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STUDIES ON SOME FACTORS AFFECTING ROYAL JELLY PRODUCTION OF HONEYBEE COLONIES, *Apis mellifera* L.

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ABSTRACT: A study was conducted in a private apiary located at El Mahala Elkobra, Gharbia to assess the impact of bee hybrid, supplemental feeding and the strength of production unit (bee colony) on royal jelly production in addition, queen cup material, age of grafted bee larva and royal jelly harvest interval were also taken in consideration. The results clear that the highest royal jelly production was attained when grafting one day old larvae. However, the differences between Carniolan and Italian hybrids in royal jelly production was insignificant. In addition, stronger colonies yielded higher royal jelly production than weaker ones in both honeybee hybrids. Using queen cups of honeybee wax produced the highest royal jelly yield followed by bee wax coated plastic cups, whereas plastic cups came in the last class. Regarding the effect of supplemental feeding (AD3E, Vit.B complex, Amino) in syrup and in patty, it is clear that AD3E induced the highest effect on royal jelly production, follow by Amino then Vit. B complex. Offering the additives in syrup was more potent than in patty the highest royal jelly yield was gained when harvesting process was made at 3 days post grafting. Mean while severe reduction in royal jelly yield was dectected when harvesting was taken place at 4 days post grafting.

Key wards: Carniolan hybrid Harvest interval, Bee wax queen cups Royal jelly, Larval age, Grafting larvae, *Apis mellifera* L. Pollen substitute, Supplements, Number of grafts

INTRODUCTION

Honeybees are considered the most important useful economic insects due to their vital role in pollinating the crops, in addition to the colony protucts which maximize the beekeeper income. Royal jelly, in particular, is characterized by its high nutritional and medicinal value. Therefore, it has become one of the most important bee product, especially in the communities of higher culture.

Nowadays, many beekeepers directed their apiaries towards royal jelly production to gain higher income due to its higher marketprice and to the rapid production cycle, especially it could be produced all the year round. Therefore, great effects are paid to maximize the colony royal jelly production. From this standpoint, the present research was designed to study the impact of some factors affecting the production process of royal jelly, including bee hybrid, supplemental feeding and colony strength as well as some technical measures such as queen cup material, age of grafted bee larva and royal jelly harvest interval.

MATERIALS AND METHODS

The present investigation was carried out in a private apiary located at El- Mahala El- Kobera, Gharbia Governorate during the period extended from 2018- 2020. The aim was to investigate same factors affecting royal jelly production of honeybee colonies.

Materials

Honeybee colonies

Honeybee colonies of Carniolan and Italian hybrids were used in two types (groups). The first group was hybrid colonies certainly of F1

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of both hybrids were used as the source of larvae used for grafting queen cups, whereas the second group colonies were between F2- F3 of both Carniolan and Italian hybrids that were prepared and cared according to the plan of study. Such colonies were prepared as follow:

Strong colonies of both hybrids were chosen in enough numbers for the proposed experiments. Such colonies were compressed on 6 combs covered dense with bees after shaken off the covered bees on the combs inside their respective colony. Each colony was considered when containing one unsealed brood comb, 4 sealed brood combs mostly up to emerge, one comb of stored pollen, a lot of young bees shaked from other colonies.

The test colonies were fed regularly on 2:1 sucrose syrup and pollen substitute when not prevented according to the aim of the experiment. Royal jelly producing colonies were orphaned 24 hrs before the onset of any experiment (introducing grafting queen cups bearing frames which located between unsealed and sealed brood combs).

Queen cups

Three types of queen cups were used as described for each experiment as follow:

- Bee wax queen cups
- Plastic queen cups
- Plastic queen cups coated with bee wax by immersing the plastic cups in molten bee wax

The cups were fixed with molten wax on wooden bars at the rate of 15 cups/bar. Two or three bars were held on one wooden frame holding queen cups.

Grafting process

The process was performed in condition grafting room. Wet grafting method was applied by transferring larvae (1-2 days old) on diluted royal jelly with water (1 :1). Grafting process was performed using stainless grafting needle. Grafted cups on the frame holding were moved in welt thick clothes to royal jelly producing colony to avoid the adverse effects of climatic factors. Then the frame was inserted between brood combs quickly to avoid bees attack from other colonies.

