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Study of certain factors affecting queen rearing in honeybee colonies on the acceptance rate of grafted queen cells.

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Abstract

A study was conducted in the apiary of Sakha Agricultural Research station during season (2006-2007) to evaluate the certain factors affecting queen rearing in honeybee colonies on acceptance percent of grafted queen cups, the double grafting gave the best result of acceptance (88.33% and 85%) followed by wet (80% and 81.66%) for wax and plastic queen cells respectively, while the dry accepted the last order gave (65% and 63.3%), there was high significant difference between the dry and each of wet and double grafting.

As respect to the queen cells bar position indicated the lower position of bars gave the best result of acceptance (90%) followed by middle (76.66%) then upper bar which gave (53.3%) there was no difference of acceptance percent found between grafted lower of larvae aged 1 and 2 days, there was found significant differences of acceptance percent among spring, summer and late summer, whereas.

The early summer season was the best period for acceptance of queen cells followed by spring then late summer of period. The tested diets (cakes) did not affect on queen cells, where acceptance percent 41.11-56.60% and 40-98.88% for grafted larvae aged 1 and 2 days, respectively.

Key Words : queen rearing, honeybee, acceptance

Introduction

Many attempts have been made to increase the acceptance of honeybee larvae grafted into artificial queen cups, especially in commercial queen-rearing operations of *Apis mellifera*. Although a number of factors control queen cell acceptance, it is particularly necessary to understand the feeding behaviour of nurse bees and the factors that influence the feeding of grafted larvae.

Queen pheromones possibly affect the behaviour of nurse bees in provisioning queen cells with royal jelly. **Butler (1957)** demonstrated that an extract of queens, later shown by **Callow and Johnston (1960)** to contain queen substance (9-oxodec-trans-2-enoic acid), inhibits queen rearing by preventing the conversion of worker cells containing larvae into queen cells.

The material from which the artificial queen cups are made may also regulate cell acceptance and provisioning. Artificial queen cups are usually made of various sorts of wax and plastic. **Vuillaume (1956)** concluded that acceptance was not affected by the construction material: bees accepted cells made of various vegetable and mineral waxes, including paraffin, or glass or plastic. **Weiss (1967)** found that artificial queen cups made of new bees wax and of bees wax from old combs were equally acceptable.

It was known that the economic characteristics of the honeybee colony depend mainly on the quality of its queen. The queen quality, in turn, depends on both genetic and environmental factors.

Quality of the queen is not only hereditarily controlled, but also depends on the conditions in which it grows as larvae, the size and vigour of a colony of honey bees are a direct reflection of the genotype of the queen, and also of her individual size and vigour. For example, as her body weight increases, and the number of ovarioles increases. Also, part of variations that are frequently observed among many queens which inherit similar size and body conformations are the results of variations in environmental factors during rearing.

The present work aimed to study of certain factors affecting acceptance percent by honeybee colonies of larvae in artificial queen cups. These factors include: larval ages, grafting methods, grafting periods, sorts of queen cups, bars position that hold queen cells and different diets.

Materials and methods

This study was performed in the apiary of Sakha Agricultural Research station during the seasons 2006, 2007, to investigate the effects of different grafting methods, bar position, larval ages, different diets and seasons on the acceptance percent by honeybee colonies of larvae in artificial queen cups.

Preparation of the queenless nurse colonies to determine the rate of the acceptance of grafted queen cups

1- The test honeybee colonies:

Twelve F_1 Italian honeybee colonies nearly of equal strength containing at least four brood combs covered with bees were chosen in the apiary.

The queenless colonies were provided with queen cups (wax or plastic) grafted with one-day-old

worker larvae, while the other queenless colonies were grafted with two-day-old worker larvae.

