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EFFECT OF FEEDING QUEEN REARING COLONIES WITH POLLEN SUBSTITUTE AND SUPPLEMENT ON PERCENT ACCEPTANCE OF GRAFTED QUEEN CELLS, ROYAL JELLY PRODUCTION AND THE WEIGHT OF VIRGIN QUEENS

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ABSTRACT

A study was conducted in the apiary of Agriculture Faculty Al-Azhar University to evaluate the efficiency of fair pollen substitute and supplements offered to queen rearing colonies on percent acceptance of grafted queen cups with 1 and 2 days old larva production of royal jelly and the weight of virgin queens. Summarized data are as follow: The tested diets (cakes) did not affect significantly percent acceptance of grafted queen cups as it ranged between 64.64-70.89 % and 58.89-63.56 %when the grafted larvae aged 1 and 2 days, respectively compared to 68 and 63.33 % for the control .The tested diets significantly increased the royal jelly production, being the highest 34.89 and 34.84 g/ colony for those fed on the supplement maize-pollen and the substitute soy-yeast, respectively with 1 day old grafted larvae. The substitute maize- yeast was the inferior, Also, grafting the cups with two days old larvae gave worst results (range 21.93-23.90 g / colony).The same trend was also recorded in case of the royal jelly production per queen cell, ranging between 315.67- 334.0 mg / cell grafted with 1 day old larva and 246.67- 256.0 mg /cell grafted with 2 days old larva. The weight of virgin queen ranged 166.9- 183.7 mg and 159.4- 176.6 mg for the virgin queens raised in the colonies fed on the tested cakes from1 and 2 days old grafted larvae compared to 164.4 and 154.5 mg / control virgins.

INTRODUCTION

Pollen grains are the main source of protein, fat, vitamins and minerals. The protein content of pollen ranges from 7-40% (Johanson and Johanson, 1977) During dearth periods and when pollen grains are not available or in short supply, it is of advantage to provide essential nutrients in the form of pollen substitutes or supplements (Taber and Poole, 1974). Then, any appropriate protein source is satisfactory for growth of emerging bee and development of their hypopharyngeal glands and royal jelly production (Haydak and Dietz, 1965, Mohammad, 2006).

Royal jelly is secreted by the hypopharyngeal and mandibular glands of nurse worker honeybees. It is traditionally known to have some diverse nutritional and/or pharmacological functions such as hypertensive activity, antitumor activity, insulin-like action disinfectant action, (Tamura, 1985; Fujii, 1995). It is used to feed the queen bee and young larvae in the colony.

It is known that the economic characteristics of the honey bee colony are dependent mainly on the quality of its queen. The queen quality, in turn, depends on both genetic and environmental factors. The rearing conditions in which the queen grows as larva within nursing colony are the most important requirement among the environmental factors Woyke (1971) mentioned that weight of virgin queen was an important criterian in selecting better queen. Heavy queens could not only contain larger spermtheca and more ovarioles but could well be expected to continue in egg-laying for longer period.

Szabo (1973), concluded that weight of the queen at emergence could be a useful index for selecting rich oviposition queens. The present work aimed to study the effects of feeding queen rearing colonies with pollen substitute and supplements on % acceptance of grafted queen cups with one and two days old larvae, royal jelly production and the weight of resulted virgin queens.

MATERIALS AND METHODS

This study was performed in the apiary of Agriculture Faculty Al-Azhar University at Mustored during 2007 to determine the rate of acceptance of grafted queen cups with 1- and 2 days old larvae, the production of royal Jelly and the weight of virgin queens, as affected by the different food materials offered to queen rearing colonies.

Preparation of the queenless nurse colonies for royal jelly production

1. The test honeybee colonies:

Thirty F1Caraniolan honeybee colonies nearly of equal strength containing at least four brood combs covered with bees were chosen in the apiary. The selected colonies were divided into ten groups of three colonies each.

Five groups were provided with queen cups grafted with one-day old worker larvae, while the other five groups were grafted with two -day-old worker larvae. For each larval age four groups were offered one of the tested pollen supplement or the substitute, while the fifth groups was left as control.

All brood combs containing unsealed brood in the experimental colonies were removed from the brood chamber (Laidlaw and Eckert, 1950). In the center of the sealed brood combs a suitable space was left to insert a frame holding the queen wax cups.

