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Economic outcome of producing royal jelly from two honeybee hybrids rearing colony under queen right and queen less conditions through two different grafting type of larvae genotype

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Abstract:

Honeybee royal jelly is one of the most important products in the honeybee industry, it is produced from the hypopharyngeal and mandibular glands of 6-12 days old workers. it's a white creamy substance containing mainly proteins, sugars, and lipids. Also, it has many benefits for humans such as stimulating the immune system, strengthening 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. This investigation aimed to study the economic feasibility of producing royal jelly from two honeybee hybrids under queen right and queen less conditions through the different grafting larvae genotype at two years .The queen rearing colonies were provided: either queen less or queen right of the Italian or Craniolan honeybee hybrids using an inverted L- queen excluder. During the spring season (April and May) - summer season (July and August) and late summer season (August to September) for two successive years 2020 and 2021. The queen right condition produced royal jelly less than the queen less. The value of royal jelly production was recorded during the spring season while The lowest value of royal jelly production was recorded at the Late summer season. The queen less condition, the Craniolan grafting larvae and spring season can use for the best quantity of produce royal jelly.

Introduction

Royal jelly is one of the most importance products in honeybee industrial, it produced from hypopharyngeal and mandibular glands of 6-12 days old workers, (Deseyn and Billen, 2005). It's a white creamy substance contains mainly of proteins, sugars, and lipids (Schmidt, 1997). Also it has many benefits for humans such as 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 and Sahinler, 2000).

The quantity of royal jelly per cup varies depending on the time it remains in the colony. Chang (1977) obtained more royal jelly when collecting 72 hours after 12-14 hour-old larvae transference than in collects done 48 hours after larvae transference. The quantity of royal jelly per cup varies depending on the time it remains in the colony. Toledo (1997) observed that Carniolan had higher larvae acceptance

and higher royal jelly production than Africanized queens descending from: queens inseminated by Italian males; Africanized queens with Italian males; Italian queens inseminated by Italian males and Italian queens with Africanized males.

Different factors may interfere in royal jelly production: genetics; colonies internal population conditions; food flow; queen's egg-laying; and external environment related to weather conditions and food availability (Nogueira-Couto, 1992 and 1996; Toledo, 1997 and Azevedo-Benitez *et al.* 1998). Thus, it is important to study the influence of these factors on royal jelly production, and honeybee's regional adaptability. Royal jelly 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 (Mohanny, 2010 and Zeedan, 2002).

Royal jelly contains remarkable amounts of proteins, lipids, glucides, vitamins, hormones, enzymes, and mineral substances (Howe *et al.*, 1985). The composition of elements in the royal jelly are water (50% to 60%), proteins (18%), carbohydrates (15%), lipids (3% to 6%), mineral salts (1.5%), and vitamins (Trace amount) (Nagai and Inoue, 2004).

There are several factors affecting royal jelly production, the most important of them are the age of transferred larvae (Sahinler and Kaftanođlu, 1997), feeding (Fuhai *et al.*, 1993), number of transferred queen cell cups (Van-Toor and Littlejohn, 1994), harvesting interval (Fiahinler and Fiahinler, 2002), whether, the colony is queen less or queen right (Van-Toor and Littlejohn, 1994) and bee race (Shibi *et al.*,1993).

This investigation aimed to study the economic outcome of producing royal jelly from two honeybee hybrids

under queen right and queen less conditions through the different grafting larvae genotype at two years.

Materials and methods

The experiments were conducted under the conditions of Kafr El-Sheikh Governorate (With the facility of the Sakha Agricultural Research Station) during years 2020 and 2021. The hive approximately equal of strength and number of combs. The queen rearing colonies were provided: Either queen less or queen right of the Italian or Craniolan honeybee hybrids using an inverted L- queen excluder.

Procedures:

During the spring season (April and May) - summer season (July and August) and late summer season (August to September) for two successive years 2020 and 2021.

The bee colonies under the study are as follows:

1. Choose the parent colony of the Italian and Craniolan honeybee hybrids, an empty worker brood frame was but between the brood frames to force the queen to lay eggs and follow until the hatching is completed three days later. The fourth day is the first larval age (larvae of one day) which takes the shape of the crescent.

