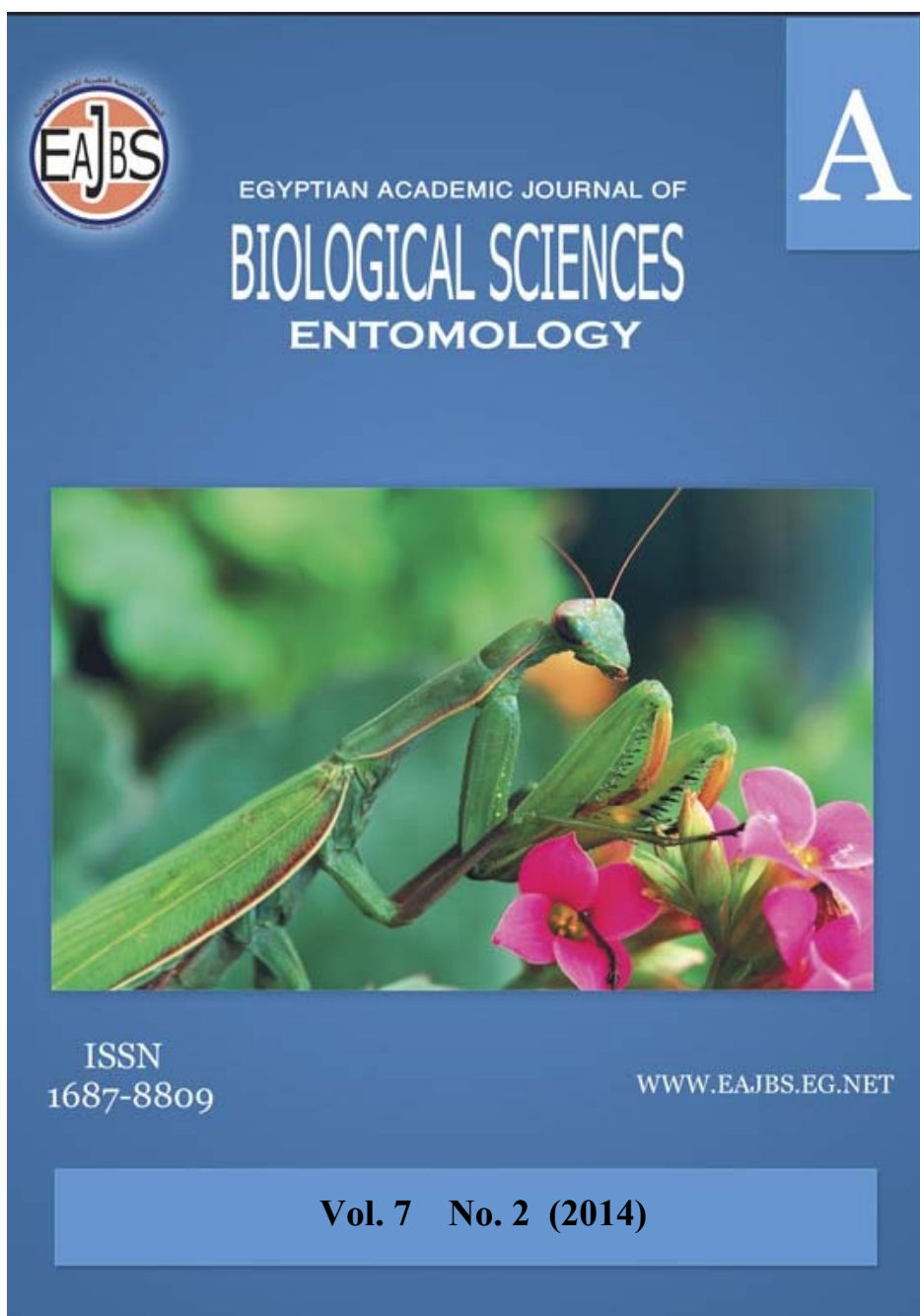


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## New Formula of Pollen Supplemental Diets to Study Honey Bee (*Apis mellifera carnica*) Attractiveness

