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## Assessment a New Pollen Supplement Diet for Honey Bee Colonies and Their Effects on some Biological Activities

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**Abstract** New pollen supplement diet, the main components of orange juice, mint oil, Turmeric and Fenugreek powders and vitamins (diet E) was prepared by us. It compared with some traditional pollen supplement diets, A (Soybean flour + Brewer's yeast+ powdered sugar + sugar syrup), B (diet A+ date pollen grains), C (diet A+ bee honey) and D (date pollen grains +powdered sugar + sugar syrup). Some biological activities, areas of sealed worker brood, mean number of combs covered with bees and consumption rates and honey yield of the tested colonies were evaluated. The obtained results showed that, there were clearly significant differences between the all tested diets and control in the mean of worker brood area during the experimental period of February and March. Diet E recorded the highest value in comparison with either the control or the other tested diets. Colonies fed with diet B and diet E resulted in the highest number of combs covered with bees being the highest with diet E. Colonies fed with diet E consumed a higher amount /2 weeks interval, with no residues of patty, than the other tested diets during the experimental period. According to the honey yield, the colonies which feed on a new artificial diet (E) produced a highly amount of citrus honey in comparison with the other tested diets and control. Application of new diet (E) as a pollen supplement diet induced the highest biological activities, consumption rate and honey yield.

**Keywords:** pollen, supplement diet, honey bees, consumption rate

### Introduction

Honey bee (*Apis mellifera* L.) colonies need pollen and nectar to fuel foraging flights, generate heat to thermoregulate their nest and to rear brood. Nectar is a carbohydrate source, while pollen supplies the bees with the protein, lipids, vitamins and minerals needed to rear larvae (DeGroot, 1953; Manning, 2001). The quality of pollen affects the number of eggs that are laid by the queen and the proportion that are reared to adults (Allen and Jeffree, 1956; Doull 1973; Hellmich and Rothenbuhler, 1986).

During the shortage or complete absence of pollen, or in the presence of only poor quality pollen, beekeepers often feed colonies on honey bees with either pollen substitute (with no pollen) or supplement (with pollen) diets (Saffari *et al.*, 2006). Commercially produced bee diets can supplement the available pollen and provide a practical method

for sustaining brood rearing in honey bee colonies. Pollen supplement diets containing 20% or more of either soybean flour or brewer's yeast are highly palatable to bees and have the nutritive requirements for their growth and reproduction (Mattila and Otis, 2006). The pollen substitute diet and pollen are equally accepted by the bees. The pollen substitute diet is thus, as highly palatable as natural pollen and easily provided as patties to colonies in standard hives (Saffari *et al.*, 2004).

The possibility of improving the efficiency of beekeeping by providing proteinaceous feed lies, in part, in the development of an effective pollen substitute to feed the colonies when pollen is scarce ( Zahra and Talal, 2008), especially in preparation for early nectar flows (Skubida *et al.*, 2008). Providing proteinaceous feed to stimulate colony strength would then help, in maximizing honey production and crop pollination, to overcome pesticide damage and resistance to parasites and diseases, and for package-bee production flows (Skubida *et al.*, 2008).

The purpose of our study was to assess a new pollen supplement diet by measuring consumption and colony growth (brood and adult populations) and comparing it with the traditional diets.

## **Materials and methods**

The trial of this study was conducted for 12 weeks from November 2013 until March 2014 in a private apiary at Belbees region, Sharkia Governorate, Egypt. The trial was ended just before moving the honey bee colonies into citrus trees flowering, and then the supplemental feeding was no longer necessary. Carniolian hybrid bee (*Apis mellifera carinica*) colonies of about equal strength containing at least four combs covered with bees and headed by new open mated carniolian hybrid queens were used for this study. The tested colonies were classified into six groups; three colonies were used for each treatment and control. Four groups were fed with traditional pollen supplement diets and the last diet was prepared as a new diet as follows:

- 100g of diet A (40g Soybean flour + 20g Brewer's yeast+ 40g powdered sugar +100ml sugar syrup).
- 100 g of diet B (30g Soybean flour + 15g Brewer's yeast+ 15g date pollen grains +40g powdered sugar + 100ml sugar syrup).
- 100 g of diet C (50g Soybean flour + 25g Brewer's yeast + 25g bee honey+ 100ml sugar syrup).
- 100 g of diet D (50g date pollen grains + 50g powdered sugar + 100ml sugar syrup).
- 100 g of diet E ( 10g Brewer's yeast + 1g bee honey + 8g Turmeric and Fenugreek powders + 0.5g A,D and E vitamins + 45g powdered sugar + 20ml orange juice + 10ml mint oil + 30ml sugar syrup).

