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EFFECT OF SOME FOOD ADDITIVE FORMULATIONS ON THE PERFORMANCE OF HONEYBEE, *Apis mellifera* L. COLONIES

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ABSTRACT: The present investigation was carried out in a private apiary located at Abu-Hammad District, Sharkia Governorate during 2017 and 2018 seasons. The study aimed to evaluate the effect (suitability) of four veterinarian food additive formulations on the performance of honeybee colonies. Summarized results showed that feeding honeybee colonies on sucrose syrup (1:1) enriched with the food additive formulations AD3H, VIGO I. Sel, VIGO mino vit and VIGO-FLU caused noticeable increase in colonies activities, that recorded 13.0, 9.0, 9.8 and 8.0% over the control in the area of sealed brood reared; 37.50, 1.09, 26.20 and 62.35% in drawn area of wax foundation; 26.9, 108.6, 27.8 and 100.2% in the hoarded amount of fortified syrup and 51.72, 8.62, 49.13 and 46.55% in clover honey yield for the four tested formulations, respectively.

Key words: *Apis mellifera*, hoarding behaviour, clover honey, carniolan hybrid, honeybee brood, clover flow, wax foundation.

INTRODUCTION

Honeybees are considered the most important economic insects due to their vital role in pollinating field and horticultural crops, in addition to the hive products which possess great nutritional and pharmaceutical value (Mc Gregor, 1976; Free and Williams, 1977; Yousif-Khalil *et al.*, 1989; Yousif-Khalil *et al.*, 1990; Yousif-Khalil, 1992a). Moreover, beekeeping projects yielded higher and rapid income and serve as a source of job opportunities for non-employees and the new candidates, as well as rural families to elevate the individual and national income (Hassan, 1997).

It is well known fact that the productivity of a bee colony is multi-factor governed issue. For instance, the good preparation of such colony to the new season is detrimental (successful wintering, controlling pests and diseases, artificial feeding, especially in dearth periods and for the early growth of the colonies during

winter and early spring, as well as achieving other beekeeping processes in right way and time.

However, and like other projects, bee projects are liable to be faced with some difficulties, including the invasion of natural enemies (El-Enany and Abdallah, 2016) and the infection of the pathogens of honeybee diseases (Yousif-Khalil, 1992a; Yousif-Khalil and Khattaby, 1993; Yousif-Khalil *et al.*, 2009; Chhuneja, 2014). Insecticidal pollution (Yousif-Khalil and Shalaby, 1992), Climatic factors (Alves *et al.*, 2015), and Shortage of food sources (Saffari *et al.*, 2004) are also from the difficulties that face bee projects.

Nowadays, dearth periods became longer and the inavailability of pollen became dominant. Therefore, food additives are essential to be offered to bee colonies to resolve the problem. Natural, availability, safe and cheap materials should be taken into consideration. All possible modern apicultural techniques should be applied in this respect.

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From this stand point, the present work was designed to test the impact of some veterinarian food formulations as food additives to honeybee colonies on workers and colony performance.

MATERIALS AND METHODS

The present investigations were carried out during 2017 and 2018 seasons to study the impact of some food additive formulations added to sucrose syrup offered to honeybee colonies on brood rearing activity, drawing out wax foundations, hoarding behavior and clover honey yield of the test colonies. Field experiments were carried out in a private apiary located at Abu-Hammad district, Sharkia Governorate, Egypt.

Materials

The test honeybee colonies

A total of fifteen honeybee nuclei were initiated during February 2017 and 2018, by division of strong colonies. These nuclei were distributed at random in 5 groups of 3 nuclei each. A mated young F₁ Carniolan queen (*Apis mellifera carnica* L.) was introduced into each nucleus. The introduction of mated queens into the experimental nuclei was taken place on the second day of division to ensure rapid and safe queens acceptance. A half ball netted cage was used for this purpose.

All nuclei were equalized in strength, number of combs covered with bees, brood, stored honey and bee bread.

