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The type of protein supplement or substitute and its location from brood affect honey bee colony performance

Hatem Sharaf El-Din* and Mohammad Abd Al-Wahab Abd Al-Fattah

Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza, Egypt
*Corresponding author: Sharaf85-0000@agr.cu.edu.eg

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ABSTRACT

Honey bee *Apis mellifera* L. is the main pollinator insect for the most crops worldwide beside the economic importance of its products. Honey bee colonies depend on stored food in their hives to manage the dearth periods successfully. This study compared the effect of three different patties, Bee Fonda® a pollen supplement (BF), home-made substitute (H) consists of two thirds powdered sugar and one third of powdered yeast, a home-made substitute mixed with Bee Plus® (BP) and sugar syrup (C) on colonies growth rate. The petties were introduced either between the frames (on a queen excluder of 15x20 cm^2) or above the frames. Colonies received BF between the frames had the highest mean of brood area, while colonies of BP between the frames had the highest means of honey and colonies of BP above the frames had the highest mean of bee bread areas. Regardless the patty type, patties located between frames had the highest mean of brood and honey areas, while bee bread areas were not affected. As a general mean of type of bee patties, the BF group had highest mean of brood area and the BP was superior in both of honey and bee bread means areas.

KEYWORDS: protein substitute, honeybee, colony performance, protein introduction method.

1. INTRODUCTION

Western honey bee, *Apis mellifera* L., provides pollination services for worldwide crops (Morse and Calderone, 2000; Eilers *et al.*, 2011 and Gisder *et al.*, 2011). The populations of honey bee colonies exposed to threat decline through the last few years (VanEngelsdorp *et al.*, 2012). The exact causes of that are unspecified and have been attributed to many reasons among them pesticides exposure, insufficient nutrition, parasites, pathogens, climate changes, management practices, and other stresses (Collins *et al.*, 2004; Decourtye *et al.*, 2005; Ciarlo *et al.*, 2012; Eiri and Nieh, 2012; Henry *et al.*, 2012; Frost *et al.*, 2013; Williamson and Wright, 2013 and Zhu *et al.*, 2014). Pollen plays an important role in honey bee health. Honey bee needs adequate food sources to regulate the nest temperature, rearing of brood, promote foraging flights for generation of warming up (De Grandi-Hoffman *et al.*, 2008).

Colonies used for crop pollination, often are limited to one type of nutrition (Kalev *et al.*, 2002; Keller *et al.*, 2005), which might contain small amounts of nectar and pollen (Day *et al.*, 1990). Honey bees adapt with periods of shortage in nectar and pollen by lowering their metabolism rate and decreasing their activities (Imdorf *et al.*, 1998; Pernal and Currie, 2001; Kalev *et al.*, 2002; Keller *et al.*, 2005). Less brood rearing resulting in reduction of adult bees' number which affect pollination services and honey production (Duff and Furgala, 1986; Nelson, 1987; Fewell and Winston, 1992; Herbert, 1999).

Honey bees are highly dependent on food supply stored in the hive, as the shortage in the storage is negatively affect colony performance. Many bee pests and pathogens attack honey bee colonies and if the colonies are weak, these factors lead to honey bee colonies loss.

Ideal colony nutrition, such as sufficient protein and carbohydrate stores, is helping bees to resist or tolerate many of the unsuitable conditions related to beekeeping (Hrassnigg and Crailsheim, 2005; Naug and Gibbs, 2009 and Brodschneider and Crailsheim, 2010). Nearly all protein and vitamins needed by bees are obtained from pollen, which are stored as bee bread inside the hive when pollen is not available in the environment (Mattila and Otis, 2006 and Brodschneider and Crailsheim, 2010).

Dearth periods of nectar or pollen are negatively affect honey bee colony activities, so beekeepers supply colonies with pollen substitute diets when they believe that bees are suffering from a nutrition shortage. (Sihag and Gupta, 2011; Pande and Karnatak, 2014 and Manning, 2016).

