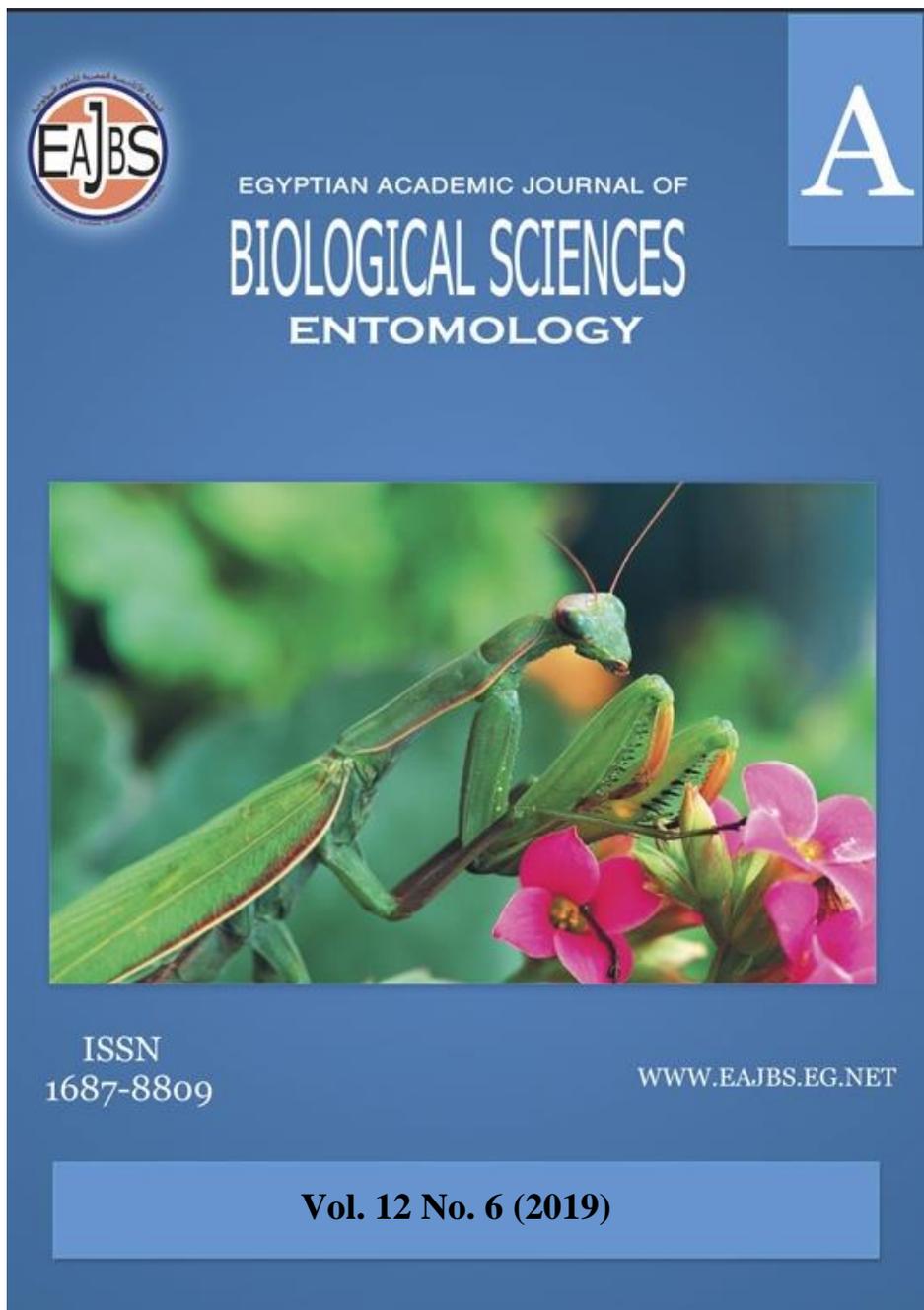


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Evaluation of Ten Supplemental Diets to Enhance Some Honey Bee (*Apis mellifera* L.) Activities during Winter Season in Egypt.

