Manipur conditions

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ABSTRACT: Five pollen substitutes were evaluated during 2022 for their effect on brood development of *Apis cerana himalaya* at Central Agricultural University, Imphal, Manipur, India. Six flours *viz*., green gram flour, soybean flour, wheat flour, rice bean flour, black gram flour and maize flour were chosen as main substrates of pollen substitutes. Five different treatments were prepared by mixing the flours with yeast and either honey solution or sugar solution. These diets were fed to *A. cerana himalaya* colonies in the form of patties on top bars during dearth period (June to August). Brood area development was observed the most in the colonies given T_3 (Rice bean flour (30%) + Yeast (30%) + Honey (40%)) with 777.66 cm^2 of brood area. The colonies given with T_5 (Maize flour (50%) + Yeast (20%) +Sugar (30%)) showed 649.13 cm^2 brood area followed by T_4(Black gram flour (50%) +Yeast (20%) +Honey (30%)) with 553.60 cm^2, T_2(Soybean flour (30%) + Wheat flour (30%) + Yeast (10%) + Sugar (30%)) showed 427.40 cm^2 and T_1 (Green gram flour (50%) + Yeast (20%) + Honey (30%)) showed 384.13 cm^2 of brood area. T_6 was control *i.e*., the colonies were only given with sugar solution which showed the least brood area as 333.93 cm^2. All the diet was found to be better as compared to control.

Keywords: *Apis cerana himalaya*, pollen substitute, brood, rice bean flour, maize flour, green gram flour, black gram flour, soybean flour, wheat flour

INTRODUCTION

Apiculture involves the controlled management of diverse honey bee species within artificial hives. Honey bees valuable not only for producing honey, beeswax, pollen, and propolis, but also for their exceptional ability to effect cross pollination in several crops of economic importance. Apiculture being closely associated with agriculture, plays a vital role in sustaining ecosystems. In beekeeping colony maintenance during dearth pesriods when adequate floral resources are not available is challenging. Scarce food resources exacerbate the decline in colony vigour. The only nutrition on which the honey bees depend are pollen and nectar from the flowers of different plants. Nectar is a carbohydrate source, while pollen supplies the bees with the proteins, lipids, vitamins, and minerals needed to rear larvae (DeGroot, 1953; Manning, 2001). The abundant availability of food source can be seen in honey flow season, whereas during dearth period there is a scarcity of food source which effect the strength of the colony.

To avoid this situation efforts should be made to formulate highly nutritional and palatable pollen substitutes for the colonies to overcome unfavourable conditions so as to strengthen the beekeeping. The necessity to formulate artificial diets for the honey bees has been undertaken by many researchers (De-Grandi *et al*. 2008; Saffari *et al*. 2010a, b; Sihag and Gupta, 2011; Morias *et al*. 2013; Gemeda, 2014; Kumar and Agrawal, 2014; Pande *et al*. 2015; Shehata, 2016; Abd el-Wahab *et al*. 2016).

Apis cerana himalaya, a subspecies of the Asian honeybee, is found in the northeastern region of India and plays a crucial role in the ecosystem. This subspecies exhibits unique adaptations to the local climate and is well-suited for beekeeping in the region (Thakur *et al*. 2012). Limited studies have been conducted on *Apis cerana himalaya* in comparison to other honeybee species. To fully understand its ecology, behaviour, and potential for sustainable development in the northeastern hill region of India, more research is necessary.

The current research work was to compare the efficacies of five different diets along with control for the brood development of *A. cerana himalya* colonies.

MATERIALS AND METHODS

Studies were conducted with the colonies of *A. cerana himalaya* during June to September,2022, considered a dearth period in Manipur. The Apiary maintained by Department of Entomology, College of Agriculture, Central Agricultural University, Imphal, Manipur. The experiment consisted of five treatments (diets), each with three replications, along with a control. Each diet

(Fig.3) weighing about 10 g was prepared freshly and provided to bee colonies at three days interval. The diets were given on the top bar of hive for consumption. The initial observation was conducted 14 days after the first feeding. Subsequently, at each 14-day interval, a new comb was selected for measuring various parameters. A measuring frame, consisting of a wire grid with squares of 1 cm (Fig.2), was used for these measurements. The parameters recorded were: Sealed brood, Unsealed brood, Eggs laid area, Total Brood area and Pollen store.The collected data were tabulated and subjected to statistical analysis (ANOVA) following Completely Randomized Design (CRD).

