STUDIES ON ROYAL JELLY PRODUCTION IN HONEYBEE COLONIES

By

HATEM AHMED SHARAF EL-DIN

B.Sc. Agric. Sci. (Plant Protection), Fac. Agric., Cairo Univ., Egypt, 2006

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Approval Committee

Dr. MOHAMMAD BAHGAT SHAWER .....
Professor of Economic Entomology, Fac. Agric., Kafr El-Sheikh University

Dr. MOHAMAD ATTIA EWIES .....
Professor of Economic Entomology, Fac. Agric., Cairo University

Dr. MOHAMMAD ABD AL-WAHHAB ABD AL-FATTAH...
Professor of Economic Entomology, Fac. Agric., Cairo University

Dr. MAHMOUD EL-SAYED NOUR .....
Professor of Economic Entomology, Fac. Agric., Cairo University.

Date: / /2010

SUPERVISION SHEET

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SUPERVISION COMMITTEE

Dr. MAHMOUD EL-SAYED NOUR
Professor of Economic Entomology, Fac. Agric., Cairo University

Dr. MOHAMMAD ABD AL-WAHHAB ABD EL-FATTAH
Professor of Economic Entomology, Fac. Agric., Cairo University

Name of Candidate: Hatem Ahmed Sharaf El-Din **Degree:** M.Sc.
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Supervisors: Dr. Mahmoud El-Sayed Nour,
Dr. Mohammad Abd Al-Wahhab Abd Al-Fattah.
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ABSTRACT

This work was carried out in the apiary of the Agricultural Experimental Station, Faculty of Agriculture, Cairo University, Giza Governorate.

This study aimed to evaluate some factors affecting royal jelly production in honeybee colonies as well as determination of amino and fatty acids percentages in some samples of the produced royal jelly.

Experiments of the firt aim were separated into colonies grafted with non related larvae and colonies grafted with related grafted larvae.

Experiments of colonies grafted with non related larvae were conducted using Egyptian race and Carniolian hybrid queenless production colonies. Results of Egyptian race colonies showed marked reduction in both mean percentage of acceptance and amount of harvested royal jelly (gm) when compared with those of Carniolian hybrid ones.

Experiments of colonies grafted with related grafted larvae were carried out using Egyptian race, Italian and Carniolian hybrids queenless production colonies. Results indicated that the Carniolian hybrid was the best for royal jelly production as the hieghest percentage of acceptance and amount of harvested royal jelly (gm) were achieved when compared with others. Italian hybrid gave higher acceptance percentage than Egyptian race but amount (gm) of harvested royal jelly was almost the same with an increase of 0.02 for the Egyptian hybrid.

In respect of amino and fatty acid percentages, the percentages of essential amino acids had their maximum values in the Egyptian race samples, followed consecutively by Italian hybrid and Carniolian hybrid samples, with an exception of Lysine acid, where Carniolian hybrid samples gave the highest amount of it, followed by Italian hybrid then the Egyptian race. The maximum values for non-essential amino acids occurred in the Egyptian race samples whereas the second and third ranks oscillated between Italian hybrid and Carniolian hybrid samples.

The maximum values of fatty acids were recorded in the Carniolian hybrid samples, whereas the second and third ranks fluctuated between Italian hybrid and Egyptian race samples except for both of 10-hydroxy-2-decanoic acid (10HDA) samples and Cis,cis-9,12- Octadecadienoic acid. Italian hybrid had the highest amount of 10HDA, followed by the Egyptian race then Carniolian hybrid samples. On the other hand, Cis,cis-9,12- Octadecadienoic acid gave the highest value in the Egyptian race samples then Carniolian and Italian hybrids, respectively.

Key words: Honeybee, colonies, royal jelly, grafted larvae, hybrid, race, amino acids, fatty acids

DEDICATION

I dedicate this work to whom my heart felt thanks; to my wife and my daughter for their patience and help, as well as to my parents for all the support they lovely offered along the period of my post graduation.

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INTRODUCTION

Honeybee colonies have been used in the advantage of humans as it used in royal jelly, honey, wax, bee venom and propolis production, besides being very important as pollinator agents (Laidlaw, 1992).

Royal jelly (RJ) is one of the most valued products of honeybee colonies and is produced by 6-12 days old honeybee workers *(Apis mellifera* L), called nurse bees (Deseyn and Billen, 2005 and Hassan and Khater, 2006)

It is secreted from the hypopharyngeal (watery) and mandibular glands (milkey). Such glands are located in the workers heads (Haydak, 1970) and direct the development of honeybee larvae into queen bees (Okamoto *et aI.*, 2003) where it is an essential food for both.

honeybee workers do not stock RJ, being immediately utilized in larval feeding. RJ has many uses such as: feeding workers and drones larvae during a certain phase (Haydak, 1970); feeding the queen during all its larval phase and adult life (Wang, 1965 and Wang and Moeller, 1969) and the cast differentiation between queen and worker bees is related strictly to differences in the feeding of the larvae.

RJ is a white creamy substance with a slightly pungent odor and taste. RJ consists naturally of a mixture of many constituents including proteins (not less than 12%), carbohydrates (not less than 12%), lipids (not less than 5.5%), moisture (not more than 66%) and 10-Hydroxy-2-decenoic acid (10 HAD) (not less than 2%), (Howe *et al.*,1985).

Humans have used RJ for a long time for it's benefits as it's believed that RJ stimulate the immune system, strengthens the body

and it's a good assistant cure for many diseases such as leukemia, cancer, high blood pressure, high cholesterol, and infertility in males and females (Krell, 1996).