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ABSTRACT

This work was carried out at the apiary of Bee Research Department, Quanter branch, Qaluobia governorate during the period from December 2016 to April 2017 to evaluate the effect of feeding honeybee colonies with ten diets as pollen substitutes on diets consumption rates, brood rearing, pollen and honey storage activities. Six Proteinaceous materials were used as follows; defatted soybean flour, chickpea flour, maize flour, pea flour, wheat germ and dried brewer's yeast, were mixed with sugar powder, bee honey and water in different proportions forming ten diets taking letters from A to J. The obtained results revealed that colonies were fed on Diet H (chickpea flour + wheat germ + dried brewer's yeast) produced the highest average of biological activities, which were 105.54 g, 363.75 inch², 134.83 inch² and 404.08 inch²/colony for diet consumption, sealed workers brood area, stored pollen area and stored honey area, respectively. On the contrary, the colonies fed on Diet C (pea flour + defatted soybean flour + dried brewer's yeast) produced the lowest average of biological activities, which were 77.0 g., 235.1 inch², 75.43 inch² and 258.39 inch²/colony for diet consumption, sealed workers brood area, stored pollen area and stored honey area, respectively. There were a direct relationship between diets consumption and each of brood rearing, pollen and honey storage activities during winter season compared with control colonies, which showed a noticeable decrease in all of these biological activities.

INTRODUCTION

Pollen grains are the male germs of flowers, rich in high quality protein, which serves as the building material for growth and tissue repair to honey bee colonies (Somerville, 2000; Alghamdi, 2002; Mishima *et al.*, 2005). Honeybee (*Apis mellifera* L.) colonies need pollen and nectar to fuel foraging flights, generate heat to thermoregulate their nest and to rear brood. Nectar is a carbohydrate source, while pollen supplies the bees with the protein, lipids, vitamins, and minerals needed to rear larvae (DeGroot, 1953; Manning, 2001). Pollen supplementary feeding plays a vital role in the life of the honeybee colony. Honeybees 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 foodstuffs provided by the beekeeper (Standifer *et al.*, 1977). The lowest pollen-gathering period was in winter within

two years of study (Younis, 2006). Testing and utilization of different materials as pollen supplements have been mainly directed toward producing brood, and less attention has been given to the pollen gathering and honey production during the spring and summer seasons of the year. The pollen is more attractive to bees than the other proteins (El-Banby and Gorgui, 1970). Honeybees consume carbohydrates composed patties faster than protein-rich patties, as high sucrose content serves as phagostimulants that attract bees more (Scheiner *et al.*, 2004; Keller *et al.*, 2005a, 2005b; Schmidt and Hanna, 2006). Various types of Brewer's yeast have found extensive application in pollen substitute formulations (Johanson and Johanson, 1977 and Herbert and Shimanuki, 1978). Colonies fed with mung bean, wheat germ, soybean, yeast, and *Sativa vulgaris* showed worker brood areas more than the control ones (EL-Shaarawi, 2001). Honey bee colonies provided with pollen substitutes of bran, yeast and chick pea reared more worker brood than in case on unfed ones (control colonies) (EL-Waseef, 2002). Soybean flour, soybean meal, wheat gluten, and bread yeast can be used as pollen supplements. The amount of food consumption was not significantly different among the treatments (Abbasian and Ebadi, 2002). Feeding honey bee colonies with pollen substitutes significantly increased the sealed brood area and egg-laying rates of Queens than control colonies which were fed on sugar syrup (1:1) only (Ghazala, 2006). Pollen substitutes can overcome a lack of natural food and reduce the weakening and loss of colonies during critical periods. Protein supplementation is a key management tool to maintain the strength of bee colonies during the period of pollen shortage (Moja, *et al.*, 2015).