Pollen supplements mixed with other ingredients also have been tested, such as honey, soybean flour, peanut meal, corn flour, brewer's yeast, powdered skim milk, and others to make a desired patty (Erickson and Herbert, 1980; Doull,

1980; Lehner, 1983; Kalev *et al.*, 2002; Keller *et al.*, 2005). Patty ingredients are important for its nutritious value and bee's acceptance (Herbert, 1999; Keller *et al.*, 2005). Bees prefer to feed on pollen supplements more readily than pollen substitutes (Standifer *et al.*, 1973), and due to their high consumption, colonies rear more brood (Keller *et al.*, 2005). Many researchers have focused on pollen patty composition, but fewer focused on the introduction method. Pollen supplements and substitute's introduction method affect the consumption and colony development (Cook and Wilkinson, 1986; Herbert, 1999; Keller *et al.*, 2005).

Mattila and Otis, (2006) found that colonies provided with pollen substitutes attained high growth and productivity. As the primary protein source utilized by colonies is pollen (Brodschneider and Crailsheim, 2010), beekeepers feed colonies pollen substitutes because the diets are more widely available and cheaper than pollen (Standifer *et al.*, 1977). Feeding colonies with pollen substitutes during dearth periods, reflects in improvements in the bees' physiological responses, such as hive weight gain, reproductive performance, disease tolerance, and the overall colony development (Morais *et al.*, 2013).

Based on the previous reports this research aimed to assess the efficiency of three different bee regimes which inserted into two different locations within honeybee colonies on their development and conservation during very dearth period of honeybee life cycle under the circumstance of Giza region.

2. MATERIALS AND METHODS

2.1 Honeybee colonies

Local Carniolan honeybee colonies, *Apis mellifera* L. of the same strength headed by mated sisters' queens were selected and maintained in the apiary yard of agricultural experimental station, Faculty of Agriculture, Cairo University, Egypt. Experiments were applied during very dearth period in this region which yearly extending from June to August 2020. Colonies had no apparent honeybee diseases, had adequate amounts of bee bread and honey stores and high number of young honeybee workers. Each colony had 8 combs covered with bees. Colonies were randomly divided into 7 groups (3 colonies each as replicates), each group received one regime of the six different treatments, while the last ones left without feeding as control group (C).

2.2. Types of honeybee patties

Three different types of honeybee patties were inserted inside the colonies in two different locations, while the control colonies were supplied only with (50:50 weight : volume) sugar syrup.
The honeybee patties types were as follows:

1- Home-made patty (H): consist of two thirds of powdered sugar and one third of powdered apiaries yeast mixed with concentrated (2:1 sugar: water) sugar syrup.
2- Bee plus patty (BP): Home-made patty mixed with Bee plus ® powder (1 g/ colony) which is a commercial product consist of carbohydrates, B-complex vitamins and essential aromatic oils. It was purchased and available in HP pharma, Egypt.
3- Bee fonda patty (BF): a commercial pollen supplements patty contains 15% powdered pollen and carbohydrates.

2.3. Honeybee patties application

Each colony was provided with 100 grams of the critical honeybee patty every 3 days. Each kind of patties was inserted into colony in two different locations; the first was above the brood frames and covered with plastic sheet (A) and the second was spread on a queen excluder, (with dimensions of 15 x 20 cm^2) and hanged between brood frames (BE). The treatments were divided to Home made patty - Above the frames, Home made patty – Between the frames, Bee plus patty – Above the frames, Bee plus patty - Between the frames, Bee fonda patty - Above the frames, Bee fonda patty - Between the frames and Control.

Each colony (including control group) was fed with 0.5 liter sugar syrup (50:50 w:v) every week during the experimental period.

2.4. Biological parameters

Brood, honey, and bee bread combs areas were measured 4 times, one before starting the feeding and after feeding every 21 days intervals for three times. Areas were measured using a grid frame divided into square centimeters. Each patch squares were counted, then the areas of each patch type were calculated and recorded for statistical analysis.

