

Research Article



Development of Pollen Substitute Diets for *Apis mellifera ligustica* Colonies and their Impact on Brood Development and Honey Production

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Abstract | Pollen substitute diets become essential when nectar and pollen are not available to honey bees. In this study, some pollen substitute diets i. e., diet 1 (soybean flour + brewer's yeast + powdered sugar + sugar syrup), diet 2 (soybean flour + brewer's yeast + powdered sugar + Fenugreek and Turmeric powders + honey + orange juice + A, D and E vitamins + sugar syrup), diet 3 (maize flour + brewer's yeast + powdered sugar + sugar syrup), diet 4 (maize flour + brewer's yeast + honey + turmeric and fenugreek powders + A, D and E vitamins + powdered sugar + orange juice + sugar syrup) and diet 5 (Control) sugar syrup were prepared and their effects were tested for various attributes of commercial apiary of honey bee, *Apis mellifera* in Kohat from June to October 2018. Data were recorded for consumption rates, sealed worker brood area, strength of honey bee colonies and honey yield. Data analysis showed significant difference among all diets tested and check (i. e. control colonies). Diet 2 induced the highest consumption rate (71.90 g per colony), maximum sealed worker brood area (1562.0 cm² per colony), mean highest maximum (12 frames covered with bees per colony), and higher (9.2 kg *Zizyphus* spp. honey per colony) as compared with control (D 5) and other tested diets. Honey bee colonies fed over Diet 2 gave a maximum economic return followed by Diet 4, Diet 1 and Diet 3, respectively. It is thus recommended that Diet 2 should be feed to *A. mellifera* colonies for commercial production of honey.

Received | November 12, 2019; **Accepted** | February 14, 2020; **Published** | June 23, 2020

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Citation | Islam, N., R. Mahmood, G. Sarwar, S. Ahmad and S. Abid. 2020. Development of pollen substitute diets for *Apis mellifera ligustica* colonies and their impact on brood development and honey production. *Pakistan Journal of Agricultural Research*, 33(2): 381-388.

DOI | <http://dx.doi.org/10.17582/journal.pjar/2020/33.2.381.388>

Keywords | Soybean flour, Maize flour, Pollen substitute, Feed consumption, Brood area, Honey production and economics

Introduction

Pollen supplementary feeding plays an important role in honey bee health and honey production. For thermo-regulation of their nest and rearing of brood, honey bees require nectar and pollen to stimulate foraging flights for generation of heat up (De Grandi-Hoffman et al., 2008). Pollen supplies proteins, fats, vitamins, and minerals for brood rearing

while nectar provides carbohydrates (Brodschneider et al., 2010). High quality nutritional sources are essential for colonies production. In absence of natural pollen sources artificial pollen diets can supplement honey bee colonies (Matilla and Otis, 2006) which is essential for young bee's development, brood rearing, reproduction and maintenance of bee colony (Manning, 2001) and honey production (Saffari et al., 2010). In lien periods (June-July) in Pakistan

beekeepers often feed honey bee colonies with pollen supplements such as defatted soybean, maize and gram flour, when natural pollen supplies are not sufficient to promote colony development and health (Saffari et al., 2010; Al-Ghamdi et al., 2011; Rashid et al., 2013). Sugar syrup as supplemental feeding is also used by majority of the beekeepers (July–August) to accelerate brood rearing and oviposition (Usha et al., 2014). Low nutritional reserves adversely affect colony performance. Colonies are attacked by various bee pests and pathogens like black ants, bee eating birds, hornets, mites (*Varroa destructor* and *Tropilaelaps clareae*) etc. and due to poor strength. These factors lead to weakening and sometimes dying of honey bee colonies. Therefore, in bee colonies management, special care must be taken.

