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Royal jelly quantity means (Mg/Cups) under different diets and bars level within two successive years

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Abstract:

Recently there has been increasing attention to the use of natural food additives. Royal jelly is regarded as one of the most important honeybee products. It's produced from the hypopharyngeal and mandibular glands of 6-12 days old workers. As it acting an essential role in honeybee growth**.** The aim of this study is to examine some factors affecting royal jelly quantity production and acceptance percentages. The experiment consisted of 18 colonies that were used as replicators distributed as follows: Six replicates fed on substitute A (Skimmed soybean flour), six replicates fed on substitute B (Chickpeas flour + medical yeast) and six replicates fed on (Maize Pollen) (C) as a control. The first year has acceptance percentages that mean more than the second with non-significant differences. While the soybean showed acceptance percentages mean more than the other diets, with significant between soybean and other treatments were non-significant. Furthermore, the middle bar has acceptance percentages that mean more than the upper and lower bars with significant differences.

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Introduction

Royal jelly is a proteinaceous secretion derived from the hypopharyngeal and mandibular glands of young worker bees. It is the only food fed to the queen throughout her lifetime and is also fed to all young larvae for the first three days after hatching. Royal jelly is being produced as a result of the grafting process and the acceptance of grafted queen cups is affected by the type of nutrition and queen cups introduced to the bees (Mohanny, 1999 and Zeedan, 2002).

There are several factors affecting royal jelly production. The age of transferred larvae is the most crucial factor (Sahinler and Kaftanoglu, 1997), feeding (Fuhai *et al*., 1993), number of transferred queen cell cups (Van-Toor and Littlejohn, 1994) and Kutluca *et al*.,1998), harvesting interval (Þahinler and Þahinler, 2002), whether, the colony is queenless or queenright and bee race (Van-Toor and Littlejohn, 1994).

The aim of this study is to examine some factors affecting royal

jelly quantity production and acceptance percentages.

Materials and methods

During the late summer season, this experiment was carried out (August and September) only in areas where natural pollen and nectar supplies are scarce for two years in a row, 2020 and 2021, processing substitutes be used under the craniolan hybrid.

1. Use of pollen substitute:

1.1. Substitute (A) skimmed soybean flour:

Ingredients: 400 grams of skimmed soybean + 300 grams of sugar powder + 100 grams of seedless paste + 100 grams of orange skin + 100 grams of Egyptian apple skin + 100 grams of carrots skin + 5 cm of linen Sowing oil. The components are mixed with sugar and fed to the experiment colonies at a rate of 100 grams each week. Plastic bags are used to wrap the replacement, with holes cut from the bottom and placed on the tops of the bee combs.

1.2. Substitute (B) chickpeas flour + medical yeast:

Ingredients: 400 grams of chickpeas + 350 grams of sugar powdered + 100 grams of seedless paste + 100 grams of medicinal yeast + 100 grams of orange skin + 100 grams of apple skin + 100 grams of carrot skin + 5 cm of cinnamon oil and be mixed with a sugar solution.

2. Use of pollen (Maize pollen) C control:

The maize pollen was supplied to the rearing colonies at a rate of 100 grams per colony per week on carton paper beneath the bee combs on the hive's floor. Pollen traps should be repeated on replacement A and B substitutes to guarantee that pollen is present in the region and to prevent the entry of bees carrying pollen from other sources or if a secondary source exists. During the trial time, sugar is administered (Pre-grafting stimulation and feeding after grafting). A sugar solution with a concentration of 1kg sugar concentrate + 1.5 water. Half a liter of the solution was supplied to each colony.

3. Procedures:

In the experiment 18 colonies were used as replicators distributed as follows:

Six replicates of substitute A. Six replicates of substitute B. Six replicates of substitute C. Each breeding frame has 15 plastic cups and is processed with three screws at three distinct locations (Upper, middle, and bottom). Two hours before the grafting, the breeding frame is exposed to the breeding colony.

4. Statistical analysis:

Using the MSTAT-C (Version 2.10) computer application, all data were analyzed using Analysis of Variance (ANOVA), followed by Duncan's multiple range test to assess the differences between the derived means. Data related to the acceptance percentage of grafting larvae in rearing colonies and the quantity mean of the royal jelly production per cup were analyzed in a split–split randomized complete block design using three replicates for each treatment.