2.5. Statistical analysis

Changes in biological parameters (brood, honey, and bee bread areas), were analyzed with Two-way analysis of variance (ANOVA) (time x treatment), followed by Duncan's Multiple Range Test using MSTAT-C program version 2.10.

3. RESULTS AND DISCUSSION

High significant differences among the interactions between time and treatments were found for all different feeding groups indicating that the change in brood, honey, and bee bread with time was dependent on the type of honeybee patty introduced to the colonies and location.

3.1. Effect of feeding type

Patty type had a significant effect on measured parameters (Table 1) as feeding honeybee with BF and H patties resulted the highest significant mean brood area followed by BP and control groups in the second rank. Bee plus patty came in the first significant rank regarding mean honey and bee bread areas followed by H group in the case of honey and by control group in the case of bee bread area.

Table 1. Effect of feeding type on means ± standard error of brood, honey, and bee bread areas (in cm.^{2})

Feeding type	Brood		Honey		Bee bread	
BP	2951.8 ± 159.2	B	3745.9 ± 426.9	A	824.4 ± 128.5	A
H	3557.2 ± 163.5	A	2783.6 ± 267.6	B	423.8 ± 125.7	C
BF	3918.4 ± 167.5	A	2456.5 ± 151.9	C	481.6 ± 84.7	BC
Control	3080.8 ± 306.6	B	1927.8 ± 80.4	D	507.6 ± 65.1	B

Values with different letter in the same column are significantly differ according to DMRT at 0.05 probability.
*BP: Bee Plus patty; *H: Home-made patty; *BF: Bee Fonda patty

3.2. Effect of patty location

The comparison between both of feeding location as general mentioned in (Table 2) showed significant differences between colonies. It was observed that the mean brood areas value in BE location was higher than A and control groups who came in the same rank. Regarding honey mean area, no significant differences found between both locations, but the BE location was superior of control group. Bee bread storage had its maximum in A location, but with no significant differences between all treatments. Generally, the group fed with patties on BE location was in the first rank regarding all parameters.

Table 2. Effect of feeding location on brood, honey, and bee bread mean areas ± standard error (in cm.^{2})

Feeding location	Brood		Honey		Bee bread	
Between frames	3727.5 ± 212.0	A	3028.3 ± 451.7	A	545.8 ±142.1	A
Above frames	3242.2 ± 192.7	B	2963.3 ± 285.1	AB	606.7 ± 133.6	A
Control	3080.8 ± 306.6	B	1928.3 ± 80.4	B	509.2 ± 65.1	A

Values with different letter in the same column are significantly differ according to DMRT at 0.05 probability.

3.3. Effect on brood area

Results in table (3) appeared that brood means areas that measured at June, 15, 2020 (before the experiments) were not significantly differed between colonies in the same day of applying different treatments (regimes) to the colonies.

Observing inspection dates mean values, the highest value of mean brood areas (3814.4cm^2) was determined at the first inspection date (July, 6) and then started to decrease significantly till the end of experiment to reach its lowest value. The interaction between feeding type and locations revealed that the highest significant brood area was registered in colonies fed with BF-BE, (4335.8 cm^2) followed by H-BE, (3742.8 cm^2) while the lowest value was observed in colonies fed with BP-A, (2798 cm^2). The results reveal that the lowest mean value of brood was recorded in control group, (1911 cm^2), while the highest value found in colonies fed with BF-BE (4851 cm^2) which represent 153.8% of the lowest value.

