Journal of

Entomology

ISSN 1812-5670

Academic
Journals Inc.

www.academicjournals.com

Journal of Entomology 10 (3): 120-135, 2013
ISSN 1812-5670 / DOI: 10.3923/je.2013.120.135

Testing the Effects of Some Pollen Substitute Diets on Colony Build up and Economics of Beekeeping with *Apis mellifera* L.

Ram C. Sihag and Manisha Gupta
Laboratory of Apidology, Department of Zoology and Aquaculture, CCS Haryana Agricultural University, Hisar-125004, India

Corresponding Author: Ram C. Sihag, Laboratory of Apidology, Department of Zoology and Aquaculture, CCS Haryana Agricultural University, Hisar-125004, India

ABSTRACT

To help the honeybee colonies to tide over harsh summer dearth period in South-Western region of Haryana (India), the efficacy of four pollen substitute diets viz. diet-1 (Soybean flour+yeast extract+honey), diet-2 (diet-1+NaCl salt), diet-3 (diet-1+salt+vitamins and minerals) and diet-4 (diet-1+vitamins and minerals) was tested on the reproduction and build up of colonies of honey bee (*Apis mellifera* L.). The diet-1 could induce reproduction and build up in the treated colonies. But, the diet-2 proved counterproductive as all the attributes of the colonies receiving the latter diet declined markedly. The diet-3 could marginally increase the values of colony attributes. However, these values remained significantly lower than those achieved under diet-1. The diet-4 proved to be the best among the four artificial pollen substitute diets. The colonies receiving the latter diet showed maximal unsealed brood area, sealed brood area, bee strength and pollen and honey stores. All the control colonies had extremely low values of these parameters, could not survive the summer dearth period and vanished by late October. The colonies receiving diet-4 could give maximal economic returns followed by, in descending order, those receiving diet-1, diet-2 and diet-3. The control colonies receiving no artificial diet died during the dearth period thus causing a complete loss of the invested capital amount. The diet-4, therefore, seems to make a sufficient pollen substitute feed for sustaining reproduction and brood rearing in honeybee (*A. mellifera*) colonies during dearth period.

Key words: *Apis mellifera*, brood, colony, dearth period, honeybee, pollen substitute, reproduction

INTRODUCTION

Seed/fruit production in crop plants is influenced by several inputs; managed pollination by honeybees is one of these inputs (Sihag, 2001). Honeybees, in fact, are excellent pollinators of several cultivated crops and help increase their seed/fruit production many fold through cross pollination (Sihag, 1986; Free, 1993). In addition to this, owing to the massive loss of wild bee pollinators, crop growers have to rely primarily on honeybee pollination (Sihag, 2001). That is why, beekeeping has become one of the essential inputs in agriculture. But, beekeeping is not a smooth process in all the months and seasons. Over the globe, almost every place has a specific period during which there is dearth of floral resources. This dearth is especially crucial in the semi-arid tropics where it coincides with very high temperature and incidence of several pests, predators and enemies (Sihag, 1990a, 1991). Though the methods of management of colonies against high temperature, pests, predators and enemies are available (Sihag, 1990a, 1991), yet the floral dearth remains a key problem for the beekeepers for the reproduction, brood rearing and ultimate survival of the colonies.

During the floral dearth period, for the sustained reproduction and buildup of the honeybee colonies, these are in dire need of an adequate and balanced artificial pollen supplement/substitute diet which is attractive as well as in palatable form (Townsend and Smith, 1969; Taber, 1978). Strong colonies are the utmost need of the crop growers for their utilization as pollinators of crops later in the flowering season. This, in turn, will depend upon an adequate supply of proteins and nutrients in the artificial diet; the former is essential for the development of hypopharyngeal glands and preparation of brood food (Doull, 1975a; Herbert and Shimanuki, 1977). In nature, pollen is the only source of proteins for the honeybee colonies for their reproduction and brood rearing activities (Doull, 1975a).

Artificial feeding of honeybee colonies during dearth period has earlier been recognized as an essential practice for the sustained survival, reproduction and brood rearing especially in the stationary beekeeping. Strong colonies during dearth period witness early buildup and more foraging bees during the subsequent floral period for augmented honey production and better pollination services. The development of a pollen supplement/substitute artificial diet has been the interest of the beekeepers/bee scientists to solve problem of floral dearth, especially in stationary beekeeping (Adbellatif *et al*., 1971; Herbert and Shimanuki, 1980, 1983; Herbert, 2000; Nabors, 2000; Van der Steen, 2007; De Grandii-Hoffman *et al*., 2008; De Jong *et al*., 2009; Saffari *et al*., 2010a; Sihag and Gupta, 2011).

Pollen at our study site is available from December to February and the period from mid May to mid July witnesses a very hot (day temperature, 44-48°C) and dry (Rh, 15-20%) floral dearth. Though little mild, this kind of situation is prevalent almost every where in India and so also in other parts of the globe. As pointed out earlier (Sihag and Gupta, 2011), engaging the honeybee colonies in pollen collection during flow period is expensive and cast heavily on their productivity. That is why, development of a pollen substitute diet is most essential for feeding honey bee colonies during dearth period.

Various materials used in the preparation of pollen substitute diets often suffer from high cost, deficiency of protein quality or other dietary requirement (Standifier *et al*., 1973), presence of toxic or pathogenic agents (Barker and Lehner, 1976; Barker, 1977) and low palatability (Herbert, 1978). Pollen traped from honeybee colonies and available commercially is nutritionally poorer and less economical than the fresh pollen (Hagedorn and Moeller, 1968). Therefore, development of a cheap and acceptable pollen substitute diet is the prime need of beekeepers especially in the developing countries where summer is very harsh and dry and there is also floral dearth combined with high temperature and incidence of several pests, predators and enemies of honeybees. Earlier, we developed and tested a pollen substitute soy based diet (Sihag and Gupta, 2011). The latter diet was acceptable and palatable to the honeybees and could also sustain the honeybee colonies leading to some honey production (Sihag and Gupta, 2011). In the present study, we aim at testing the four pollen substitute diets prepared due to addition/deletion of some ingredients to the earlier diet and at another location. We also aim at working out the economics of these diets for the benefit of beekeepers and for strengthening the importance of the study.

MATERIALS AND METHODS

This is in continuation of our earlier study (Sihag and Gupta, 2011). However, we carried out this study at CCS Haryana Agricultural University, Hisar (India), about 200 km North-western side of our earlier study site and adjacent to the Thar Desert. We adopted following methodology for this study.

Selection and equalization of *Apis mellifera* colonies: We selected twelve colonies of honey bees (*Apis mellifera* L.) on 9 July, 2004 for artificial feeding. A set of other three colonies was used as a control. Each experimental colony had five standard Langstroth frames and was equalized for the following five colony attributes:

- Unsealed brood (eggs and larvae) = 250 cm^2
- Sealed brood = 500 cm^2
- Pollen area = 50 cm^2
- Honey area = 300 cm^2
- Strength = 5 frames fully covered with bees on both sides carrying about 14000 bees (Burgett *et al.*, 1984)

Recommended colony management practices were regularly followed in all the colonies selected for the present study (Sihag, 1990a).