To combat with this situation many researchers formulated and tested various artificial diets (Saffari et al., 2006; De Grandi-Hoffman et al., 2008; Sihag and Gupta, 2011). Pande and Karnatak (2014) tested the effect of four different pollen substitutes' viz., ger chickpea, ger Horse gram, ger pea, and ger mung bean which were compared with no feeding (control). They observed more than 65% palatability and slowly increase in pollen and honey stores, in brood area and foraging activity after feeding in combination of all the tested diets. Furthermore, they reported that all diets were significantly superior over control. Usha et al. (2014) carried out research study by using flour of maize, wheat, gram and soybean as pollen substitutes for honey bee (*Apis mellifera*) colonies. They mixed flour + honey + water with all the flours used in the experiment. They found that soybean flour + honey + water was the best as pollen substitute and all other diets were accepted by honey bees and their population was increased after feeding diets. Aly et al. (2014) studied the efficiency of flours of seeds of cumin, fenugreek, anise, caraway, gram, rice, pea, fennel, coriander, white kidney beans, oats and beans by comparing with sugar candy (control). Their results showed that the highest (47.42 gm per colony/week) was recorded with Diet 1 (mixture of: 50% flour of oats + 25% flour of rice + 25 % flour of anise seeds + honey). Moreover, there was great relationship among the diet consumed and increase in sealed worker brood area. Rezaei et al. (2015) used fermented gluten meal, fermented soybean meal, soybean meal and gluten meal as supplementary diets for colonies activity and sugar syrup and pollen were used as (untreated) control. They observed significant difference in

consumption of food and brood rearing activity at ($P < 0.05$) among pollen supplementary diets and control. Amro et al. (2016) used five supplementary diets viz., soybean, pod powder, date pasta, corn gluten and Feedbee compared with untreated colonies and determined their effect on brood rearing activity and the amount of diet consumption. They found that highest consumption was recorded with diet 3 (Date paste) with 213.2 g/colony followed by diet 4 (Feedbee) 173.6 g/colony, diet 2 (Mesquite) 124.1 g/colony, diet 5 (Corn gluten) 95.7 g/colony and diet 1 (Soybean meal) 87.4 g/colony in 42 days respectively. They further noted that maximum worker brood 1066.7 cells/colony were recorded in the control group followed by diet 4 (Feedbee) 174.7 sealed brood cells per colony.

Little work on the influence of pollen substitute diets on *A. mellifera* L. colonies has been done in Pakistan. Rashid et al. (2013) reported that honey bee colonies treated with supplemental gram diet produced higher honey yield than colonies that were fed on brewer's yeast, maize flour and pollen. Consequently, gram supplemental diet with pollen is recommended to be a good substitute for pollen grains. Sabir et al. (2000) reported that maize flour + vitamin B-complex + Glycine are most suitable diet with 416.14 square inches brood area recorded in the hives provided with this diet combination.

It is necessary to supply pollen substitutes to honey bee colonies for survival and development which is calculated through diet consumption or measuring area of worker brood (Sihag and Gupta, 2013; Kumar et al., 2013; Kumar and Agrawal, 2014; Morias et al., 2013; Pande et al., 2015; Gameda, 2014; Shehata, 2016). Keeping the above factors in mind, present study was conducted with the aim to prepare and test a formulation from locally available protein sources that would be preferably palatable for bees and to determine their nutritional effects on brood development and honey production in *A. mellifera* colonies.

Materials and Methods

The study was conducted on commercial bee farm at Kohat, Khyber Pakhtunkhwa province (Pakistan) from June to October, 2018. Pollen substitute diets were fed to honey bee colonies from 4th June to 5th August 2018 and after that stopped till Ber honey extraction

because of the availability of maize and millets pollens. Main steps of experiment were as below:

Selection and equalization of Apis mellifera colonies

Forty-five honey bees, *A. mellifera* L. colonies kept in standard Langstroth hives were chosen for experimental purpose of four pollen supplemental diets and control. For consistency, each colony of honey bee consisted of five frames which were fully covered with bees on both sides. These bee colonies were equalized according to their attributes. Each selected colony had two frames with unsealed and sealed worker brood cells, 40 cm² pollen area and one frame honey. For this study, regularly recommended colony management practices were followed in selected bee colonies. All colonies were randomly assigned to 5 pollen supplemental diets groups including control and each colony was numbered and labeled.