Table 3. Effect of different treatments on means of brood areas (in cm.²)

Inspection dates	BP-BE	BP-A	H-BE	H-A	BF-BE	BF-A	Control	Mean ± SE
15/06/2020 (0 time)	3658 DEF	3741 BCDE	3664 DEF	3691 CDE	3619 DEF	3689 CDE	3871 BCDE	3704.7 ± 34.2 ab
06/07/2020	2652 GHI	2350 HI	4597AB	3916 BCDE	4851 A	4073 ABCD	4262 ABCD	3814.4 ± 223.8 a
27/07/2020	3505 DEF	2809 FGH	3614 DEF	3469 DEFG	4540 ABC	3596 DEF	1911 I	3349.1 ± 181.9 b
17/08/2020	2609 HI	2292 HI	3096 EFGH	2410 HI	4333 ABCD	2647 GHI	2279 HI	2809.4 ± 177.4 c
Mean ± SE	3106.0 ± 148.8 (cd)	2798.0 ± 195.6 (d)	3742.8 ± 171.7 (b)	3371.5 ±175.4 (bc)	4335.8 ±290.6 (a)	3501.3 ± 161.8 (bc)	3080.8 ± 306.6 (cd)	3419.4 ± 94.0

Values with different letters CAPITAL, small or (between brackets) in the same column or row are significantly different according to DMRT at 0.05 probability

*BP-BE: Bee plus patty between frames; *BP-A: Bee plus patty above the frames; *H-BE: Home-made patty between frames; *H-A: Home-made patty above the frames; *BF-BE: Bee fonda between frames; *BF-A: Bee fonda patty above the frames

The change percentage of mean brood areas in different treatments comparing to the control group was also calculated and illustrated in Fig. (1) by comparing the treatments brood area in the last inspection dates. Data showed that colonies fed with BF-BE had highest increase percentage (90.1%), followed by H-BE group with ratio of 35.8%.

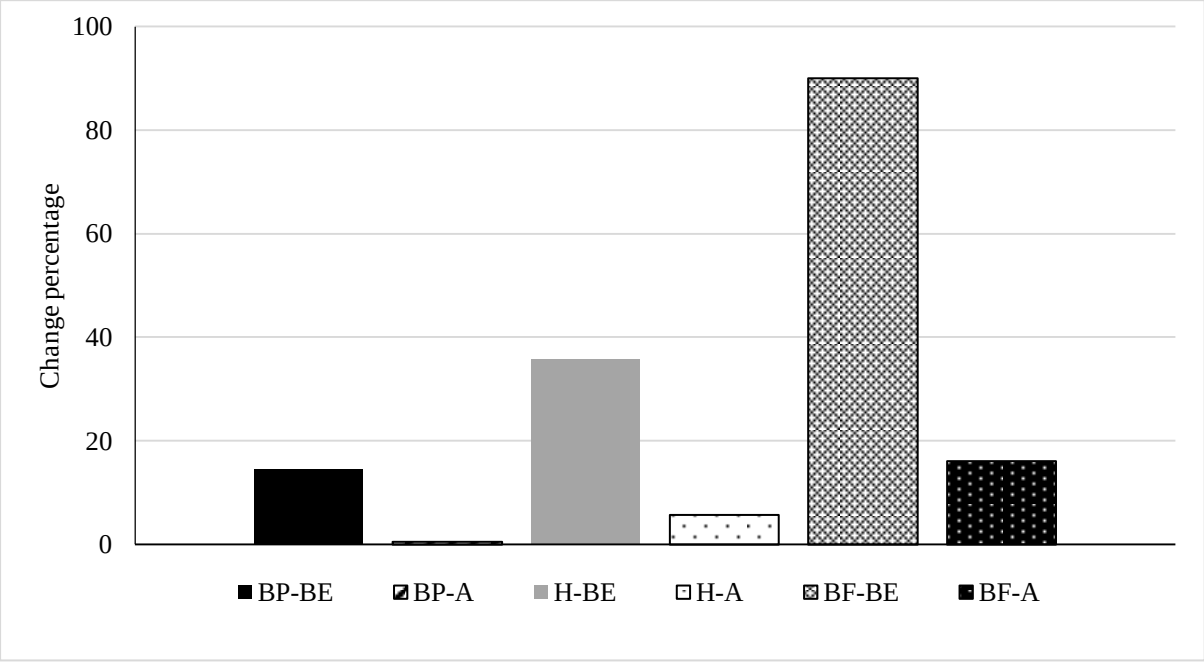

Fig. 1. Change in different treatment brood area means comparing to control group

*BP-BE: Bee plus patty between frames; *BP-A: Bee plus patty above the frames; *H-BE: Home-made patty between frames; *H-A: Home-made patty above the frames; *BF-BE: Bee fonda between frames; *BF-A: Bee fonda patty above the frames

3.4. Effect on honey storage

Results in (Table 4) showed that honey means areas that measured at June, 15, 2020 (before the experiments began) were not significantly differed between colonies in the same day of applying different treatments to the colonies.