Preparation and feeding of various pollen substitute diets: Efficacy of four pollen substitute diets on the reproduction and build up of *Apis mellifera* colonies were evaluated. The colonies deprived of an artificial pollen substitute diet acted as a control.

There was a basic principle behind the preparation of an artificial pollen substitute diet for the honey bee colonies for floral dearth that the diet should contain a bulk of proteins and carbohydrates and also vitamins and minerals/salts. Our earlier study revealed that a slurry formulation was most acceptable to the bees; accordingly the diet was prepared. Feeding a slurry formulation inside the hive was less wasteful and more acceptable; therefore, diets were made available to the bees by directly introducing these in to the frames, as suggested by Sihag and Gupta (2011). To eliminate/inactivate the anti-nutritional factors (ANFs) present in soybean seeds, the seeds were hydrothermically treated at 151 lb (121°C for 15 min) (Sihag and Gupta, 2011).

Each candidate pollen substitute diet was given to a set of three colonies. Every week 100 g of pollen substitute diet and one litre of 50% sugar syrup were given to each colony. The control colonies received one litre of 50% sugar syrup per week only. All the colonies had newly mated and laying queens which were raised in February 2004.

Measurement of various colony parameters

Measurement of brood, pollen and honey stores: Unsealed brood, sealed (worker and drone) brood, pollen and honey stores were measured in terms of area (cm^2). Observations were recorded at an interval of 21 days from 9 July 2004 to 16 March 2005. The above colony parameters were measured using a frame sized wire-grid, which could fit well on the four wooden bars of the comb frames, when placed on it. The wire-grid consisted of squares each having 6.45 cm^2 area (Chhuneja *et al.*, 1993a). The wire grid was placed on the comb for measuring various parameters and numbers of squares of wire grid covering the different parameters were counted on all the combs in a colony. From this total; brood, pollen and honey areas were obtained. Honey stores were calculated by following Chhuneja *et al.* (1993a). Ten frames with different areas of honey stores were selected at random from different colonies. The area of honey in every comb was measured with the help of wire grid. The honey in those combs was then squeezed out and weighed. The total weight of the honey (kg) squeezed out was divided with the total area of honey (cm^2) which came out to be 1.25×10^{-3} kg cm^{-2}. For measuring the quantity of honey in a colony, the area of honey store (measured with the help of wire grid), was multiplied with a 1.25×10^{-3} factor to get weight (kg) of honey present in the colony.

Measurement of bee strength: Total bee strength was measured in terms of frames actually covered by bees (Burgett *et al.*, 1984). Bee strength of all the experimental colonies was recorded at an interval of 21 days from 9 July 2004 to 16 March 2005.

Derivation of correlations between different attributes of experimental colonies: Correlations between any two attributes of the colonies feeding a particular diet were derived following Snedecor and Cochran (1989). For example, between unsealed brood versus sealed brood, colony strength, pollen stores and honey store. While doing so, the initial fixed values were discarded to minimize the experimental biases.

Determining the economics of beekeeping with colonies fed pollen substitute diets: Net profit was calculated from colonies fed various pollen substitute diets. Only loss was observed in the colonies where these could not sustain due to the lack/inadequacy of food/diet.

Working out the gross expenditure: Investment was calculated on the basis of cost of honeybee colonies, honey store present in the colonies at the start of the study in July, quantity of various pollen substitute diets and sugar fed to the experimental colonies and the number of wax-foundation sheets used. The prevailing rates of various commodities in Indian Rupee (1 Rs ~ 0.020 US$ or 0.015€) are listed below:

- Cost of soybean flour @ Rs. 25 kg^{-1}
- Cost of honey @ Rs. 140 kg^{-1}
- Cost of yeast extract @ Rs. 2034 kg^{-1}
- Cost of multivitamins @ Rs. 2 500 mg^{-1}
- Cost of wax foundation sheet @ Rs. 10 per piece
- Cost of honeybee colony @ Rs. 400 $frame^{-1}$

The cost of honeybee colonies, honey present in the colonies at the start of the study in July, total quantity of diet fed to the colonies and the number of wax-foundation sheets used were multiplied with their respective rates and the resulting total amount was taken as the gross expenditure.

Each colony was given 2100 g of pollen substitute and 11 kg of sugar from 9 July, 2004 to 2 Dec. 2004. Therefore, to each colony, 1.260 kg soybean @ Rs. 25 per kg (amounting to Rs. 31.50); 0.785 kg honey @ Rs. 140 per kg (amounting to Rs. 109.90) and 0.105 kg yeast extract @ Rs. 2034 per kg (amounting to Rs. 213.17) and 11 kg of sugar @ Rs. 30 per kg (amounting to Rs. 330) were given. Besides these, the growing colonies were provided with wax foundation sheets @ Rs. 10 per sheet.

Working out the gross returns: Returns were calculated on the basis of honey bee strength and honey store per colony at the end of this study in March, 2005. The prevailing rates of various commodities were as listed below:

- Cost of honey (raw) @ Rs. 140 kg^{-1}
- Cost of surplus frames @ Rs. 400 $frame^{-1}$

These rates were multiplied with the total bee strength and the total honey present in the colonies at the end of the experiment in March, 2005 and the resulting total was taken as the gross return.

Working out the net profit: Net profit was calculated by subtracting gross expenditure from gross return.

Statistical analysis: All the experiments were laid down in completely randomized design following Snedecor and Cochran (1989). A significant F-value for the given degrees of freedom in the F-test ($p \leq 0.05$) prompted to derive critical differences. The differences among the mean values of data recorded for a date were compared with the derived respective critical difference and the comparisons among the diets were recorded.

RESULTS

The effect of artificial diets on different colony attributes was studied and the results are presented below:

Effect of various pollen substitute diets on the unsealed brood area of honey bee colonies: General pattern of the unsealed brood in the colonies under different feeding treatments and on different dates is shown in Table 1. All the colonies receiving pollen substitute diets started egg laying and brood rearing with these diets. However, level of unsealed brood remained maximal in colonies receiving diet-4 followed by, in descending order, those receiving diet-1, diet-3 and diet-2. This pattern continued till August 2004. Within two months of feeding of artificial diets, a clear pattern could emerge on their effect on brood production in the colonies (Table 1). From September onwards, unsealed brood area continued to increase in all the colonies. However, the colonies receiving diet-4 had maximal unsealed brood followed by, in descending order, those receiving diet-1, diet-3 and diet-2. Significant differences were seen in the unsealed brood area of the colonies receiving various pollen substitute diets, but in the control colonies (receiving no pollen substitute diet), it was significantly lower than that of the other colonies ($p \leq 0.05$, ANOVA, Table 1).

In February, unsealed brood area increased tremendously and the peaks were achieved in all the colonies. However, the colonies receiving diet-4 had significantly more unsealed brood area as compared to the colonies receiving diet-1, diet-2 and diet-3 ($p \leq 0.05$, ANOVA, Table 1).