Harvesting royal jelly

Harvesting royal jelly was made at three harvest intervals i.e. 2, 3and 4 days post grafting unless defined according to the experiment. On royal jelly collection the frames holding queen cups were moved quickly to grafting room after discharding (dislodging) bees covering using bee brush. There after the tips of the cups were carefully shaved off using sharp razor blade, then larvae were removed and royal jelly was collected and weighed. Three harvests (batches) were made from each colony then mother queen were re-introduced in their respective colonies to avoid laying workers existence.

Nutritional supplements

The following nutritional food additives were used mixed in pollen substitute patty or dissolved in sucrose syrup (1:1) to feed royal jelly producing honeybee colonies

AD_3H

The product is manufactured by United Company for Supplements in liquid form in which each one liter contains Vitamin A (100000000 IU), Vitamin D3 (20000000 mg) and Vitamin E (40000 mg).

Amino

This supplement is composed of: Lysine (440 mg), praline (1700mg), L-arginine (480 mg), panthoenol (4000 mg), Thropine(80 mg), Glutamic acid (1000 mg), Glycine (2600 mg), phenylalanine (200 mg), Methonine (80 mg), Aspartic acid (500 mg), Sereine (100 mg) Alanine (1000 mg), Essential amino acid (260 mg).

Vit. B

Complex produced by Agri-vit Company for Vitamins and Food Supplements Cairo, Egypt Each liter contains Vit. B1 (2000 mg), Vit.B2 (10000 mg), Vit. B 6 (5000 mg), Vit. B 12 (20 mg), pantothenic acid (20000 mg), Nicotinic acid (50000 mg), Folic acid (1000 mg) and Vit.K3 (5000 mg) dissolved in one liter of distilled water.

The Experiments

Investigating the effect of age of grafted larva and harvest interval on royal jelly yield

This experiment was carried out during spring season of 2018. Honeybee worker larva of one and 2 days old of F1 Carniolan and F1

Italian hybrid colonies were grafted in 30 bee wax queen cups for each hybrid. Two bars holding (bearing) 15 grafted cups each were held on one frame that inserted in the prepared orphaned colonies of both hybrids. Royal jelly was collected at 2, 3 and 4 days post grafting then weighed using electronic balance of 2 decimals and recorded as mg/ cup.

Investigating the effect of queen cup material and colony strength on royal jelly production

The present experiment was performed during summer season of 2019. Twelve honeybee colonies were used (six Carniolan and six Italian hybrid colonies). The colonies of each hybrid were divided into two groups of 3 colonies each as follow:

Group 1 colonies were compressed colonies of 4 combs covered with bees (nucleus level). Group 2 colonies were compressed on six combs covered with bees. All the test colonies of the two hybrids were orphaned 24 hrs before inserting grafted queen cups with one day old larva of the same subspecies.

Queen cups were made of bee wax, plastic and plastic coated with molten bee wax. Royal jelly was harvested 3 days post grafting and expressed as mg/ cup.

Investigating the effect of nutritional supplements on royal jelly production of honeybee colonies

This experiment was made on Carniolan and Italian hybrid colonies. Three supplements were tested i.e. AD3E, Vit.B complex and Amino. The supplements were offered to the test colonies dissolved in sucrose syrup (1:1) and in pollen substitute patty placed in perforated plastic saccules to be put on the top bar of the test colonies at the rate of 300 g/colony and renewed every 4 days to avoid its dryness. Wooden frame bearing 30 bee wax queen cup grafted with one day old was inserted between open and sealed brood combs. Royal jelly yield was harvested at 2, 3 and 4 days post grafting and the yield was expressed as mg / cup.

Analysis of variance was made normally according to **Snedecor and Cochran (1967)** methods, that calculated according to COSTAT computer program (**Anonymous, 2005**).

RESULTS AND DISCUSSION

Effect of Age of Grafted Larva and Harvest Interval on Royal Jelly Production

Data presented in Table 1 clear that the mean royal jelly production (mg/ queen cup attained 353, 398.33 and 296 mg/ cup when grafting 1 day old Carniolan larva and 290.67, 358 and 246.33 mg/ cup for 2 days old grafted Carniolan hybrid larva and when harvesting process was performed at 2, 3 and 4 days post grafting, respectively. The corresponding quantities for Italian hybrid colonies were 390.67, 386.67 and 308.33 mg/cup grafted with one day old larva and 331.67, 349.33 and 260 mg/cup grafted with 2- days Italian hybrid larva. Analysis of data detected significant differences between the 3 harvest intervals where collecting RJ at 4 days post grafting showed the least significant quantity of harvested royal jelly, meanwhile collecting royal jelly at 3 days post grafting yielded the highest significant royal jelly quantity. In addition, the younger the grafted larva, the higher significant the royal jelly yield. On the other hand, the differences between.