Observing inspection dates mean values, the highest value of mean honey areas (3729.9 cm2) was determined at the second inspection date (July, 27), while the lowest value was observed before the experiments began. The interaction between feeding type and locations revealed that the highest significant honey area was registered in colonies fed with BP-BE, (4241.3 cm2), while the lowest values were observed in control and BF-BE groups, (1928 and 1892.3 cm2, respectively). The results reveal that the highest mean value (5751 cm2) found in colonies of BP-BE group which represent 297.16% of the lowest mean value of honey (1448 cm2) as it was recorded in BP-A group before the experiments start.

Table 4. Effect of different treatments on means of honey areas (in cm2)

Inspection dates	BP-BE	BP-A	H-BE	H-A	BF-BE	BF-A	Control	Mean ± SE
15/06/2020 (0 time)	1528 PQ	1448 Q	1595 OPQ	1543 OPQ	1603 OPQ	1624 NOPQ	1637 NOP	1568.3 ± 23.5 d
06/07/2020	4545 C	3289 FG	2453 J	2812 I	2042 LM	3188 GH	2298 JK	2946.7 ± 179.9 c
27/07/2020	5751 A	4638 C	4676 C	3444 EF	2206 KL	3600 DE	1794 N	3729.9 ± 298.7 a
17/08/2020	5141 B	3626 D	3074 H	2672 I	1718 NO	3665 D	1983 M	3125.6 ± 245.6 b
Mean ± SE	4241.3 ± 494.1 (a)	3250.3 ± 373.3 (b)	2949.5 ±340.4 (c)	2617.8 ± 208.4 (d)	1892.3 ± 76.1 (e)	3019.3 ± 251.5 (c)	1928.0 ±80.4 (e)	2842.6 ± 136.1

Values with different letters CAPITAL, small or (between brackets) in the same column or row are significantly different according to DMRT at 0.05 probability

*BP-BE: Bee plus patty between frames; *BP-A: Bee plus patty above the frames; *H-BE: Home-made patty between frames; *H-A: Home-made patty above the frames; *BF-BE: Bee fonda between frames; *BF-A: Bee fonda patty above the frames

The change percentage of mean honey areas in different treatments comparing to the control group (Fig. 2) showed that colonies fed with BP-BE had highest increase percentage (159.3%), followed by BP-A group with ratio of 82.9%. Colonies of BF-BE recorded 13.45% decrease in honey areas comparing to control group.

