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Impact of using pollen substitute on honeybee *Apis mellifera* (Hymenoptera: Apidae) colonies in Egypt

Elnabawy, E. M.[1]; Elashmawy, A. A.[1]; Gawad, A.A.[2,3] and Rakha, O. M.[1]

[1] *Department of Economic Entomology, Faculty of Agriculture, Kafrelsheikh University.*

[2] *Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.*

[3] *Faculty of Desert and Environmental Agricultural, Matrouh University.*

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Abstract:

This study was conducted in the apiary at Bassiun City, Gharbia Governorate during the banana blooming season from 1st August 2019 to 10th October 2019 to find out the effects of using a pollen substitute to honeybee *Apis mellifera* L. (Hymenoptera: Apidae) colonies. Also, honeybee colonies which fed on pollen substitutes could produce more sealed brood area and more honey yield at the end of the blooming season. So, the utility of pollen substitutes during the banana flowering period is recommended for honeybee colonies and higher brood, bee population per colony, production of royal jelly, the queen rearing, the weight of virgin queens, stored pollen and honey production.

Introduction

Honeybee *Apis mellifera* L. (Hymenoptera: Apidae) use nectar and pollen of different flowering plants and the availability of flowering plants is very essential for honeybee to become colony strength (Shawer *et al*., 2019). Pollen is considered as the essential source of amino acids, lipids, vitamins, and essential minerals in the nutrition of the honeybee colonies and the development of honeybee require these nutritive elements which ingested with nectar and water for their growth. One colony of honeybee require approximately 18 kg of pollen yearly to supply them with protein (Crailsheim *et al*., 1992). The young honeybees feed on stored pollen grains, which were influenced by some microbes and this means bee bread. The honeybee workers need a large amount of pollen for protein within the first six day of adulthood, which will encourage their full development.

Many biological activities of honeybees are increased by feeding on a protein source, particularly during the shortage times because it stimulates the different glands in charge of secretion of larval foods (Barker, 1971). Also, the incomplete amount of protein in the bee diet is a big problem, during the hypopharyngeal glands development (Funari *et al*., 2003). Consequently, the reproduction and production of the honeybee colonies are influenced (Pereira *et al*., 2006). Also, Haydak (1949) showed that the ability of brood-rearing of the honeybees without the pollen is mostly impaired because the different glands responsible for producing the food by nurse honeybees keep non-functional and undeveloped.

When the weather circumstances are inappropriate for the honeybee colonies and during the dearth period. During that time, the colony needs a pollen substitute, which means change the use of pollen by other substitutes. The most famous substitution contains the mix of soybean flour, brewer's yeast, and corn flour (House, 1961; Feng, 2002 and Saffari *et al*., 2004). Feeding plays an essential role in defense against different pathogens and in preserving the gut fitness of honeybee colonies (Ritz and Gardner, 2006). The main agents affecting the feeding and its acceptable of pollen substitutes contain the level of lipids and protein (De Groot, 1953 and Schmidt *et al.*, 1987). The pollen substitutes help the honeybee colonies in preserving them with the appropriate total brood area (Imdrof *et al*., 1988; Awad, 1998 and Mohammad, 2002). The Pollen trapping has some impacts on the colonies of the honeybee. Such as, the activities of foraging of pollen trapped bee colonies was declined over *a* period, although the proportion of forager return rate which gathering the pollen grains did not influence (Duff and Furgala,1986). McLellan (1974) and Cook (1985) indicated the pollen trapping did not change brood area, while Nelson *et al*., (1987) found a slight impact on the sealed brood area, and in Alberta, Canada, they indicated that trapping pollen decreased the total honey yield with 11 to 31%.

The chance of enhancing the beekeeping performance by adding protein source in pollen substitute to feed the honeybee colonies especially when pollen is rare (Zahra and Talal, 2008), particularly for the early blooming season (Skubida *et al*., 2008) and they showed that adding a source of protein to encourage the strength of honeybee colony and assist, enlarge honey yield. Also, the abundance of pollen enhances the development of glands which responsible for larval food production and it can produce enough amount of royal jelly which required for rearing of queen larvae (Svoboda *et al*., 1986).

This aims of study was to estimate the effectiveness of pollen substitute in boosting brood rearing areas, bee numbers per colony, production of royal jelly, the queen rearing, the weight of virgin queens, honey production and stored pollen.

Materials and methods

This work was done at Bassiun City, Gharbia Governorate, (1813 feddan), through the banana blooming season during the period from 1st August 2019 to 10th October 2019 to study the impacts of using pollen substitute to honeybee colonies.