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### ABSTRACT

The present work aimed to examine the attractiveness of ten new pollen supplemental diets to honey bees (*Apis mellifera*) through evaluating the consumption rate, besides their efficiency in supporting the colony through measuring colony growth (sealed workers brood area). Twelve materials were used and mixed with honey bee in different proportions forming diets taking numbers from 1 to 10. These materials were flours of: gram seeds, beans seeds, pea seeds, fenugreek seeds, coriander seeds, cumin seeds, anise seeds, white kidney beans seeds, caraway seeds, rice seeds, fennel seeds and oats. Diets were consumed at rates that were comparable to the control (sugar candy). Generally differences among consumption rates of supplemental pollen diets were highly significant. The highest consumed amounts were recorded in colonies fed on diet (1); 47.42gm/ colony in average, mix. (2); 43.35 gm/ colony in each week. While the lowest consumption rate (27.3 gm/ colony) in each week was recorded for diet no. 10 in average. There was a significant relationship between the amount of diet consumed and the increase in workers sealed brood area. Where colonies fed with mixture diets from gram seeds (Diet 2), dried pea with rice, fennel and fenugreek (Diet 3), dried beans seeds with fennel seeds (Diet 4) and oats, rice, anise seeds (Diet 1), showed 53.71%, 38.44%, 35.98% and 5.85% more brood area than control ones.

**Keywords:** honeybee, pollen supplement, sealed worker brood areas, phagostimulation, oat flour, fennel seeds flour, anise seeds flour, gram flour, fenugreek flour.

### INTRODUCTION

Pollen supplementary feeding plays a vital role in life of honey bee colony. Honey bees require protein (amino acids), carbohydrates (sugars), lipids (fatty acids, sterols), vitamins, minerals (salts) and water to survive. These nutrients must be in the diet in a definite qualitative and quantitative ratio for optimum nutrition. Adult bees obtain their dietary protein from either the collected pollen or the nitrogenous food stuffs provided by the beekeeper (Standifer *et al.*, 1977).

Supplemental feeding may help colony survival or brood rearing and colony development (Mohanna, 1977; Standifer *et al.*, 1977; El-Shemy, 1977 and Mohanna, 1999).

Bee pollen contains all the essential components of life. In addition, it corrects failings due to deficient or unbalanced nutrition (Royden Browen, 1994).

Honey bee colonies can be stimulated to increase in population even in the absence of sufficient foraging, through providing sugar syrup. Honey bees can deal with periods of shortage in nectar and pollen within their foraging range, by lowering their metabolism and reducing tasks and activities in the hive, such as foraging and brood rearing (Pernal and Currie, 2001; Kalev *et al.*, 2002; Keller *et al.*, 2005b). Less brood rearing eventually reduces the number of adult bees, including foragers, and may consequently affect pollination efficiency and honey yields (Duff and Furgala, 1986; Nelson, 1987; Fewell and Winston, 1992; Herbet, 1992; Herbert, 1999).

Adult bees can survive on carbohydrate (that is honey of sucrose) and water, however, proteins, lipids, or fats, minerals, and vitamins are necessary for young bees' growth and development, as well as rearing larvae and reproduction. Nectar and honey dew are the chief sources of supply for carbohydrates in the diet of bees, and pollen provides all other indispensable constituents (Stranger and Laidlaw, 1974; Dietz, 1975; Johanson and Johanson, 1976, 1977).

If nectar is insufficient, beekeepers can supply the bees with sugar syrup, which is a routine beekeeping practice. During the shortage or complete absence of pollen, or in the presence of only poor quality pollen, beekeepers often feed colonies of honey bees with either pollen substitute (with no pollen) or supplement (with pollen) diets in order to strengthen the colony. These are ideal materials that provide required nutrients to bees (Saffari *et al.*, 2004; Zahra and Talal, 2008; Al-Ghamdi *et al.* 2011; Rashid *et al.* 2013).

A wide range of plant and animal protein sources have been tested as substitutes for pollen, including soybean flour, peanut meal, whole wheat flour, brewer's yeast, corn flour, ground dry fish, powdered skim milk, egg albumin, and others (Erickson and Herbert, 1980; Doull, 1980; Lehner, 1983; Kalev *et al.*, 2002). Supplements contain bee-collected pollen mixed with other ingredients, for example soybean flour and honey, in order to form the desired patty consistency (Kalev *et al.*, 2002; Keller *et al.*, 2005b). Patty composition is important both for its nutritional value and for its effect on how readily bees consume it (Herbert, 1999). Bees tend to consume pollen supplements more readily than pollen substitutes and as a result of the higher consumption, to rear more brood (Standifer *et al.*, 1973; Keller *et al.*, 2005b).