The new prepared diet was assessment by comparison with the other traditional diets. All tested diets were fed in patties that directly placed over the brood nest of tested bee colonies and covered with plastic sheets to avoid drying (add to fed these colonies with sugar syrup). All tested diets were fed to colonies at 2 weeks intervals and each colony was provided with 100g/ tested diet. Before feeding the colonies, the unconsumed portions of the patty diets were removed from the colony and weighed to estimate consumption.

The biological activities were determined where areas of sealed worker brood were measured at 12 days intervals by inch square according to Fresnay, 1962. Adult bee populations were made by estimating the number of combs covered with bees (DeGrandi-Hoffman *et al.*, 2008). At the end of the citrus trees flowering season, honey yield was determined by weighting the combs before and after the honey extraction process. The weight difference was considered as the amount of harvestable honey (Rashid *et al.*, 2012).

### ***Statistical analysis***

The obtained data were subjected to analysis of variance (ANOVA) through SPSS computer program. Means were compared using Duncan's Multiple Range tests.

### **Results**

Regardless of the treatments, the highest mean of biological activities was recorded during February and March compared with the results at the beginning of the experiment. As shown in Table 1 the mean area of sealed worker brood (inch<sup>2</sup>) and number of queen cups did not differ among treatment and control colonies in December and January. Although, the mean area of worker brood increased slightly during January, diet E recorded the highest value (61.66 inch<sup>2</sup>) in comparison with either the control or the tested diets. Results obtained in Table 1 show that providing bee colonies with diet E induced the highest amount of worker brood area (131.1 and 285.8 inch<sup>2</sup>) followed by diet A (120.0 and 153 inch<sup>2</sup>) and Diet B (70.55 and 281.6 inch<sup>2</sup>) in February and March respectively. There were clearly significant differences between the all tested diets and control in the mean of worker brood area during the experimental period of February and March.

With regard to the mean number of combs covered with bees, data in Table (1) shows that the highest numbers resulted by feeding with D and E diets (4.0 combs) in January and significantly differed with the other tested diets and control. Irrespective of the treatments the highest mean number of combs covered with bees were observed in the last two months

of the trail (February and March) Further and regardless of the time diet B and diet E resulted in the highest number of combs covered with bees being the highest with diet E. In comparison with the control, there are significant variations between the different tested diets were recorded in the almost experimental period.

Concerning the number of queen cups produced owing to the various tested treatments, Table 1 reveals that no significant differences between the tested diets and control were recorded during the most experimental periods. In February and March colonies fed with diet B results a significant number of queen cups (1.22 and 1.16) in comparison with control and other tested diets.

The effect of feeding with different tested diets on the mean number of eggs laid by queen/ open worker brood cell indicated that no significant differences were found between tested diets at the beginning of the experiment (Table 1). While, the queen bees in the colonies which feed with diet E laid a significant number of worker brood cells has more one eggs (2.22 and 0.5) in February and March, respectively.

Data in Table 1 shows that there are significant differences between the tested diets in the mean amount of consumption by bee colonies during the experimental periods. Colonies fed with diet E consumed a significant amount (100 g.) /2 weeks interval with no residues of patty. Colonies fed with A and C diets consumed lower amount than the other diets /2 weeks interval during the experimental period (Table 1).

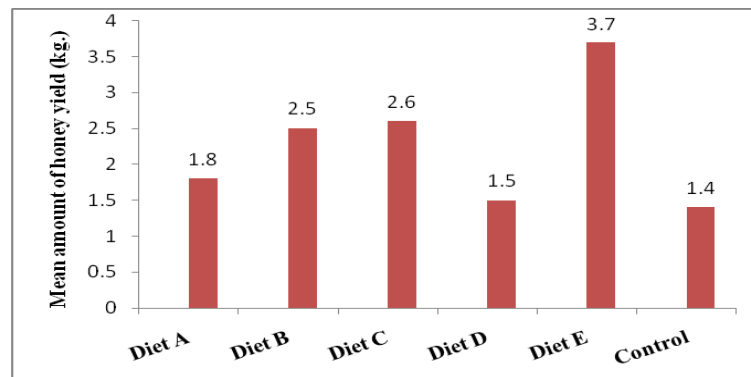
After flowering of the citrus trees the collected honey by bee workers for each experimental colony was harvesting and weight (Fig.1). Total mean of honey yield shows that the colonies which feed on a new artificial diet (diet E) produced a highly amount of citrus honey (3.7kg/colony) followed by diets C and B (2.6 and 2.5) in comparison with control colonies (1.4kg/colony).

