



The impact of pollen trapping on royal jelly production in honeybee *Apis mellifera* L., colonies

Mohanny, K.M. ^{1*}, Aslam I. Ragab ² and Shahira A. Gad ¹

¹ Plant Protection Department, Faculty of Agriculture, South Valley University, 83523 Qena, Egypt.

² Medical surgical nursing Department, Faculty of Nursing, South Valley University, 83523 Qena, Egypt.

Abstract

Royal jelly (RJ) is a yellowish-white acidic glandular secretion produced by worker honeybees. RJ is fed to worker and drone bees in the first three days of their larval stage, but for five days in queen larvae and after development as adult. Globally, RJ is mightily demanded and utilized commercially in supplementary medicines and cosmetics. The present work was conducted to study the impact of pollen trapping of queen less-honeybee, *Apis mellifera*, colonies on acceptance rate of grafted queen cups, RJ production, i.e., amount per cup, and total colony production. Obtained results showed that non-trapped colonies averaged 54.186% acceptance rate of cell cups /colony, 0.124 mg RJ/ cup, and 3.012 g RJ/ colony, while those deprived of pollens were severely affected, averaged 23.799%, 0.099mg, and 1.073g for the same parameters, respectively. Significant differences between values of trapped and non-trapped colonies were found.

Keywords: Honeybee pollen traps; Grafting; Royal jelly; Production.

1. Introduction

RJ is a yellowish-white proteinaceous secretion of hypopharyngeal, mandibular, postcerebral, and thoracic glands of young nurse bees (Ahmad *et al.*, 2021). RJ has a pH between 3.4 and 4.5, it is acidic, and of a sweet-sour taste (Ramanathan *et al.*, 2018). RJ is a food source for queen bees throughout their full larval phase and for worker and drone larvae for only their first three days (Li *et al.*, 2010). RJ contributes to the special characteristics of queen bees, i.e., their lifespan, prolific fecundity, and superior cognitive and memory abilities (Pyrzanowska *et al.*, 2014). Moreover, RJ potentializes singular supply of a valuable natural substance used in health-improving and cosmetics (Ramanathan *et al.*, 2018; Ahmad *et al.*, 2020). RJ is a crucial functional food that has a variety of positive

biological effects. RJ is worldwide employed in commercial medicines, and nutritional supplements. RJ is beneficial as an antibacterial, anti-inflammatory, vasodilator, hypotensive, antiseptic, antioxidant, anti-hypercholesterolemic, and anticancer agent, due to its bioactive compounds, e.g., fatty acids, proteins, and phenolics (Ramadan and Al-Ghamdi, 2012). As RJ is crucially market-demanded compared to other bee products, i.e., honey, pollen, and propolis, RJ may be manufactured on a commercial scale. Nevertheless, RJ has become a significant money source for beekeepers worldwide (Ramadan and Al-Ghamdi, 2012; Ghramh *et al.*, 2020). For instance, China harvests and exports 4000 tones RJ annually, making up more than 90% of the world's RJ production (Altaye *et al.*, 2019). Numerous biotic and abiotic factors impact quantity and quality of RJ (Murat, 2020) especially nutritional factors (Xun *et al.*, 2020; Qi *et al.*, 2021). Compared to any other natural

*Corresponding author: Mohanny, K.M

Email: karem.mohanny@agr.svu.edu.eg

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or artificial food, pollen is the key of RJ constituents, e.g., amino acids, carbs, and vitamins, which significantly alter RJ composition. The current study aimed to determine the impact of pollen trapping on the monthly productivity of RJ.

2. Materials and Methods

The study was conducted in a private apiary at the farm of the Faculty of Agriculture and the Department of Plant Protection at South Valley University during April-June, 2020. The hybrid of the carniolan bee race *Apis mellifera* L. was chosen to start the planned experiment.

2.1. Preparation of colonies

Six honeybee colonies headed by young-equal-aged hybrid Carniolan, *A. m. cornice*, queens were used. Colonies reared in standard Langstroth's wooden hives, were utmost equal in strength (each colony had nearly three sealed worker brood combs). The mother queens and unsealed brood were excluded. The colonies were prepared one day before larvae were grafted. Stored pollen discs were also removed from cells treated with pollen traps, then considered queen less units enforced for royal jelly production. Tested colonies were daily fed with sucrose syrup (two sugar: one water). Dry grafting technique (Doolittle, 1888) was followed. The colonies were divided into two equal subgroups (three colonies each). The 1st group was deprived of pollen by placing pollen traps, at the entrances of their hives during the study period. The pollen trap used was a wooden box, with a horizontal plastic strip perforated with four holes per inch, allowing only workers to pass. The pollen loads carried in the pollen baskets of their fallen hind legs, were removed by means of a horizontal wire mesh in the collection tray (Mesbah *et al.*, 2017). while, the

2nd group was normal colonies (control) during the same period.