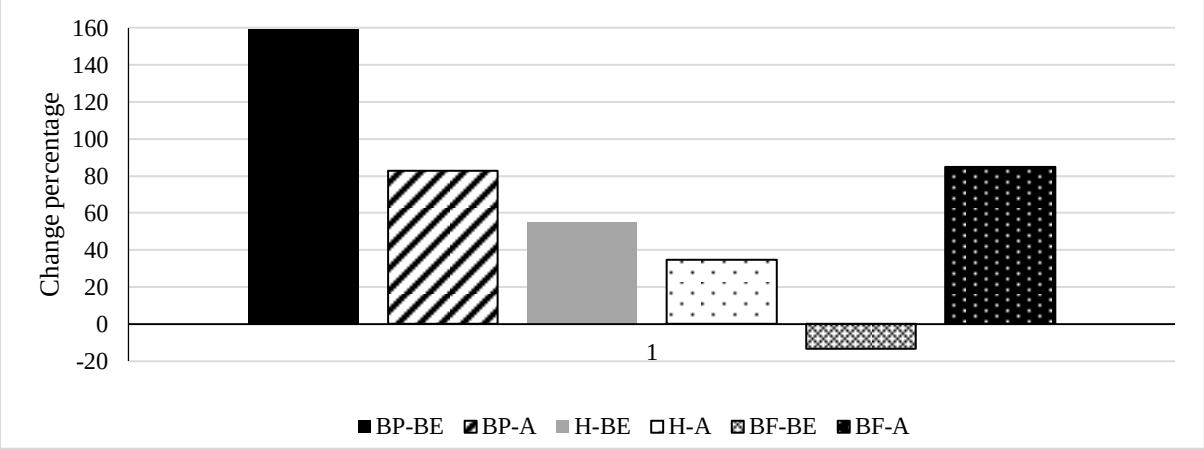

Fig 2. Change in different treatment honey area means comparing to control group

*BP-BE: Bee plus patty between frames; *BP-A: Bee plus patty above the frames; *H-BE: Home-made patty between frames; *H-A: Home-made patty above the frames; *BF-BE: Bee fonda between frames; *BF-A: Bee fonda patty above the frames

3.3. Effect on bee bread storage

Results in (Table 5) appeared that bee bread means areas that measured at June, 15, 2020 were not significantly differed between colonies.

Regarding inspection dates mean values, the highest value of mean bee bread areas (1094.3cm^2) was determined at the first inspection date (July, 6), then started to decrease significantly till the last inspection date. The interaction between feeding type and locations revealed that the highest

Table 5. Effect of different treatments on means of bee bread areas (in cm2)

Inspection dates	BP-BE	BP-A	H-BE	H-A	BF-BE	BF-A	Control	Mean ± SE
15/06/2020 (0 time)	599.3 FG	596.7 FG	581.7 FG	624.7 EFG	615 EFG	590.3 FG	573.3 FG	597.3 ± 6.7 b
06/07/2020	1767 A	1352 B	898.4 D	1144 C	803.4 DE	891 D	804.5 DE	1094.3 ± 81.8 a
27/07/2020	0 K	1092 C	0 K	140.9 IJK	550.7 FG	89.83 JK	440 GH	330.5 ± 82.7 c
17/08/2020	453.7 GH	734.1 DEF	0 K	0 K	277.7 HI	35.07 JK	212.7 IJ	244.8 57.2 c
Mean ± SE	705.0 ± 197.0 (b)	943.7 ± 90.2 (a)	370.0 ± 116.6 (e)	477.4 ± 135.9 (cd)	561.7 ± 58.0 (c)	401.6 ± 123.1 (de)	507.6 ± 65.1 (c)	566.7 ± 48.3

Values with different letters CAPITAL, small or (between brackets) in the same column or row are significantly different according to DMRT at 0.05 probability

*BP-BE: Bee plus patty between frames; *BP-A: Bee plus patty above the frames; *H-BE: Home-made patty between frames; *H-A: Home-made patty above the frames; *BF-BE: Bee fonda between frames; *BF-A: Bee fonda patty above the frames

significant bee bread area was registered in colonies fed with BP-A followed by BP-BE in the second rank, (943.7 and 705 cm^2, respectively), while the lowest value was observed in H-BE group, (370 cm^2). The results reveal that the lowest mean value of bee bread was recorded in BP-BE, H-BE and H-A groups (0 cm^2), while the highest value was in BP-BE group with 1767 cm^2.

The change percentage of mean bee bread areas in different treatments comparing to the control group (Fig. 3) showed that colonies fed with BP-A had highest increase percentage (245.1%), followed by BP-BE group with ratio of 113.3%. Colonies of both H-BE and H-A groups did not have any stored bee bread recording -100% decrease comparing to control group.