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 or plastic 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 the natural queen cells were destroyed. These colonies were provided continuously with 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 queen frame which workers had been just emerged in a special cage provided with queen excluders on both side. The cage was placed in the center of brood nest of the colony, the wet grafting technique was followed according to **(Laidlaw, 1979)**, using queen cups were previously prepared after **(Doolittle, 1909)**, three types of worker larvae grafting, dry, wet and double larvae grafting were used. Fifteen or ten cell cups were fixed on a wooden bar, using melted wax, and two bars were fitted into each frame. The frame with grafted cups (wax or plastic), inserted between brood combs in the cell building colony to let the bees clean and prepare the queen cells in the different cups.

3- Preparing of the diets for colonies used for queenless colonies the components of pollen substitutes cakes are presented in table 1 for queen rearing.

Table 1. The constituents of pollen substitutes.

Percentage of feeding types %	Pollen substitutes	
	* Soybean	* Chick-pea
50	Soybean	Chick- pea
20	Orange shell juice	Orange shell juice
10	Apple and carrot peels juice	Apple and carrot peels juice
10	-	Drawer yeast powder
	20% Agwa	10% Agwa

* Adding 5 ml anise oil for different pollen substitutes when make as cake.

Pastes (Cake) of pollen substitute were prepared 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 100g 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. Control colonies fed with natural pollen during that season.

Statistical analysis:

Data obtained from the experiments of queen rearing were statistically analyzed according to **Snedecor (1957)** methods.

Results and Discussion

Effect of certain factors affecting on queen rearing in honeybee colonies on the acceptance percent of grafted queen cells were conducted as follows:

1. Effect of different larval grafting methods on the acceptance during (2006) season.

Table 2. Effect of different larval grafting methods on the acceptance during (2006) season.

Grafting methods	Sort queen cups	No. of grafted queen cells	Acceptance Replicates			Total	Mean ± S.E	Percentage %
			R_1	R_2	R_3			
Dry	Wax	60	13	12	14	39	13.00±0.58b	65.00
	Plastic	60	12	12	14	38	12.66±0.67b	63.30
Wet	Wax	60	15	17	16	48	16.00±0.59a	80.00
	Plastic	60	15	18	16	49	16.33±0.88a	81.66
Double	Wax	60	17	18	18	53	17.66±0.33a	88.33
	Plastic	50	15	19	17	51	17.00±1.15a	85.00

L.SD at 0.05 = 2.296 **Value F = 7.973***

Means marked with different letters were significantly differ at 0.05 level of probability.

Data presented in table (2) clear that the double grafting gave the best result of acceptance for wax and plastic queen cups (88.33 and 85%), followed by wet (80 and 81.66%) then dry which gave (65 and 63.30%) respectively, there were high significant difference only between the dry and each of double and wet grafting methods.

Our obtained results are in agreement with these obtained by **Diab (1986)** who stated that the double grafting method gave the best result of queen cells acceptance (60%), followed by wet and dry methods of grafting (52.22 and 42.22%) respectively, with high significant differences between them. Also, **El-Hanafy (1991)** who found that wet grafting method gave significant better results (81.7%) than dry grafting (50.8%) in the acceptance of grafted larvae. Also, **Dedei-S (1994)** reported that it was a significant different, in weight of virgin queen, number of accepted larvae, number of emerged queens. All these parameters were higher in the double grafting technique.

However, **Wongsiri, *et al.*, (1989)** found that there was no significant difference between single grafting and double grafting in the number of accepted cells.

2. Effect of different bar position on larval acceptance during (2006) season.

Table 3. Effect of different positions of bars on the acceptance during (2006) season.

Position of bars	Type of queen cups	No. of grafted queen cells	Acceptance Replicates			Total	Means. S.E.±	Percentage %
			R_1	R_2	R_3			
Upper	Wax	30	6	5	5	16	5.33±0.3333c	53.3
	Plastic	30	7	6	5	18	6.00±0.5774bc	60.0
Middle	Wax	30	7	8	8	23	7.66±0.3333ab	76.6
	Plastic	30	6	9	10	25	8.33±1.2019a	83.3
Lower	Wax	30	8	9	10	27	9.00±0.5774a	90.0
	Plastic	30	8	9	9	26	8.66±0.3333a	86.6

L.SD at 0.05 = 1.966 **Value F = 5.536***

Means marked with different letters were significantly differ at 0.05 level of probability.