Rashid *et al.* (2013) reported that honey bee colonies treated with supplemental gram diet produced higher honey yield than colonies that were fed on brewer's yeast, maize flour and pollen. Consequently, Gram supplemental diet with pollen is recommended to be a good substitute for pollen grains during the dearth period.

Honey bees consume carbohydrates composed patties faster than protein rich patties, as high sucrose content serve as phagostimulants that attract bees more (Scheiner *et al.*, 2004; Keller *et al.*, 2005a, 2005b; Schmidt and Hanna, 2006).

Waller *et al.* (1970) found that certain substances may increase the palatability of pollen substitutes such as soy flour, anise, fennel and milk.

The quality of food collected by honeybees has an important relationship to the overall hive development, and special attention must be given to the role that food plays on the development of the hypopharyngeal glands (HG). HG of *Apis mellifera* (Hymenoptera: Apidae) workers have been morphologically and physiologically studied due to their importance on the production of royal jelly (Gatehouse *et al.*, 2004; Cruz-Landim, 2009; Pinto, *et al.*, 2012).

Thus, such vital role of these glands provides motivation for improving the current knowledge on the development of adequate diets that stimulate the development of the glands, with the aim of empowering the production of this apicultural product of great

nutritional properties. In this context, the aim of this study was to evaluate the effect of different diets to know influence of new formulations of supplement feeding on honey bee colonies brood rearing all year round.

## MATERIALS AND METHODS

This work was carried out in Plant Protection Department's Apiary, Faculty of Agriculture, South Valley University during dearth period from February to July 2012. Carniolan hybrid bee race *Apis mellifera carnica* was chosen to initiate the planned experiments on, in order to evaluate:

### **Evaluation of the feeding preference degree and consumption rate for ten diets numbered from 1 to 10 by honey bee workers:**

- Thirty three honey bee colonies of equal strength nearly, divided into eleven groups; each group was fed with ten supplemental diets, while the eleventh group was fed on sugar candy and used as control. These diets composed of the following materials:
- Mixture of: 50% Flour of Oats (*Avena sativa*) + 25% Flour of Rice (*Oryza sativa*) + 25 % Flour of Anise Seeds (*Pimpinella anisum*) + honey (diet No. 1).
- Mixture of: 50% Flour of Fennel Seeds (*Foeniculum vulgare*) + 50% Flour of Gram Seeds (*Cicer arietinum*) + honey (diet No. 2).
- Mixture of: 25% Flour of Rice (*Oryza sativa*) + 40% Flour of Pea Seeds (*Pisum sativum*) + 25% Flour of Fennel Seeds (*Foeniculum vulgare*) + 10% Flour of Fenugreek Seeds (*Trigonella foenumgraecum*) + honey (diet No. 3).
- Mixture of: 50% Flour of Beans Seeds (*Vigna cowpea*) + 50% Flour of Fennel Seeds (*Foeniculum vulgare*) + honey (diet No. 4).
- Mixture of: 15% Flower of Fenugreek Seeds (*Trigonella foenumgraecum*) + 50% Flour of Dried Beans (*Vigna cowpea*) + 35% Flour of Fennel Seeds (*Foeniculum vulgare*) + honey (diet No. 5).
- Mixture of: 50% Flour of Dried White Kidney Beans Seeds (*Phaseolus vulgaris*) + 25% Flour of Dried Seeds (*Pisum sativum*) + 25% Flour of Coriander Seeds (*Coriandrum sativum*) + honey (diet No. 6).
- Mixture of: 50% Flour of Dried White kidney Bean Seeds (*Phaseolus vulgaris*) + 50% Flour of Caraway Seeds (*Carum carvi*) + honey (diet No. 7).
- Mixture of: 35% Flour of Beans Seeds (*Vigna cowpea*) + 35% Flour of Gram Seeds (*Cicer arietinum*) + 30% Flour of Coriander (*Coriandrum sativum*) + honey (diet No. 8).
- Mixture of: 50% Flour of Gram Seeds (*Cicer arietinum*) + 10% Flour of Fenugreek Seeds (*Trigonella foenumgraecum*) + 40% Flour of Cumin Seeds (*Cuminum cyminum*) + honey (diet No. 9).
- Mixture of: 50% Flour of Pea Seeds (*Pisum sativum*) + 50% Flour of Caraway Seeds (*Carum carvi*) + honey (diet No. 10).