1. Activities of honeybee colonies:

Sixteen of honeybee colonies, roughly same-strength and each contained 10,000 individuals and open mated sister queens (*A.mellifera carnica*) and they were used in this investigation to study the effect of pollen substitutes in banana flowers season as an assistant factor for honeybees during the dearth period in Egypt. Colonies were divided into two equal groups. Group 1 fed on a diet of 120g brewer yeast paste (70g dry yeast + 50g honey) per colony. Group 2 did not receive any pollen substitutes (control). The diet was placed in waxed paper to prevent moisture loss and put directly over the brood nest placed. The area ($inch^2$) of stored pollen and worker sealed brood were measured at 12 days intervals using an empty standard frame divided into square inches. By the end of the season, the number of combs covered with bees/colony was recorded to determine the colony population. And the population size per colony was counted as one well-covered comb of about 2000 bees on both sides (Taha, 2005). By the end of the flow season, the honey yield was estimated by the

difference between the weight of honeycombs before and after extraction. The number of incoming bees/min, number of pollen foragers/min was counted on the peak hours flying weekly during the period from 15th August until 2nd of October.

2. Queen rearing and royal jelly production:

Doolittle (1909) method was used for queen rearing. The experiment of queen rearing was done by using queenless rearing colonies for queen rearing and royal jelly production through the flowering period of banana during September. Eight colonies were divided into two equal groups, group 1 fed on previous pollen substitute while group 2 did not receive any pollen substitutes (Control). Every colony was provided with 30 wax cups and grafted with 24 hours-old larvae for queen rearing and 45 wax cups for royal jelly production. After three days, royal jelly was collected and weighted (mg). Repined queen cells were taken to emergence cages until the new queens were emerged and then weighted (mg).

3. Statistical analyses:

SPSS software (SPSS, 2006) was used to analyze the data and the statistical differences between the two treatments were determined by one-way analysis of variance (ANOVA).

Results and discussion

One of the essential nurturant materials of honeybee colonies is pollen substitute for many causes, maintain continued honeybee colony growth in times and places of a scarcity of nectar and pollen grains, to protect colonies with best numbers in the time of nectar flows, make honeybee colonies to high numbers for production of package-bee, and supply sufficient food reservoir for overwintering honeybee colonies.

The results this study showed that the effect of the use of the pollen substitutes on the brood rearing area, the stored pollen grains and the bee numbers per colony as shown in Table (1). There was a statistically significant difference among treatments on the brood rearing activity as determined by one-way ANOVA ($F(1, 54) = 42.43$, $p \leq 0.001$). Also, the differences between the two treatments in bee numbers per colony were statistically significant ($F(1, 54) = 8.70$, $p \leq 0.005$). The brood-rearing in honeybee without the protein source (pollen) is mostly declined because the glands which are responsible for food production by nurse honeybees become undeveloped and non-functional (Haydak, 1949) so, the brood-rearing and bee numbers per colony increased with pollen substitutes treatment. And there were statistically significant differences in the stored pollen grains between the two tested groups as determined by one-way ANOVA ($F(1, 54) = 42.17$, $p \leq 0.001$).

The data in Table (2) showed that the colonies which fed on the pollen substitutes could produce significantly more honey yield compared with control as determined by one-way ANOVA ($F(1, 6) = 59.87$, $p \leq 0.001$). The honeybee colonies which consumed the greater amount of pollen substitutes could produce more of brood area and more bee numbers per colony resulted in significantly more honey yield. So that stronger honeybee colony store more honey yield compared with the weak one (Chhuneja *et al*., 1992 and Kumar *et al*., 1995).

The data in Table (3) showed that the impact of using pollen substitute on royal jelly production and there was a statistically significant difference in royal jelly production between the two tested treatments as determined by one-way ANOVA ($F(1, 22) = 9.93$, $p \leq 0.005$). Also, the results in Table (4) indicated that there was a statistically significant difference in percentage of accepted larvae between groups as determined by one-way ANOVA ($F(1, 4) = 30.25$, $p \leq 0.005$).

Also, there was a statistically significant difference in the weight of virgin queens between treatments as indicated by one-way ANOVA ($F(1, 22) = 17.17$, $p \leq 0.001$). The queen acceptance depends on some factors such as weather factors, particularly the temperature, humidity and length of daytime. Also, the food quality and availability are very essential for honeybee hives, such as the plenty of pollen increases the growth of larval food production glands and can produce an appropriate amount of royal jelly which required for producing queen larvae of good bodies (Svoboda *et al*., 1986).

Table (1): The impact of pollen substitute on some biological activities of honeybee colonies.