Pollen substitute diets preparation and feeding

Three colonies were used for each diet with three replications. Four pollen substitute diets efficiency on diets consumption, sealed worker brood area, number of frames covered with bees and production of Ber honey were recorded in experimental honey bee colonies. Honey bee colonies which were not fed pollen substitute diet were kept as control treatment. While preparing pollen substitute diet during floral dearth was taken care that diet must have proteins, carbohydrates, vitamins and minerals/salts for the honey bees. Each replication of pollen substitute diet and control consisted of three honey bee colonies. The experimental bee colonies randomly contained five groups of pollen substitute diets and control as follows:

Diet 1 = 100 g (40 g soybean flour + 20 g Brewer's yeast + 40 g powdered sugar + 150 ml sugar syrup.

Diet 2 = 100 g (30 g soybean flour + 15 g Brewer's yeast + 5 g honey + 20 g powdered sugar + 9.5 g powder of Fenugreek and Turmeric + 20 ml orange juice + 0.5 g A, D and E vitamins + 150 ml sugar syrup.

Diet 3 = 100 g (40 g maize flour + 20 g Brewer's yeast + 40 g powdered sugar + 150 ml sugar syrup.

Diet 4 = 100 g (30 g maize flour + 15 g Brewer's yeast + 5 g honey + 20 g powdered sugar + 9.5 g powder of Fenugreek and Turmeric + 20 ml orange juice + 0.5 g A, D and E vitamins + 150 ml sugar syrup.

Diet 5 = Control (1 liter of 50% sugar syrup).

All the diets were placed on brood frames in experimental honey bee colonies given in patties

which were covered in the plastic sheets for avoiding drying. Hundred grams diet of each pollen substitute was fed to a honey bee colony from 4th June to 5th August at seven-day intervals. Each pollen substitute diet was applied 10 times to each colony. One-liter sugar syrup 50% per week was given to control bee colonies only. In all bee colonies, queens were newly mated and raised during April 2018.

Data parameters

Diet consumption: Net weight of pollen supplemental diets consumed with in treatments after feeding 10 times to each colony (June 4 to August 2018) was recorded for each treatment by calculating the differences in diet weight before and after 6 days feeding in gram per colony (Amro et al., 2016).

Measurement of brood area: Sealed worker brood area was noted after two weeks by using measuring frame having wire grid with divisions giving an area of one square inch each (Seeley and Mikheyev, 2003; Amir and Peveling, 2004; Sabir et al., 2000) and then converted into cm² by multiplying with 2.54 according to (Abd El-Halim et al., 2006). This sealed brood was used as criteria for judging the development of colonies.

Measurement of strength of honey bee colonies: Total strength of bee colonies was measured by recording total frames which were fully covered by bees (Burgett et al., 1984). Data of total bee frames were noted after placing honey bee colonies on *Zizyphus* spp. flora during August, 2018.

Honey production: At the end of the Ber nectar flow season, data on production of honey in kg/colony were observed to compare yield of honey in colonies fed on pollen supplemental diets with control colonies to examine impact of pollen diets used in this study (Aziz et al., 2015).

Economic analysis

The net profit from colonies treated with pollen substitute diets was calculated.

Gross expenditure: On the basis of quantity of pollen substitute diets and sugar given to honey bee colonies and Ber honey production in kg/colony, investment rate was calculated (Tables 1, 2 and 3). Prevailing rates of various commodities in Pakistani Rupees were as under:

Table 1: Diet consumption vs honey yield kg per colony.

Consumption per colony gross returns								
Diets	Soybean (gm)	Yeast (g)	Sugar (gm)	Honey (g)	Turmeric and fenugreek (g)	Multi Vit. (g)	Orange juice (ml)	Honey (kg)
Diet 1	400	200	1150	-	-	-	-	6.5
Diet 2	300	150	950	50	95	5	200	9.0

Table 2: Diet consumption vs honey yield kg per colony.