Plant seeds were grinded until flour, and then mixed very well with honey according to the previously mentioned quantities. Afterwards, these ready to use diets were spread on a numbered plastic sheet having dimensions 66.6 x 66.6 x 2.2 mm in size; in order to increase surface area exposed to largest number of bees as much as possible, and kept in the refrigerator for 48 hours. Later, they were introduced to the experimented colonies at early morning at 8:00 AM every along the experiment period. These colonies were divided into three groups, of three colonies each, 100 grams of the diets were provided to each colony at 7 days interval, first group was fed on the pollen supplement No. 1 and the second till the tenth group were fed on pollen supplements 2, 3, 4, 5, 6, 7, 8, 9 and 10, respectively. The consumption rate of the diets was calculated by the difference between the weight of the diets

before and after consumption on daily basis for 13 weeks, for approximately four months, starting from the end of February 2012 till the beginning of July 2012.

**The impact of introducing supplemental feeding of different dietary pollen supplemental mixtures on sealed workers brood areas (in cm<sup>2</sup>):**

Fifteen honey bee colonies of similar strength nearly, the experimented colonies were divided into four groups each group included three colonies. The groups were fed on the above mentioned diets which illustrated in the second periods, diets 1, 2, 3 and 4 respectively. While the 5th group, was fed on sugar candy and used as a control.

The areas of sealed worker brood was measured at 13 days intervals, measurements of sealed brood areas of the workers were recorded at 13 days intervals using a plastic sheet (equal to standard Langstrouth frame) divided into squares.

**N. B:** sucrose syrup (2 sugar: 1 water) was continuously provided daily to each colony under investigation. The obtained data were tabulated and were analyzed using computer based software SPSS to calculate the least significant difference (L.S.D.) and the differences between treatments were tested by Duncan's multiple range test (Duncan, 1955).

## RESULTS AND DISCUSSION

### **Evaluation of the feeding preference degree and consumption rate for different mixtures diets by honey bee workers:**

Results in Table (1) showed significant differences among the attractiveness of different pollen supplements. The amount consumed from diets of pollen supplements were 47.42, 43.35, 40.09, 37.51, 35.30, 33.49, 31.91, 30.40, 28.89 and 27.36 for the diets 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, respectively and 25.32 for the control. There was statically significant difference between the first six diets versus the control diet. Also, it is clear from the same table that bees consumed Diet 1 by the highest rate (47.42 gm/ colony) in average, followed by Diet 2 that was 43.35gm/ colony. While, the lowest consumption rate (27.36 gm/ colony in average) was recorded for Diet 10.

Observations on consumption and preference of the supplemental pollen diets for the experimental period (13 weeks) indicated that was consumed by the highest rate (995.86 gm) with average 47.42 followed by 910.26 with average 43.35, which means that both Diet (1) and Diet (2) may be have a considerable amount of attractive volatile components which may not found in the other diets.

Pollen supplemental feeding has an efficient impact on the overall honey bee activities within the colony. It decompensate pollen shortage during severe conditions such as presence of a threatening predator, empowering bees to survive while suffering from either internal or external parasites, or during passing through dearth period and shortage of pollen especially in winter or raining seasons. It is effective in stimulating brood rearing and increasing honey, but it must be palatable (attractive) to bees and nutritious; thus our findings agreed with those described by (Standifer *et al.*, 1973; Mattila and Otis 2006; Nabors, 2000; DeGrandi-Hoffman *et al.*, 2010).