Fig.1. Top bar feeding

Fig. 2. Measuring frame

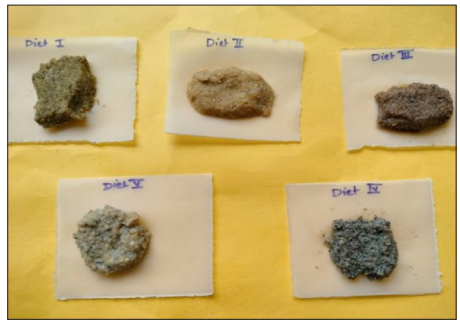

Fig. 3. Pollen Substitutes (Diets)

RESULTS AND DISCUSSION

The results revealed that feeding pollen substitutes to the bee colonies increased the brood area much faster compared to the control colonies which was only given with sugar solution (2:1).

Effect of diet formulation on sealed brood area

The effect of various diet formulations on sealed brood area is shown in table 1 Maximum sealed brood area (336.60 cm^2) was recorded in the colonies fed with T_3 (Green gram flour (50%) + Yeast (20%) + Honey (30%)), followed by 223.30 cm^2 when colonies with fed with T_5 (Maize flour (50%) + Yeast (20%) +Sugar (30%)). Other treatments i.e., T_1, T_2, and T_4 also shows increase in the sealed brood area as 127.33 cm^2, 135.00 cm^2 and 203.33 cm^2, respectively. The least development (116.73 cm^2) was seen in the colonies taken as control which was only given with sugar solution of ratio 2:1. The above findings on sealed brood area can be compared with the work of Kumari and Kumar (2020) who reported that the colonies treated with pollen substitute containing Defatted Soy flour and Parched Gram shows 1938.3 cm^2 sealed brood in *Apis mellifera*.

Effect of diet formulation on Unsealed brood area

The data given in table 2 on effect of feeding diet formulations on Unsealed brood revealed that maximum of unsealed brood was 219.25 cm^2 when the colonies were fed with T_3 followed by 207.75 cm^2 when the colonies were given with T_5. The colonies showed increase in unsealed brood area from first feeding to the last feed. The colonies also showed increase in unsealed brood area when given with T_1, T_2 and T_4, respectively. The data can be compared with the finding of Kumar and Agrawal (2014), who reported a maximum unsealed area of 65% when given with diet containing soy flour. Pandey (2008) also studied the effect of various diets on unsealed broods and reported maximum area under unsealed brood was found to be 21.67% in treatment containing Mandua + honey + Yeast extract + Multivitamins + Pollen pellets.

Effect of diet formulation on egg area

The colonies fed with T_3 showed more area (221.26 cm^2) where eggs are laid followed by the colonies fed with T_5 showed 214.53 cm^2. The mean observation recorded for T_1, T_2 and T_4 were 133.33 cm^2, 140.53 cm^2 and 148.53 cm^2, respectively. Colonies provided with only sugar solution showed less result. However, all the colonies which were given with pollen substitute showed good result as compared to the control colonies. The data can be compared with the finding of Kumar and Agrawal (2014), who reported maximum egg laying as 76% when given with a diet containing soy flour.

Effect of diet formulation on total brood area

Total brood was calculated by adding sealed brood, unsealed brood and egg area of all the colonies. Maximum brood area was recorded as 777.66 cm^2 when the colonies were given with $_{T3}$ containing Rice bean flour whereas least brood area (333.93 cm^2) was recorded in control colonies. All other treatments showed significant increase in brood area as compared to

the control colonies. When the colonies were treatment with T_5 it showed 649.13 cm^2 brood area. T_2 and T_1 showed 427.40 cm^2 and 384.13 cm^2 of brood area. The present study is comparable with the findings of Kumar *et al.* (2013), who reported that diet 3 (defatted soy four, brewer's yeast and soy protein hydrolysate powder) proved to be most effective with 2155.3 cm^2 brood area, 5.8 total bee covered frames and 11509 bee population. A similar study was again conducted by Kumar *et al.* (2013), who reported that the maximum (peak) amount of sealed brood area was observed in the colonies given diet 3 (723.4 cm2 per colony).