The queenless colonies were fed on sugar syrup at least 3 days prior to grafting and throughout the cell building period. The colonies were examined before inserting the grafting cells and all the natural queen cells were destroyed. These colonies were provided continuously with sealed brood combs.

2. Grafting technique:

In order to obtained larvae at the proper age (24 hours) (Woyke, 1971), a prolific queen of a selected colony was confined with a marked empty worker comb frame which workers had been just emerged in a special cage provided with queen excluders on both sides. The cage was placed in the center of the brood nest of the colony.

The wet grafting technique was followed according to (Laidlaw,1975), using queen cups were previously prepared after (Doolittle, 1909).

Fifteen cell cups were fixed on a wooden bar, using melted wax, two bars were fitted into the frame. The frame with grafted cups was then inserted between brood combs in the cell building colony to let the bees clean and prepare the queen cell cups.

To give quantity of the royal jelly produced, the cells were removed from the colony when the larvae were 3.5 days-old the age at which greatest a mount of royal jelly was obtained (El-Sherif *et al.*, 1994). The accepted cells produced by different diets were counted, their larvae were removed and the royal jelly was collected in dark glass vials, then weighed, for each treatment.

3. Production of virgin queens:

One day after inserting the frame of grafted cups in the test colonies the successful queen cells, being accepted by the bees, were collected and prefixed to wooden bars, then returned to the middle part for completion of nourishment. Ripe queen cells (after 9 days of grafting) were carefully removed from the bars, and each queen cell was put under semi-spherical cage on unripe honey comb, fifteen virgen queens of each diet were weighed immediately after emergence then the average weight / virgin was calculated.

4. Preparing of the diets.

The components of pollen substitutes and supplements cakes are presented in Table 1.

Table (1): The constituents of pollen substitute and supplement diets (cake).

Percentage	Pollen substitutes		Pollen supplements	
	Soybean-yeast	Maize -yeast	Broad bean-pollen	Maize -pollen
50	Soybean	Maize	Broad bean	Maize
20	melon shell juice	Orange shell juice	Mandarin shell juice	Apple shell juice
10	Orange shell juice	Melon shell juice	Apple shell juice	Mandarin shell Juice
10	Yeast	Yeast	Pollen	Pollen
10	Agwa	Agwa	Agwa	Agwa

Adding 5mL sesame oil for different diets

Paste (cake) of pollen supplement and substitute were prepared as usual by mixing the ingredients(10 %) in form of paste using sugar syrup. Powdered sugar was added until the cake became semisolid. The cake was packed in perforated saccules containing 100 g each. Each experimental colony was offered one saccule / week. Feeding the test colonies on the cakes was started 3 weeks prior to the onset of the experiments.

Statistical analysis:

Data obtained were statistically analyzed according to Snedecor (1957) methods.

RESULTS AND DISCUSSION

Effect of larval age and food materials provided to bee colonies

1. Percent acceptance of grafted queen cups:

Data presented in Table2 clear that the mean % acceptance of grafted queen cups with one day old larvae recorded70.89, 64.64, 70.0 and 69.56 %for the colonies fed on soy-yeast, maiz- yeast, pollen-broad bean, pollen-maize cakes, respectively. From the obtained data clear that with two days old grafted larva were 61.56, 58.89, 63.56 and 62.22 %. The data of acceptance in control colonies attained 68.0 and 63.33 % for the two larval ages, respectively. The difference between the diets and the control were insignificant. However, significant differences were detected between the two ages of grafted larvae. Obtained results are in accordance with those of. Sahinler, *et al.*, (1997) who found that feeding colonies with pollen substitute increased the acceptance rates significantly ($P<0.01$) in queenless cell builders. The age of the larvae was also important on the acceptance of cells. The acceptance rates of 1 or 2 days old larvae were higher than that of 3 days old larvae in queenless.

Also, Sharaf El-Din *et al.* (1999) found that feeding colonies with yeast gave the highest result of acceptance (85.50) followed by soybean (82.20%), semidry dry date (77. 80%), mandarin cortex jam (73.30%) and sugar syrup (61.10%) respectively. However, Hanna (1963) concluded that the larval age had no significant effects on the percentage of acceptance. The percentage of cell acceptance was higher when one and two days old larva were used than when cells larva not more than 24 hours old were used.