Data obtained in the entire study showed that the honey bees were attracted and consumed to Diet 1; oats, rice and anise in high rates and very fast regardless its nutritional value, because the diet composed mainly of carbohydrates (high sucrose content) that serve as phagostimulatnts that increases honey bees response toward this patty according to (Scheiner *et al.*; 2004; Keller *et al.*; 2005a, 2005b; Schmidt and Hanna, 2006). In addition, the presence of anise increased palatability of the diet, accordingly positively affected the consumption rate. The second highly consumed amount is that of Diet 2; that composed of gram (rich protein content) with fennel; which increases the palatability of the diet (Waller *et al.*, 1970). Similarly, consumption of the third diet was significantly high due to the presence

of rice, fennel, fenugreek and peas and that is relatively confirms to results published by (Mohanny *et al.*, 2008).

Table 1: The Degree of Preference and Rate for Dietary Pollen Supplemental Mixture Diets (in gm) from February to July 2012.

Date	No. of Pollen Supplemental Diets										
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	Control
20-02-12	29.42	27.61	26.34	24.77	23.37	22.26	21.23	20.36	19.43	18.25	19.30
27-Feb	28.407	26.67	26.40	25.81	23.96	22.91	22.33	21.19	20.56	19.41	20.90
06-Mar	29.713	28.19	27.00	25.06	23.25	22.26	21.32	20.48	19.42	18.39	20.94
13-Mar	26.99	25.47	24.94	24.02	23.16	22.21	21.16	20.40	19.51	18.30	18.67
20-Mar	27.343	26.65	26.32	25.26	24.85	23.65	22.85	21.66	20.78	19.60	15.00
27-Mar	29.633	27.76	26.67	25.73	24.42	23.04	22.00	20.94	19.81	18.49	21.00
03-Apr	49.733	46.10	43.00	40.46	38.15	36.27	34.52	32.61	30.81	29.25	30.00
10-Apr	55.477	46.77	43.17	40.86	38.68	36.83	35.16	33.53	31.97	30.50	32.80
17-Apr	58.567	52.08	45.96	42.06	39.12	36.73	34.75	33.29	31.60	30.04	27.96
21-Apr	55.653	49.63	46.14	43.50	41.19	39.07	36.93	35.07	33.29	31.71	30.56
28-Apr	64.433	58.17	52.60	49.26	46.47	44.70	42.79	41.13	38.65	36.43	31.00
01-May	71.8	64.87	59.38	55.47	52.47	50.00	47.57	45.30	43.08	40.67	23.50
08-May	60.466	49.85	45.23	41.63	39.73	37.93	36.47	35.03	33.55	32.00	33.60
15-May	65.5	60.00	53.62	49.48	46.15	43.38	41.10	39.03	37.04	35.54	31.91
22-May	70.34	65.37	60.69	56.60	52.94	50.01	47.51	45.18	42.51	40.17	29
29-May	80.63	76.07	70.34	61.44	55.93	52.10	48.71	46.38	44.02	41.88	26.6
05-Jun	76.2	69.76	60.25	56.34	52.90	49.91	47.57	44.56	41.95	39.71	35.12
12-Jun	29.79	27.68	25.61	24.45	23.00	21.74	20.96	20.01	18.93	17.68	21.9
19-Jun	28.29	26.88	25.53	24.88	23.64	22.63	21.54	20.51	19.67	18.58	22
26-Jun	27.55	26.84	26.28	25.38	23.98	22.66	21.76	20.94	20.09	19.16	15.9
02-Jul	29.93	27.82	26.48	25.23	24.00	22.97	21.89	20.90	19.93	18.84	24.09
Total	995.86	910.26	841.95	787.7	741.37	703.25	670.14	638.49	606.59	574.60	531.75
Average	47.42	43.35	40.09	37.51	35.30	33.49	31.91	30.40	28.89	27.36	25.32
LSD	22.97*	18.89*	15.64*	13.06*	10.85*	9.03*	7.46	5.95	4.43	2.91	-

\* The mean difference is significant at the 0.05 level.

- The shaded area in the table represents cessation in the foraging flights due to annual flights of Bee-eater bird; *Merops* sp.