Table 1. Effect of diet formulation on sealed brood area (cm^2)

Diet	Treatment	DATE					Mean
		14 June	**28 June**	**12 July**	**26 July**	**9 Sep**	
T_1	Green gram flour (50%) + Yeast (20%) + Honey (30%)	103.33	112.00	134.33	141.33	148.33	127.33
T_2	Soybean flour (30%) + Wheat flour (30%) + Yeast (10%) + Sugar (30%)	108.33	122.33	140.00	152.33	163.33	135.00
T_3	Rice bean flour (30%) + Yeast (30%) + Honey (40%)	311.00	327.00	345.00	346.66	352.66	336.60
T_4	Black gram flour (50%) +Yeast (20%) +Honey (30%)	128.00	184.33	223.33	236.00	244.33	203.33
T_5	Maize flour (50%) + Yeast (20%) +Sugar (30%)	185.00	208.00	229.00	242.33	251.33	223.3
T_6	Sugar solution (2:1)	46.66	109.33	131.00	137.66	146.00	116.73
	SE(m)	2.47	2.12	2.45	2.17	2.50	0.79
	$CD_{(0.05)}$	7.70	6.61	7.62	6.75	7.79	2.47

Table 2. Effect of diet formulation on Unsealed brood area(cm^2)

Diet	Treatment	Day after treatment					Mean
		14 June	**28 June**	**12 July**	**26 July**	**9 sep**	
T_1	Green gram flour (50%) + Yeast (20%) + Honey (30%)	75.33	111.33	121.66	143.66	164	119.62
T_2	Soybean flour (30%) + Wheat flour (30%) + Yeast (10%) + Sugar (30%)	117.33	132.00	152.00	164.66	187	150.83
T_3	Rice bean flour (30%) + Yeast (30%) + Honey (40%)	187.33	192.00	208.00	245.33	266.33	219.25
T_4	Black gram flour (50%) +Yeast (20%) +Honey (30%)	160.33	182.00	195.00	225.33	246	203.73
T_5	Maize flour (50%) + Yeast (20%) +Sugar (30%)	179.00	187.33	202.00	231.33	257.33	207.75
T_6	Sugar solution (2:1)	47.33	96.66	119.33	129.33	136	109.60
	SE(m)	1.55	1.73	1.92	2.05	2.43	2.01
	$CD_{(0.05)}$	4.85	5.39	5.99	6.40	7.58	6.28

Table 3. Effect of diet formulation on Egg-laid area(cm^2)

Diet	Treatment	Day after treatment					Mean
		14 June	**28 June**	**12 July**	**26 July**	**9 Sep**	
T_1	Green gram flour (50%) + Yeast (20%) + Honey (30%)	110.00	134.00	136.00	138.00	148.66	133.33
T_2	Soybean flour (30%) + Wheat flour (30%) + Yeast (10%) + Sugar (30%)	112.33	136.66	146.66	152.00	155.00	140.53
T_3	Rice bean flour (30%) + Yeast (30%) + Honey (40%)	199.66	210.33	214.00	233.66	248.66	221.26
T_4	Black gram flour (50%) +Yeast (20%) +Honey (30%)	113.33	144.00	149.33	166.33	169.66	148.53
T_5	Maize flour (50%) + Yeast (20%) +Sugar (30%)	197.66	200.00	203.66	226.66	244.66	214.53
T_6	Sugar solution (2:1)	98.00	110.00	11.66	118.66	125.66	112.8
	SE(m)	4.23	3.07	3.26	2.96	2.92	3.14
	$CD_{(0.05)}$	13.20	9.56	10.18	9.24	9.10	9.81

Table 4. Effect of diet formulation on Total Brood area(cm^2)

Diet	Treatment	Day after Treatment					Mean
		14 June	**28 June**	**12 July**	**26 July**	**9 Sep**	
T_1	Green gram flour (50%) + Yeast (20%) + Honey (30%)	288.66	356.00	392.00	423.00	461.00	384.13
T_2	Soybean flour (30%) + Wheat flour (30%) + Yeast (10%) + Sugar (30%)	338.00	386.00	438.66	469.00	505.33	427.40
T_3	Rice bean flour (30%) + Yeast (30%) + Honey (40%)	698.66	729.33	767.00	825.66	867.66	777.66
T_4	Black gram flour (50%) +Yeast (20%) +Honey (30%)	402.33	510.33	567.66	627.66	660.00	553.6
T_5	Maize flour (50%) + Yeast (20%) +Sugar (30%)	562.00	595.33	634.66	700.33	753.33	649.13
T_6	Sugar solution (2:1)	192.00	322.33	362.00	385.66	407.66	333.93
	SE(m)	5.44	5.26	3.44	6.42	7.57	4.77
	$CD_{(0.05)}$	16.95	16.40	10.71	20.00	23.60	14.86