The aim of this study was to evaluate the effect of ten mixtures of diets as pollen substitutes on diets consumption rates, brood rearing, pollen, and honey storage during winter season.

MATERIALS AND METHODS

This work was carried out in Plant Protection Research Institute at the apiary of Bee Research Department, Quanter branch, Qaluobia governorate during the period 8/12/2016 - 6/4/ 2017 to evaluate the effect of feeding honeybee colonies with ten mixture diets as pollen substitutes on food consumption rates, brood-rearing rates, honey, and pollen storage.

Experimental Colonies:

Thirty-three honeybee colonies (Carniolan hybrid bee race *Apis mellifera carnica*) with relatively the same strengths were used. Each contained 5 combs (2 combs of sealed and unsealed brood, 2 combs of stored honey and pollen and one empty comb) covered with bees. The bee colonies were divided into 11 groups, of three colonies each, ten of them were received experimental diets and the eleventh used as control. Empty combs and frames provided with a new wax foundation were added when necessary.

Tested Diets:

Six materials that are rich in their protein contents and available in the local area were selected to make ten mixtures of diets (Table 1) to test them as pollen substitute.

Plant seeds (chickpea, maize and pea) were grinded until flour, however defatted soybean flour, wheat germ and dried brewer's yeast are available in local markets in a suitable form. Proteinaceous materials for each diet were mixed very well with sugar powder and bee honey and water according to the previously mentioned quantities in Table (1) before using.

Table 1: Description of diet ingredients.

Materials	Composition of the diets / 1 Kg.									
	A	B	C	D	E	F	G	H	I	J
Defatted Soybean flour (g.)	62	62	62	62						
Chickpea flour (g.)				246	154			154		154
Maize flour (g.)		246			154	154	154			
Pea flour (g.)			246				154		154	154
Wheat germ (g.)	246					154		154	154	
Dried Brewer's yeast (g.)	62	62	62	62	62	62	62	62	62	62
Sugar powder (g.)	315	315	315	315	315	315	315	315	315	315
Bee Honey (ml)	251	251	251	251	251	251	251	251	251	251
Water (ml)	64	64	64	64	64	64	64	64	64	64
Total (g.)	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

Treatment of Honeybee Colonies:

Every honeybee colony was received 150g. of the diet + one litre of sugar syrup (1:1) weekly during the experimental period. While, control colonies were received just one litre of sugar syrup (1:1) weekly for each one. All tested diets were fed in patties that placed directly over the brood nest of tested bee colonies and covered with plastic sheets to avoid drying. Consumption rates of diets were measured by weighting unconsumed portions every 7 days intervals.

Measurements of Biological Activities:

Areas of sealed workers brood cells, honey cells and pollen grains cells (bee bread) were measured in square inches by using a typical Langstroth wireframe divided into square inches before the beginning of the experiment and after feeding at 13 days intervals till the end of the experiment.

Statistical Analysis:

The experimental design for all the experiments mentioned above was a completely randomized design (CRD). ANOVA was performed and means were compared by using Duncan's multiple range tests at 5% level of probability (Duncan, 1955) with the SAS 9.1.3 programme (SAS Institute, 2004).

RESULTS AND DISCUSSION**The Consumption Rates Of Diets :**

Results in Table (2) Showed significant differences among the attractiveness of different diets, except Diets D and F there were no significant differences between them and Diets A and E.

The mean amounts of consumed diets (pollen substitutes) during the experimental period were 95.85, 83.43, 77.0, 94.12, 92.36, 94.63, 87.61, 105.54, 88.48 and 84.37 g./colony for the Diets A to J, respectively.

Also, it is clear from the same table and Fig. (1) that, bees consumed Diet H significantly by the highest rate with average 105.54 g./colony, followed by Diet A that was 95.85 g./colony. While, the lowest consumption rate was recorded for Diet C with average 77.0 g. /colony.

Table (2): Rate of food consumed (g.) by honeybee colonies fed on ten types of diets during winter season 2016-2017.