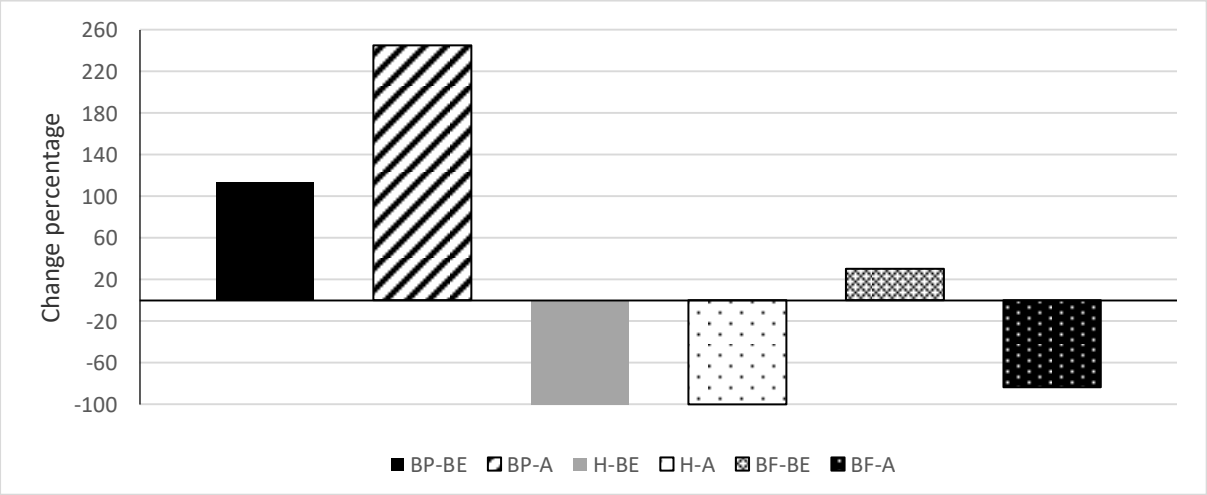

Fig 3. Change in different treatment bee bread area means comparing to control group

*BP-BE: Bee plus patty between frames; *BP-A: Bee plus patty above the frames; *H-BE: Home-made patty between frames; *H-A: Home-made patty above the frames; *BF-BE: Bee fonda between frames; *BF-A: Bee fonda patty above the frames

4. DISCUSSION

Pollen substitutes and supplements had proved to be essentially for honeybee colonies during dearth period especially if would be followed by Autumn season to maintain and stimulate the early buildup of honeybee colonies. Not only amount but also type and application method are important (Kumar *et al.*, 2013; Morais *et al.*, 2013; Kumar and Agrawal, 2014; Pande *et al.*, 2015; Shehata, 2016; Adgaba *et al.*, 2020; Ullah *et al.*, 2021)

Many researchers revealed the significant effect of pollen substitutes towards better over-wintering, growth rate and honey storage of colonies. However, natural pollen with the lowest crude protein percentage reported to perform better than pollen substitutes with a higher crude protein percentage (Matilla and Otis, 2006; Oliver, 2014).

In this research we compared the effect of one commercial pollen supplement and two pollen substitutes on the honeybee colonies development during dearth period. Results indicated that after 21 days of continuous supply of different patties, the mean of brood area of the first inspection date was the highest then it started to decrease significantly till the end of experiment which ensure the importance of supply colonies with protein nutrition.

The pollen supplement patty introduced between the frames had the highest mean of brood area at the first inspection and also as general mean of the other treatments, which in agreement with Avni *et al.*, 2009 and Adgaba *et al.*, 2020 who revealed the role of the introduction method in colonies growth rate. Despite adding vitamins and minerals to patties should enhance the effect of patties (Sihag and Gupta, 2013), adding of Bee Plus® spuriously decreased the effect of home-made patty on colonies growth to be in the same rank with control colonies. The decrease effect of Bee plus® brings up a question on how the product is interacting with regular patty which need more investigation.