In March, unsealed brood area in all the colonies was lower than that in February. This was perhaps due to the decline in the bee forage in this region. But, the colonies receiving diet-4 (protein+carbohydrate+vitamins and minerals) had significantly more brood area as compared to the colonies receiving diet-1 (protein+carbohydrate), diet-2 (protein+carbohydrate+salt) and diet-3 (protein+carbohydrate+vitamins and minerals+salt).

Table 1: Effect of various pollen substitute diets on unsealed brood area in the colonies of honey bee (*A. mellifera*)

	Unsealed brood area (cm²) in the colonies fed pollen substitute diets*					
Date	Diet 1	Diet 2	Diet 3	Diet 4	Control	CD (p≤0.05)
09 July, 04	250.00±0.00[a]	250.00±0.00[a]	250.00±0.00[a]	250.00±0.00[a]	250.00±0.00[a]	NS
29 July, 04	345.00±21.43[b]	113.33±8.22[c]	113.33±7.74[c]	448.33±24.84[a]	51.66±3.33[d]	21.08
19 Aug., 04	251.66±15.18[b]	203.33±12.56[c]	243.33±15.28[b]	470.00±26.22[a]	32.24±2.17[d]	14.42
09 Sept., 04	658.33±36.88[b]	453.33±26.74[d]	541.66±31.47[c]	925.00±52.83[a]	16.73±0.99[e]	35.50
01 Oct., 04	991.66±57.49[b]	603.33±34.37[d]	830.00±59.23[c]	1297.33±74.76[a]	00.00±0.00	57.30
22 Oct., 04	1316.66±81.63[b]	840.00±55.88[d]	920.00±54.97[c]	1600.00±89.46[a]	00.00**	71.95
12 Nov., 04	1666.33±94.52[b]	1166.66±66.25[d]	1413.33±84.33[c]	1950.00±113.56[a]		97.58
02 Dec., 04	2016.66±118.37[b]	1250.00±73.65[d]	1520.00±87.88[c]	2535.00±149.69[a]		101.95
22 Dec., 04	2436.66±137.87[b]	1446.66±83.34[d]	1785.00±92.57[c]	3316.00±193.72[a]		121.97
12 Jan., 05	2725.00±152.45[b]	1700.00±96.87[d]	2133.33±118.45[c]	4676.66±244.87[a]		145.63
02 Feb., 05	3008.33±178.54[b]	1950.00±114.34	2266.66±149.75[c]	6105.00±345.66[a]		169.47
23 Feb., 05	3503.33±203.46[b]	2316.66±154.77[d]	2486.66±141.22[c]	7515.00±434.88[a]		178.35
16 Mar., 05	2823.33±157.73[b]	1533.33±85.41[d]	1900.00±110.48[c]	7390.00±413.68[ab]		150.86

*Mean±SD of three replications, **Colonies died, NS: Non-significant, Means in a row with dissimilar letters differ significantly

Table 2: Effect of various pollen substitute diets on sealed brood area in the colonies of honey bee (*A. mellifera*)

	Sealed brood area (cm²) in the colonies fed pollen substitute diets*					
Date	Diet 1	Diet 2	Diet 3	Diet 4	Control	CD (p≤0.05)
09 July, 04	500.00±0.00[a]	500.00±0.00[a]	500.00±0.00[a]	500.00±0.00[a]	500.00±0.00[a]	NS
29 July, 04	246.66±17.49[a]	248.33±15.88[a]	244.66±16.53[a]	247.66±18.67[a]	248.50±17.89[a]	NS
19 Aug., 04	320.00±21.88[b]	106.66±7.88[c]	098.33±6.54[c]	423.33±27.22[a]	45.32±2.47[d]	18.88
09 Sep., 04	225.33±13.32[b]	213.66±12.88[c]	224.33±13.45[c]	447.66±25.71[a]	18.18±1.22[d]	14.42
01 Oct., 04	608.00±35.88[b]	408.66±23.97[d]	512.00±30.11[c]	904.33±54.65[a]	12.64±0.88[e]	34.50
22 Oct., 04	959.33±57.12[b]	562.33±34.25[d]	803.33±48.64[c]	1258.00±81.75[a]	0.00±0.00**	49.30
12 Nov., 04	1286.66±85.67[b]	795.33±51.87[d]	893.33±52.77[c]	1553.33±93.34[a]		71.95
02 Dec., 04	1603.33±101.29[b]	1115.00±64.53[d]	1382.66±92.22[c]	1903.33±108.66[a]		93.43
22 Dec., 04	1972.33±112.83[b]	1226.66±71.24[d]	1496.66±87.88[c]	2513.33±154.33[a]		113.75
12 Jan., 05	2386.66±154.32[b]	1423.33±89.76[d]	1764.33±115.31[c]	3300.66±198.42[a]		123.97
02 Feb., 05	2683.33±173.71[b]	1674.33±108.22[d]	2106.66±119.66[c]	4660.00±286.68[a]		147.63
23 Feb., 05	2975.66±302.45[b]	1935.00±121.23[d]	2241.33±125.85[c]	6087.33±385.22[a]		158.74
16 Mar., 05	3477.66±188.99[b]	2175.33±124.32[d]	2463.00±138.88[c]	7495.00±413.68[a]		184.37

*Mean±SD of three replications, **Colonies died, NS: Non-significant, Means in a row with dissimilar letters differ significantly

Effect of various pollen substitute diets on the sealed brood area of honey bee colonies: General pattern of sealed brood present in the colonies under different treatments and on different dates is shown in Table 2. The differences in the sealed brood area under different pollen substitute diets were significant ($p \leq 0.05$, ANOVA). The colonies receiving diet-4 had maximal sealed brood followed by, in descending order, those receiving diet-1, diet-3 and diet-2. This pattern was observed throughout the experimental period. Sealed brood area of control colonies declined to a very low level of 12.64 cm^2 in early October and all these colonies died in late October.

Effect of various pollen substitute diets on the strength of honey bee colonies: Table 3 presents the general pattern of bee strength in the colonies under different feeding treatments. Significant differences were observed in the bee strength of colonies receiving different pollen

Table 3: Effect of various pollen substitute diets on the strength of colonies of honey bee

Date	Strength (in terms of frames actually covered by bees) of colonies fed various pollen substitute diets*					
	Diet 1	Diet 2	Diet 3	Diet 4	Control	CD ($p \leq 0.05$)
09 July, 04	5.00±0.00[a]	5.00±0.00[a]	5.00±0.00[a]	5.00±0.00[a]	5.00±0.00[a]	NS
29 July, 04	7.33±0.45[a]	7.00±0.44[a]	7.00±0.43[a]	7.33±0.46[a]	7.00±0.45[a]	NS
19 Aug., 04	6.33±0.38[b]	5.33±0.32	5.33±0.34[c]	7.33±0.46[a]	6.33±0.41[d]	0.32
09 Sept., 04	5.33±0.33[b]	5.33±0.31	5.33±0.29[c]	7.33±0.44[a]	4.00±0.25[c]	0.28
01 Oct., 04	5.33±0.33[b]	4.33±0.25	4.33±0.23[c]	7.33±0.47[a]	1.33±0.08[d]	0.26
22 Oct., 04	5.00±0.31[b]	3.66±0.22	3.33±0.21[c]	7.33±0.49[a]	0±0.00**	0.24
12 Nov., 04	4.33±0.26[b]	3.33±0.23	333±0.22[c]	7.66±0.52[a]		0.21
02 Dec., 04	5.33±0.35[b]	3.66±0.23	3.33±0.22[c]	8.33±0.50[a]		0.27
22 Dec., 04	6.33±0.47[b]	4.00±0.37	4.33±0.26[c]	10.33±0.61[a]		0.33
12 Jan., 05	7.00±0.44[b]	5.33±0.35	5.00±0.32[c]	13.33±0.88[a]		0.36
02 Feb., 05	9.33±0.0.57[b]	6.00±0.37	7.33±0.48[c]	16.66±1.02[a]		0.48
23 Feb., 05	11.33±0.68[b]	7.33±0.47	9.33±0.56[c]	20.66±1.31[a]		0.61
16 Mar., 05	14.00±0.83[b]	10.33±0.62	11.33±0.69[c]	23.33±1.42[a]		0.72