The test food additive formulations used

The test food additive formulations used in the present work were:

AD3E : that composed of vitamins A, D3 and E as follow:

Vitam. A 1000000 iu

Vitam. D3..... 200.000 iu

Vitam. E..... 2000 mg

VIGO I. Sel that composed of:

Vitamin E acetate (Tocopherol-a)... 20000 iu

Sodium selenite..... 50 µg

VIGO mino Vitam. that composed of :

| | |
|----------------------------|-------------|
| Lysine | 440 mg |
| L-arginine..... | 480 mg |
| Thropin | 80 mg |
| Glycine..... | 2600 mg |
| Methonine..... | 80 mg |
| Serein..... | 100 mg |
| Essential amino acid..... | 260 mg |
| Proline..... | 1700 mg |
| Panthenol..... | 4000 mg |
| Glutamic acid..... | 1000 mg |
| Phenylalanine..... | 200 mg |
| Aspartic acid..... | 500 mg |
| Alanine..... | 1000 mg |
| VIGO-FLU that composed of: | |
| B. glucan..... | 100 mg |
| Ascorbic acid | 25 mg |
| Vitamin A | 5000.000 iu |
| Vitamin E | 2500 mg |
| Vitamin D | 5000 iu |
| Vitamin K..... | 10 mg |

The test formulations were used at the rate of 1 ml/liter sucrose syrup.

Control colonies were offered sucrose syrup without any additives.

Methods

Measuring brood rearing activity

Brood rearing activity of the experimental colonies fed on the test food additive formulations was followed during clover flow period of 2017 and 2018 seasons by measuring the areas of sealed worker cells present in each colony separately at 12 day intervals, using rigid plastic sheets divided into square inches. The bees covering each brood comb were firstly shaken off to allow easier and safer measuring with lower bee kill (**Kasim *et al.*, 2017**), then sealed brood area in both sides of the comb was measured. The total sealed brood area per colony was then calculated.

Drawing out wax foundation

Each test colony was provided with one wax foundation sheet frame that was inserted between brood combs during clover flow period of 2017 and 2018 seasons. Additional wax foundation sheets were added to the test colonies when necessary. The area on both sides of wax foundation frame drawn out during flow period of clover was measured by means of a transparent rigid plastic sheet divided into square inches.

Evaluating hoarding behavior

Hoarding behavior of the test colonies was evaluated under field conditions during summer season of 2017 and 2018, post harvesting clover honey yield according to **Khater (1998)** method by measuring the quantity of sucrose syrup hoarded by bee colonies per unit of time from outdoor feeders to the wax combs.

Sucrose syrup of (1:1) (without and with 1ml of the test food additive formulations) was prepared as usual and offered to the test colonies in wide mouth graduated bottles at the rate of 1000 ml. A thin wooden piece of a suitable size was put in each bottle to be floated on the surface of the syrup during feeding to protect workers from sinking. The quantities of hoarded (sucked) sucrose syrup from each feeder was measured and recorded after 1, 2, 3, 4, 5 and 6 hours from offering the syrup. Additional known quantities of treated and untreated sucrose syrup were added when needed to the test colonies.

Clover honey yield

Clover flow period was started at the beginning of May and lasted to the first week of June when honey yield was estimated for each experimental colony solely. Honey yield was estimated as the difference in the weight of honey combs before and after honey extraction.

Statistical Analysis

Data obtained were statistically analyzed according to **Snedecor and Cochran (1967)** methods, that calculated according to COSTAT computer program (**Anonymous, 2005**).

RESULTS AND DISCUSSION

Total Sealed Brood Area

Results presented in Table 1 indicate that the mean total sealed brood area in the test colonies fed on the additive formulations AD3E, VIGO I.Sel, VIGO mino Vitam. and VIGO-FLU as well as control colonies fed on sugar syrup only during clover flow period attained 1544.5, 1331.0, 1396.0, 1399 and 1187.0 inch²/colony in 2017 season; 1046.5, 982.0, 1135.0, 1057.0 and 1001.0 inch²/colony in 2018 season, respectively. The respective two years mean total sealed brood area were 1295.5, 1156.5, 1265.5, 1228.0 and 1094 inch²/colony. It is obvious that the test food additive formulations increased the mean total sealed brood area during May by 18.39, 5.71, 15.67 and 12.11% over that measured in control colonies.