Total mean of the different biological activities for colonies that received different treatment diets was shown in Fig. 2. The colonies receiving diet E had a maximal area of sealed worker brood as well as mean number of combs covered with bees followed by B and A diets. While, colonies feed with D and C diets recorded the lowest value. According to the pattern in Fig. 2C there is an increasing in the total mean number of queen cups in colonies fed with diet B and control. While, the other tested diets recorded the lowest value. General patterns of mean number of worker brood cells with one egg in Fig. 2D revealed that, colonies provided with diet E recorded the highest total mean followed by diet A&B as compared to the other tested diets and control. A highly total mean amount of tested diets was consumed by colonies that fed on diet E followed by diet B and D respectively. Colonies fed with diet C consumed the lowest amount (Fig. 2E).

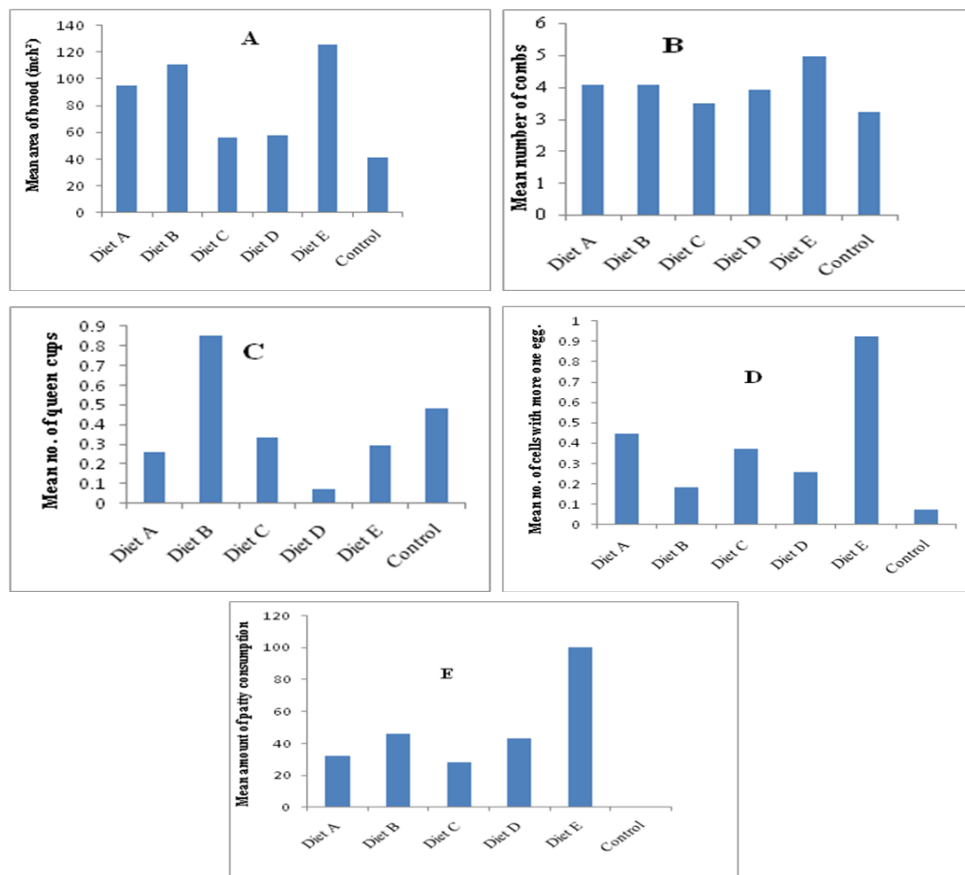
**Table 1.** The effect of different pollen supplement diets on some biological activities.