Consumption per colony gross returns								
Diets	Maize (gm)	Yeast (g)	Sugar (gm)	Honey (g)	Turmeric and fenugreek (g)	Multi Vit. (g)	Orange juice (ml)	Honey (kg)
Diet 3	400	200	1150	-	-	-	-	5.5
Diet 4	300	150	950	50	95	5	200	7.5

Table 3: Economics of using pollen substitute diets in beekeeping.

Diets	Gross investment per colony (Rs.)	Gross return per colony (Rs.)	Profit (Rs.)	Profit (%)
Diet 1	275	2200	1925	33.85
Diet 2	354.34	4700	4345.66	52.22
Diet 3	279	1200	921	21.82
Diet 4	357.34	3200	2842.66	42.67

S. No.	Ingredients	Cost (Rs./kg/Liter)
1	Soybean flour	60
2	Maize flour	70
3	Brewer's yeast	910
4	Honey (<i>Acacia modesta</i>)	550
5	Sugar	60
6	Turmeric and fenugreek powder	800
7	Orange Juice	200
8	Cost of multivitamin	19.67

The cost of diet was multiplied with the rates of different materials used in the diets and the total amount was taken as its gross expenditure.

Gross returns: It was calculated from the total produced Ber honey in kg per colony fed with pollen substitute diet and market price per kg of Ber honey during October, 2018. Whole sale market Ber honey price per kg was @ Rs. 1000.

This whole sale market rate was multiplied with honey produced in kg and the amount in rupees was the gross return.

Net profit: Gross expenditure was subtracted from gross return to calculate the net profit.

Statistical analysis of data

This experiment was carried out by using a two factor factorial under Completely Randomized Design (CRD) having three replications. Statistical analysis including diet consumption, sealed worker brood area, frames covered with bees and honey production per colony was analyzed by two ways Analysis of Variance (ANOVA). For data analysis, statistics software version 8.1 was used. Least Significant Difference (LSD) at $p \leq 0.05$ was used for the separation of means.

Results and Discussion

Effectiveness of four pollen substitute diets were tested in honey bee, *A. mellifera* colonies to determined diet consumption rate, their effect on worker brood area, frames covered with bees and Ber honey production (Figures 1, 2, 3 and 4) and the results are presented below:

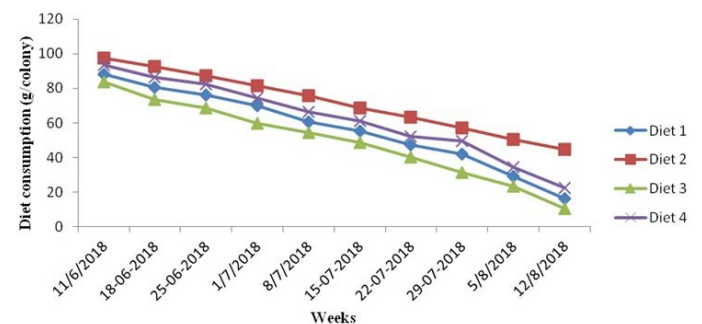


Figure 1: Pollen substitute diet consumption (g/colony).

Diet consumption

Mean data for consumption rate are presented in Figure 1. It showed that Diet 2 was maximally consumed (71.90 gm) by honey bees per week. The next preferred diet was Diet 4 with the mean consumption of 62.3 gm per week. Mean consumption of other diets

were 56.60 gm and 49.44 gm per colony for Diet 1 and Diet 3, respectively. Minimum consumption was observed for Diet 3 (49.44 gm) which was statistically lower from all other diets. Significant differences were found in the mean diet consumption among tested colonies (Figure 1). It is clear from weekly consumption data of pollen substitute diets that during the month of June and July when natural pollen was not available for honey bees in the field, their rate of consumption of diets was higher, but the availability of natural pollen to honey bees after second week of July, the pollen substitute diets consumption was gradually decreased. Same results have been noted by Saffari et al. (2004); Mattila and Otis (2006) and De Grandi-Hoffman et al. (2008) who reported protein supplements consumed at higher rates as compared to that of pollen/pollen supplements.