2.2. RJ production

Queen cell cups made of non-toxic plastic were used. A plastic grafting pen (10cm length) was used to pick up 24h-larvae from brood combs to cell cups that fixed in a wooden frame (45 cup/colony) which was left in prepared colony for 12-24 h to be cleaned by bees before grafting. After 3 days of grafting, trapped and non-trapped grafted colonies were inspected. Number of successful queen cells was recorded (% acceptance/ colony). RJ/ cup was collected after removing each larva with a suitable wooden stick and was kept in a vial/ cup. In the lab, RJ was weighed sensitive scale with three decimal places, then stored in clean dark glass container at freezing temperature (-18°C). Tools utilized in this experiment were clean and in standard sizes.

2.3. Statistical analysis

The data obtained were analyzed statistically using T test. Mean value were compared using analysis of variance and a multiple comparison test according to the least significant difference (LSD) (Snedecor & Cochran)1957).

3. Results

Obtained data in Tables (1) show successful Rate of queen Cell Cups in trapped and non-trapped grafted honeybee colonies. Successful rate (percentage average of total acceptance) of queen cell cups in non-trapped group, were 56.22, 53.38 & 53.037 % in April, May, and June, respectively. These values decreased in trapped group being 27.55, 22.76 & 21.18 % for the same periods, respectively. Values in the two groups were significantly different.

Table 1. Acceptance rates (%) of grafted queen cell cups in non-trapped and trapped colonies during April-June, 2020 at Qena, Egypt.

Date	Non-trapped colonies				Trapped colonies			
	Acceptance of queen cups				Acceptance of queen cups			
	colonies No.				colonies No.			
	1	2	3	%	1	2	3	%
1/4/2020	25	26	24	55.556	11	13	11	25.926
4	30	26	27	61.481	14	11	18	31.851
7	25	22	25	53.333	13	10	11	25.185
10	31	27	32	66.667	15	15	17	34.815
13	24	26	18	50.37	10	12	9	22.963
16	23	24	27	54.814	12	10	16	28.148
19	20	23	21	47.407	11	13	12	26.667
22	27	22	24	54.074	13	9	11	24.444
25	30	28	25	61.462	14	13	11	28.148
28	22	25	30	57.037	11	13	13	27.407
Total	257	249	253	562.201	124	119	129	275.554
1-May	21	15	22	24.963	11	7	9	20
4	26	24	28	57.778	9	9	11	21.481
7	25	23	27	55.556	12	11	13	26.667
10	25	24	24	54.074	11	10	11	23.704
13	27	29	24	59.259	11	11	10	23.704
16	27	30	28	62.963	12	13	12	27.407
19	30	24	25	58.519	12	10	10	23.704
22	20	22	25	49.63	7	8	11	19.259
28	27	24	26	57.037	12	11	11	25.185
31	25	22	25	53.333	11	10	11	23.704
Total	278	258	281	587.186	119	109	120	250.371
3-Jun	23	21	25	51.111	10	8	7	18.519
6	25	26	27	57.778	11	12	11	25.185
9	20	17	23	44.444	7	5	10	16.296
12	24	19	20	46.667	11	5	7	17.037
15	27	23	27	57.037	11	7	11	21.481
18	25	25	27	57.037	6	12	11	21.481
21	25	27	26	57.778	10	11	12	24.444
24	25	26	23	54.815	12	11	10	24.444
27	24	23	25	53.333	12	9	10	22.963
30	24	20	24	50.37	9	8	10	20
Total	242	227	247	530.37	99	88	99	211.85
Grand Total	777	734	781	1679.757	342	316	348	737.775
Mean	25.064	23.677	25.194	54.186	11.032	10.194	11.226	23.799

Data in Table (2) show the amounts of RJ produced per cup of the grafted queen cells in each colony when pollen traps were used or not. In non-trapped colonies, average RJ amounts were 0.133, 0.122 & 0.116 mg/ cup during

April, May, and June, respectively. However, in trapped colonies these values were noticeably low being 0.102, 0.096 & 0.099 mg/ cup for the same months, respectively with significant differences between the two groups.