Carniolan and Italian hybrid colonies in the collected quantities of royal jelly were insignificant (Table 1). Obtained results are supported by those of **Muli *et al.* (2005)** who reported insignificant difference in royal jelly production between *A.m monticola* and *A.m scutella*. However, larval age, feeding and harvest time showed significant differences in royal jelly production between races. On the other hand, **Chen Chi Tung (2000), Toledo and Mouro (2005) and Garcia and Couto (2005)** reported significant variations in royal jelly production between races. Similarly, **Sahinler and Kaftanuglu (2005)** measured 3748 , 325 ± 6.7 and 200 ± 1.1 mg royal jelly/ cup for Carniolan, *A.m carnica, M*ugla bees, *A.m anatolica* and *A.m caucasica*, respectively.

Of course, the younger the larval age on grafting, the heavier the resulted queen weight and the greater the royal jelly yield. However, many researches have had reported varied results in this respect that may be due to the varied experimental conditions (biological, climatic, processing, evaluating conditions). For instance, **Abd El-Salam *et al.* (1980) and Chang *et al.* (1993)** agreed that grafting one day

Table 1. Effect of honeyhee hybrid, age of grafted larva and harvest interval on royal jelly production (mg/cup) during spring season of 2018

Bee hybrid	Age of grafted larva (day)	Harvest interval (days)			
		2	3	4	Mean
Carniolan	1	353	398.33	296	349.11
	2	321.835	378.165	246.33	271.165
Italian	1	390.67	386.67	308.33	361.89
	2	331.67	349.33	260.0	313.67
Mean	Carniolan	371.835	378.165	271.165	321.22
	Italian	361.17	368.0	284.15	337.77
LSD 0.05 for		Bee hybrid = ns Age of grafted larva = 16.418 Harvest interval = 26.107 Interaction bt.3factors =40.215			

old larva yielded the highest royal jelly quantity than other ages. Meanwhile, **El Sherif *et al.* (1994)** stated that royal jelly collected after grafting 72 hrs old was higher than collected from the cups grafted with 12hrs larva. **However, Ahmed (1995**) reported that grafting with 12hrs old larva or less is the best for royal jelly production than the older larva. **Gilles-Fert (2003)** stated that grafting 2 days old larva and colleting royal jelly after 3 days increased royal jelly production. Regarding the effect of harvest interval (time) post grafting, it seems that it possessed greater effect on royal jelly production. Generally, many researches statements agreed that 3 days post grafting is considered the best time for collecting the highest possible royal jelly quantity per cup and per colony. For instance, **El Din and El Samni (1990)** harvested 343.6+- 8.87 and 428.46 + - 8.75 mg/ royal jelly/cup after 2 and 3 days post grafting. Similary, Cheng and Chang (1993) harvested 553.1+- 79.1 and 379.5+-50 mg/ cup at 3 and 2 days post grafting, respectively. The same trend was also reported **Sahinler and Sahinler (2002), Kumova *et al.* (2005)**, **Baungratz *et al*. (2005), Sajwan and Mall (2008) and Guo YaHui *et al.* (2015)**. Moreover, **Singh and Tiwari (2013)** went so far, indicating that royal jelly yield was found as 190.5% higher in 72 hrs harvesting time interval over that of 48 hrs.

Effect of Colony Strength and Queen Cup Material on Royal Jelly Production

Data presented in Table 2 indicated that the mean royal jelly yield (mg/cup) recorded 300.33, 238.67 and 284.67 mg/cup in compressed Carniolan colonies with 4 frames covered with bees and 359, 377.33 and 350.33 mg/cup for compressed stronger Carniolan hybrid colonies for bee wax, plastic and plastic cups coated with bee wax, respectively. The corresponding figures for Italian hybrid colonies were 315.6, 235.33 and 287.33 mg/cup for compressed median colonies and 384.33, 346, and 388.67 mg/cup for compressed stronger ones. The sqme trend was also reported by **Rana *et al*. (1996)**.