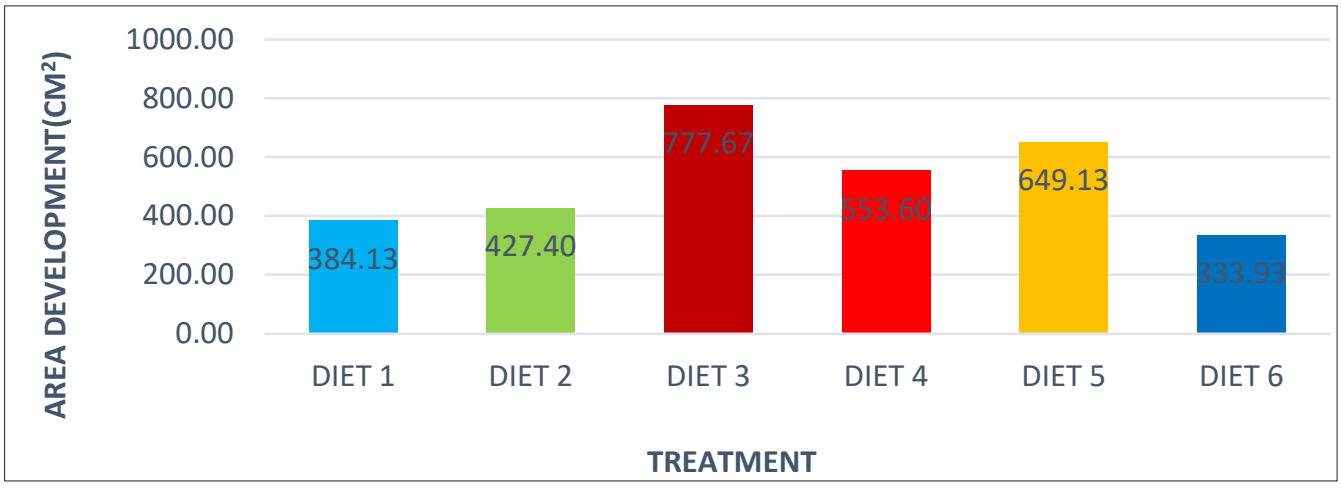

Fig. 4. Diagrammatic representation of brood area (cm²)

Table 5. Effect of diet formulation on Pollen store area (cm²)

DIET	TREATMENT	DATE					MEAN
		14 JUN	**28 JUN**	**12 JUL**	**26 JUL**	**9 SEP**	
T_1	Green gram flour (50%) + Yeast (20%) + Honey (30%)	19.50	22.00	25.00	29.00	33.00	25.70
T_2	Soybean flour (30%) + Wheat flour (30%) + Yeast (10%) + Sugar (30%)	20.66	23.00	26.00	31.00	34.00	26.66
T_3	Rice bean flour (30%) + Yeast (30%) + Honey (40%)	26.33	27.00	31.66	37.00	40.00	32.40
T_4	Black gram flour (50%) +Yeast (20%) +Honey (30%)	22.00	24.00	28.67	31.33	36.00	28.73
T_5	Maize flour (50%) + Yeast (20%) +Sugar (30%)	23.66	24.66	29.67	34.00	38.00	30.00
T_6	Sugar solution (2:1)	17.66	20.00	24.00	28.00	29.00	23.73
	SE(m)	0.69	0.64	0.75	0.64	0.58	0.68
	$CD_{(0.05)}$	2.22	1.97	2.30	1.97	1.78	2.08

Effect of diet formulation on pollen store area

Pollen is the main source of protein to honey bees, which get scarce during dearth period. The area under pollen store was measured for each colony to analysis the best treatment showing pollen store. Data collected in Table 6 and fig 6 revealed that though the pollen store area was less compared to other above parameters maximum area was seen in T_3 i.e., 32.40 cm² followed by 30.00 cm² of area under T_5. Among the other treatments T_4 showed 28.75 cm², T_2 showed 26.66 cm² and T_1 showed 25.70 cm². Only 23.73 cm² area of honey store was measured from colonies taken as control. The present data, which shows that all the pollen substitutes had pollen storage when compared with the control, is in accordance with the work of Vijayakumari et al. (2021), who reported that 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 cm²), followed by T_1 – Bee pollen (65%) + Honey (34%) + multivitamin (1%) of about (61.62 cm²). The other pollen substitute had a smaller pollen storage than T_4 and T_1. The control colonies were observed to have the least amount of

pollen storage area compared to the other treatments (44.62cm^2).

Based on findings, it can be concluded that Diet containing Rice bean flour shows best result to increase the brood rearing activity during the dearth period. Our research shows that providing sufficient nutrition can sustain bee colonies and encourage brood rearing, even during unfavorable conditions when rearing rates are at their lowest.

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