Table 2. Average numbers of successful grafted queen cells (cup/ colony) and average amounts of produced royal jelly (mg/ cup) in non-trapped and trapped colonies during April-June, 2020 at Qena, Egypt.

Date	Non-trapped colonies		Trapped colonies	
	mean number of successful queen cell cups/colony	Mean of quantity R.J. / queen cell cups	mean number of successful queen cell cups/colony	Mean of quantity R.J. / queen cell cups
1/4/2020	25	0.13	11.667	0.094
4	27.667	0.127	14.333	0.107
7	24	0.129	11.333	0.101
10	30	0.113	15.667	0.11
13	22.667	0.108	10.333	0.086
16	24.667	0.13	12.667	0.11
19	15.333	0.173	12	0.093
22	24.333	0.14	11	0.11
25	27.667	0.145	12.667	0.11
28	25.667	0.139	12.333	0.1
Total	247.001	1.334	124	1.021
1-May	19.333	0.127	9	0.092
4	26	0.116	9.667	0.105
7	25	.138	12	0.101
10	24.333	0.12	10.667	0.096
13	26.667	0.12	10.667	0.094
16	28.333	0.126	12.333	0.097
19	26.333	0.112	10.333	0.095
22	22.333	0.109	8.667	0.094
25	24.333	0.12	10.333	0.09
28	25.667	0.125	11.333	0.094
31	24	0.125	10.667	0.094
Total	322.332	1.246	115.667	1.052
3-Jun	23	0.107	8.333	0.101
6	26	0.113	11.333	0.097
9	20	0.104	7.333	0.101
12	21	0.124	7.667	0.109
15	25.667	0.117	9.667	0.103
18	25.667	0.117	9.667	0.107
21	26	0.12	11	0.096
24	24.667	0.129	11	0.092
27	24	0.117	10.333	0.091
30	22.667	0.112	9	0.092
Total	238.668	1.16	95.333	0.989
Grand total	758.001	3.832	335	3.062
Mean	24.452	0.124	10.806	0.099

Obtained data in Tables (3) show the average values of produced RJ in trapped and non-trapped grafted honeybee colonies. Average monthly total amounts of RJ in non-trapped group were 3.25, 3.01 & 2.77 g/ colony in April,

May, and June, respectively. While, those values decreased in trapped group being 1.27, 1.00 and 0.94 g/ colony for the same months, respectively.

Table 3. Amounts (g) of royal jelly produced in non-trapped and trapped colonies during April-June, 2020 at Qena, Egypt.

Date	Non-trapped colonies Royal jelly production colonies No.				Trapped colonies Royal jelly production colonies No.			
	1	2	3	Mean	1	2	3	Mean
1/4/2020	3.301	3.612	2.811	3.241	1.103	1.204	1.001	1.102
4	4.013	3.452	3.048	3.504	1.434	1.191	1.991	1.538
7	3.113	2.875	3.325	3.104	1.311	0.998	1.108	1.139
10	4.408	3.998	4.744	3.383	1.681	1.512	1.991	1.728
13	2.877	3.011	1.482	2.456	0.959	1.221	0.494	0.891
16	3.025	2.868	3.752	3.215	1.312	0.956	1.921	1.396
19	2.453	3.001	2.526	2.66	1.001	1.331	1.003	1.111
22	3.854	2.887	3.471	3.404	1.521	0.962	1.157	1.213
25	4.223	4.13	3.662	4.005	1.601	1.376	1.22	1.399
28	2.885	3.547	4.251	3.561	1.001	1.191	1.525	1.239
Total	34.152	33.381	33.072	32.533	12.924	11.942	13.411	12.756
1-May	2.551	1.812	2.99	2.451	1.001	0.604	0.881	0.828
4	3.013	2.665	3.556	3.013	0.981	0.888	1.185	1.018
7	3.387	3.015	3.951	3.451	1.229	1.005	1.415	1.216
10	2.893	2.853	3.002	2.916	1.001	0.951	1.112	1.021
13	3.115	3.564	2.94	3.206	1.038	1.001	0.977	1.005
16	3.352	3.842	3.501	3.565	1.117	1.311	1.167	1.198
19	3.654	2.55	2.652	2.952	1.218	0.855	0.884	0.985
22	2.112	2.209	3.002	2.441	0.704	0.736	1.006	0.815
28	3.507	2.815	3.293	3.205	1.111	1.002	1.097	1.07
31	3.002	2.752	3.279	3.011	1.006	0.917	1.093	1.005
Total	33.679	30.333	35.529	33.121	11.443	10.022	11.818	11.091
3-Jun	2.452	2.255	2.646	2.451	0.911	0.851	0.772	0.844
6	2.778	2.933	3.112	2.941	1.001	1.201	1.103	1.101
9	2.001	1.691	2.551	2.081	0.767	0.563	0.901	0.743
12	3.183	1.999	2.651	2.611	1.061	0.577	0.883	0.84
15	3.225	2.558	3.22	3.001	1.075	0.852	1.073	1
18	2.991	2.977	3.005	2.991	0.887	1.221	1.001	1.036
21	2.991	3.215	3.127	3.111	0.997	1.071	1.11	1.059
24	3.311	3.51	2.722	3.181	1.154	1.002	0.907	1.021
27	2.988	2.561	2.884	2.811	1.001	0.853	0.961	0.938
30	2.765	2.063	2.771	2.533	0.883	0.687	0.9236	0.8312
Total	28.685	25.762	28.689	27.712	9.737	8.878	9.634	9.413
Grand Total	96.534	89.476	97.29	93.666	34.104	30.843	34.864	33.26
Mean	3.114	2.886	3.138	3.012	1.1	0.995	1.125	1.073