Data presented in Table (3), showed that there were bars significant differences between each of (lower and middle) and upper positions, while no significant difference were found between the wax and plastic queen cups, regarding the acceptance percentages of queen cells. The lower bars position gave the best result of acceptance 9 queen cells out of 10 (90%) followed by middle bar 8.33 queen cells (83.3%) then upper bar which gave only 6 accepted queen cells (60%).

These results agree with **Orosi-Pal (1957)** concluded that the cells on lower row were more accepted than on the upper one. **Ali (1994)** who found that the highest number of accepted queen cells was found on the bottom position followed by the middle level, then the top one. According to **Shah (2000)** the percentage of accepted larvae that were grafted with dilute royal jelly among groups showed statistical difference between acceptance of 1- and 2- day- old larvae among of upper and lower bar of the grafted frames. The groups accepted more two-day-old larvae as compared to one-day-old larvae.

3. Effect of different seasons on the acceptance during (2007) season.

Table 4. Effect of different seasons on the acceptance during (2007) season.

Larval ages	Different periods	No. of grafted queen cups	Type queen cups	Replicates			Total	Means.	Percentage %
				R_1	R_2	R_3			
One day	Spring	90	Wax	18	24	21	63	21.00ab	70.00
		90	Plastic	21	29	21	71	23.66a	78.88
	Summer	90	Wax	17	18	21	56	18.66abc	62.20
		90	Plastic	26	18	24	68	22.66a	75.53
	Late summer	90	Wax	14	14	12	40	13.33c	44.43
		90	Plastic	17	11	18	46	15.33bc	51.00
Two days	Spring	90	Wax	20	14	18	52	17.33abc	57.76
		90	Plastic	23	12	18	53	17.66abc	58.88
	Summer	90	Wax	20	11	15	46	15.33bc	51.11
		90	Plastic	23	18	12	53	17.66abc	56.66
	Late summer	90	Wax	15	12	13	40	13.33c	44.43
		90	Plastic	15	13	14	42	14.00bc	46.66

L.SD at 0.05 = 6.178 **Value F = 2.734***

Means marked with different letters were significantly differ at 0.05 level of probability.

Data in table (4) showed that the highest mean number of accepted queen cells was recorded (23.66) in one day old larvae grafted in plastic cups during spring season and the lowest mean number recorded during late summer (13.33) for the same larval age in

wax cups. There was a significant difference of accepted queen cups during sparing and late summer.

According to **El-Din-Haes (1999)** showed that the acceptance and body weight were gradually increased during the season, probably due to the increase in the food resource around the apiary. Also, **Zeedan (2002)** who stated that there were significant differences in the mean of accepted larvae between both spring (84.2%) and summer (82.3%) from one side and both autumn (73.4%) and winter (71.1%) from the other one. Also, **Hammad (2007)** recorded that the mean number of queen cells produced during spring season was higher than in summer one.

While, Abd Al-Fattah and Shemy (1996) found that the plastic queen cups caused significant increase in acceptance percentage. They added that non-significant differences were found between plastic and wax queen cups for the percentages of queen cells.

4. Effect of larval age and food materials provided to queenless colonies during late summer (2007) season.

Table 5. Effect of larval age and food materials provided to queenless colonies during late summer (2007) season.

Larval ages	Different diets	No. of grafted queen cups	Type queen cups	Replicates			Total	Means.	Percentage %
				R_1	R_2	R_3			
One day	Natural pollen grains	90	Wax	12	13	14	39	13.00bc	43.33
		90	Plastic	14	13	13	40	13.33bc	44.44
	Soybean	90	Wax	14	15	15	44	14.66b	48.88
		90	Plastic	16	17	18	51	17.00a	56.66
	Chick pea	90	Wax	13	11	13	37	12.33cd	41.11
		90	Plastic	14	12	11	37	12.33cd	41.11
Two days	Natural pollen grains	90	Wax	11	12	10	33	11.00d	36.66
		90	Plastic	12	11	13	36	12.00cd	40.00
	Soybean	90	Wax	14	13	12	39	13.00bc	43.33
		90	Plastic	14	15	15	44	14.66b	48.88
	Chick pea	90	Wax	12	11	12	35	11.66cd	38.88
		90	Plastic	13	12	11	36	12.00cd	40.00

L.SD at 0.05 = 1.613 **Value F = 9.000*****

Means marked with different letters were significantly differ at 0.05 level of probability.