Analysis of variance cleared that the highest significant sealed brood area was recorded with AD3E in 2017 season and VIGO mino Vitam. in 2018 season. On the other hand, the least significant sealed brood area was measured in control colonies in 2017 and VIGO I.Sel fed colonies in 2018 season. In this respect, **Hussein (1979)** recorded 32% increase in sealed brood area (above the control) in honeybee colonies offered Vitam. in sugar syrup. **Kasim et al. (2017)** measured a total of 1310.65 in²/sealed brood area/Carniolan hybrid colony during Egyptian clover flow period. In addition, **Elbassiouny (2006)** counted 523 sealed brood cells in vitamin-supplemented honeybee colonies compared to 219 cells reared in control.

Regarding vitamins supplementation and their impact on brood rearing activity of honeybee colonies, **Standifer (1980)** reported that vitamins are necessary for initiating brood rearing activity and for glandular development and secretions, especially royal jelly for young larvae and the queen, especially Vitam. B complex and Vitam. C. The same trend was also reported by **Verma and Rhogat (1982)**, **Zahra and Talal (2008)** and **Andi and Ahmadi (2014)**. Also, **Feng et al. (2011)** reported that Vitam. A supplemented in diet improved the sealed brood quantity.

Concerning amino acid supplementation and their effect on brood rearing activity, **Garcia et al. (1986)** and **Cuoto et al. (1988)** reported that

Table 1. Total sealed brood area (inch²/colony) reared by the test colonies offered the test food additive formulations during Egyptian clover flow period of 2017 and 2018 seasons

| Food additive | Season | | Two years mean | Over the control (%) |
|---------------------|--------|--------|----------------|----------------------|
| | 2017 | 2018 | | |
| AD3E | 1544.5 | 1046.5 | 1295.5 | 18.39 |
| VIGO I.Sel | 1331.0 | 982.0 | 1156.5 | 5.71 |
| VIGO mino vit | 1396.0 | 1135.0 | 1265.5 | 15.67 |
| VIGO-FLU | 1399.0 | 1057.0 | 1228.0 | 12.24 |
| Control | 1187.0 | 1001.0 | 1094 | |
| LSD _{0.05} | 40.9 | 23.1 | | |

amino acids did not increase brood rearing activity. On the contrary, **Mohebodini *et al.* (2013)** stated that adding 200 ppm thiamine to honeybee colonies increased significantly sealed brood area during summer but not spring season. Also, **Wilde *et al.* (2014)** found that addition of immune bee solution (amino acids) stimulated brood production and comb building.

Drawing Out Wax Foundation

The drawn out area of wax foundation inserted between brood comb in the test colonies during Egyptian clover flow period of 2017 and 2018 seasons were measured using rigid transparent plastic sheet divided into square inches. Results obtained are presented in Table 2.

Obtained results indicated that the mean total drawn out area of wax foundation by the test colonies offered the additive formulations AD3E, VIGO I.Sel, VIGO mino Vitam. and VIGO-FLU as well as control colonies received no additives reached 202.15, 138.80, 184.60, 231.00 and 137.50 inches²/colony in 2017 season, and 150.50, 120.10, 138.60, 184.80 and 118.60 inches²/colony in 2018 season, respectively. The respective two years mean drawn out area of wax foundation attained 176.32, 129.45, 161.60, 207.90 and 128.70 inches²/colony.

Statistical analysis of data detected significant differences between the treatments in both seasons of study. For instance, the colonies offered vitamins drew out the highest significant mean area of wax foundation in both seasons, realizing 62.20% over the control. The additive AD3E came in the second class (37.50%)

followed by the additive VIGO mino Vitam. (26.20% over the control). On the other hand, control colonies and those received VIGO I.Sel showed the least drawn out area of wax foundation in both seasons.