*Mean±SD of three replications, **Colonies died, NS: Non-significant, Means in a row with dissimilar letters differ significantly

substitute diets as well as on different days ($p \leq 0.05$, ANOVA, Table 3). In the colonies receiving diet-1, diet-2 and diet-3 the bee strength increased from 5 frames in July to 7 frames in August. Thereafter, the bee strength kept on declining from August to early November. The control colonies, however, died in late October. The colonies receiving diet-4, however, kept the bee strength at 7 frames from late July to early November. This indicates the better efficacy of the latter diet over the others and also its better suitability as pollen substitute diet during dearth period. It also speaks of the adequacy of diet-4 and inadequacy of diet-1, diet-2 and diet-3. Colonies receiving diet-1 and diet-4 started build up in early December, where as colonies receiving diet-2 and diet-3 did so in late December. Thereafter, a regular build up continued in all the test colonies receiving any of the four diets. However, colonies receiving diet-4 attained bee strength of 23 frames at the end of the experiment in March, 2005 (Table 3).

Effect of various pollen substitute diets on the pollen storage in the honey bee colonies: General pattern of pollen storage in the colonies under different feeding treatments and on different dates is shown in Table 4. In the beginning of the experiment, all the experimental colonies were equalized at 50 cm^2 area of pollen storage. In July, a decrease in pollen area was observed in all the experimental colonies but this decrease was more in the control colonies as compared to those receiving pollen substitute diets. Among the latter too, colonies receiving diet-4 gathered/preserved more pollen as compared to those receiving diet-1, diet-2 and diet-3. The pattern of storage was similar for all the dates; colonies receiving diet-4 gathered maximal pollen followed by those receiving diet-1, diet-3 and diet-2; the differences among diets of all the dates were significant ($p \leq 0.05$, ANOVA, Table 4).

In August, pollen area decreased drastically to a very low level in all the colonies. Decrease in the pollen area in colonies receiving various pollen substitute diets showed that bees consumed stored pollen too along with the given diet. However, significant differences were seen in the pollen area of different colonies receiving various diets; the control colonies had lower pollen area as compared to the colonies supplied with various pollen substitute diets.

Table 4: Effect of various pollen substitute diets on pollen storage in the colonies of honey bee (*A. mellifera*)

	Pollen area (cm^2) in the colonies fed various pollen substitute diets*					
Date	Diet 1	Diet 2	Diet 3	Diet 4	Control	CD ($p \le 0.05$)
09 July, 04	50.00 ± 0.00^{a}	50.00 ± 0.00^{a}	50.00 ± 0.00^{a}	50.00 ± 0.00^{a}	50.00 ± 0.00^{a}	NS
29 July, 04	46.66 ± 2.91^{a}	40.00 ± 2.42^{c}	43.33 ± 2.46^{b}	46.66 ± 2.81^{a}	31.66 ± 1.92^{d}	2.57
19 Aug., 04	2.33 ± 0.15^{b}	1.66 ± 0.11^{d}	1.83 ± 0.12^{c}	2.66 ± 0.18^{a}	01.83 ± 0.11^{e}	0.12
09 Sep., 04	2.56 ± 0.17^{b}	1.36 ± 0.08^{d}	1.53 ± 0.08^{c}	3.26 ± 0.20^{a}	01.06 ± 0.07^{d}	0.14
01 Oct., 04	3.40 ± 0.23^{b}	2.26 ± 0.15^{d}	2.73 ± 0.18^{c}	5.53 ± 0.33^{a}	00.00 ± 0.00	0.19
22 Oct., 04	13.33 ± 0.09^{b}	6.66 ± 0.42^{d}	8.33 ± 0.52^{c}	16.66 ± 1.01^{a}	00.00**	0.71
12 Nov., 04	19.66 ± 1.21^{b}	13.66 ± 0.91^{d}	15.33 ± 0.94^{c}	32.66 ± 2.01^{a}		0.96
02 Dec., 04	28.00 ± 1.94^{b}	18.66 ± 1.21^{d}	22.33 ± 1.34^{c}	55.33 ± 3.35^{a}		1.59
22 Dec., 04	64.00 ± 3.91^{b}	40.00 ± 2.52^{d}	45.00 ± 2.83^{c}	128.00 ± 8.23^{a}		3.30
12 Jan., 05	77.33 ± 4.58^{b}	47.00 ± 2.96^{d}	52.66 ± 3.23^{c}	163.00 ± 9.86^{a}		3.95
02 Feb., 05	84.66 ± 5.32^{b}	52.00 ± 3.23^{d}	57.33 ± 3.87^{c}	170.00 ± 11.21^{a}		4.49
23 Feb., 05	121.33 ± 8.03^{b}	60.00 ± 3.65^{d}	68.66 ± 4.86^{c}	252.00 ± 16.87^{a}		6.44
16 Mar., 05	102.66 ± 6.88^{b}	41.66 ± 2.68^{d}	46.66 ± 2.99^{c}	230.00 ± 15.76^{a}		5.22

*Mean±SD of three replications, **Colonies died, NS: Non-significant, Means in a row with dissimilar letters differ significantly

In early October, pollen area increased in all except in the control colonies. The latter had no pollen store at this time. Pollen area was maximal in colonies receiving diet-4. In November too, significant differences in pollen stores were observed in colonies receiving different diets; but colonies receiving diet-2 (protein+carbohydrate+salt) had significantly smaller pollen area ($p \le 0.05$, ANOVA, Table 4). The colonies receiving diet-4 had maximal pollen area followed by those receiving diet-1, diet-3 and diet-2. Starting in early October, the colonies continued adding to the pollen storage till the peaks were achieved in late February. There was some decline in the pollen storage in all the colonies in mid-March. It was evident that unsealed brood area was also maximal in colonies receiving diet-4 (Table 1).

At the end of experiment (i.e. in March, 2005), pollen storage was maximal in colonies receiving diet-4 (protein+carbohydrate+vitamin) i.e. 230 cm^2; followed by that on diet-1 (protein+carbohydrate) i.e. 102.66 cm^2; diet-3 (protein+carbohydrate+vitamin+salt) i.e. 46.66 cm^2 and it was least in the colonies receiving diet-2 (protein+carbohydrate+salt) i.e. 41.66 cm^2.