Obtained results indicated that the highest significant royal jelly yield was produced in bee wax cup followed by plastic cups coated with bee wax. On the contrary , plastic cups showed the least royal jelly quantity ,especially in 4-frames covered with bees producer colonies, while 6- frames ones did not manifest significant differences between the 3 types of queen cup material in general. In connection, **Karldag and Genic (2009)** stated that queen cup material is considered one of the main factors affecting royal jelly production. **In (1999)** Durmas and Guler harvested 401.26± 3.38 and 371.04 ± 8.7 mg/pure wax and commercial wax cups, respectively. On the contrary, **EL-Din and El Sammi (1990)** and **Chhuneja and Gill (2012)** reported that rubber and PVC cups produced higher royal jelly than wax cups, respectively.

Effect of Supplemental Feeding on Royal Jelly Production

The mean royal jelly yield in Carniolan and Italian hybrid colonies (mg/cup) harvested after 2,3 and 4 days post – grafting one day old larvae in bee wax queen cups that inserted in colonies offered AD3E, Vit.B. complex and Amino in syrup and in patty was studied in comparison

Table 2. Effect of bee hybrid, colony strength and queen cup material on royal jelly yield (mg/cup) harvested at 3 days post- grafting one day old larva during summer season of 2019

Bee hybrid	No: combs covered with bees	Cup material			Mean
		Bee wax	Plastic	Plastic coated with bee wax	
Carniolan	4	300.33	238.67	284.67	274.446
	6	354	377.33	350.33	362.22
Italian	4	315.67	235.33	287.33	279.44
	6	384.33	346.0	338.67	355.33
Mean	Carniolan	329.665	308	317.5	318.38
	Italian	350	290.665	313	317.88
LSD 0.05 for	Bee hybrid = ns Colony strength = 13.897 Queen cup material = 17.020 Interaction bt.3 factors = 34.040				

with the royal jelly yield of colonies of the two hybrids offered sucrose syrup or patty without any additives. Data obtained in Table 3. indicated that the mean royal jelly yield/cup recorded 321, 343 and 219.33 mg/cup for AD3E in syrup offered to Carniolan hybrid colonies with harvest intervals of 2,3 and 4 days post-grafting ,respectively. The corresponding figures for AD3E added to the patty were 298, 317.67 and 212.33 mg/cup. Regarding Vit.B complex in syrup ,it resulted in royal jelly yield of 281, 34.67 and 244.33 mg/cup when collected at 2,3 and 4 days post grafting. The respective figures for Vit.B complex offered in patty were 268, 323.67 and 23,33 mg/cup. Concerning the royal jelly yield with Amino in syrup ,it recorded 325, 352.33 and 224 mg/cup when harvested at 2,3 and 4 days post grafting from Carniolan hybrid colonies.Royal jelly yield with Amino in patty under the same circumstaces recorded 256, 297 and 211.33 mg/cup Carniolan hybrid colonies received patty alone yielded 244.33, 252.33 and 202 mg royal jelly/cup meanwhile those receive sucrose syrup (1:1) alone produced the lightest royal jelly yield, recording 206.33, 225 and 178 mg/cup when collected at 2,3 and 4 days after grafting , respectively (Table 3). The picture for Italian hybrid colonies received the nutritional supplements in sucrose syrup or in patty (pollen substitute) was not greatly differed as the statistical analysis revealed insignificant differences between the two hybrids. For instance, royal jelly yield (mg/cup) for Italian colonies recorded 310.67, 325.33 and 237.33 for AD3E in syrup and 324.67, 327 and 217.67 mg/cup for AD3E in patty , 275, 313 and 231.67 for vit B complex in syrup , 266.67 , 347 and 226 mg/cup for vit B complex in patty, 278.67, 358.33 and 234.67 mg/cup for for Amino in syrup 259, 278 and 190 mg/cup for Amino in patty when harvest intervals were 2,3 and 4 days, respectively.

Discussing the data obtained revealed the following:

It is clear, in general that offering the supplements in syrup caused more inducement to the glands secreting royal jelly thus the yield became higher than when offered in patty. This phenomenon could be attributed to the rapid ingestion (take up) of the supplements in the syrup quicker than in the patty.