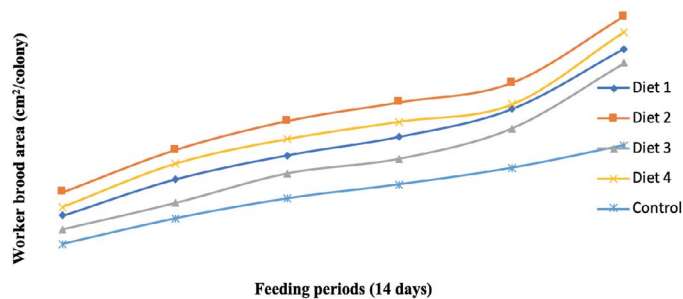


Figure 2: Impact of pollen substitute diets on worker brood area (cm²/colony).

Worker brood area

Sealed worker brood area noted after 14 day intervals in bee colonies fed with pollen substitute diets is presented in Figure 2. Initial area of worker brood in all equalized colonies was 397.52 cm². Mean data indicated that bee colonies who received pollen substitute diets started rearing of worker brood. Maximum area of sealed worker brood was recorded in Diet 2 which was followed by Diet 4, 1 and 3. This trend continued till the end of August 2018. There was a clear indication in increasing worker brood area within one and a half month of feeding of pollen substitute diets. As a whole, during the second week of July onwards, increased area of worker brood was observed in all the colonies used in this experiment. However, maximum area of worker brood was recorded in Diet 2 (1562.0 cm²/colony) statistically significant from other diets given Diet 4 (1419.4 cm² per colony), Diet 1 and Diet 3 values being 1314.3 and 1160.5 cm² per colony respectively. Minimum area of worker brood 884.3 cm²/colony was observed in the control colonies. Colonies receiving different

pollen substitute diets were significantly different from each other as compared to control. Similar results have been observed by Castangnino et al. (2004); De Grandi Hoffman et al. (2008); Saffari et al. (2010) and Sihag and Gupta (2011) who get better results by using pollen substitutes and supplements for increasing brood area.

Honey bee strength

Figure 3 presents impact of pollen substitute diets on combs covered with bees during the month of August before honey harvesting of Ber. The results indicated that maximum (12.0 bee frames/colony) bee strength was recorded in the double story colonies fed with Diet 2 while Diet 4 (10.0 bee frames/colony) different significantly from those colonies which were treated with pollen substitute diets and those not received pollen substitute diets. The total bee frames per colony noted with Diet 1 and Diet 3 were 9 and 8 bee frames/colony. Total frame 7/colony observed in control colonies was lower as compared diets tested colonies. The results are in line with those of Moustafa (2000); Saffari et al. (2006); De Grandi-Hoffman et al. (2008) and Sihag and Gupta (2013), who observed that supplement diets produced more honey bee frames in comparison to non-supplemented fed control colonies.

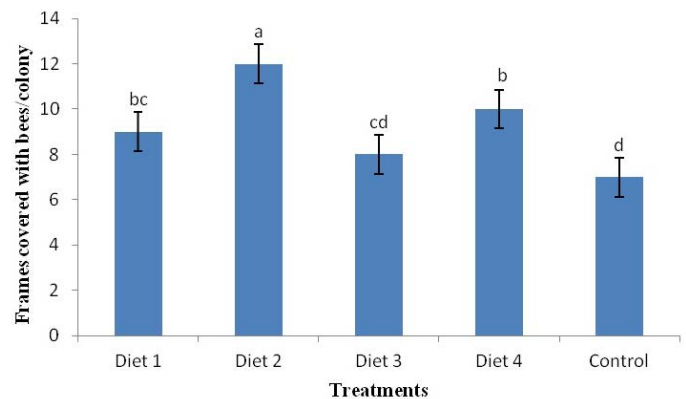


Figure 3: Mean number of frames covered with bees per colony.