Effect of various pollen substitute diets on the honey storage in honeybee colonies: General pattern of honey area in the colonies under different treatments and on different dates is shown in Table 5. In the beginning of experiment, the honey area in each colony was 300 cm^2. From July to early October, no natural flora was present in this region. Therefore, honey area showed a decline in all the colonies. But colonies receiving diet-4 (protein+carbohydrate+vitamins and minerals) had significantly more honey area as compared to the other colonies ($p \le 0.05$, ANOVA, Table 5). Minimal level of honey area was observed in the control colonies which died in late October (Table 5).

Honey area started increasing in late October. Colonies receiving diet-4 had significantly more honey area as compared to the other colonies. In December, honey area sharply increased and colonies feeding on diet-4 (protein+carbohydrate+vitamins and minerals) had significantly more honey area as compared to that of other colonies ($p \le 0.05$, ANOVA, Table 5). From December to March, honey area further increased and the colonies provided with diet-4 (during dearth period) collected significantly more honey as compared to the other colonies. It is also evident that bee strength in colonies receiving diet-4 was also maximal from August to March (Table 3).

Table 5: Effect of various pollen substitute diets on honey storage in the colonies of honey bee (*A. mellifera*)

	Honey area (cm^2) in the colonies fed various pollen substitute diets*					
Date	Diet 1	Diet 2	Diet 3	Diet 4	Control	CD ($p\leq0.05$)
09 July, 04	300.00±0.00[a]	300.00±0.00[a]	300.00±0.00[a]	300.00±0.00[a]	300.00±0.00[a]	NS
29 July, 04	161.66±9.88[b]	140.00±8.89[d]	148.33±9.11[c]	226.66±14.33[a]	161.66±9.83[b]	7.84
19 Aug., 04	141.66±8.89[b]	88.33±5.88[d]	126.66±8.11[c]	211.66±13.87[a]	110.00±6.98[e]	7.46
09 Sep., 04	80.33±5.11[b]	60.83±5.89[d]	65.66±4.11[c]	100.33±6.54[a]	08.33±0.51[e]	4.60
01 Oct., 04	48.00±3.12[b]	30.66±1.98[d]	36.00±2.54[c]	60.66±3.98[a]	00.00±0.00	2.72
22 Oct., 04	61.00±3.97[b]	47.00±3.11[d]	56.66±3.96[c]	84.00±5.32[a]	00.00**	3.21
12 Nov., 04	96.00±6.11[b]	62.50±3.98[d]	93.33±6.11[c]	100.33±6.76[a]		4.96
02 Dec., 04	201.33±12.65[b]	125.66±7.98[d]	190.66±12.13[c]	232.33±13.88[a]		11.27
22 Dec., 04	550.00±33.45[b]	402.50±24.87[d]	510.00±31.22[c]	800.00±65.43[a]		31.60
12 Jan., 05	1558.33±94.54[b]	1133.33±68.99[d]	1275.00±74.33[c]	2916.66±178.98[a]		76.59
02 Feb., 05	3150.00±191.73	2160.66±131.11[d]	2500.00±152.43[c]	6025.00±358.76[a]		170.60
23 Feb., 05	3633.33±221.71[b]	2300.00±143.56[d]	2883.33±173.73[c]	8132.66±632.33[a]		198.54
16 Mar., 05	3820.00±231.53[b]	2553.33±162.69[d]	3000.00±178.48[c]	9800.00±592.37[a]		205.21

*Mean±SD of three replications, **Colonies died, NS: Non-significant, Means in a row with dissimilar letters differ significantly

The colonies receiving diet-4 had maximal bee strength and produced more honey than the other colonies receiving other pollen substitute diets which had lower bee strengths (Table 3, 5).

Correlations among the colony attributes: Very high positive and significant correlations existed among the five colony attributes viz. colony strength, unsealed brood, sealed brood, pollen stores and honey stores in all the colonies receiving pollen substitute diets ($p\leq0.05$, t-test, Table 6). Larger bee strength seemed to help production of greater quantities of honey, as very high positive and significant correlations existed between colony strength and honey production in all the colonies receiving pollen substitute diets ($p\leq0.05$, t-test, Table 6).

Economics of beekeeping with colonies fed pollen substitute diets: The data in Table 7 show the amount of material consumed and produced in colonies receiving different diets. The colonies consumed equal quantities of diets. However, the number of wax foundation sheets utilized was different and so were the bee frames and honey produced. The data regarding the economics of beekeeping with *Apis mellifera* colonies receiving various pollen substitute diets during dearth are presented in Table 8. The expenditures incurred on diets were little different i.e. Rs. 2000 per colony was invested on control colonies, whereas this cost was Rs. 2444.67 per colony for those receiving diet-1, Rs. 2404.67 per colony for those receiving diet-2, Rs.2418.67 per colony for those receiving diet-3 and Rs.2530.67 per colony for those receiving diet-4. Gross return was maximal in colonies receiving diet-4 (Rs. 8775.00 per colony) followed by those receiving diet-1 (Rs. 4233.50 per colony), diet-3 (Rs. 2925.00 per colony) and diet-2 (Rs. 2446.74 per colony). Maximal profit was drawn from the colonies receiving diet-4 (Rs. 6244.33 per colony) followed, in descending order, by those receiving diet-1 (Rs. 1788.83 per colony), diet-3 (Rs. 0506.33 per colony) and diet-2 (Rs. 0042.07 per colony). But the colonies provided with latter two diets gave better results than the control colonies. Control colonies vanished in late October and incurred a net loss of Rs. 2000 per colony.

Table 6: Correlation matrix between different attributes of honey bee (*A. mellifera*) colonies receiving different diets

	Values of correlation coefficient (r) for different colony attributes				
Colony attributes	1	2	3	4	5
Diet 1					
1	1.000	0.975**	0.962**	0.961**	0.943**
2		1.000	0.967**	0.964**	0.938**
3			1.000	0.958**	0.968**
4				1.000	0.935**
5					1.000
Diet 2					
1	1.000	0.973**	0.964**	0.958**	0.939**
2		1.000	0.969**	0.961**	0.941**
3			1.000	0.954**	0.963**
4				1.000	0.941**
5					1.000
Diet 3					
1	1.000	0.971**	0.969**	0.961**	0.943**
2		1.000	0.971**	0.965**	0.945**
3			1.000	0.959**	0.964**
4				1.000	0.946**
5					1.000
Diet 4					
1	1.000	0.976**	0.963**	0.955*	0.939**
2		1.000	0.973**	0.959**	0.938**
3			1.000	0.951**	0.958**
4				1.000	0.951**
5					1.000

1: Unsealed brood, 2: Sealed brood, 3: Colony strength, 4: Pollen stores, 5: Honey stores, **($p<0.01$, t-test)

Table 7: Consumption and yield of different commodities by the colonies of honey bee (*Apis mellifera*) fed various pollen substitute diets