Date	Diets									
	(A) Wheat germ + Soybean	(B) Maize + Soybean	(C) Pea + Soybean	(D) Chickpea + Soybean	(E) Maize + Chick pea	(F) Maize + Wheat germ	(G) Maize + Pea	(H) Chickpea + Wheat germ	(I) Pea + Wheat germ	(J) Chickpea + Pea
15-Dec-16	72.62	62.13	58.59	69.85	66.02	73.27	71.49	86.19	65.50	65.15
22-Dec-16	76.18	64.54	63.45	75.03	73.14	75.29	72.58	84.93	73.59	66.54
29-Dec-16	78.79	62.51	61.53	72.94	89.21	72.94	67.67	81.53	71.67	63.65
5-Jan-17	83.58	67.37	63.03	79.68	85.64	79.68	65.89	84.18	74.54	61.00
12-Jan-17	76.22	65.91	64.83	76.59	86.73	78.04	62.68	93.25	77.51	58.03
19-Jan-17	99.00	69.68	72.18	78.72	86.13	91.62	85.63	97.21	79.62	79.67
26-Jan-17	69.23	73.45	73.48	91.62	88.85	82.28	84.98	99.52	83.96	78.09
2-Feb-17	88.60	79.32	67.06	82.28	87.49	92.20	85.14	97.48	84.51	79.90
9-Feb-17	90.22	85.33	63.92	92.20	84.36	98.79	89.33	97.95	87.33	83.67
16-Feb-17	91.83	85.20	70.86	98.10	88.02	94.53	87.00	98.41	87.45	83.75
23-Feb-17	107.80	89.78	86.02	98.79	91.67	78.72	91.24	103.27	88.45	85.14
2-Mar-17	102.78	94.90	81.36	94.53	97.30	98.10	98.46	108.59	93.70	90.56
9-Mar-17	112.82	98.31	76.23	103.06	100.33	104.08	102.64	116.47	98.23	95.66
16-Mar-17	109.70	95.20	83.87	104.08	102.67	103.06	97.12	123.77	98.79	102.87
23-Mar-17	116.67	100.00	95.93	117.38	107.40	117.05	101.67	136.72	107.30	105.20
30-Mar-17	123.78	109.35	112.00	130.67	114.79	130.67	109.11	139.67	113.80	115.23
6-Apr-17	129.67	115.26	114.69	134.53	120.45	138.34	116.78	145.02	118.13	120.23
Average	95.85 b	83.43 e	77.00 f	94.12 bc	92.36 c	94.63 bc	87.61 d	105.54 a	88.48 d	84.37 e

Means in a row with dissimilar letters differ significantly at 0.05 level of probability

Brood Rearing Activity:

Results obtained in Table (3) stated highly significant differences in brood rearing activity between control colonies and all treated colonies with tested pollen substitutes. The mean areas of sealed workers brood were 342.65, 266.37, 235.10, 329.43, 280.31, 303.82, 256.23, 363.75, 298.45, 290.01 and 171.98 inch²/colony, for the Diets A to J and Control colonies, respectively.

Also, the same table and Fig. (1) clearly showed that, colonies fed by Diet H were significantly produced the highest rate of sealed workers brood with average 363.75 inch²/colony, followed by Diet A that was 342.65 inch²/colony. While, the lowest brood-rearing rate was recorded for colonies fed by Diet C with average 235.10 inch²/colony and control colonies (treated by sugar syrup only) with average 171.98 inch²/colony.

Pollen Storage Activity:

Data tabulated in Table (4) showed highly significant differences in pollen grains storage between control colonies and all experimental colonies with tested diets. The mean areas of stored pollen grains were 119.63, 97.52, 75.43, 110.35, 100.61, 114.01, 87.30, 134.83, 100.33, 102.43 and 61.53 inch²/colony, for the colonies fed with Diets A to J and control colonies, respectively.