2. Processing of grafting frames with three strips arranged in three different locations (Upper, middle, lower) each frame carrying 45 plastic cups. The rearing frame is then exposed to the rearing colony two hours before the grafting.

3. Colonies without queens (Queen less):

The queens were removed, and the colonies became without queens (queen less). The strength of the colonies were Eight combs covered with bees divided into five sealed brood combs plus three honey and pollen combs + plastic feed.

4. Colonies with queens (Queen right):

Inverted L-shaped excluder was used. Each colony has 11 combs covered with bees divided as follows: two sealed brood combs + one honey and pollen comb) under the Queen's reservation + a vertical queen excluder + four sealed and open brood combs, the open combs close to the sealed (Two sealed brood) + two honey and pollen combs) in addition to plastic feed. The rearing process in the honey box.

Twelve honey bee colonies were used in the experiment of the Italian and Craniolan honeybee hybrid distributed as follows:

Six replicates of the Italian honey bee hybrid was divided to three replicates without queen and three replicates with queens. Also, Six replicates of the Craniolan honey bee hybrid were divided to three replicates without a queen and three replicates with queens.

The queens of the rearing colonies were removed 48 hours the before inserted the graphing from either for queen less or queen right colonies. The method of Doolittle was applied 1909 - wet method of grafting (1 gram of royal jelly: 1 cm distilled water) (1909)

Procedures:

1. Exposing the grafting frames that carry the plastic cups to the breeding colony for two hours before the grafting. Provide nutrition to the rearing colony

2. Rearing was carried out in the brood chamber for the queen less. In the case of the queen right, rearing was carried out in honey supers under the inverted L-shaped excluder

3. Grafting the one day larvae were transferred into the plastic cups by the grafting needle and then the rearing frame that carried three wooden bars placed between the sealed brood combs in both queen less and queen right chamber.

4. The nutrition

Sugar solution with concentrate of 1kg sugar: 1.5 water. Each colony fed on half a liter of the solution.

5. On the same date of grafting, after 72 hours the rearing frames were raised from the rearing colonies and removing the larvae from the plastic cups by a plastic needle, then collecting the royal jelly with a wooden spoon, and collecting the royal jelly according to the successful royal cups. The royal jelly containers were weighed empty and filled then numbered with a code number, the capacity of each container was five grams. Each queen cell was weighed (1-5 cups or less). The number of cups is divided by the number of cups to find the average per cup. The royal jelly was saved in the fridge.

The grafting process is repeated every three days to bring new crews in the royal cups. During the experiment, five grafting process were taken for all seasons and also in all experiments for 2020 and 2021 years.

Results and discussion

Data in Table (1) indicated the royal jelly quantity means (gm/colony/year) produced from Craniolan hybrid rearing colony under queen right and queen less of grafted Italian and Craniolan genotype larvae during different seasons in year 2015. At the spring season the Italian grafting larvae in the queen right produced 21.32 gm less than the queen lees which produced 24.32 gm. As the Craniolan grafting larvae in the queen right produced 23.83 gm less than the queen lees which produced 26.17 gm.

At the summer season, the Craniolan grafting larvae in the queen right produced 19.97 gm less than the queen lees which produced 21.89 gm. Moreover, the Italian grafting larvae in the queen right produced 15.99 gm less than the queen lees which produced 22.46 gm.

At the late summer season, the Italian grafting larvae in the queen right produced 13.00 gm less than the queen lees which produced 13.85 gm. However, the Carniolan grafting larvae

in the queen right produced 15.55 gm less than the queen lees which produced 16.36 gm.

Table (1):Royal jelly quantity means (gm/ colony/year) produced from Carniolan hybrids rearing colony under queen right and queen-less of grafted Italian and Carniolan genotype larvae during different seasons in year 2020.