	Consumptions/colony				Gross returns/colony	
Diet	Soybean (kg)	Honey (kg)	Yeast (kg)	Sheets* (No.)	Bee frames (No.)	Honey (kg)
Diet-1	1.260	0.785	0.105	9	9	4.525
Diet-2	1.260	0.785	0.105	5	5	3.191
Diet-3	1.260	0.785	0.105	6	6	3.750
Diet-4	1.260	0.785	0.105	18	18	11.250

*Wax foundation sheets

Table 8: Economics of beekeeping with colonies of honey bee (*Apis mellifera*) fed various pollen substitute diets

Diet	Gross investment per colony (Rs.)*	Gross return per colony (Rs.)	Profit/loss (Rs.)	Profit/loss (%)
Diet-1	2444.67	4233.50	+1788.83	+73.17
Diet-2	2404.67	2446.74	+0042.07	+01.75
Diet-3	2418.67	2925.00	+0506.33	+20.93
Diet-4	2530.67	8775.00	+6244.33	+246.75
Control	2000.00	0.0**	-2000.00	-100.00

*Rs. 1000 per colony as hive cost are common for all the colonies, hence not included, The variations in expenditure are due to differences in the costs of the diets and the number of wax foundation sheets given to the colonies, **Colonies died in October, (+): Profit, (-): Loss

DISCUSSION

A comparison between the colonies receiving various pollen substitute diets with control colonies could infer that pollen substitute diets helped sustaining the brood production (reproduction) in various colonies during dearth period which later on resulted in to fast build-up of colonies in favourable period in the subsequent months (when climate was suitable and pollen and nectar from *Brassica juncea* crop became available in plenty). However, brood production in the control colonies regularly declined till it completely ceased in early October. Consequently, none of the control colonies could survive the dearth period and the latter vanished in late October.

In the beginning of experiment in July, decrease in unsealed brood area even in the presence of pollen substitute diet was observed which was due to the incidence of extremely hot and dry climate in this region. The intense heat during this period could be responsible for hampered egg laying, as a negative correlation has been reported to exist between brood rearing and ambient temperature (Singh, 1962).

Although pollen substitute diet was given to the colonies in July to November, yet brood rearing was less in these months as compared to that in December to March, when pollen and nectar were available from raya (*Brassica juncea*), one of the major winter crops of this region. This shows that despite being rich in several essential food ingredients, the pollen substitute diets are less effective for brood rearing as compared to the naturally available pollen and nectar. The occurrence of harsh climatic conditions in June-July and non-availability of bee forage from mid-May to November in this region (Sihag 1990b, 1991) may be a reason for lower brood rearing in these months. It was interesting to note that the colonies receiving diets containing salt (diet-2 and diet-3) could rear less brood as compared to those receiving other diets, i.e. diet-1 and diet-4. Maurizio (1946) observed that feeding of sugar water to bees, which included small amount of salt (0.5 to 0.7% NaCl) resulted in the reduction of their longevity. Piskovoi *et al.* (1964) showed that addition of 0.125% salt to the food caused the death of caged bees in 17 days and those that were fed 1% salt in food died on 4th day. Temnov (1958) found that salts present in honeydew were detrimental to bees and caused reduction in their longevity. This probably is the reason for the lower sealed brood area in colonies receiving diet-2 and diet-3. Sealed brood area was maximal in colonies receiving diet-4 (protein+carbohydrate+vitamins and minerals). This shows that vitamins are vital for brood rearing. Diet-3 was not that effective in brood rearing though it contained vitamins because it also contained 0.5% NaCl which seemed to act as a deterrent to brood rearing.

Higher strength of colonies receiving diet-4 (protein+carbohydrate+vitamins and minerals) can be attributed to higher brood area in these colonies. Very high and significant correlations existed between colony strength and unsealed brood/sealed brood in all the colonies receiving pollen substitute diets ($p \leq 0.05$, t-test, Table 6). Chhuneja *et al.* (1993b) reported that larger areas of brood production and the lower mortalities of the sealed brood so produced due to an effective pollen substitute caused significant increase in colony population. Dodologlu *et al.* (2004) reported that there was a direct relation between brood production and the number of frames of bees and an increase in brood activity caused an increase in the number of mature bees.

Significant differences were seen in the pollen area of different colonies receiving various diets; the control colonies had lower pollen area as compared to the colonies supplied with various pollen substitute diets. Earlier reports suggest that in a free choice comparison, bees ate considerably more pollen than pollen substitute (Haydak, 1970). The bees apparently did not collect or consume pollen substitute in sufficient quantities, either because it lacked attractiveness (Waller *et al.*, 1970) or for other reasons (Standifier *et al.*, 1978).

In early October, pollen area increased in all except in the control colonies. The latter had no pollen store at this time. Pollen area was maximal in colonies receiving diet-4. During this period, some pollen became available from guava (*Psidium guajava* L.). Free and Williams (1971) reported that provisioning of pollen substitute diet, particularly the one consumed in higher quantities, inside the honeybee colonies, reduced the bees' instinct for pollen collection from the fields and bees returned to the hive with lighter loads. However, Nabors (2000) reported that consumption of pollen substitute diet (yeast+soy flour+sugar) decreased as pollen became available and the use of pollen substitute finally subsided after 15 days of pollen availability. The present results could not corroborate the latter findings. This was perhaps due to the fact that guava is not a major pollen source of this region.

In March, unsealed brood area in all the colonies was lower than that in February. This was due to the decline in the bee forage in this region (Sihag, 1990b).

Very high and significant correlations existed between colony strength and unsealed brood/sealed brood and pollen and honey stores in all the colonies receiving pollen substitute diets ($p \leq 0.05$, t-test, Table 6). Jordan (1961) reported that brood less colonies would collect pollen, but not as much as colonies with brood. Barker (1971) reported that the degree of pollen collecting activity is related to the amount of unsealed brood present in a hive. Al-Tikrity *et al.* (1972) also found a positive correlation between the amount of unsealed brood and the amount of pollen collected. They also reported that queen right colonies with large amount of unsealed brood collected more pollen as compared to colonies without queen and brood. The present results corroborate these findings.

Like wise, larger bee strength seemed to help production of greater quantities of honey, as very high and significant correlations existed between colony strength and honey production in all the colonies receiving pollen substitute diets ($p \leq 0.05$, t-test, Table 6). It can be inferred that larger bee strength helped production of greater quantities of honey. The colonies receiving diet-4 had maximal bee strength and produced more honey than the other colonies receiving other pollen substitute diets which had lower bee strengths (Table 3, 5). Singh (1962) also recommended maintenance of strong colonies before nectar flow for higher honey productivity, as strength of worker bees in a honey bee colony before a flow season was considered to be one of the most important factors influencing the honey production. Kumar *et al.* (1995) studied the effect of strength of worker bees on honey production in *Apis mellifera* colonies. They found that colonies of 10-frame bee strength yielded significantly higher amount of honey as compared to colonies of 4, 6 and 8 frame bee strength. Adbellatif *et al.* (1971) have also reported the increased honey production from colonies feeding pollen substitute during dearth period. Chhuneja *et al.* (1992) reported that higher consumption of pollen substitute diet (Brewers' yeast+Guar meal) resulted in higher production of brood and more populous colonies produced significantly more honey. It is contented that stronger colonies store more honey as compared to the weaker colonies (Kumar *et al.*, 1995).