Results and discussion

Data in Table (1) presented the royal jelly quantity mean (mg/cup) from different diets under different bars level during two successive years (2020 and 2021) during late summer (August and September). During the first year, the highest royal jelly quantity means were (156, 134.5, and 153) recorded in the middle/upper bars dieted on soybean and lower on pollen respectively. However, the lowest royal jelly quantity means were (124.4, 155.6, and 147.2) recorded in the upper/lower bars dieted on chickpea and middle bars dieted on soybean respectively. During the second year, the highest royal jelly quantity means were (154.6, 150.2, and 130.9) recorded in the lower, middle and upper bars dieted on soybean, respectively. However, the lowest royal jelly quantity

means were (127.7, 142.3, and 139.6) recorded in the upper/lower and middle bars dieted on chickpea and pollen respectively.

Table (1): The royal jelly quantity means (Mg/Cups) from different diets under different bars level during two successive years (2020 and 2021) during late summer (August and September).

Diet	First year				Second year				mean s.e±/ diet
	Upper	middle	Lower	AV± s.e	Upper	middle	Lower	AV± s.e	
Soybean	134.5	156	147.2	145.9±8.28	130.9	150.2	154.6	145.2±8.40	145.6±5.73 A
Chickpea	124.4	155.6	151.5	143.8±15.09	127.7	143.8	142.3	137.9±7.33	140.9±8.26 A
Pollen	130.4	155.8	153	146.4±13.36	129	139.6	142.8	137.1±6.78	141.8±7.61 A
Mean	129.8	155.8	150.6	145.4±7A	129.2	144.5	146.6	140.1±4.41A	142.7±4.16

Mean/ bars level upper129.4±3.850 B middle 150.2±5.710 A lower148.6±7.221 A150.2±5.710 A
L.S.D value for years at 5 %=18.435 L.S.D value for different diets at 5 %=5.786
L.S.D value for level of bars at 5 %=5.645

The results could be concluded that the first year has royal jelly quantity mean more than the second with non-significant differences. While the soybean showed royal jelly quantity mean more than the other diets, with non-significant differences between all diet's treatment. Moreover, the middle bar has a royal jelly quantity means more than the upper and lower bars with significant differences between upper and middle/lower, and non-significant differences between middle and lower bars.

Data in Table (2) presented the grafted queen cups acceptance percentage mean using different diets and bars levels for two successive years (2020 and 2021) in late summer (August and September). During the first year, the highest acceptance percentage means were (76.9 - 70.7, and 66) recorded in the middle bar fed on pollen, lower and upper diet on soybean respectively. but the lowest acceptance percentage means were (60.4 - 67.2, and 70.7) recorded in the upper bar fed on pollen, lower bar fed on pollen, and lower bar fed on soybean respectively. During the second year, the highest acceptance percentage means were (70.6 - 70.5, and 65.3) recorded in the middle bar fed on pollen, lower soybean, and upper soybean respectively. but the lowest acceptance percentage means were (61.6 - 67.9, and 69.2) recorded in the upper and lower bars fed on pollen and middle bar fed on chickpea respectively.

Table (2): Grafted queen cups acceptance percentages Mean using different diets and bars level of two successive years (2020 and 2021) at late summer (August and September).

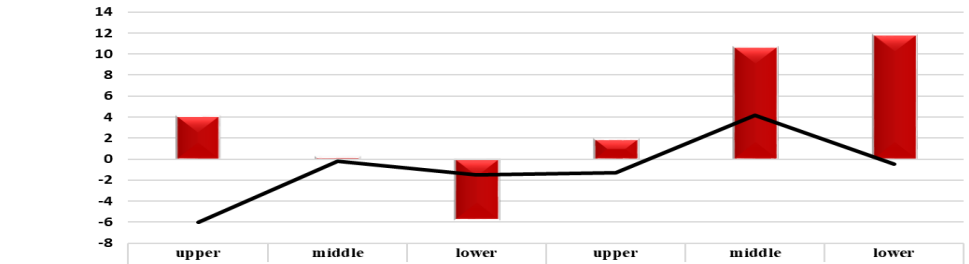
Diet	First year				Second year				Mean s.e±/ Diet
	Upper	Middle	Lower	AV± s.e	Upper	Middle	Lower	AV± s.e	
Soybean	66	70.7	70.7	70.8±3.86	65.3	69.8	70.5	68.5±2.04	69.7±2.19A
Chickpea	64.3	75.7	69.4	69.8±3.71	64.7	69.2	69	67.6±1.69	68.7±2.05AB
Pollen	60.4	76.9	67.2	68.2±5.16	61.6	70.6	67.9	66.7±3.00	67.4±2.92B
Mean	63.6	76.1	69.4	69.6±2.42A	63.95	69.8	69.1	67.6±1.32A	68.6±1.39