Rearing colony	Grafting Larvae genotype	Seasons						Mean /Colony /Year	
		Spring		Summer		Late Summer		Queen right	Queen less
		Queen right	Queen less	Queen right	Queen less	Queen right	Queen less		
Carniolan	Italian	21.32	24.32	15.99	22.46	13.00	13.85	50.31	60.63
Hybrid	Carniolan	23.83	26.17	19.97	21.89	15.55	16.36	59.35	64.42
Means		22.6	25.3	17.98	22.2	14.3	15.1	54.85	62.5

Data in Table (2) indicated the royal jelly quantity means (gm/ colony/year) produced from Italian hybrid rearing colony under queen right and queen-less of grafted Italian and Carniolan genotype larvae during different seasons in year 2016. At the spring season the Italian grafting larvae in the queen right produced 29.00 gm less than the queen lees which produced 31.10 gm. As the Carniolan grafting larvae in the queen right produced 27.04 gm more than the queen lees which produced 23.90 gm.

At the summer season, the Italian grafting larvae in the queen right produced 22.76 gm less than the queen lees which produced 27.50 gm. As the Carniolan grafting larvae in the queen right produced 20.32 gm less than the queen lees which produced 21.28 gm. At the late summer season, the Italian grafting larvae in the queen right produced 16.93 gm less than the queen lees which produced 17.79 gm. As the Carniolan grafting larvae in the queen right produced 13.31 gm less than the queen lees which produced 14.70 gm.

Table (2): royal jelly quantity means (gm/ colony) produced from Italian hybrids rearing colony under queen right and queen-less of grafted Italian and Carniolan genotype larvae during different seasons in year 2021.

Rearing colony	Grafting Larvae genotype	Seasons						Mean /Colony /Year	
		Spring		Summer		Late Summer		Queen right	Queen less
		Queen right	Queen less	Queen right	Queen less	Queen right	Queen less		
Italian	Italian	29.00	31.10	22.76	27.50	16.93	17.79	68.69	76.39
Hybrid	Carniolan	27.04	23.90	20.32	21.28	13.31	14.70	60.67	59.9
Means		28.02	27.5	21.55	24.4	15.12	16.25	64.68	68.15

Data in Table (3) indicated the royal jelly quantity means (gm/ colony/year) produced from Italian hybrid rearing colony under queen right and queen-less of grafted Italian and Carniolan genotype larvae during different seasons in year 2016. At the spring season the Italian grafting larvae in the queen right produced 27.66 gm more than the queen lees which produced 26.69 gm. As the Carniolan grafting larvae in the queen

right produced 24.88 gm less than the queen lees which produced 25.00 gm. At the summer season, the Italian grafting larvae in the queen right produced 22.34 gm more than the queen lees which produced 19.65 gm.

As the Carniolan grafting larvae in the queen right produced 22.91 gm more than the queen lees which produced 20.62 gm. At the late summer season, the Italian grafting larvae in the queen right produced 15.00 gm more

than the queen lees which produced 14.03 gm. As the Craniolan grafting larvae in the queen right produced

12.34 gm less than the queen lees which produced 12.99 gm.

Table (3): Royal jelly quantity means (gm/ colony) produced from Italian hybrid rearing colony under queen right and queen-less of grafted Italian and Carniolan genotype larvae during different seasons in year 2020.

Rearing colony hybrid	Grafting larvae genotype	Seasons						Mean /Colony /Year	
		Spring		Summer		Late Summer		Queen right	Queen less
		Queen right	Queen less	Queen right	Queen less	Queen right	Queen less		
Italian Hybrid	Italian	27.66	26.69	22.34	19.65	15.00	14.03	65.00	60.37
	Carniolan	24.88	25.00	22.91	20.62	12.34	12.99	60.13	58.1
Means		26.3	25.8	22.6	20.10	13.67	13.51	62.6	59.23

Data in Table (4) indicated the royal jelly quantity means (gm/ colony) produced from Italian hybrid rearing colony under queen right and queen-less of grafted Italian and Carniolan genotype larvae during different seasons in year 2016. At the spring season the Italian grafting larvae in the queen right produced 23.49 gm more than the queen lees which produced 22.41 gm. As the Craniolan grafting larvae in the queen right produced 24.28 gm less than the queen lees which produced 23.89 gm.

At the summer season, the Italian grafting larvae in the queen right produced 22.54 gm more than the queen lees which produced 15.34 gm. As the Craniolan grafting larvae in the queen right produced 19.57 gm more than the queen lees which produced 18.02 gm. At the late summer season, the Italian grafting larvae in the queen right produced 11.82 gm less than the queen lees which produced 12.30 gm. As the Craniolan grafting larvae in the queen right produced 14.00 gm less than the queen lees which produced 13.23 gm.