To prepare these diets many kinds of materials in diverse combinations have been used, for example, skimmed milk (Zaytoon *et al.*, 1988), milk products like whey and wheast (Herbert and Shimanuki, 1979), soy products (Kulencervic *et al.*, 1982), brewer's yeast (Adbellatif *et al.*, 1971), fish meal (Chalmers, 1980) and meat scraps (Herbert and Shimanuki, 1979). Several studies have earlier been made reporting the preparation of pollen supplement/pollen substitute diets (Adbellatif *et al.*, 1971; Herbert and Shimanuki, 1980, 1983; Herbert, 2000; Nabors, 2000;

Van der Steen, 2007; De Grandii-Hoffman *et al*., 2008; De Jong *et al*., 2009; Saffari *et al*., 2010a). But, none of these could match the pollen in their efficiency to effect buildup and pollen and honey stores in the colonies except the one prepared by Saffari *et al*. (2010b).

We used soy based pollen substitute diets. This is because, soy products are still the most popular ingredients of honeybee diet, some of these diets seemed to be even better than pollen in their attractiveness as well as in palatability to honeybees (De Grandii-Hoffman *et al*., 2008; De Jong *et al*., 2009; Saffari *et al*., 2010a, b). Some authors, however, have reported the poor efficiency of artificial diets in colony build up and stores (Herbert and Shimanuki, 1979; Kulencervic *et al*., 1982; Zaytoon *et al*., 1988; Chhuneja *et al*., 1993a, b; Schmidt and Hanna, 2006; Saffari *et al*., 2010a).

The pollen substitute artificial diet must contain required ingredients, texture and consistency that are attractive and palatable to honeybees (Herbert and Shimanuki, 1979; Saffari *et al*., 2010a). It must have nutritional values and be free from anti-nutritional factors (Herbert, 2000; Saffari *et al*., 2010a). Above all, it must be easy to prepare and economically viable to the beekeepers. Therefore, a cheap/low cost and acceptable pollen substitute diet is one of the prime needs of the beekeepers to sustain beekeeping. We have taken care of all these aspects while preparing the pollen substitute diets, as is evident from our earlier study (Sihag and Gupta, 2011).

Use of pollen substitute diets has many advantages, e.g., production of queens and package bees (Doull, 1975b), early buildup of colonies for their utilization in the pollination of crops to be in blooms in the subsequent flowering season (Wyndham, 1973; Doull, 1975b; Free, 1993), to meet out the dearth caused by environmental factors (Taber and Poole, 1974; Doull, 1975b) and to overcome the scarcity of pollen when bees are working on nectar rich but pollen deficient sources such as eucalyptus and alfalfa (Johansson and Johansson, 1977). A regular provisioning of pollen substitute is considered to markedly enhance the colony performance (Doull, 1975b; Kleinschmidt and Kondos, 1978). Provision of pollen substitute may also be useful to overcome the damage caused to the colonies due to pesticides poisoning (Wyndham, 1973). Our motive behind the preparation and testing of these diets was to meet all these challenges.

Maximal gain from colonies receiving diet-4 was due to the maximal bee strength in these colonies during nectar flow period. The bee strength was in decreasing order in colonies receiving diet-1, diet-3 and diet-2 and so were the profits received from these colonies. Therefore, diet-4 seemed to be the best among the four tested diets of this study for the sustenance, buildup and economic returns of the colonies.

CONCLUSION

The honey bee colonies in semi-arid tropics of India, with five frames or lesser strength, if not given any pollen substitute diet during dearth period, cannot survive and would result in net loss equivalent to the total investment. A palatable diet containing proteins, carbohydrates, vitamins and minerals (e.g. diet-4 of our study) was found to be highly useful in attaining an excellent bee strength and pollen and honey reserves. This diet, therefore, seemed to be a suitable and economically viable pollen substitute for the honeybee colonies during the floral dearth period. This diet helped maintaining the colony strength during the dearth period that resulted in excellent build up and honey production during nectar-pollen flow period. Addition of salts in to the diet seemed to be counter productive. Therrefore, we recommend the commercial production and large scale utilization of diet-4 of this study for the sustained reproduction and buildup of honeybee colonies during floral dearth period.

ACKNOWLEDGMENTS

We are thankful to Dr. R.K.Lakra, the then Project Co-ordinator, AICRP (Honeybees) for providing apiary facility and Ms Reena for typing the manuscript. Manisha Gupta is thankful to CCS Haryana Agricultural University for the Merit Stipend during the course of this study.

REFERENCES

Adbellatif, M.A., F.H. El-Gaisar and N.M. Mohanna, 1971. Three forms of yeast as a pollen substitute. Am. Bee J., 111: 14-15.

Al-Tikrity, W.S., A.W. Benton, R.C. Hillman and W.W. Clarke, 1972. The relationship between amount of unsealed brood in honeybee colonies and their pollen collection. J. Apic. Res., 11: 9-12.

Barker, R.J., 1971. The influence of food inside the hive on pollen collection by a honey bee colony. J. Apicult. Res., 10: 23-26.

Barker, R.J. and Y. Lehner, 1976. Milk sugar poisons honeybees. Am. Bee J., 116: 322-332.

Barker, R.J., 1977. Some carbohydrates found in pollen and pollen substitutes are toxic to honeybees. J. Nutr., 107: 1859-1862.

Burgett, D.M., G.C. Fisher, D.F. Mayer and C.A. Johansen, 1984. Evaluating honeybee colonies for pollination: A guide for growers and beekeepers. PNW Extn. Publ. No. 245, Oregon, Idaho, Washington, USA., pp: 1-8.

Chalmers, W.T., 1980. Fish meals as pollen-protein substitutes for honeybees. Bee World, 54: 89-95.

Chhuneja, P.K., H.S. Brar and N.P. Goyal, 1992. Studies on some pollen substitutes fed as moist-patty to *Apis mellifera* L. colonies 1. Preparation and consumption. Indian Bee J., 54: 48-57.

Chhuneja, P.K., H.S. Brar and N.P. Goyal, 1993a. Studies on some pollen substitutes fed as moist-patty to *Apis mellifera* L. colonies 2. Effect on colony development. Indian Bee J., 55: 17-25.

Chhuneja, P.K., H.S. Brar and N.P. Goyal, 1993b. Studies on some pollen substitutes fed as moist-patty to *Apis mellifera* L. colonies 3. Effect on honey storage, pollen load and wax production. Indian Bee J., 55: 26-30.

De Grandii-Hoffman, G., G. Wardell, F.A. Segura, T. Rinderer, R. Danka and J. Pettis, 2008. Comparisons of pollen substitute diets for honey bees: Consumption rates by colonies and effects on brood and adult populations. J. Apicult. Res., 47: 265-270.

De Jong, D., E.J. da Silva, P.G. Kevan and J.L. Atkinson, 2009. Pollen substitutes increase honey bee haemolymph protein levels as much as or more than does pollen. J. Apicult. Res., 48: 34-37.