Mean/ bars level upper 63.7±1.28 C middle 73.0±2.18A lower 69.1±1.25 B
L.S.D value for years at 5 %= 4.08 L.S.D value for different diets at 5 %= 0.93
L.S.D value for level of bars at 5 %= 1.38

The data in Figure (1) illustrated the differences between treatment and control in royal jelly quantity means (Mg/Cup) from Italian and crniolan hybrids with different diets within two successive years (2020 and 2021) during late summer. The first year on soybean diet showed means more than the control in booth upper and middle bars (4.1 / 0.2) respectively. But on the chickpea diet, all bars (upper, middle, and lower) were less than control (-6 / –

0.2 / -1.5), respectively. The second year showed means more than control in soybean diet (1.9 / 10.6 / 11.8) in all bars (upper, middle, and lower) respectively. However, in the chickpea diet the middle bar has a higher mean than the control (4.2). As both upper and lower bars have meant less than the control (-1.3 / -0.5), respectively.

Fig (1): Differences between treatment and control in the royal jelly quantity mean (mg/cup) from different diets of bars level during two successive years (2020 and 2021) during late summer

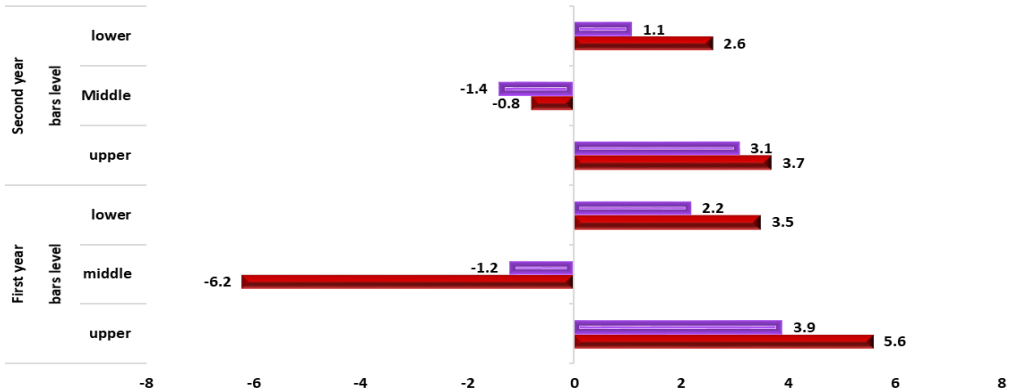


	First year			Second year		
	upper	middle	lower	upper	middle	lower
Soybean	4.1	0.2	-5.8	1.9	10.6	11.8
Chickpea	-6	-0.2	-1.5	-1.3	4.2	-0.5

The data in Figure (2) illustrated the differences between treatment and control in grafted queen cups acceptance percentage Mean using different diets and bars level for two successive years (2020 and 2021) in late summer. The first year showed means more than the control in booth diet soybean and chickpea (5.6 -3.9) in upper bars respectively. But the middle bars were less than the control (-1.2 / -6.2) in chickpea and soybean respectively. The lower bar showed means more than control (3.5 – 2.2) soybean and chickpea, respectively.

The second year showed means more than the control in booth diet soybean and chickpea (3.7-3.1) respectively in the upper bar. However, the middle bars were less than the control (-1.4 & -0.8) chickpea and soybean respectively. While the lower bar showed means more than control (2.6 -1.1) for soybean and chickpea respectively.

Fig (2):grafted queen cups acceptance percentages Mean using different diets and bars level of two successive years (2020 and 2021) at late summer.



	First year bars level			Second year bars level		
	upper	middle	lower	upper	Middle	lower
Chickpea	3.9	-1.2	2.2	3.1	-1.4	1.1
Soybean	5.6	-6.2	3.5	3.7	-0.8	2.6

The soybean showed acceptance percentages mean more than the other diets, with significance between soybean and other treatments being non-significant. Many researchers have supported these findings. Szymas and Przybyl (1995) mentioned how feeding with different pollen substitutes affected different tissues of the honeybee. Potato protein, soybean meal, yeast, low-fat powdered milk, powdered chicken eggs, extruded maize, and vitamin mix. The institute for the development of pharyngeal glands, fat body, and ovaries is similar to that of the bees fed with bee bread. The haemolymph protein and quality of royal jelly produced by honeybee colonies fed only on pollen substitutes compared with those of natural colonies. These pollen substitutes were chickpea, milk, and defatted soy flour. Total sugar amounts of crude protein and 10-hydroxy-2- decenoic acid varied slightly but within the reported range for natural royal jelly. Ideally, no differences were noted in the characters of queen bees reared on the royal jelly (Zaytoon, 2002).