The dominance of vitamins on other additives in enhancing drawing out wax foundation may be due to their role in carbohydrate, lipid and protein metabolism. Such role is of a great importance for wax production *via* carbohydrate synthesis. This statement is supported by that of **Elbassiouny (2006)** who found that compressed are traditional honeybee colonies drew out 4.31 and 1.61 wax foundations when offered vitamin supplement as compared to 2.1 and 0.79 comb for control colonies, respectively. Moreover, the test colonies were more active in drawing out wax foundation in 2017 season than in 2018. This phenomenon is commonly taken place and depending upon floral and climatic factors as well as the intrinsic conditions of the colonies, in addition to the abundance or absence of natural enemies (wasps, *Merops* spp). All these variables make one season more suitable than another for bees foraging activity and wax secretion and building of wax combs. In connection, **Muller (1992)** recorded seasonal variation in the amount of wax secreted by bee colonies. Moreover, **Kasim *et al.* (2017)** reported seasonal and racial variation in the area of wax foundation drew out by bee colonies between clover and citrus flow periods and between Carniolan and Italian hybrid honeybee colonies. Also, **Szabo (1977)** added that the number of combs built greatly determined by the weight of bees in the colony and prevailing air temperature.

Table 2. Drawn out area (inch²/colony) of wax foundation by honeybee colonies offered AD3E, VIGO I.Sel, VIGO mino Vitam. and VIGO-FLU additive formulations during Egyptian clover flow period of 2017 and 2018 seasons

| Food additive | Season | | Two years mean | Over the control (%) |
|---------------------|--------|--------|----------------|----------------------|
| | 2017 | 2018 | | |
| AD3E | 202.15 | 150.50 | 176.32 | 37.50 |
| VIGO I.Sel | 138.80 | 120.10 | 129.45 | 1.09 |
| VIGO mino | 184.60 | 138.60 | 161.60 | 26.20 |
| VIGO-FLU | 231.00 | 184.80 | 207.90 | 62.35 |
| Control | 137.50 | 118.60 | 128.05 | |
| LSD _{0.05} | 12.60 | 14.50 | | |

Hoarding Behavior

The hoarded quantity of sucrose syrup enriched with the test additive formulations by the test honeybee colonies was measured at 1 hour intervals, for 6 successive hours. Results obtained are recorded in Table 3.

Obtained results indicated that the mean hoarded quantity of syrup after one hour attained 0.0, 19.33, 0.0, 23.0 and 0.0 ml sucrose syrup fortified with the additives AD3E, VIGO I.Sel, VIGO mino Vitam. and VIGO-FLU as well as control colonies fed sucrose syrup only, respectively. The respective hoarded quantities during in the second hour were 0.0, 64.3, 0.0, 57.0 and 0.0 ml. The hoarded quantity of sugar enriched with the additive formulations AD3E, VIGO I.Sel, VIGO mino Vitam. and VIGO-FLU as well as clean sucrose syrup (control) during the third hour attained 40.0, 371.6, 59.3, 434.0 and 0.0 ml, respectively. The corresponding quantities hoarded during the 4th hour jumped to reach 241.6, 476.6, 178.3, 351.6 and 121.6 ml.

The hoarded quantity during the 5th hour attained 481.6, 560.0, 428.3, 541.6 and 348.3 ml for the four tested formulations and the control. Moreover, the corresponding hoarded amount during the 6th hour of the fortified syrup and the clean sucrose syrup reached 545.0, 651.6, 651.1, 655.0 and 560.0 ml.

The respective total hoarded quantities of the treated and control syrup recorded (summed) 1308.2, 2143.43, 1317.0, 2062.2 and 1029.9 ml. It is obvious that the test food additive formulations induced bee colonies to suck and

store (hoard) sucrose syrup fortified with the test additives, so they recorded percent increase in the hoarded quantities of 26.9, 108.1, 27.8 and 100.2% over the control (sucrose syrup without additives).