2. Royal jelly production:

The quantities of royal jelly obtained from queenless colonies fed on the (based upon % acceptance of a total of 150 grafted cups) recorded 34.84, 30.62, 33.88 and 34.89 g / colony with one day old larvae; and 22.89, 21.93, 23.31 and 23.90 g / colony when the grafted larvae aged 2 days for the test colonies fed on soy- yeast, maize- yeast, pollen-broad bean, and pollen- maize cakes, respectively. Control colonies produced 29.10 and 24.49 g / colonies with the two larval ages, respectively. Analysis of variance manifested significant differences between the diets, especially with one day old larva . Similarly, the differences between the two larval ages were highly significant.

Obtained results are in agreement with that El-Sherif *et al.* (1996) found that amount of royal jelly produced in four successive batches differed according to the type of food colonies supplied with a 50% sucrose syrup beside sucrose-yeast cake fortified with pollen significantly produced the highest amount of royal jelly (30.76g/colony) followed by colonies supplied with a 50% sucrose syrup beside sucrose-yeast cake (26.74g/colony), colonies supplied with suspension of pollen in honey syrup (26.65 g/colony) or honey syrup beside sucrose-pollen cake (25.49 g/colony). Also, Hammad (2007) found that the amount of royal jelly was significantly higher in colonies fed on liquid yeast diet (*Candida troicalis*) at 25 % conc., than in control colonies. Also, the current results agree with those reported by Serag El-Dien *et al.* (2009),who stated that the royal jelly was highest in colonies fed on paste cake,soybean-yeast (92 gm / group) comparing with the other diets.

3. The quantity of royal jelly / queen cell (mg):

The quantities of royal jelly obtained from queen cells collected from queenless colonies fed with the test diets recorded 328.0, 315.67, 320.0 and 334.0 mg / cell grafted with one day larva, and 249.0, 246.67, 248.0 and 256.0 mg / cell grafted with two days larva and introduced in the test colonies fed on soy-yeast, maize- yeast, pollen- broad bean and pollen-maize cakes, respectively. Queen cells of control colonies produced 286.0 and 260.0 mg / cell grafted with one and two days old larvae, respectively. The tested diets increased significantly the quantity of royal jelly / cell over the control when the grafted larva aged one day old only .In addition, the differences between the two larval ages were highly significant in flavor of one day old larva(Table 2).

Sahinler *et al.* (1997) stated that the average royal jelly yields in queenless (mg/cell) were 189 when bees were fed only on sugar syrup and 226, with pollen substitute. Durmus and Guler (1999) found that the average amount of royal jelly were 369.6 and 402mg/cup at 10 and 20 frames respectively, 401.26 and 371.04mg/cup with pure and commercial wax cups.

Cheng *et al.*, (2002) concluded that in order to produce high yields of royal jelly from a new strain of honey bees, They used methods involves 8 steps maintaining a high-yielding strain of honey bee, using specially developed equipment, relenting the royal jelly, production period, mastering skills of manipulation, supplementary feeding, adjusting the structure of the combs, using the appropriate number of queen combs per colony and rearing larvae.

4. Mean weight of virgin queens:

The mean weight of virgin queens produced from the colonies provided with the tested diets recorded 164.4, 179.7, 183.7, 171.7 and 166.9 mg when the grafted larvae was of 1 day old, and 159.5, 173.3, 176.6, 164.33 and 159.4 mg with two days old larva introduced in the colonies fed on sugar syrup control, soy - yeast, maize - yeast, pollen-broad bean and pollen- maize, respectively (Table 2). Analysis of variance clear that the differences between the diets and larval age were mostly significant.

Mohamed Asmaa (2003) found that a significant differences between the means of fresh body weight of newly emerged virgin queens, the highest means were found in colonies fed on drone larva and fish powder (172.62 and 169.96mg) respectively. The lowest means were found in colonies fed on lints and control (152.29 and 141.47mg) respectively.

From the obtained results, it could be suggested that feeding honey bee colonies with diets (Maize-yeast), (Maize-pollen), and (Soybeen-yeast) were very efficient on producing more amount of royal jelly during dearth period of flowering plants before and after main period of nectar flow at Mustored region.