Table (4) royal jelly quantity means produced from Italian and Carniolan hybrids rearing colony under queen right and queen-less of grafted Italian and Craniolan genotype larvae during different seasons in year 2021.

Rearing colony hybrid	Grafting larvae genotype	Seasons						Mean /Colony /Year	
		Spring		Summer		Late Summer		Queen right	Queen less
		Queen right	Queen less	Queen right	Queen less	Queen right	Queen less		
Carniolan Hybrid	Italian	23.49	22.41	22.54	15.34	11.82	12.30	57.85	50.1
	Carniolan	24.28	23.89	19.57	18.02	14.00	13.23	57.85	55.14
Means		23.9	23.15	21.1	16.68	12.91	12.8	57.85	52.62

From the obtained results it could be suggested that, under the Craniolan rearing colony in year 2020 the queen right condition produced royal jelly less than the queen less. Whoever the Craniolan grafting larvae produced royal jelly more than the Italian one. The highest value of royal jelly production was recorded at the spring season while The lowest value of royal jelly production was recorded at the Late summer season. under the

Italian rearing colony in year 2021. As the queen less condition produced royal jelly more than the queen right. Whoever the Italian grafting larvae produced royal jelly more than the Craniolan one. The highest value of royal jelly production was recorded at the spring season while The lowest value of royal jelly production was recorded at the Late summer season. under the Craniolan rearing colony in year 2020 the queen right condition

produced royal jelly more than the queen less. Whoever the Italian grafting larvae produced royal jelly more than the Carniolan one. The highest value of royal jelly production was recorded at the spring season while The lowest value of royal jelly production was recorded at the late summer season.

Under the Italian rearing colony in year 2021 the queen right condition produced royal jelly more than the queen less. Whoever the Carniolan grafting larvae produced royal jelly more than the Italian one. The highest value of royal jelly production was recorded at the spring season while The lowest value of royal jelly production was recorded at the Late summer season this may be due to the differences between season in weather conditions and the differences between hybrids.

From the obtained result the following conclusion can be drawing. The queen less condition, the Carniolan grafting larvae and spring season can use for the best quantity of produce royal jelly. Many authors discussed these points . Descendants from highly royal jelly productive colonies had a higher acceptance of transferred larvae and also deposited more royal jelly per cup (Azevedo, 1996). Toledo (1997) observed that Carniolan had higher larvae acceptance and higher royal jelly production than Africanized queens descending from: queens inseminated by Italian males; Africanized queens with Italian males; Italian queens inseminated by Italian males and Italian queens with Africanized males. Different factors may interfere in royal jelly production: genetics; colonies internal population conditions; food flow; queen's egg-laying; and external environment related to weather conditions and food availability (Nogueira-Couto, 1992 and 1996; Toledo, 1997 and Azevedo-Benitez *et al.*, 1998). The average acceptance rates throughout the season were 88.2% and

72.1% in queen less and queen right cell builders, respectively (Sahinler and Kaftanođlu, 1997). Kaftanođlu and Kumova (1990) reported that, the average acceptance rates for queen less cell builders were 87.1%, 75.9% and 77.59%, respectively. Royal jelly production in April was 9.2% greater than that in May, 17.7% greater than that in June, 41.9% greater than that in July, 65.1% greater than that in August and 103% greater than that in September. These results showed that royal jelly production decreased significantly over time. This was due to the shortage of fresh pollen during summer and weakness of the queen less cell builders due to ageing. Öztürk (1997) reported that the average royal jelly yield for queen less cell builders was 0.180 g and 0.303 g, respectively.