Discussing the data obtained clear that all test additive formulations attracted bee foragers to suck and hoard the treated syrup more than the clean sucrose syrup (without additives). Moreover the highest attraction was recorded with VIGO I.Sel- treated syrup that followed closely by VIGO-FLU- treated syrup. However, VIGO I.Sel killed a lot of bees in other experiment, which means that bee foragers could not discriminate VIGO I.Sel in the syrup which proves that VIGO I.Sel is dangerous and must not be used in bees feeding. **Hladun *et al.* (2012) and Hladun *et al.* (2013)** came to the same conclusion, reporting that bees may not avoid selenium compounds in the plant tissues which exposed bees to adverse effects.

Regarding the enhancing effect of vitamins on bees hoarding behavior, **Khater(1998)** stated that the addition of vitamin c (lemon juice) to sucrose syrup increased significantly the hoarded amount of the syrup fortified with vitam. C. Similarly **Elbassiouny (2016)** reported that honeybee colonies fed on vitamins plus pollen, pollen and syrup only hoarded 236, 220 and 191 ml sucrose syrup/3 days, respectively.

Generally, hoarding capacity of a honeybee colony is a good indicator of expected honey production, that usually depends upon in breeding and selection in beekeeping domain. The rapid transmitting of the syrup from the feeders

Table 3. The hoarded volume of sucrose syrup enriched with the test food additive formulations from outdoor feeders by honeybee colonies

| Food additive | Time passed (hour) after offering the syrup | | | | | | Total hoarded quantity (ml) | Over the control (%) |
|---------------------|---|-------|-------|-------|-------|-------|-----------------------------|----------------------|
| | 12 noon | 1 pm | 2 pm | 3 pm | 4 pm | 5 pm | | |
| AD3H | 0.0 | 0.0 | 40.0 | 241.6 | 481.6 | 545.0 | 1308.2 | 26.9 |
| VIGO I. sel | 19.33 | 64.3 | 371.6 | 476.6 | 560.0 | 651.6 | 2143.4 | 108.1 |
| VIGO mino vitam. | 0.0 | 0.0 | 59.3 | 178.3 | 428.3 | 651.1 | 1317.0 | 27.8 |
| VIGO-FLU | 23.0 | 57.0 | 434.0 | 351.6 | 541.6 | 655.0 | 2062.2 | 100.2 |
| Control | 0.0 | 0.0 | 0.0 | 121.6 | 348.3 | 560.0 | 1029.9 | |
| LSD _{0.05} | 6.72 | 10.86 | 35.56 | 43.56 | 39.92 | 57.76 | | |

into the combs is of a great importance, as it prevents robbing between colonies and avoid the fermentation of the syrup due to long lasting in the feeders which help in attracting the natural enemies (wasps) to attack the colonies, especially the weak ones. This behavior is a multi-factor governed one, such as attractiveness of the syrup (Khater, 1998) the area of empty combs (Rindere and Baxter, 1978; Rinderer *et al.*, 1982; Pham-Delegue *et al.*, 1984) and the concentrations of sugar in the syrup (Sylvester, 1978).

Clover Honey Yield

Results presented in Table 4 indicate that the mean clover honey yield produced by the test colonies fed sucrose syrup fortified with the additive formulations AD3E, VIGO I.Sel, VIGO mino Vitam. and VIGO-FLU as well as control colonies fed on sucrose syrup only attained 9.0, 7.6, 9.2, 9.0 and 6.0 kg/colony in 2017 season and 8.6, 5.0, 8.1, 8.0 and 5.6 kg/colony for test additives and the control, respectively in 2018 season. The respective two years mean clover honey yield harvested from honeybee colonies fed on test additives recorded 8.80, 6.30, 8.65, 8.50 and 5.80 kg/colony. Analysis of variance cleared that the additives AD3E, VIGO mino Vitam. and VIGO-FLU produced significantly higher clover honey yield as compared to that produced from the colonies received VIGO I.Sel. On the other hand, control colonies yielded the least significant honey yield in 2017 season. The same trend was also detected in 2018 season except that the difference between VIGO I.Sel additive and control was insignificant.

