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## Impact of some Pollen Substitutes in Liquid Form on the Biological Activities of Honey Bee Colonies

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**Abstract** This study was conducted to evaluate the effects some commercial liquid formulations of pollen substitute on some biological activities of honey bee colonies. Sixteen colonies of Carniolan hybrid bee (*Apis mellifera carinica*) colonies of about equal strength and headed by new open mated Carniolan hybrid queens were used for this study. Three groups of honey bee colonies were used to determine the effect three commercial pollen substitutes, Star (Egyptian product), Royal king 4 H (German product) and Royal star (German product) on the biological activities of honey bee colonies. There are significant increases in the mean area of sealed brood in colonies fed with Royal 4H and Royal star more than control colonies during January to March 2014. While, no significant differences between colonies treated with Star formulation and control. During the experimental period Royal 4H formulation recorded a higher significant in the mean number of combs covered with bees in compare with Royal star formulation and sugar syrup (control). Colonies fed with Royal 4H and Royal star recorded significant mean amount of diet consumption by bee colonies during the most experimental periods. The highest significant number of open worker cells contain more one egg, laid by queen, was observed in colonies fed with Royal 4H diet. Feeding with Royal 4H recorded the highest significant number of open worker cells contain more one egg laid by queen especially in December (0.87cell) and February (0.58cell) periods. Also, the results show that the colonies treated by tested diets had a higher number of worker cells which included more one egg than control colonies in the most period of experiment.

**Keywords:** Pollen substitute, Royal king 4H, Royal star, bee consumption

### Introduction

During the floral dearth period, for the sustained reproduction and build of the honey bee 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). Artificial feeding of honey bee 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.

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Strong colonies during dearth period witness early build up 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 (Abdel-Latif *et al.*, 1971; Herbert and Shimanuki, 1983; Herbert, 2000; Van der Steen, 2007; De Grandi-Hoffman *et al.*, 2008; De Jong *et al.*, 2009; Saffari *et al.*, 2010; Sihag and Gupta, 2011). The artificial diets of pollen substitute were provided to bee colonies in a liquid or patty forms (De Grandi-Hoffman, *et al.*, 2008). Artificial diets of a pure liquid culture of yeast (*Candida tropicalis*) in different concentrations as pollen grains substitute were used to feed honey bee colonies. Application of 25% and 50% of yeast culture results the highest significant amount of worker brood during the floral dearth period (Abd El-Wahab and Gomaa, 2005).

The purpose of our study was to evaluate different commercially available bee diets in liquid formulations by measuring consumption and their effects on some biological activities of honey bee colonies.

## **Materials and methods**

Sixteen colonies were chosen from a privet apiary at Belbees region, Sharkia Governorate, Egypt, during the winter season from 28<sup>th</sup> November to March 15<sup>th</sup>, 2013-2014. Carniolan hybrid bee (*Apis mellifera carinica*) colonies of about equal strength containing at least four combs covered with bees and headed by new open mated Carniolan hybrid queens were used for this study.

Colonies were divided into 4 groups; three groups were used to study the effect of commercial pollen substitutes in liquid formulations on the honey bee biological activities. The rest group was used as control and supplied with sugar solution only. The tested commercial pollen substitutes were Star (Egyptian product), Royal king 4 H (German product) and Royal star (German product). These formulations contain some vitamins as A.D.E.C. B composite, and amino acids and proteins. All tested diets were fed to colonies at 2 weeks intervals and each colony was provided with 2cm<sup>3</sup> of diet/1L. of sugar syrup in a plastic feeder. Before feeding the colonies, the unconsumed portions of the diets were removed from the colony and measured 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 estimated by counting 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 data has been obtained from different treatments were subjected to analysis of variance (ANOVA) by using SPSS (Statistical Package for the Social Sciences) computer program. Means were compared using Duncan's Multiple Range Test.

### Results

Results presented in Table (1) showed significant increasing in the mean area of sealed brood in colonies which fed with Royal 4H and Royal star in compare with control colonies during January to March 2014. While, no significant differences recorded between colonies treated with Star formulation and control. During the experimental period Royal 4H formulation recorded extremely significant in the mean number of combs covered with bees comparing with Royal star formulation and sugar syrup (control). Insignificant differences were found in the mean number of queen cells and queen cups between the colonies treated with the most different tested diets and control. In general Royal 4H formulation recorded the highest mean number of queen cups (1.50 and 2.0) and queen cells (0.8 and 1.25) during February and March 2014, respectively.

The colonies which provided with Royal 4H recorded noteworthy number of open worker cells contain more one egg laid by queen especially in December (0.87cell) and February (0.58cell) periods. The colonies which provided with Royal 4H recorded the highest significant number of open worker cells contain more one egg laid by queen especially in December (0.87cell) and February (0.58cell) periods. Also, the results show that the colonies treated by tested diets had a higher number of worker cells which included more one egg than control colonies in the most period of experiment.