Obtained data in Tables (4) show statistically significant differences between Average acceptance rates of grafted queen cell cups (%/

colony) and average amounts of produced royal jelly (g/ colony) in non-trapped and trapped colonies during April-June, 2020.

Table 4. Average acceptance rates of grafted queen cell cups (%/ colony) and average amounts of produced royal jelly (g/ colony) in non-trapped and trapped colonies during April-June, 2020 at Qena, Egypt.

Months	Non-trapped colonies		Trapped colonies	
	Royal jelly production	Acceptance of queen cups	Royal jelly production	Acceptance of queen cups
April	3.25a	56.22	1.27d	27.55
May	3.01b	53.38	1.00e	22.76
June	2.77c	53.037	0.94e	21.18
LSD 0.05			0.5	
Sig 0.05			0.000	
F 0.05			209.504	

Values of varied letters are significantly different

4. Discussion

It is obvious that RJ production was not affected in normal colonies (without pollen traps) compared to those of pollen-deprived (with pollen traps) (Tables 1-4). Normally, there is a direct proportional relationship between the amount of available pollen and colony performances including secretion of RJ. The obtained results are consistent with those of (Kongpitak *et al.*, 1990) showed that the amount of pollen is a limiting factor for RJ production. This relationship depends on pollen demands inside the hive. Regardless the amount of RJ per cup, produced RJ was obviously higher in non-trapped colonies compared to that of trapped ones. Moreover, successful rate of grafted queen cell cups (larval acceptance) was also high. The current findings are in general agreement with those of (Khan and Ghramh, 2022) revealed a substantial difference between two bee stocks in larval acceptance rate, RJ yield per colony, and per cell cup. (Wytrychowski *et al.*, 2013) found that two bee stocks fed on a natural pollen diet had much higher rates of larvae acceptance and RJ yield. The current findings shows that RJ production was descendingly greater in April,

May and June may be due to decrease in available pollens. In this respect, (Serag and Dien, 2004) indicated that the production of the largest amount of royal jelly was during the period from (May 15 to July 15), followed by the period (1 July to August 30) while the period from (February 15 to April 15) is the lowest. also (Şahinler and Kaftanoğlu, 2005) indicated that output of RJ in April was 9.2% higher than those in May, 17.7% in June, 41.9 in July, 65.1 in August, and 103% in September

5. Conclusion

Larval acceptance rate, production of royal jelly, i.e. average amount per grafted cell and per colony was higher in colonies without pollen traps than those of trapped colonies. There is a direct relationship between available pollen and royal jelly production, acceptance rate of grafted queen cell cups. Obtained results also showed that values of these tested parameters were high in April, May, and then June, due to a decrease in amount of regional available pollens.

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Authors' Contributions

All authors contributed to this research.

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Institutional Review Board Statement

All Institutional Review Board Statements are confirmed and approved.

Data Availability Statement

Data presented in this study are available at fair request from the respective author.

Ethics Approval and Consent to Participate

This work carried out at the plant protection department and followed all the department instructions.

Consent for Publication

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

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