Clover honey yield in the present work is in parallel with that recorded by Kasim *et al.* (2017) who harvested clover honey yield of 8.9 kg/colony for Carniolan hybrid colonies and 9.9 kg/colony for Italian bees; Similarly, Khattaby *et al.* (2018) gained between 5-7 kg clover honey/colony. Also, El-Nagar *et al.* (2019) gathered between 4 to 6 kg clover honey/colony fed on other food additives.

Although the experimental colonies of all treatments placed in the same location, being nearly similar in strength, headed by sister queens and received the same beekeeping practices, yet they produced varied clover honey yield. This proves, undoubtedly that this variation is due to the varied additives offered to the test colonies. The variation in honey yield from one season to another could be attributed to many factors such as botanical factors that represented by plant race, flowering period and intensity, volume and quality of secreted nectar also soil factors including the fertility and rate of irrigation. Meteorological factors also have their impact, especially air temperature, wind speed and relative humidity which play reciprocal role on both bee flight and nectar secretion and its persistent. The occurrence of natural enemies (wasps, *Merops*) that play a detrimental impact on bees foraging and nectar gathering, subsequently honey production. Similar statements were also reported by many authors such as Brandeburgo and Concalves (1989), Perez-Pineiro (1986), Abdallah (1999), Racys (2002), Kasim *et al.* (2017), Khattaby *et al.* (2018) and El-Nagar *et al.* (2019).

Table 4. Clover honey yield (kg/colony) produced by honeybee colonies offered AD3E, VIGO I.Sel, VIGO mino Vitam. and VIGO-FLU during 2017 and 2018 seasons

| Food additive | Season | | Two years mean | Over the control (%) |
|---------------|--------|------|----------------|----------------------|
| | 2017 | 2018 | | |
| AD3E | 9.00 | 8.60 | 8.80 | 51.72 |
| VIGO I Sel. | 7.60 | 5.00 | 6.30 | 8.62 |
| VIGO mino vit | 9.20 | 8.10 | 8.65 | 49.13 |
| VIGO-FLU | 9.00 | 8.00 | 8.50 | 46.55 |
| Control | 6.00 | 5.60 | 5.80 | |
| LSD 0.05 | 0.60 | 0.78 | | |

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تأثير بعض الإضافات الغذائية المصنعة على أداء طوائف نحل العسل

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أجريت هذه الدراسة في منزل خاص ناحية مركز أبوحماد، محافظة الشرقية خلال عامي ٢٠١٧ و ٢٠١٨ لتقييم تأثير أربعة إضافات غذائية بيطرية مصنعة على أداء طوائف نحل العسل وقد أظهرت النتائج أن تغذية طوائف نحل العسل مع محلول سكري (١:١) مدعم بالإضافات الغذائية AD3H و VIGO I.Sel و VIGO .mino Vitam و VIGO-FLO قد أحدثت زيادة واضحة في نشاط الطوائف والذي سجل نسباً بلغت ١٣,٠ ، ٩,١ ، ٩,٨ ، ٨,٠ % زيادة عن المقارنة في نشاط تربية الحضنة و ٣٧,٥٠ ، ١,٠٩ ، ٢٦,٠ ، ٦٢,٣٥ % زيادة عن المقارنة في مساحة الأساس الممطوط و ٢٦,٩ ، ١٠٨,١ ، ٢٧,٨ و ١٠٠,٢ % زيادة في كمية المحلول السكري المدعم بالإضافات والذي تم نقله من الغذايات للأقراص و ٥١,٧٢ ، ٨,٦٢ ، ٤٩,١٣ و ٤٦,٥٥ % زيادة في محصول عسل البرسيم كمتوسط لعامي الدراسة للإضافات الأربعة المختبرة على الترتيب.

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