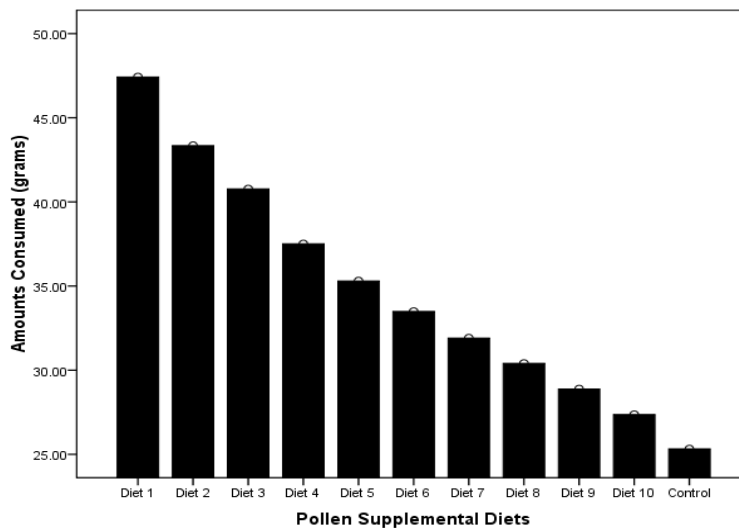


Fig. 1: Average amounts of diets consumed by colonies during seven days intervals and for the 21-week study period. (L.S.D. post hoc test, P<0.05)

Likewise, 4<sup>th</sup> diet that fennel beans. However, when the proportion of fenugreek from 10% to 15% in the 5<sup>th</sup> diet in a mix. with beans and fennel the consumption declined, this might be due to its bitter taste. Finally, the presence of anise and fennel increased honey bee response towards the offered diets due to their aromatic characteristics, thus leading to high consumption rates of the overall offered diet.

Interestingly, during of presence *Merops* sp., consumption rate of each diet in the study increased; as honey bees were not able to conduct out-hive activities such as foraging and such severe condition caused bees to consume more of the offered diets inside the colony. Indeed, the Bee- eater bird (*Merops* sp.) usually visits twice a year; during Spring (optimum time of many honey bee activities such as queen rearing and extensive foraging flights) and Autumn, and stays nearby apiaries for a period that exceeds a month or six weeks, threatening field tasks for bees.

### The impact of introducing supplemental feeding of different dietary pollen supplemental diets on sealed workers brood areas (in cm<sup>2</sup>):

The obtained results presented in Table (2) indicated a considerable increasing in workers sealed brood areas after supplying the diets.

The increase of workers sealed brood areas is represented by the following descending order of percentage 53.71%, 38.44%, 35.98% and 5.85% for diet number 2, 3, 4, and 1 compared with control treatment respectively.

Table 2: Supplemental Feeding on Different Diets and Sealed Workers Brood Areas (in cm<sup>2</sup>) February to July 2012.

Date	Pollen Supplemental Diets				
	No. (1)	No. (2)	No. (3)	No. (4)	Control
2-3-2014	1260	2880	3150	1890	1715
14-Mar	1800	3060.5	2700	3015	1890
22-Mar	2340.5	4140	1620	2880	2290
04-Apr	585.66	900	720	630	495.5
17-Apr	540	855	585	495	360
30-Apr	360.66	540.66	500.66	540	315
13-May	450	810	495	450.5	225
26-May	315.5	585	450	315	180
08-Jun	1890	2800	2700	2520	1620
21-Jun	1260.6	5400	3600	3150	1080
<b>Total</b>	10802.92	21971.16	16520.66	15885.5	10170.5
<b>Average</b>	1080.29	2197.1	1652.1	1588.55	1017.05
<b>% increasing</b>	5.85	53.71	38.44	35.98	-
<b>LSD</b>	63.24	1180.07*	635.02	571.50	-

\* The mean difference is significant at the 0.05 level.

- The shaded area in the table represents cessation in the foraging flights due to annual flights of Bee- eater bird; *Merops* sp.

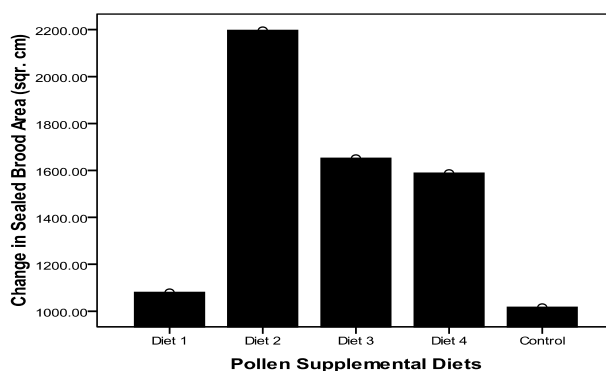


Fig. 2: Average change in workers sealed brood area during thirteen days intervals and for the 10-week study period. (L.S.D. post hoc test,  $P < 0.05$ ).