Also, Table (4) and Fig. (1) demonstrated that, colonies fed by Diet H were significantly produced the highest rate of pollen grains storage with average 134.83 inch²/colony, followed by Diet A that was 119.63 inch²/colony. While, the lowest pollen grains storage rate was recorded for colonies fed by Diet C with average 75.43 inch²/colony and control colonies with average 61.53 inch²/colony.

Table (3): Mean areas (inch²) of sealed workers brood in honeybee colonies fed on ten types of diets during winter season 2016-2017

Date	Diets										Control
	(A) Wheat germ + Soybean	(B) Maize + Soybean	(C) Pea + Soybean	(D) Chickpea + Soybean	(E) Maize + Chickpea	(F) Maize + Wheat germ	(G) Maize + Pea	(H) Chickpea + Wheat germ	(I) Pea + Wheat germ	(J) Chickpea + Pea	
Before feeding											
8-Dec-16	179.67	185.00	189.00	182.67	175.00	178.67	180.33	173.67	173.00	175.43	182.00
After feeding											
21-Dec-16	212.34	165.00	167.33	238.34	200.00	213.20	170.33	226.50	210.30	219.32	135.56
3-Jan-17	221.50	193.33	161.96	232.67	174.75	220.33	183.67	278.33	223.00	215.34	128.00
16-Jan-17	286.00	205.00	176.33	271.00	212.33	249.98	193.67	291.50	225.50	262.12	145.56
29-Jan-17	295.33	208.00	188.33	275.33	212.82	260.63	197.67	356.83	249.10	237.61	143.33
11-Feb-17	336.24	237.66	198.67	347.33	289.36	333.39	199.67	407.33	262.12	242.80	161.78
24-Feb-17	430.10	317.67	220.00	342.67	311.88	349.05	333.67	425.67	332.43	331.56	163.11
9-Mar-17	450.23	356.19	302.33	403.33	375.57	374.13	330.33	449.00	406.50	347.23	200.78
22-Mar-17	482.99	352.50	326.00	472.33	398.35	424.87	384.33	463.34	440.12	426.91	221.00
4-Apr-17	532.11	443.33	421.00	528.66	453.07	433.97	388.67	565.33	462.43	441.80	238.67
Average	342.65 b	266.37 ef	235.10 g	329.43 b	280.31 de	303.82 c	256.23 f	363.75 a	298.45 cd	290.01 cd	171.98 h

Means in a row with dissimilar letters differ significantly at 0.05 level of probability

Table (4): Mean areas (inch²) of stored pollen in honeybee colonies fed on ten types of diets during winter season 2016-2017.

Date	Diets										Control
	(A) Wheat germ + Soybean	(B) Maize + Soybean	(C) Pea + Soybean	(D) Chickpea + Soybean	(E) Maize + Chickpea	(F) Maize + Wheat germ	(G) Maize + Pea	(H) Chickpea + Wheat germ	(I) Pea + Wheat germ	(J) Chickpea + Pea	
Before feeding											
8-Dec-16	111.67	106.67	107.33	108.00	117.81	102.49	121.34	119.88	104.90	109.15	112.33
After feeding											
21-Dec-16	100.00	96.67	82.67	93.87	117.24	105.92	118.33	130.13	109.92	97.68	67.67
3-Jan-17	131.66	110.00	95.00	134.67	124.12	123.49	116.00	140.78	131.14	112.39	76.33
16-Jan-17	121.67	82.50	73.33	104.67	91.68	111.60	78.33	121.22	98.77	99.46	63.00
29-Jan-17	99.17	59.75	53.33	93.34	80.53	97.91	47.34	106.13	79.56	87.32	43.67
11-Feb-17	94.67	57.00	52.07	82.00	75.82	97.42	43.33	102.68	70.11	84.52	32.33
24-Feb-17	122.48	111.67	66.66	107.66	97.19	121.40	78.33	137.97	93.36	102.77	34.67
9-Mar-17	136.33	117.66	78.33	123.34	107.80	130.22	91.00	165.01	104.22	111.43	54.33
22-Mar-17	123.33	109.00	58.87	106.33	82.41	113.73	72.31	143.07	95.07	103.05	50.00
4-Apr-17	155.33	124.33	86.67	149.67	111.52	135.93	106.67	181.40	116.22	116.50	81.00
Average	119.63 b	97.52 d	75.43 f	110.35 c	100.61 d	114.01 bc	87.30 e	134.83 a	100.33 d	102.43 d	61.53 g

Means in a row with dissimilar letters differ significantly at 0.05 level of probability.