Month	Treatment	Mean area of sealed worker brood	Mean number of combs covered with bees	Mean number of queen cups	Mean number of queen cells	Mean number of worker cells with more one of queen eggs	Mean amount of food consumption (g.)
December	Diet A	59.16 a	4.50 ab	0.33 a	0.00	0.00 a	39.16 c
	Diet B	44.16 a	3.66 b	0.00 a	0.00	0.50 a	58.33 b
	Diet C	38.33 a	3.50 b	0.00 a	0.00	0.16 a	17.50 d
	Diet D	71.66 a	4.33 ab	0.00 a	0.00	0.00 a	63.33 b
	Diet E	52.00 a	5.83 a	0.00 a	0.00	0.00 a	100.00 a
	Control	41.66 a	3.50 b	0.50 a	0.00	0.33 a	0.00 e
	<b>F value</b>	1.80 <sup>NS</sup>	2.907*	1.293 <sup>NS</sup>		2.182 <sup>NS</sup>	48.325**
January	Diet A	36.00 a	3.50 ab	0.00 a	0.00	1.50 a	30.83 c
	Diet B	37.50 a	3.16 bc	0.83 a	0.00	0.33 b	44.16 bc
	Diet C	29.33 a	3.33 abc	0.00 a	0.00	0.83 a	30.83 c
	Diet D	50.33 a	4.00 a	0.00 a	0.00	0.00 b	46.66 b
	Diet E	61.66 a	4.00 a	0.16 a	0.00	0.33 b	100.00 a
	Control	21.50 a	2.66 c	0.50 a	0.16	0.00 b	0.00 d
	<b>F value</b>	2.217 <sup>NS</sup>	5.259**	1.925 <sup>NS</sup>	1.000 <sup>NS</sup>	4.615**	49.302**
February	Diet A	120.0 ab	3.88 abc	0.11 b	0.00 a	0.22 a	25.55 c
	Diet B	70.55 abc	4.11 ab	1.22 a	0.00 a	0.00 a	40.00 b
	Diet C	51.11 bc	3.44 bc	0.44 ab	0.00 a	0.22 a	37.77 bc
	Diet D	44.66 bc	3.44 bc	0.00 b	0.00 a	0.44 a	35.55 bc
	Diet E	131.1 a	4.55 a	0.33 b	0.00 a	2.22 b	100.00 a
	Control	34.66 c	3.11 c	0.55 ab	0.11 a	0.00 a	0.00 d
	<b>F value</b>	2.599*	2.912*	2.465*	1.000 <sup>NS</sup>	10.129**	50.206**
March	Diet A	153.00 bc	4.50 a	0.66 a	0.00 a	0.16 a	41.66 b
	Diet B	281.66 a	5.33 a	1.16 a	0.00 a	0.00 a	41.66 b
	Diet C	109.66 c	3.83 a	0.83 a	0.50 a	0.33 a	15.00 c
	Diet D	72.00 c	4.16 a	0.33 a	0.00 a	0.50 a	20.00 bc
	Diet E	285.83 a	5.66 a	0.66 a	0.00 a	0.50 a	100.00 a
	Control	71.00 c	3.66 a	0.33 a	0.00 a	0.00 a	0.00 c
	<b>F value</b>	5.212**	2.235 <sup>NS</sup>	0.419 <sup>NS</sup>	2.143 <sup>NS</sup>	1.036 <sup>NS</sup>	25.388**

NS: Non-significant, Means in a row with dissimilar letters differ significantly at 0.05 level of probability.



**Figure 1.** Mean amount of honey yield (Kg.) affected by different tested diets.



**Figure 2.** Total mean for effecting the pollen substitute diets (as a patties) on the different biological activities, A) mean area of brood, B) mean no. of combs covered with bees, C) mean no. of queen cups, D) mean no. of cells with more one egg and mean amount of cake consumption.

## Discussion

Pollen substitute/supplement artificial diets can be effective in stimulating honey bee colonies to rear brood (Mattila and Otis, 2006, Nabors, 2000 and Standifer *et al.*, 1973), but they must be both palatable to bees and nutritious. All diets tested here were not equally effective in stimulating brood rearing or number of combs covered with bees. Whereas, providing bee colonies with diet E induced the highest amount of worker brood area followed by diet A and Diet B especially in the period of February and March. 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. Tested diets were differed in their consumption by bees, diet E was completely consumed in comparison with the other diets. DeGrandi – Hoffman *et al.*, (2008) suggested that differences in the nutritional quality of the diets (i.e., amounts of protein and carbohydrate) and perhaps the digestibility and accessibility of their nutrients to worker bees influence the amount of brood that can be reared even when consumption rates are similar.

Colonies receiving diet E had maximal bee strength and produced more honey than the other colonies receiving other pollen supplement diets which had lower bee strengths (Fig. 2). Abdellatif *et al.*, (1971) have 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 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 weak colonies (Kumar *et al.*, 1995).

## Conclusion

The honey bee colonies in Egypt, 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 (Diet E 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 supplement for the honey bee 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. Therefore, we recommend the commercial production and large scale utilization of diet E of this study for the sustained reproduction and build up of honey bee colonies during floral dearth period.

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