Data in Table (5) showed that there were significant differences between soybean and each of (natural pollen and chick pea) on the acceptance percentages of queen cell cups. The feeding with soybean gave the best result of acceptance with grafted wax cups, (14.66 and 13.00) and plastic (17 and 14.66) queen cups with one and two old days respectively, while the lowest percentages of accepted queen cells were recorded with natural pollen chick pea (12.33 queen cell out of 20) for one day old larva. The difference between the diets and the control were significant, as well as 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 larvae was also important on the acceptance of cells.

Also, **Sharaf El-Din *et al.* (1999)** found that feeding colonies with yeast gave the height result of acceptance (85.50%) followed by soybean (82.20%) semidry date (77.80%), mandarin cortex jam (73.30%) and sugar syrup (61.10%) respectively. Also, **Shehata (2009)** found that the tested diets 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 control. However, **Hanna (1963)** concluded that the larval age had no significant effects on the percentage of acceptance.

Finally queen cell acceptance may be controlled by different factors such as larval grafting methods, cell queen cups, feeding and rearing season these factors quality of queen cell affect professioning through larval development and the quality of queen produced. Acceptance of grafting larvae in the good indication of the colony condition.

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دراسة بعض العوامل المؤثرة فى طوائف تربية الملكات على نسب قبول الكئووس الملكية المطعومة

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الملخص

أجريت هذه الدراسة فى منحل محطة البحوث الزراعية- بسخا (كفرالشيخ) وذلك لتقييم بعض العوامل المؤثرة فى طوائف تربية ملكات نحل العسل على نسب قبول الكئووس الملكية المطعومة مثل : نوع التطعيم، وضع الكئووس على السدابة، العمر اليرقى، فترات التربية، وكذلك التغذية على بعض بدائل حبوب اللقاح (دقيق فول الصويا، دقيق الحمص+ الخميرة)، نوع الكأس (شمعى- بلاستيكى).

ويمكن تلخيص النتائج المتحصل عليها كما يلى :

- التطعيم المزدوج أعطى أفضل النتائج بنسبة قبول 88.33% للكئووس الشمعية، و85% للبلاستيكية، مع وجود فروق معنوية بين التطعيم الجاف وكل من التطعيم المبتل والمزدوج، وقد وجدت فروق معنوية بين أوضاع الكئووس الشمعية العلوية وكل من الوسطى والسفلية أعطت أفضل نتائج بنسبة 90% للكئووس الشمعية، و86.6% للبلاستيك، وبالنسبة للعمر اليرقى يوم ويومان فان أفضل النتائج كانت لعمر يوم فى الربيع والصيف والصيف المتأخر مقارنة بالتربية من يرقات عمر يومين فأن اليرقات عمر يوم أعطت أفضل النتائج فى المواسم المختلفة.

- عن تأثير التغذية على نسب قبول الكئووس المطعومة بيرقات عمر يوم أو يومين فأن التغذية على دقيق فول الصويا أعطت أفضل النتائج (56.6%، 48.88% للكئووس البلاستيك عمر يرقى 1، 2 يوم على التوالى) خلال فترة الصيف المتأخر ويليه حبوب اللقاح الطبيعية ثم دقيق الحمص+ الخميرة.

- وأوضحت النتائج أيضاً عدم وجود فروق معنوية بين نسبة القبول فى الكئووس الملكية المطعومة فى كل من الكئووس الشمعية والبلاستيكية.