The acceptance rates in the Carniolan bee genotype were 7.9% and 16.7% more than in the Muđla and Caucasian genotypes, respectively. Similarly, royal jelly yield in the Carniolan bee genotype was 14.46% and 86% more than that in the Muđla and Caucasian genotypes, respectively. The honeybee colonies can maintain their life even in adverse weather conditions. They are active during the whole year, but the degrees of these activities differ from time to time, from period to period and from season to another. These differences are due to the variation in the environmental conditions, which includes both weather factors and sources of nectar and Pollen, which considered the main food for these colonies. The brood rearing activity of honeybee colonies depends upon many factors, among them temperature and the collection of pollen and nectar, on one side and the colony population (Farrar, 1944; Larie and Brand, 1958; Townsend and Smith, 1969 and Keith, 1975) in the other. Mohanna (1969) reported that, the breeder colony had a limited capacity to

rear queens, and the obtained number of successful queen cells differed from one month to another. The number of sealed queen cells differed from 10 cells in March to 25 sealed queen cells in July. The highest percentage of a successful queen cell (Average 71.8%) was obtained in July. The lowest average percentage occurred during March average (45.80%). During May, the average percentage of successful queen cells was 60%, but was only 47% in September.

Eweis (1974) found that the largest amount of royal jelly production for younger worker colonies was obtained during the spring (11.82 and 8.84 g), the late summer (14.23 and 10.95 g) and early summer (11.28 and 7.8 g) through 1970 and 1971, respectively. The lowest amount for the old worker bees (4.8 and 2.47 g) queen less normal colonies (4.59 and 3.56 g) and small worker age colonies (7.83 and 3.77 g) were showed during the autumn season in 1970 and 1971. Mouatadid (1978) reported that the queens reared during the honey flow (June-July) were significantly heavier (196,0 mg) than those reared at other times (159.5 mg). Nasr (1979) found that the mean weights of newly emerged queens during spring, summer and autumn seasons were 196.6 mg, 185.6 mg and 177.9 mg, respectively.

Krol (1985) found that during the first 6 days of queen larval development, differences were observed between Causasian, Camiolan, Italian and north European honey bee races. Carnelians built the largest and heaviest queen cells and put most royal jelly in each cell. The best time of the year for queen production was at the end of May and in early August. In all the races, the amount of royal jelly in the cell was maximal one at the fourth day of larval development.

El-Sarrag (1993) found that queens were successfully reared in

February (92%) from grafted larvae and during March – June (82%). The least favorable periods (48%) were in August – September, 28% in December – January. He added that the mortality of embryos was significantly correlated with temperature and with pollen collection. The most suitable seasons for rearing queens could be descending ordered as follows: spring, summer and late summer. Ismail (2001) found that queen rearing began when the brood nest was congested and nectar and pollen were abundant, obtained. March was the maximum month for queen cups production. El-Wassef (2002) indicated that the mean number produced queen cups were higher in spring – 1998 (16.77/col.) and winter (5.741/col.) than in summer (2.89/col.) and autumn (0.2/col.) in the indoor colonies in closed area. While, in the second experimental period (1999-2000) the mean number of produced queen cups were higher in winter (3.71/col.) and summer (3.481/col.) than in spring (3/col.) and autumn (0.29/col.) under the same condition. The fed colonies were active in building more queen cups than unfed ones and higher building queen cups in March.

Shoreit *et al* (2002) found that the maximum total of queen cells and queen cups was found during February – April period or swarming period. Minimum construction of queen cells and cups was observed during November and December. No constructed queen cells were observed during June, October and November, while queen cups can be observed every month maximum population of adult bees was found during the period from July till September. Maximum stored pollen or bee bread was observed inside colonies during August – October period, while minimum stored pollen was noticed during December, November and May.

Zeedan (2002) showed that the highest number of building queen cups was during March (24.6 queen cups/colony) while the lowest number was during December (0.9 queen cups /colony). The building queen's cups could be arranged in descending order as follows: during spring (38.4%) followed by summer (26.4%) winter (20.1%) and autumn (15.1%) seasons. He added 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, and he also, added that the heaviest queens were produced during summer (178.7mg/queen). This value was significantly higher than during the other seasons which were 175.6, 171.8 and 168.2 mg/ queen in spring, autumn and winter, respectively. Abd Al-Fattah *et al.* (2003) under the environmental conditions of North Sinai region determined that the highest rate of acceptance (82.8%) occurred during summer. Significant difference was found between summer and other seasons. The lowest significant percentage appeared in winter (36.1%) while moderate results for the acceptance larvae were noticed during spring (70.0%) and autumn (72.8%) seasons with insignificant differences between them.

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