In the same trend the change percentage of mean brood areas comparing to the control group revealed that introducing pollen supplement between frames is considered the best way to avoid honeybee colonies dwindling as colonies of this group had a 90.1% more brood area, this was in agreement with findings of Vasquez and Olofsson in 2009, Mattila *et al.* in 2012 and also confirmed by Adgaba *et al.*, 2020 who feed honey bee colonies using pollen powder packed in the frame cells and compared with other patties and found increase of about 86.3% more brood than the patty-form.

Honey mean storage took an increasing trend during experiment period which could be refer to the continuous supply with sugar syrup. The two ways of introducing Bee plus® patty to honeybee colonies resulted in the first and second rank of honey mean areas, which could be a result of the decrease in brood rearing in these colonies especially when data revealed that the Bee fonda patty who had the highest value of brood rearing had also the lowest value in honey storage. Naturally the change in the BP group was the highest comparing to other patties, these suggestions were in the same trend of Adgaba *et al.*, 2020.

The bee bread means areas reached its highest value in the first inspection date and decreased significantly in the following dates which confirm the existence of dearth period and the importance of protein feeding. In the same pervious trend, the BP group had the highest area and change of bee bread for probably the same reason of the decrease in reared brood which consume the most of food storage.

Feeding colonies between the frames had the best effect on brood rearing and honey storage regardless of patties type, while bee bread storage was not affected by patty introduction method. Patty type also had a significant effect on the reared brood area as both of BF and H patties was in same first rank followed by BP and control groups in the same rank. The BP also had the highest value of honey and bee bread mean areas regardless the introduction method.

5. CONCLUSION

Results revealed that the best way to preserve the colony development is supplying it with bee fonda patty between the brood frames which encouraged the brood rearing and consequently colony development growth rate.

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الملخص العربي

نوع الغذاء البروتيني المدعم او البديل وموضعه بالنسبة للحضنة يؤثر على تقدم طوائف نحل العسل

حاتم شرف الدين و محمد عبد الوهاب عبد الفتاح

قسم الحشرات الاقتصادية والمبيدات – كلية الزراعه – جامعة القاهرة – مصر

تعتبر حشرات نحل العسل الملقح الرئيسي لمعظم المحاصيل الهامة في العالم إلى جانب أهمية منتجاته الغذائية والعلاجية، وتعتمد طوائفه على العسل وخبز النحل المخزن في الخلايا لتستطيع البقاء على قيد الحياة خلال فترات القحط الغذائي بنجاح. قارنت هذه الدراسة تأثير ثلاثة أنواع مختلفة من التغذية البروتينية المجهزة في صورة عجائن مع المحلول السكري على معدل نمو الطوائف، ١- Bee Fonda® (BF) وهو مكمل حبوب اللقاح، ٢- بديل حبوب لقاح منزلي الصنع (H) يتكون ثلثي اجزائه من السكر المطحون والثلث من مسحوق خميرة المناحل الجافة، ٣- البديل المنزلي مخلوط مع Bee Plus® و٤- المحلول السكري فقط بنسبة (١ سكر : ١ماء). تم وضع العجائن داخل الخلايا في موضعين: الاول بين اقراص الحضنة على شريحة من حاجز ملكات ابعادها (١٥ x ٢٠ سم^{٢}) أو توضع الوجبة الغذائية فوق اقراص الحضنة. الطوائف التي تلقت BF بين الاقراص كان لها أعلى متوسط لمساحة حضنة الشغالات، بينما الطوائف التي تلقت BP بين الاقراص حققت أعلى متوسط من مساحة العسل المخزون. الطوائف التي تلقت BP فوق الاقراص حققت أعلى متوسط لمساحة خبز النحل المخزنة. وعلى ذلك فإن إدخال العجائن بين اقراص الحضنة كان له أعلى متوسط لمساحات الحضنة والعسل، بينما لم تتأثر مساحات خبز النحل. و من جهة تأثير نوع العجينة فان مجموعة الطوائف التي تم تغذيتها على BF حققت أعلى متوسط من الحضنة المرباة اما مجموعة الطوائف التي تم تغذيتها على BP فقد احرزت تقدما في كل من مساحات العسل وخبز النحل المخزن.