Dodologlu, A., C. Dulger and F. Genc, 2004. Colony condition and bee behaviour in honey bees (*Apis mellifera* L.) housed in wooden or polystyrene hives and fed bee cake or syrup. J. Apicult. Res., 43: 3-8.

Doull, K.M., 1975a. Pollen supplements, I. Relationships between supplements pollen and brood rearing. Am. Bee J., 115: 14-15.

Doull, K.M., 1975b. Pollen supplements. III. Making effective use of supplementary feeding. Am. Bee J., 115: 88-89.

Free, J.B. and I.H. William, 1971. The effect of giving pollen and pollen supplement to honeybee colonies on the amount of pollen collected. J. Apicult. Res., 10: 87-90.

Free, J.B., 1993. Insect Pollination of Crops. Academic Press, London.

Hagedorn, H.H. and F.E. Moeller, 1968. Effect of the age of pollen used in pollen supplements on their nutritive value for the honeybee. I. Effect on thoracic weight, development of hypopharyngeal glands and brood rearing. J. Apic. Res., 7: 89-95.

Haydak, M.H., 1970. Honeybee nutrition. Ann. Rev. Entomol., 15: 143-156.

Herbert, Jr. E.W. and H. Shimanuki, 1977. Brood rearing capability of caged honeybees fed synthetic diets. J. Apic. Res., 16: 150-153.

Herbert, Jr. E.W., 1978. Consumption and brood rearing by caged honeybees fed pollen substitutes fortified with various sugars. J. Apic. Res., 17: 27-31.

Herbert, Jr. E.W. and H. Shimanuki, 1979. Brood rearing and honey production by colonies of free flying honeybees fed wheast and whey-yeast or sugar syrup. Am. Bee J., 119: 833-836.

Herbert, Jr. E.W. and H. Shimanuki, 1980. An evaluation of seven potential pollen substitutes for honeybees. Am. Bee J., 120: 349-350.

Herbert, Jr. E.W. and H. Shimanuki, 1983. Effect of mid-season change in diet on diet consumption and brood rearing by caged honey bees. Apidologie, 14: 119-125.

Herbert, Jr. E.W., 2000. Honey Bee Nutrition. In: The Hive and the Honeybee, Graham, J.M. (Ed.). Dadant and Sons, Hamilton Illinois, pp: 197-233.

Johansson, T.S.K. and M.P. Johansson, 1977. Feeding honeybee pollen and pollen substitute. Bee World, 58: 105-115.

Jordan, R., 1961. Auch brutlose *Volker tragen* Pollenein. Bienenvater, 82: 305-306.

Kleinschmidt, G.J. and A.C. Kondos, 1978. The effect of dietary protein on colony performance. Aust. Beekeeper, 79: 251-257.

Kulencervic, J.M., W.C. Rothebuhler and T. Rinderer, 1982. Effect of certain protein sources given to honeybee colonies in Flordia. Am. Bee J., 122: 181-189.

Kumar, J., S. Srivastava and N.P. Kashyap, 1995. Effect of strength of worker bees on honey production in *Apis mellifera* colonies. Indian Bee J., 57: 174-176.

Maurizio, A., 1946. Beobachtungen uber die lebensdaucer und den Futtervenbrauch gefangen gehaltener Bienen. Beih. Schweiz. Bienenztg., 2: 1-48.

Nabors, R., 2000. Effect of spring feeding pollen substitute to colonies of *Apis mellifera* L. Am. Bee J., 140: 322-323.

Piskovoi, F.R., V.A. Shibidko and K.K. Borisov, 1964. Poisoning bees with common salt. Pchelovodstvo, H1: 13-15.

Saffari, A., P.J. Kevan and J. Atkinson, 2010a. Consumption of three dry pollen substitutes in commercial apiaries. J. Apic. Sci., 54: 13-20.

Saffari, A., P.J. Kevan and J. Atkinson, 2010b. Palatability and consumption of patty: formulated pollen and pollen substitutes and their effects on honeybee colony performances. J. Apic. Sci., 54: 63-71.

Schmidt, J.O. and A. Hanna, 2006. Chemical nature of phagostimulants in pollen attractive to honeybees. J. Insect. Behav., 19: 521-532.

Sihag R.C. and M. Gupta, 2011. Development of an artificial pollen substitute/supplement diet for colonies of honeybee (*Apis mellifera* L.) to tide over the dearth season. J. Apic. Sci., 55: 15-29.

Sihag, R.C., 1986. Insect pollination increases seed production in cruciferous and umbelliferous crops. J. Apic. Res., 25: 121-126.

Sihag, R.C., 1990a. Seasonal management of honeybee (*Apis mellifera*) colonies in Haryana. Indian Bee J., 52: 51-56.

Sihag, R.C., 1990b. Ecology of European honeybee (*Apis mellifera* L.) in semi-arid sub-tropical climates. 1. Melliferous flora and over-seasoning of the colonies. Korean J. Apic., 5: 31-43.

Sihag, R.C., 1991. Ecology of European honeybee (*Apis mellifera* L.) in semi-arid sub-tropical climates. 2. Seasonal incidence of diseases, pests, predators and enemies. Korean J. Apic., 6: 16-26.

Sihag, R.C., 2001. Why should beekeeping be utilized as an input in agriculture? Curr. Sci., 81: 1514-1516.

Singh, S., 1962. Beekeeping in India. International Center for Abduction Research, New Delhi, pp: 214.

Snedecor, G.W. and W.G. Cochran, 1989. Statistical Methods. 6th Edn., Oxford & IBH Publishing Co., New Delhi.

Standifier, L.N., F.E. Moeller, N.M. Kauffeld, E.W.J. Herbert Jr. and H. Shimanuki, 1978. Supplemental feeding of honeybee colonies. United States Department of Agriculture Information Bulletin No. 413, pp: 1-8.

Standifier, L.N., M.H. Haydak, J.P. Mills and M.D. Levin, 1973. Influence of pollen in artificial diets on food consumption and brood production in honey bee colonies. Am. Bee J., 113: 94-95.

Taber, S. and H.K. Poole, 1974. Rearing and mating of queen and drone honeybee in winter. Am. Bee J., 114: 18-19.

Taber, S., 1978. Rearing honey bees when you need them. Am. Bee J., 118: 408-411.

Temnov, V.A., 1958. Composition and toxicity of honeydew. Proceedings of the 17th International Beekeeping Congress, September 15-23, 1958, Rome, Italy, pp: 657-662.

Townsend, G.F. and M.V. Smith, 1969. Pollen storage for bee feed. Am. Bee J., 109: 14-15.

Van der Steen, J., 2007. Effect of a home made pollen substitute on honey bee colony development. J. Apic. Res., 46: 114-119.

Waller, G.D., M.H. Haydak and M.D. Levin, 1970. Increasing the palatability of pollen substitutes. Am. Bee J., 110: 302-304.

Wyndham, R.J., 1973. Interest in bee feeding soaring in California. Am. Bee J., Vol. 113.

Zaytoon, A.A., M. Matsuka and M. Saaki, 1988. Feeding efficiency of pollen substitutes in a honey bee: Effect of feeding site on royal jelly and queen production. Applied Entomol. Zool., 23: 481-487.