**Table 1.** Impact of three commercial pollen substitutes in liquid formulations, on the biological activities of honey bee colonies.

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 (mL)
December	Star	16.25 b	2.75 b	0.00 a	0.00 a	0.00 b	100.00
	Royal 4H	26.87 ab	3.37 a	0.25 a	0.25 a	0.87 a	100.00
	Royal star	37.50 a	3.37 a	0.00 a	0.00 a	0.25 b	100.00
	Control	40.75 a	3.50 a	0.50 a	0.00 a	0.25 b	100.00
	F value	5.109**	3.540*	2.33 <sup>NS</sup>	2.33 <sup>NS</sup>	3.96*	--
January	Star	24.12 b	2.75 b	0.12 a	0.00 a	0.37 a	84.37 b

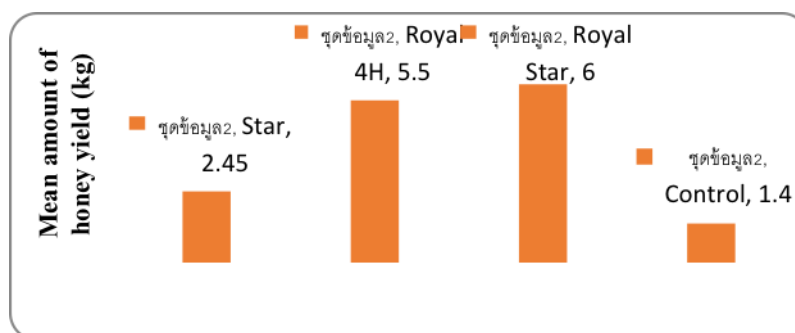
	Royal 4H	98.25 a	4.37 a	0.50 a	0.00 a	0.00 a	93.75 ab
	Royal star	96.87 a	3.75 a	0.00 a	0.00 a	0.25 a	100.00 a
	Control	21.75 b	2.75 b	0.50 a	0.25 a	0.00 a	80.37 b
	F value	9.09**	9.96**	1.36 <sup>NS</sup>	2.33 <sup>NS</sup>	1.46 <sup>NS</sup>	83.72**
<b>February</b>	Star	75.08 b	3.91 bc	0.25 b	0.00 a	0.33 ab	88.75 b
	Royal 4H	169.58 a	5.91 a	1.50 a	0.08 a	0.58 a	100.00 a
	Royal star	188.75 a	4.75 b	0.75 b	0.00 a	0.41 ab	100.00 a
	Control	36.83 b	3.16 c	0.58 b	0.08 a	0.00 b	79.70 b
	F value	11.44**	12.28**	4.05*	0.66 <sup>NS</sup>	3.02*	262.99**
<b>March</b>	Star	192.50 ab	5.50 b	0.50 bc	0.00 a	0.12 a	93.75 a
	Royal 4H	330.00 a	7.25 a	2.00 a	1.25 a	0.00 a	100.00 a
	Royal star	280.62 a	6.12 ab	1.87 ab	0.00 a	0.12 a	100.00 a
	Control	71.37 b	3.62 c	0.25 c	0.00 a	0.00a	71.70 b
	F value	4.170*	7.137**	3.53*	1.57 <sup>NS</sup>	0.66 <sup>NS</sup>	524.80**

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

Moreover, Colonies fed with Royal 4H and Royal star recorded significant mean amount of diet consumption by bee colonies during the most experimental periods Table(1). In contrast colonies fed with Star and sugar syrup (control) consumed a lowest value especially in February and March months.