Honey Storage Activity:

Data recorded in Table (5) showed highly significant differences in honey storage activity between control colonies and all treated colonies with tested diets. The mean areas of stored honey (inch²) were 399.94, 303.79, 258.39, 396.50, 316.14, 321.22, 269.23, 404.08, 321.24, 310.28 and 226.82 inch²/colony, for the colonies treated with Diets A to J and control colonies, respectively.

Also, it is clear from the same table and Figure (1) that, colonies fed by Diet H has produced the highest rate of honey storage with average 404.08 inch²/ colony, followed by Diet A that was 399.94 inch²/colony and Diet D that was 396.50 inch²/colony without

significant differences among them. While, the lowest honey storage rate was recorded for colonies treated by Diet C with average 258.39 inch²/colony and control colonies with average 226.82 inch²/colony.

Table (5): Mean areas (inch²) of stored honey in honeybee colonies fed on ten types of diets during winter season 2016-2017.

Date	Diets										Control
	(A) Wheat germ + Soybean	(B) Maize + Soybean	(C) Pea + Soybean	(D) Chickpea + Soybean	(E) Maize + Chickpea	(F) Maize + Wheat germ	(G) Maize + Pea	(H) Chickpea + Wheat germ	(I) Pea + Wheat germ	(J) Chickpea + Pea	
Before feeding											
8-Dec-16	317.00	310.33	337.33	316.33	319.67	332.67	340.67	315.00	321.67	307.23	320.66
After feeding											
21-Dec-16	410.33	239.00	271.67	387.00	245.33	354.00	244.67	402.67	370.00	228.25	271.67
3-Jan-17	434.17	276.34	288.00	325.67	323.67	392.00	183.00	341.67	321.00	313.85	177.50
16-Jan-17	403.18	259.88	237.00	310.67	328.67	339.00	224.33	399.50	270.17	235.50	127.50
29-Jan-17	298.33	280.02	170.00	361.67	283.00	327.67	157.00	299.33	262.47	274.12	156.75
11-Feb-17	311.18	271.00	145.00	339.00	270.00	192.33	160.00	322.00	270.15	276.25	136.75
24-Feb-17	321.67	278.00	212.67	348.33	299.67	207.67	258.67	340.50	285.20	285.78	197.07
9-Mar-17	428.23	297.67	271.23	498.33	296.67	288.50	339.00	443.34	351.42	382.23	253.53
22-Mar-17	525.20	367.35	315.33	510.00	396.67	330.33	370.67	540.33	360.54	385.65	303.43
4-Apr-17	550.11	458.33	335.67	568.00	398.00	448.00	414.33	636.50	399.80	413.90	323.37
Average	399.94 a	303.79 b	258.39 c	396.50 a	316.14 b	321.22 b	269.23 c	404.08 a	321.24 b	310.28 b	226.82 d

Means in a row with dissimilar letters differ significantly at 0.05 level of probability.