Day	1st August		13thAugust		25th August		5th September		17th September		29th September		11th October	
Treat-ment	D.	C.	D.	C.	D.	C.	D.	C.	D.	C.	D.	C.	D.	C.
Work-er sealed brood	592.50 ±1.18	595.00 ± 21.01	608.75± 14.19	625.00 ±26.29	662.50 ±17.50	607.50 ±26.88	665.00 ± 15.54	540.00 ±24.83	715.00 ±11.90	550.00 ±26.45	717.50 ±6.29	532.50± 26.88	670.00 ±12.24	508.75 ±28.01
Store d pollen area	93.75± 2.39	96.25± 4.54	101.25± 3.14	95.00± 6.45	110.00 ± 7.07	88.75± 4.88	127.50 ± 11.08	95.00± 6.45	152.50 ±8.53	80.00± 4.08	140.00 ±9.12	76.25±8. 50	115.00 ±6.45	70.00± 4.08
No. honey - bee /colon y	9550.0 0±210. 15	9750.0 0± 478.71	10375.0 0± 554.33	10175. 00± 283.94	11000. 00± 408.24	11125. 00± 426.95	12500. 00± 500.00	11875. 00± 657.84	13125. 00± 314.57	11625. 00± 375.00	14750. 00± 478.71	11500.0 0± 353.55	15750. 00± 478.71	11125. 00± 426.95

C. Control D. Diet

T**able (2): The influence of using pollen substitute to honeybee colonies on honey production.**

Parameters	Honey yield (banana season)	
	Diet	Control
1st September	115.00±13.22	95.00±6.45
15th September	242.50±24.62	150.00±17.79
30th September	400.00±20.41	255.00±21.01
Before extract	550.00±20.41	362.50±23.93
Kg.	4.82±0.11	3.00±0.20

Table (3) : The impact of using pollen substitute to honeybee colonies on royal jelly production.

Parameters	1st September		15th September		30th September	
	Diet	Control	Diet	Control	Diet	Control
Number of queen cells	45	45	45	45	45	45
Percentage accepted larvae %	73%	68%	78%	66%	75%	67%
The weight of royal jelly (mg)/cup± SE	109.25±3.25	105.00± 4.56	117.50± 1.04	108.75± 3.14	118.25± 1.18	106.75± 3025

Table (4): The influence of using pollen substitute on queen rearing.

Parameters	1st September		15th September		30th September	
	Diet	Control	Diet	Control	Diet	Control
Number of queen cells	30	30	30	30	30	30
Percentage accepted larvae %	73%	66%	76%	63%	73%	60%
The weight of virgin queens (mg)± SE	167.50± 3.22	161.25± 1.25	172.50± 3.22	165.00± 2.04	176.25± 2.39	162.50± 2.50

The data in Table (5) showed the effect of using pollen on the flight activities of bee workers and there was a statistically significant difference in the numbers of incoming bee between the treatments as determined by one-way ANOVA ($F(1,14) = 16.38$, $p = .001$). Also, by using one-way ANOVA, there was a significant difference in the numbers of pollen foragers between the tested treatments ($F(1,14) = 94.01$, $p = .00$). The colonies which fed on pollen substitutes produced more of brood rearing area resulted in more bee numbers per hive and resulted in significant numbers of incoming bee per minute and significant numbers of pollen foragers per minute. The foraging activity is influenced by brood rearing activity and honeybee colony strength (Abou-Shaara *et al*., 2013), and the abundance of pollen (Weidenmuller and Tautz, 2002).

Table (5) : The effect of using pollen substitute on the flight activity of honeybee.

Date	Numbers of incoming bee/min± SE		Numbers of pollen foragers/min± SE	
	Pollen substitute	Control	Pollen substitute	Control
15th August	89.12±2.55	84.50±2.09	29.25±1.22	26.50±1.19
22nd August	87.87±2.27	82.00±1.47	30.00±1.56	25.87±1.38
29th August	88.75±1.61	81.00±2.59	31.37±1.32	26.62±1.19
6th September	90.75±2.31	85.37±2.34	32.00±1.28	26.00±1.43
13rd September	95.37±3.35	87.75±2.29	33.00±1.03	26.12±1.35
20th September	98.55±3.65	90.12±2.08	33.50±1.13	25.62±1.65
27th September	98.87±3.33	86.87±1.66	34.12±1.18	25.62±1.55
2nd October	96.37±3.97	85.87±1.52	32.75±1.16	25.25±1.41

The results of this study showed that the use of pollen substitutes could increase colonies strength with higher brood production, bee numbers per colony, royal jelly production, the queen rearing, the weight of virgin queens, honey production and stored pollen.

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