RJ is being produced as a result of grafting process, and the acceptance of grafted queen cups is being affected by type of nutrition and queen cups introduced to the bees (Zeedan, 2002). Moreover, acceptance percentage of queen cells was significantly higher when the grafted larvae were less than 48 hrs old (Abd Al-Fattah *et al*., 2003 b).. Mouro and Toledo (2004) found that Carniolan hybrid showed a higher production of RJ per colony, in three-day collect when compared to the Africanized honeybees and the Carniolan hybrid also had a higher percentage of larvae acceptance compared to the Africanized honeybees.

Furthermore, the total quantity of RJ produced by the colony was higher in queenless colonies than in queenright ones (Ibrahim, 2002). Not only that , but RJ production is also being affected by bee race (Saleh, 1999).

RJ is being commonly produced from the apiaries in Egypt for its low costs and high price, since the price of one Kg could reach 6000 E.P. Natural RJ could be either stored under refrigeration for a period extending from 18-24 months at – 20 ºC after packing or stored at 0 – 5 ºC and to save it's efficacy for one year.

As results of the increasing interest of RJ in human health, the numbers of reports in the area of authentication and quality control have increased within the past few years. Methods have been developed to characterize the quality of RJ by determination of general

parameters as the main way to definite the quality of RJ is the chemical analysis of its content specially the percentages of amino acids and 10 HDA.

Different factors may interfere in RJ production: genetics; colonies internal population conditions; food flow and external environment related to weather conditions (Nogueira-Couto, 1992 and Azevedo-Benitez *et al.*, 1998). Thus, it is important to study the influence of these factors on RJ production, and honeybee's regional adaptability. The aim of this study was to investigate some factors affecting the quantity and quality of produced RJ at Giza region as follow:

1. Evaluation of some factors affecting royal jelly production in honeybee colonies.
2. Determination of amino and fatty acid percentages in samples of the produced royal jelly.

REVIEW OF LITERATURES

1. Methods of production

As Root (1945) in USA, reported in his book that in order to rear good queens, it is necessary to have number of young -one day old- larvae. Some authorities say that larvae at any time under three days old from the egg will make good queens.

Snelgrove (1946) in England, used pure bees-wax in his artificial queen-cups, but this wax must be fresh and best light color. He also used 24 hrs. larvae for rearing queens.

Muller (1952) in Scandinavian region, grafted larvae within 24 hours of emergence.

Chauvin and Vuillaume (1955) in Paris, thought that the acceptance of artificial queen cells was poor when the wax used in making them contained queen extracts, which apparently inhibited feeding of the grafted larvae.

Wilson (1955) in USA, placed about forty cells grafted with twelve to fifteen hours old in swarm box. After twenty four hours, the cells were transferred to a nursery colony. When the larvae were between 3-4 days old, the greatest volume of royal jelly was obtained.

Vullaume (1956) reported that propolis mixed in the wax was an inhibitor for queen cell acceptance.

Nienan (1958) proved that beeswax grafting compound yielded better results than did asphalt emulsions

Eckert and Shaw (1960) in USA, mentioned that strong colonies

will start and finish 30 to 45 good queen cells.

Baryes (1963) gave a method that required the proper preparation for a number of colonies. The first colony where queen cups and eggs were produced had not more than 30-40 queen cells, of which 10 - 12 were put into queen right colonies for rearing and finally into queenless nuclei for emergence.

Inoue and Inoue (1963) in Japan, grafted larvae about 20 hrs-old into the cups and concluded that the plastic cups and wax cups were accepted (90.3, 75.8%, respectively). Plastic cups were superior except in the yield of royal jelly per cup.

Abdel-Rahman (1964) in Egypt, indicated that capacity of the colony to accept cell cups was averaged 18 cell cups in Alexandria and 34 in Gharbia. The highest records were occurred from March to September, while the number accepted in February was clearly limited.

The same author (1967 b) found that artificial queen cups made of new beeswax and of beeswax from old combs were equally acceptable.

Ibrahim *et al.* (1969) in Egypt, reached a maximum of about 45 larvae at one time to the cups.

Mohanna (1969) in Egypt, found that the breeder colony has a limited capacity to obtain numbers of successful queen cells differed from one month to another. The highest percentage of successful queen cells (averaged 71.8%) obtained in July. During this month, the total number of introduced cells was 120 cups, and the accepted number of queen cells was 86. The lowest average percentage of successful queen cells occurred during March (45.8%). During May, the average

percentage of successful queen cells was 60%, as opposed to 57% only in September. He mentioned that rearing queens from worker larvae aged more than 48 hours proved to be less successful.

Lensky (1971) demonstrated that close contact of the queen with the grafted larvae had no inhibitory effect upon their acceptance and early rearing during late summer and winter. The acceptance percentage of grafted larvae by colonies with free and confined queens were 48-69 and 37-73%, respectively.

Wilson (1971) placed about forty cells grafted with 12 to 15 hrs. old in swarm box. After 24 hrs., the cells were transferred to a nursery colony. When the larvae were between 3-4 days old, the greatest volume of royal jelly was obtained.

Weiss (1974) in Germany, found that the percentage acceptance of double transplanted cells was 89% whereas that of single-transplanted cells was 72%, where the bees were given a choice between equal numbers of both presentations. He did not find this difference to be a significant one.

Rekos (1976) in Italy, achieved best results for queen rearing when larvae were transferred into prepared cups made from wax.

Moneim and Seleem (1978) in Egypt, found that the percentage of successful queen cells in preliminary grafting with one day old larvae.

Ebadi and Gary (1980) in USA, indicated some factors affecting the acceptance of grafted queen cells. Acceptance was low (32%) when queen pheromone (9-oxodec-trans-2-enoic acid) was present in the wax of artificial queen cups. Statistically, significant