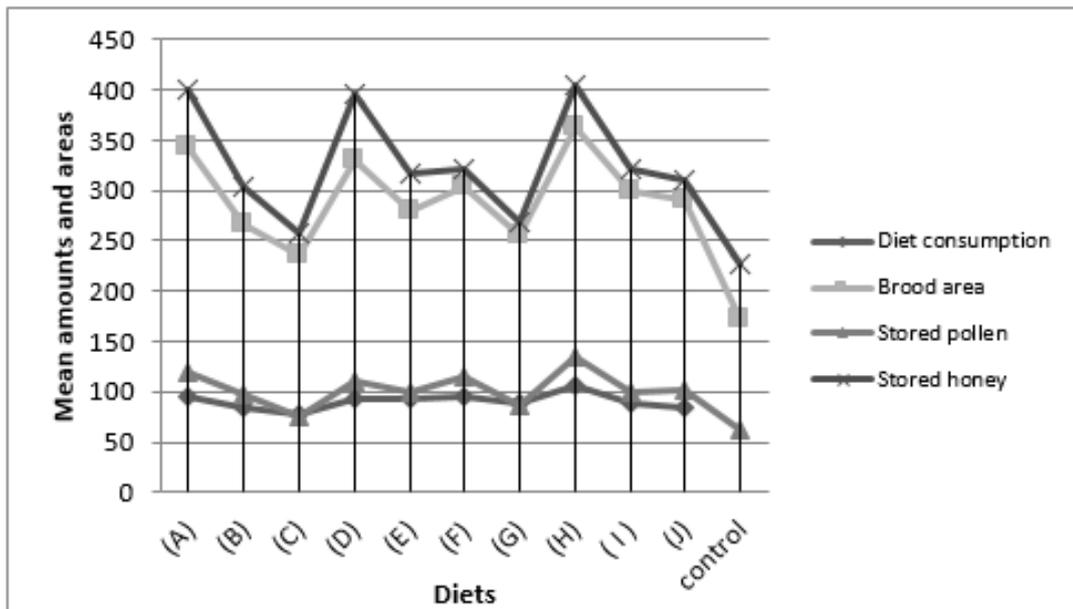


Fig. 1: Mean amounts (g.) of diets consumption in relation with mean areas (inch²) of worker brood, stored pollen and honey during winter season 2016-2017.

From results in Figure 1 and Tables 2, 3, 4 and 5 it was observed that there was a continuous increase in consumption rates of different pollen substitutes which has an efficient impact on the overall honey bee activities within the colony especially at the beginning of March due to the improvement of weather conditions. Also it is very clear that, there was a

direct relationship between diets consumption and each of brood rearing, pollen storage and honey storage during winter season compared with control colonies which showed a noticeable decrease in all biological activities. Thus our findings agreed with those described by (Standifer *et al.*, 1973; Nabors, 2000; Mattila and Otis 2006 and DeGrandi-Hoffman *et al.*, 2010). Feeding colonies with pollen substitute cake beside sugar syrup stimulate queens to lay more eggs and to workers encourage rear more brood, this increase in brood rearing reflects on colony population and pollen and honey production (Nabors, 2000; Ghazala, 2006 and Ghazala and Nowar, 2013). Pollen substitute feeding has an efficient impact on the overall honey bee activities within the colony. It compensates pollen shortage during severe conditions passing through the 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 attractive to bees and nutritious (Aly, *et al.*, 2014).