In addition, harvesting royal jelly after 3 days of grafting one days old larva in bee wax queen cups proved to be the best time. Moreover, all the 3 test supplements, induced significantly royal jelly production, at any harvest interval whatever offered in syrup or in patty (pollen substitute) more than patty or syrup without any supplements and this is may be due to the active role of the vitamins and amino acids constituting the supplements which inhance some biochemical reactions, especially in brood food glands which, in turn, induced royal jelly secretion.

Table 3. Effect of supplemental feeding of Carniolan and Italian hybrid colonies on royal jelly production (mg/cup) at different harvest intervals during 2020

Bee hybrid	Carniolan hybrid			Italian hybrid		
Type of feeding	Harvest interval (days)					
	2	3	4	2	3	4
AD3E in syrup	**321**	343.67	219.33	310.67	325.33	237.33
AD3E in patty	**298**	317.67	212.33	324.67	327.0	217.67
Vit. B. complex in syrup	**281**	321.67	244.33	275.0	331.0	231.67
Vit. B. complex in patty	**286**	323.67	231.33	266.67	347.0	226
Amino in syrup	**325**	325.33	224.0	278.67	358.33	234.67
Amino in patty	**256**	297.0	211.33	259.0	278	190
Patty	**244.33**	252.33	202.0	246.67	243.33	169.33
Sucrose syrup (1:1)	**206.33**	225	178.0	198	220.67	172.67
LSD 0.05 for	Bee hybrid = ns Type of feeding = 17.388 Harvest interval = 10.648 Interaction bt.3 factors = 42.593					

In addition, the patty alone is more potent in inhancing royal jelly production than sucrose syrup alone, of course due to the patty contents of nutritional food staffs needed for the glands development and royal jelly secretion. In connection, **Sahinler and Kaftanuglu (1997)** found that bee colonies received pollen substitute produced more royal jelly than those fed on sucrose syrup. However, **Toledo *et al*. (2010)** reported insignificant difference in royal jelly production between colonies received protein diet (35% crude protein) and control colonies did not receive protein diet. Regarding the role of the supplements offernd to royal jelly producing coloneis in the literature, many authors reported positive effect on royal jelly production. For instance, **Sahinler *et al*. (2005)** clear that supplementing producing coloneis with sucrose syrup and vit E caused the highest positive effect followed by pollen substitute + Vit.E while pollen substitute and sucrose syrup came later. In addition, **Garcia and Couto (2005) and Karldag and Genic (2009)** reported great effect of the supplements on royal jelly production, the positive effect of Vit. E on royal jelly production was also reported by **Zayton *et al*. (1988), Chang *et al.* (1993), Van Toor (1994) and Feng Qian Qian *et al*. (2011)**. Also, **Chhuneja and Gill (2012)** reported that Vit B6 as supplement increased significantly royal jelly production.

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دراســـــات على بعض العوامل المؤثرة على إنتاج الغذاء الملكي في طوائف نحل العســـــــل

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قسم وقاية النبات - كلية الزراعة - جامعة الزقازيق - مصر

أجريت هذه الدراسة في منحل خاص بناحية المحلة الكبرى - غربية لتقييم تأثير بعض العوامل الداخلية والخارجية على إنتاج الغذاء الملكي ، وقد أظهرت النتائج انه تم تسجيل أعلى إنتاج للغذاء الملكي عند تطعيم يرقات عمر يوم في كؤوس مصنوعة من شمع النحل وعند جمع الغذاء بعد 3 ايام من التطعيم في طوائف قوية ، في حين لم تظهر أي اختلافات معنوية بين الهجينين قيد الدراسة. هذا وقد أنتجت الطوائف القوية غذاء ملكي أعلى من تلك الضعيفة في كلا الهجينين وقد اعطت الكؤوس المصنوعة من شمع النحل محصولاً أعلى من الغذاء الملكي تلاها تلك المصنوعة من البلاستيك والمبطنة بشمع النحل في حين سُجل اقل إنتاج للكؤوس البلاستيك، إضافة إلى ذلك أحدثت الإضافات الغذائية أد3هـ وفيتامين ب المركب ومركب أمينو زيادة كبيرة في محصول الغذاء الملكي وذلك مقارنةً بمحصول الطوائف التي تغذت على بديل اللقاح او المحلول السكري بدون إضافات غذائية وبصفة أعلى عند إضافتها للمحلول السكري مقارنةً بعجينة بديل اللقاح.

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