Honey production

Production of Ber honey differed significantly when the bee colonies fed with pollen substitute diets. The deposited honey was harvested and weighted after 48 days of supplied pollen substitute diets. Strong and healthy bee colonies produced higher yield of honey as compared to weak colonies. Maximum frames covered with bees and more honey harvested was recorded by colonies fed over Diet 2 which also had the highest pollen substitute diets consumption as compared to other colonies tested in this experiment

(Figure 3). Highest honey yield (9.2 kg per colony) was harvested from the colonies fed with Diet 2 but the lowest (4.3 kg/colony) honey yield extracted from the control colonies without providing them pollen substitute diets (Figure 4). The colonies given with Diet 4 and Diet 1 also yielded better honey yield (7.5 kg/colony) and (6.5 kg/colony) in comparison with check bee colonies. Honey yield results are in confirmation with the results of Sabir et al. (2000); De Grandi-Hoffman et al. (2008) and Rashid et al. (2013) who reported higher honey yield in colonies provided different pollen substitute diets.

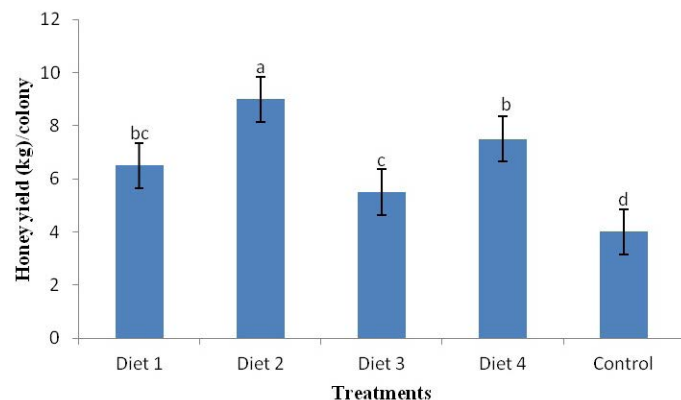


Figure 4: Mean honey yield in kg per colony.

Economic analysis

Tables 1 and 2 presents quantity and materials used in pollen substitute diets and Bee honey produced in colonies receiving different pollen diets. Diets were consumed in unequal quantities by honey bee colonies. Honey yield produced after feeding diets was different. Results indicated that cost for Diet 1 was Rs. 275, for Diet 2 was Rs. 748.4 and for Diet 3 it was Rs.279 while for Diet 4 it cost Rs. 751.4 per colony. Maximum gross return was recorded for bee colonies who were given Diet 2 @ Rs. 4700 followed by Diet 4, @ Rs. 3200 for Diet 1, @ Rs. 2200 and for Diet 3, @ Rs. 1200 per colony. Maximum profit was recorded for colonies receiving Diet 2 with Rs. 3951.16 followed by Diet 4, (Rs. 2448.6), for Diet 1 (Rs.1925) and for Diet 3, (Rs. 921) per colony.

Conclusions and Recommendations

It is concluded from the present study that pollen substitute diet should be supplied to honey bee *Apis mellifera* colonies during dearth period in Pakistan for survival and better honey yield. From the present finding Diet 2 (30 g soybean flour + 15 g Brewer's yeast + 5 g honey + 20 g powdered sugar + 9.5 g powder of Fenugreek and Turmeric + 20 ml orange juice +

0.5 g A, D and E vitamins + 150 ml sugar syrup) was found highly useful for attaining maximum bee strength and honey production. Maximum profit (Rs. 3951.16) was recorded for colonies receiving Diet 2. It is recommended for commercial production of honey.

Author's Contribution

Noor Islam designed the study, collected and analyzed data while Saleem Abid helped him in data analysis. Noor Islam conducted research and wrote the article. Ghulam Sarwar did review and contributed in graph preparation. Rashid Mahmood supervised the work, evaluated and edited the manuscript. Sarfaraz Ahmad did critical revision of the article and contributed in finalization of "Results and Discussion".

Conflict of interest

All authors have no conflict in this research study.

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