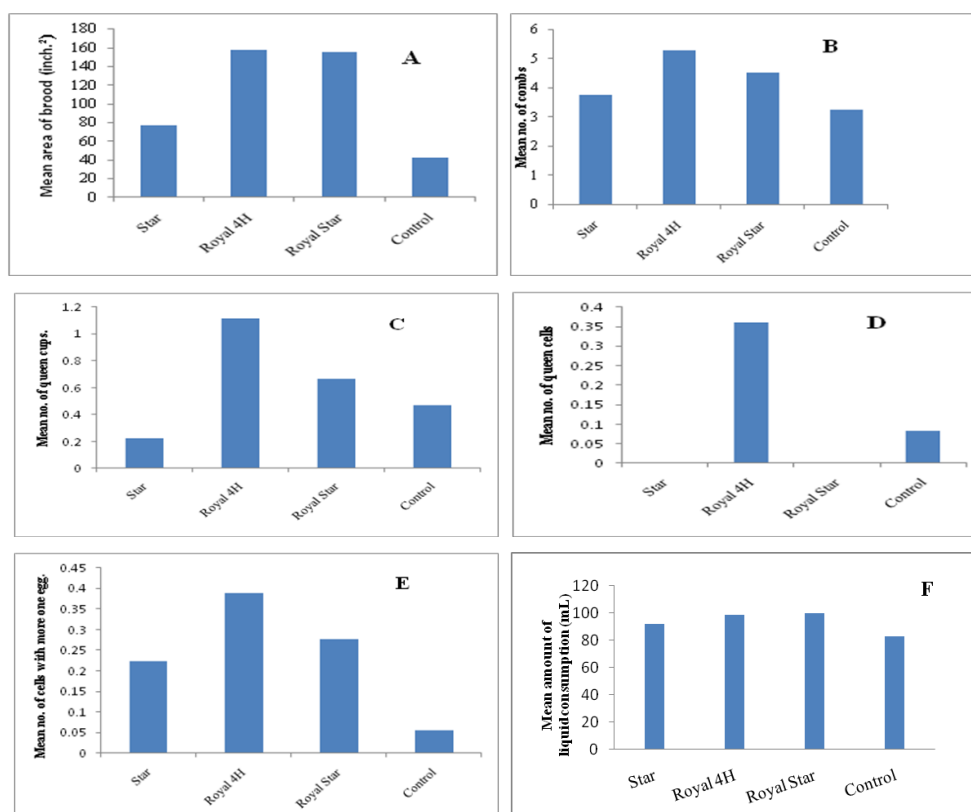
These results may explain that feed the honey bee colonies with the pollen substitutes in liquid formulations improve the honey bee biological activities during the active season. Also, the increasing of their consumption by bee workers indicates for suitable to feed bee. Bee honey which collected by bee workers from each experimental colony after flowering of the citrus trees was harvesting and weighting Fig.(1).

Total mean of honey yield shows that the colonies which fed on Royal Star diet produced a highly amount of citrus honey (6.00kg/colony) followed by Royal 4H diet (5.5 kg/colony) and then Star diet (2.45 kg/colony) in comparison with control colonies (1.4kg/colony).



**Figure 1.** Effect of different kinds of diets provided to honey bee on the mean amount of honey yield.

Influence of different treatments of diets on various biological activities of honey bee colonies was presented in Fig.(2). Total mean of the different biological activities for colonies that received different treatment diets was shown in Fig. (2). The colonies receiving Royal king 4H and royal star diets had a maximal area of sealed worker brood as well as mean number of combs covered with bees while, the Star diet recorded the lowest values followed by control colonies (Fig. 2 A and B). According to the pattern in Fig. 2C there is an increasing in the total mean number of queen cups in colonies fed with Royal king 4H followed by Royal Star and Star diets. General patterns of mean number of worker brood cells contain one queen egg in Fig. 2E revealed that, colonies provided with Royal King 4H diet recorded the highest total mean followed by diet Royal Star and Star diets as compared to the control colonies.



**Figure 2.** Influence of the pollen substitute (as a syrup) on the different biological activities of honey bee., A) mean area of brood, B) mean no., of combs covered with bees, C) mean no., of queen cups, D) mean no., of queen cells, E) mean number of cells with more one egg and the last (F) mean amount of syrup consumption.

The study showed that colonies of honey bee were differed in the rate of consuming the tested diets. Colonies have been fed on Royal Star diet recorded highly mean amount of consuming, followed by Royal king 4H and Star diets, respectively. Colonies fed with control diet (sugar syrup) consumed the lowest amount (Fig. 2F).

## Discussion

In our study the results indicate that feeding honey bee on a commercial pollen substitute (Royal king 4H and Royal star) produced a highly amount of brood and honey production. Herbert, (1992) recorded that, supplying an alternative artificial source of proteins (pollen substitutes) is a viable alternative to promote colony maintenance, development and multiplication. Also, honey bee colonies require nutritional supplementation to keep the colonies healthy, especially during dearth periods or when more bees are needed for pollination (Brodschneider and Crailsheim, 2010). Our results are in coincidence with some previous studies about the feeding of honey bee colonies on commercial pollen substitute called Feed bee (Saffari *et al.*, 2004, Saffari *et al.*, 2010 and Van Der Steen, 2007). In the other study DeGrandi-Hoffman *et al.*, 2008 found that feed honey bee colonies with commercial diets of pollen substitute in liquid form did not differ from those food pollen cakes. Abd El-Wahab and Gomaa, (2005) have been tested to feed honey bee colonies on the artificial diets of pure liquid culture of yeast in different concentrations as pollen grains substitute. They found that application of 25% and 50% of the yeast culture resulted the highest significant amount of worker brood and mean number of combs covered with bees in comparison with other treatments of traditional artificial diets in cake form. Although these pollen substitute diets gave good biological activities to those honey bee colonies, the results showed that this kind of feeding pushed the queen to lay more than one egg in each worker brood cell. Therefore, feeding honey bee colonies with commercial diets in liquid formulations may be causes a negative effect on the queen's activity specially their laying eggs. Abd El-Wahab *et al.*, (2016) stated that feeding honey bee colonies with artificial diets which contains some vitamins and yeast in cake form, resulted a significant number of worker brood cells has more than one egg, which laid by queen.

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