Table (2): Effect of different diets and larval age on percent of acceptance queen cup, mean amount of royal jelly (both colony and queen cup) and weight of virgin queens.

Characters		Mean numbers and percentage of queen cup				Mean amount of royal jelly				Mean weight of virgin queen	
Larval ag / Diet		One day		Two days		One day	Two days	One day	Two days	One day	Two days
		Accepted	%	Accepted	%	g/ colony	g/ colony	/colony mg	mg/ colony	mg	mg
Soy bean - yeast		106.33	70.89	92.33	61.56	34.84	22.89	328	249	179.7	173.3
Maize -Yeast		97	64.67	88.33	58.89	30.63	21.6	315.67	246.67	183.7	176.6
Broad bean-Pollen		105	70	94	63.56	33.64	23.31	320	248	171.7	164.33
Maize -Pollen		104.33	69.56	93.33	62.22	34.89	23.91	334	256	166.9	159.4
Control		102	68	95	63.33	29.16	24.67	286	260	164.4	159.5
Total		514.66		462.99		162.8	116.38	1583.67	1259	866.4	833.03
Mean		102.932		92.598		32.56	23.276	316.734	251.8	173.28	166.606
LSD for age	5%	6.12				1.6		10.25		3.46	
	1%	8.15				2.5		13.66		4.62	
LSD for diets	5%					2.5		22.91		5.48	
	1%					3.3		30.55		7.30	

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تأثيرتغذية طوائف تربية الملكات ببدائل ومكملات حبوب اللقاح على قبول الكؤوس الملكية المطعومة وانتاج الغذاء الملكى ووزن الملكات العذارى

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أجريت هذه الدراسة في منحل كلية الزراعة- جامعة الازهر لتقييم فعالية أربعة بدائل ومكملات حبوب اللقاح كوجبات لطوائف تربية الملكات على نسبة قبول الكؤوس المطعومة ببرقات شغالات عمر ١و ٢ يوم وإنتاج الطوائف من الغذاء الملكى وأيضا وزن العذارى الناتجة ويمكن تلخيص النتائج المتحصل عليها كما يلى:-

لم تؤثر الوجبات(العجائن) على نسبة قبول الكؤوس المطعومة ببرقات عمر يوم أو يومين حيث تراوحت نسبة القبول بين٦٤,٦٤-٧٠,٨٩ % للعمر اليرقي الأول و ٥٨,٨٩-٦٣,٥٦ % للعمر اليرقي الثانى مقارنة بـ ٦٨,٠ و ٦٣,٣٣ % فى طوائف المقارنة لكلا العمرين على التوالي.

أدت تغذية الطوائف على العجائن المختبرة الى زيادة معنوية فى انتاج تلك الطوائف من الغذاء الملكى والذى بلغ اقصاة ٣٤,٨٩ و ٣٤,٨٤ جرام/ طائفة فى الطوائف التى تغذت على مكمل حبوب اللقاح- ذرة والبديل صويا- خميرة على الترتيب عندما كان عمر اليرقات المطعومة يوم واحد وكان بديل ذرة- خميرة الادنى٠ علاوة علىَ ذلك فقد اعطت الطوائف التى تم امدادها ببرقات عمر يومين نتائج اقل تراوحت بين ٢١,٩٣ -٢٣,٢٠ جرام / طائفة٠

وقد لوحظ نفس الاتجاه فى محتوى البيت الملكى من الغذاء الملكى حيث تراوحت من٣١٥,٦٧- ٣٣٤,٠ ملجم لليرقات عمر يوم و٢٤٦,٦٧ - ٢٥٦,٠ ملجم / بيت مطعوم ببرقة عمر يومين وذلك للطوائف التى تغذت على العجائن المختبرة ٠

تراوح وزن الملكات العذارى من ١٦٦,٩- ١٨٣,٧ ملجم و ١٥٩,٤ - ١٧٦,٦ ملجم للعذارى الناتجة فى الطوائف المختبرة ومع اليرقات المطعومة عمر يوم و يومين على الترتيب فى حين كانت أوزان العذارى المقارنة ١٦٤,٤ و ١٥٩,٥ ملجم لليرقات المطعومة عمر يوم ويومين على الترتيب وكانت الفروق معنوية