Our results observed the middle bar has acceptance percentages that mean more than the upper and lower bars with significant differences. But In Brazil, the bar positions had an insignificant effect on the acceptance percentage of larvae. But the middle position resulted in the greater weight of royal jelly for queen cells than positions in front and close to the entrance and at the rear and far from the entrance. The higher percentage of larvae acceptances were associated with the greater weight of royal jelly for queen cells and heavier larvae produced in both genetics (Albarracín *et al.*, 2006). El-Barbary (2007) revealed that during summer the mean weight of queens that emerged from cells located in the middle position of the rearing frame was significantly heavier than that produced on the edge position. Similar results were obtained during spring. The position at which the queen cell presented within the building colony has an effect on the weight of the resulted queens throughout various seasons of the year. The queen cells that were positioned in the middle areas of the rearing frame gave a number of queens with heavy weight in relative frequencies significantly higher than those resulting from cells presented on the edge areas of the rearing frame. The intermediate queen weight was significantly less during summer than those obtained during spring for the middle position. For the edge position, a little increase was recorded during summer than spring for the relative queen weight distribution during the summer and spring seasons. However, the lightest queens were frequented in a significant rate in the peripheral areas than in the middle ones it is, also noticed from these results that the frequency percentages of heavy queens were significantly higher during the summer season than those that occurred during the spring season.

The results of Abd Al-Fattah *et al.* (2003) are in agreement with our findings. They determined that the highest acceptance percentage occurred during summer. Significant difference was found between summer and other seasons. The lowest significant percentage appeared in winter. While moderate results for the acceptance larvae were noticed during the spring and autumn seasons with insignificant differences between them in the North Sinai region. Also, Genc *et al*. (2005) observed the maximum acceptance percentage was in dry grafting in July and grafting with the addition of royal jelly in July and August, respectively.

The highest percentage of accepted queen cells in the two years, 2002 & 2003 respectively obtained from colonies located in the Rasheed

region during the citrus flowering period. While the lowest percentage of accepted queen cells were recorded during the squash blooming period in Kafr EL-Sheikh region (Shawer *et al*., 2007). Guler and Alpay (2005) found the mean acceptance percentage of *A. mellifera Carnica* was higher than *A. mellifera liguistica* grafted larvae from May to August. Later, Erdogan *et al.* (2017) reported that the average acceptance percentage was similar, but the amount of royal jelly per cell was significant in the Aegean ecotypes and Italian x Aegean crossbred honey bees, respectively. The differences between the colonies for the number of grafted larvae, percentage, and amount of royal jelly per cell were significant. As the number of grafted larvae increased, the rate of acceptance of colonies decreased, but the total royal jelly production of colonies increased. The Aegean ecotype was found to be suitable for royal jelly production under south Aegean region conditions. Here Figure (1) illustrated the differences between treatment and control in royal jelly quantity means (Mg/Cup) from Italian and crniolan hybrids with different diets within two successive years (2020 and 2021) during late summer. These results are in agreement with Saleh (1999), who stated that the crniolan race was better than the Italian race in royal jelly production. As the highest acceptance percentage in queenless colonies was in April. As the lowest in September. Ibrahim (2002) showed the total quantity of royal jelly produced by the colony was higher in queenless colonies than in queenright ones. Not only that but royal jelly production is also being affected by the bee race. Worker larvae grafted at the age of 12 hours were more acceptable and the queen cells gave more royal jelly than older ones. The best time for collecting royal jelly from queen cells was 3 days after the grafting process (Khattab *et al.*, 1998).

The results could be concluded that the first year has acceptance percentages that mean more than the second with non-significant differences. While the soybean showed acceptance percentages mean more than the other diets with significant between soybean and chickpea/pollen, the differences between chickpea and pollen were non-significant. Moreover, the middle bar has acceptance percentages that mean more than the upper and lower bars with significant differences. The best acceptance percentage was recorded in the soybean diet. While the best bar level for acceptance percentage was the upper bar.

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