As for Table (2), it may be clear that the percentage of increasing of sealed brood areas is significantly high for colonies fed on diet number (2); it reaches 53.71%, followed by colonies fed on the third diet (38.44). The percentage of increasing in workers sealed brood areas in colonies fed diet number (4) is also significantly high; as it is 35.98, while colonies fed on diet number (1) recorded the lowest percentage (5.85) comparing to control colonies.

According to obtained results, it could be concluded that supplying honey bee colonies with pollen supplements has a very positive effect on activity of the colonies in general. As in Table (2), gram and fennel diet highly and effectively stimulated bees to rear more brood and increase sealed brood area, this agrees with (Waller *et al.*, 1970, Rashid *et al.* 2013), this might be due to the high protein content in gram and phagostimulative effect of fennel that induced high royal jelly production, resulting in increasing the ability of nursing workers to feed and rear more brood. Accordingly, colonies fed on Diet 3; rice, fenugreek and fennel along with peas (which is known to have high protein level) produced significantly larger sealed brood area than colonies fed on control diet composed of sugar candy. Similarly, diet of beans and fennel enhanced brood rearing and caused bees to produce more brood, relatively equal to Diet 4. While, the lowest brood area was produced in colonies that fed on the carbohydrate rich diet and no protein source.

In addition to nutrition quality, brood rearing is also influenced by the quality of the queen. There might have been some variations among queens in the number of eggs they could potentially lay per day and this might have affected the obtained results. According to the amounts of diet consumed, there is a strong relationship between consumed amounts and increasing in sealed brood area, concluding that still type of diet being fed and its preference for bees have a strong influence on brood production.

The shaded area of the table represents brood rearing during the presence of Bee-eater, it is obvious from data that sealed brood area declined hardly, as the offered diet along with sugar syrup helped honey bees to only sustain life, but not in producing more brood. However, bees tended to store honey during that time as several frames of honey were present in the colony.

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### ARABIC SUMMARY

#### وصفات جديدة لوجبات مكملة لحبوب اللقاح لدراسة إنجذاب النحل (*Apis mellifera*)

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تناولت هذه الدراسة دراسة إنجذاب نحل العسل لعشرة وجبات مكملة لحبوب اللقاح من خلال تحديد معدل إستهلاك هذه الوجبات، بالإضافة إلى كفاءتها في دعم الخلية من خلال قياس الزيادة في مساحة الحضنة المغلقة للشغالات. حيث تم إستخدام نسب مختلفة من إثنى عشر مادة في هذه الدراسة بعد خلطها بالعسل وذلك لتحضير الوجبات المرقمة من ١ إلى ١٠. إشتملت هذه المواد على دقيق بذور كل من: الحمص، اللوبيا، البسلة، الفاصوليا، الحلبة، الشمر، الينسون، الكراوية، الأرز، الكمون، الشوفان والكسبرة.

عند مقارنة معدل إستهلاك خلايا نحل العسل لهذه الوجبات وجدنا أنه توجد فروق معنوية فيما بينها، حيث سجلت الوجبة ١ أعلى معدل إستهلاك (٤٧.٤٢)، يليها الوجبة الثانية بمتوسط إستهلاك ٤٣.٣٥، بينما كان أقل معدل إستهلاك في خلايا النحل التي تغذت على الوجبة ١٠.

علاوة على ذلك، وجدنا أنه هناك علاقة معنوية بين معدل إستهلاك الوجبات والنسبة المئوية للزيادة في مساحة الحضنة المغلقة للشغالات وصلت نسبتها إلى ٥٣.٧١%، ٣٨.٤٤%، ٣٥.٩٨% و ٥.٨٥% للوجبات ٢، ٣، ٤ ثم ١ على التوالي. من ذلك نستنتج أن أفضل هذه الوجبات كانت وجبة الحمص والشمر يليها وجبة الأرز مع البسلة، الشمر والحلبة ثم وجبة اللوبيا والشمر وأخيرا وجبة الشوفان مع الأرز والينسون.