According to obtained results it could be concluded that, the colonies were fed on Diet H (based on chickpea flour, wheat germ and dried brewer's yeast) produced the highest rates of biological activities, followed by Diet A (based on of wheat germ, defatted soybean flour and dried brewer's yeast), and then Diet D (based on chickpea flour, defatted soybean flour and dried brewer's yeast). On the contrary, the colonies fed on Diet C (based on pea flour, defatted soybean flour and dried brewer's yeast) was the lowest one. Thus our findings agreed with those described by Wallace, *et al.*, (2016) who's showed that, Chickpea flour contains 21g protein, 53g carbohydrates, 10g crude fiber, 6g fat, and 356 calories. Kent, *et al.* (1967) mentioned that, wheat germ contains humidity 9-13%, protein 22-32%, fat 6-11%, fiber 2.5-1.8%, ash 4-5% and carbohydrates 35-45%. Hammad, (2000) found that, the chemical compositions of soybean flour as followed: humidity 6.58%, protein 50.88%, fat 5.41%, fiber 6.54%, and ash 6.73%. Atallah, (1975) showed that, the chemical compositions of Dried Brewer's yeast as follows: humidity 9.5%, crude protein 40.5%, other extracts 1.5% and ash 6.25% in addition to vitamin B1, B2, and nicotinic acid. El-sherif *et al.*, (2017) noticed that, feeding the honeybee colonies on Brewer's yeast-chickpea cake fortified with 4.2% pollen as a protein supplement plus sugar syrup (2:1) at 3-day intervals for two month of spring season produced higher brood-rearing rate than the other feeding regimes in the experiment, and caused an increase of storing honey and storing pollen. Mohanny, (1999) and Younis, (2006) found that, the consumption of pollen substitute based on Wheat germ was higher than other diets during different seasons. Also, it is the best pollen substitute, as it increases the bee's activities, especially in the lack of pollen grains sources. Abusabbah, *et al.*, (2012) resulted that, honey production was about 25.7, 23.8, 21.4, 16.8 and 12.1 for chickpea with yeast, compressed date, maize flour with yeast and pollen, respectively when compared with those before feedings. Donald, (1976) found that, Pea protein concentrate was the poorest diet in longevity and brood rearing. Thus our findings disagreed with Dastouri, *et al.* (2007) who's said that, the best performance was in pea and pollen treatments; without any significant difference between them.

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ARABIC SUMMARY

تقييم عشرة مكملات غذائية لتحسين بعض أنشطة نحل العسل خلال موسم الشتاء في مصر

محمد سمير يونس

قسم بحوث النحل – معهد بحوث وقاية النباتات – مركز البحوث الزراعية.

أجري هذا العمل في منحل فرع قسم بحوث النحل بالقناطر بمحافظة القليوبية خلال الفترة من ديسمبر 2016 وحتى إبريل 2017 لتقييم تأثير تغذية طوائف نحل العسل بعشرة وجبات كبدائل لحبوب اللقاح على معدلات إستهلاك الغذاء وتربية الحضنة وتخزين حبوب اللقاح والعسل وقد تم إستخدام ستة مواد بروتينية وهي دقيق فول الصويا منزوع الدهن، دقيق الحمص، دقيق الذرة، دقيق البسلة، جنين القمح والخميرة الجافة الغير نشطة حيث تم خلطها بنسب مختلفة مع السكر المطحون والعسل والماء لتكوين 10 خلطات سميت من (A) وحتى (J). وقد أوضحت النتائج أن الطوائف المغذاة بالوجبة H (دقيق الحمص، جنين القمح والخميرة الجافة غير النشطة) أعطت أعلى معدلات الأنشطة الحيوية حيث كانت المتوسطات 105,54 جرام، 363,75 بوصة²، 134,83 بوصة² و 404,08 بوصة² لكل طائفة، وذلك لكل من معدل إستهلاك الوجبة، مساحة حضنة الشغالات، مساحة حبوب اللقاح المخزنة و مساحة العسل المخزنة علي التوالي. بينما أعطت الطوائف المغذاة بالوجبة C (دقيق البسلة، دقيق فول الصويا منزوع الدهن والخميرة الجافة الغير نشطة) أقل معدلات الأنشطة الحيوية حيث كانت المتوسطات 77 جرام، 235,1 بوصة²، 75,43 بوصة² و 258,39 بوصة² لكل طائفة، وذلك لكل من معدل إستهلاك الوجبة، مساحة حضنة الشغالات، مساحة حبوب اللقاح المخزنة و مساحة العسل المخزنة علي التوالي. وقد لوحظ وجود علاقة مباشرة بين معدلات إستهلاك الوجبات وكل من تربية الحضنة وتخزين حبوب اللقاح والعسل خلال موسم الشتاء مقارنة بالطوائف غير المعاملة والتي أظهرت نقص ملحوظ في كل هذه الأنشطة الحيوية.