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The impact of caging the queens during the flow season on some biological activities of honeybee colonies

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ABSTRACT

This study was achieved in a private apiary located in a banana farm in Sa El Hagar, Basioun, Gharbia, Egypt from August 15, 2019 to May 25, 2020, including the banana (*Musa* sp., Musaceae) flow season (August and September) and extend to Egyptian clover (*Trifolium alexandrinum* L., Fabaceae) flow season (May). The study aimed to evaluate the effect of confining the queen during the banana flow season on the brood rearing, honey yield, and activation of worker's ovaries. Also, we determined the negative impact of caging the queen during the banana flow season on the activity of the colony in brood rearing, storing pollen, and honey yield after releasing the queen on 5 October, extending to the next flow season in May. The obtained results showed that the honeybee colonies with the caged queen produced significantly more honey yield and less brood production than the free queen ones during the banana flow season. Also, the caging of the queen did not affect the colony strength after releasing the queen despite the partial development of the ovaries of some workers, but they did not lay eggs. In addition, releasing the queens suppressed the ovaries of the laying workers. It can be concluded that caging the queen during the banana flow season helps the colonies to produce more honey yield without effect on the colony strength after releasing the queen despite the ovaries development of few workers without egg-laying.

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1. Introduction

The amount of the harvested honey yield reflects the status of the colony. The honey yield depends on some factors such as the availability of floral resources (Taha, 2000; Helal et al., 2003; Taha et al., 2006), colony strength (Jevtic et al., 2009; Taha and AL-Kahtani, 2013; Kasangaki et al., 2018), subspecies of the bees (Taha and Al-Kahtani, 2019), and season (Taha, 2014; Brar et al., 2018; Taha and Al-Kahtani, 2020). Also, feeding on proteinaceous diets (Taha, 2015b; Puškadija et al., 2017), and age of comb (Taha and El-Sanat, 2007; Taha and Al-Kahtani, 2020) were reportedly affected. In addition, Valle et al. (2004) and Wakjira et al. (2020) used two-queen colonies to gain high honey yield.

There are both qualitative and quantitative differences between the flowering plant species with regards to nectar and pollen production; some of them supply both nectar and pollen abundantly during flowering, and others provide nectar or pollen for brood rearing (Taha, 2015a; Kebede and Gebrechistosb, 2016; Taha et al., 2019; Al-Kahtani et al., 2020). The banana plants have reportedly been considered an essential nectar resource for honeybees, and beekeepers could get a good honey yield during its flow season (Taha, 2007; Shaver et al., 2019).

The honeybee colonies should have a large bee population, especially at the beginning of the honey flow season. The honey yield was less in the colonies that reared more brood area during the honey flow season (Genç and Aksoy, 1993; Taha and AL-Kahtani, 2013; Gąbka, 2014). Schneider and Blyther (1988) showed that the honeybee (*Apis mellifera scutellata*) usually stores a small amount of food and uses about 78% of the comb area for brood rearing. Also, Harbo (1993) indicated that about 6.5 kg of honey is needed to rear about 40,000 honeybee workers. So, if the honeybee colony reared brood during the honey flow season, a great quantity of honey would be consumed for the brood rearing. A balance between the brood rearing and honey yield is critical. This might be achieved by limiting the continuous egg laying by confin-

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ing the queen throughout the peak of nectar flows and directing honeybee workers towards collecting and storing nectar to produce high honey yield (Adgaba et al., 2013; Zaghloul et al., 2017).

The amount of pollen and sealed brood areas in the honeybee colony reflects its strength level and can be used to predict the honey yield. Many previous studies have shown a positive relationship between stored pollen, sealed brood area, and honey yield (Shoreirt et al., 2002; Taha, 2005; Jevtic et al., 2009; Taha, 2015b; Taha and Al-Kahtani, 2019, 2020). The rapid increase of ovaries activation following caging the queen of honeybee colonies strongly indicates that confinement of the queen to one portion of the hive disrupts the pheromonal signals that naturally curtail the worker ovaries activation (Free, 1987; Taha, 2013). Also, confining the queen to a half of the hive results both in a reduction in the contact rate of honeybee workers with the queen and prevents the queen from laying the egg, thereby reducing the workers' exposure to brood pheromones (Holmes et al., 2014). In the absence of a honeybee queen and unsealed brood, some bee workers activate their ovaries, and after 5–46 days begin laying eggs (Mohammedi et al., 1998; Taha, 2013). Queen pheromones that regulate ovaries' development would be essential when no brood was found in the colony, such as during natural periods of dearth, winter, or queen replacement (Hoover et al., 2003).

Starting the flow season with strong colonies helps get a high honey yield (Taha and AL-Kahtani, 2013). In the condition of weak colonies as the status in most Middle East, we can use new treatments that curtail brood rearing during the flow season to increase the colony honey yield. This study was designed to determine the effect of caging the queen during the banana flow season on the brood production, honey yield, and activation of worker's ovaries. Also, we monitored the negative impact of caging the queen during the banana flow season on the activity of the colony in brood rearing, storing pollen, and honey yield after releasing the queen on 5 October, extending to the next flow season (Egyptian clover season) in May.

2. Material and methods

2.1. Experimental site and design

This study was carried out at a private apiary located in a banana farm (45 ha) in Sa El Hagar (30° 57' 53" N, 30° 46' 6" E; 12 m above sea level), Basioun, Gharbia, Egypt. The experiment extended from August 15, 2019 to May 25, 2020, including the banana (*Musa sp.*, Musaceae) flow season (20 August–5 October) and the Egyptian clover (*Trifolium alexandrinum* L., Fabaceae) flow season (May). Twenty hybrid Carniolan honeybees (*Apis mellifera carnica*) colonies of the same strength (each 12,000 bees) and food storage were used for this study. All colonies were requeened by newly open mated sister queens. The colonies were divided into two equal groups. The honeybee queens in the first group were caged with queen cages (4 × 6 cm) during the banana flow season. The queens were caged on 5/9/2019 and released after the banana flow season on 5/10/2020. The queens in the second group were left free. We continued to measure the activity of the colonies in storing pollen, brood rearing, and honey yield until the end of the next flow season (Egyptian clover) in May to determine the negative effect of caging the queens. The colonies in the two groups were exposed to routine work during the experimental period.

2.2. Worker sealed brood area

The areas (inch²) of stored pollen and worker sealed brood were measured at twelve days interval using an empty standard Lang-

stroth frame divided into square inches. The measurements continued until the end of the next honey flow season (Egyptian clover) at the end of May.

2.3. Sampling and dissection of laying workers

Three weeks after caging the queens, we started monitoring the laying workers in the colonies on 25 September and continued until 10 October, i.e., the six days after releasing the queens. Hundred honeybee workers were randomly collected from each colony twice a week, and each worker was examined to determine the ovaries' development according to Hess (1942). The workers were pinned on a wax dish and submerged in ranger solution under a binocular. The abdomens of the bees were dissected by making an incision along the lateral and anterior side of the abdomen. The development of both ovaries was recorded.

2.4. Honey yield

The area of capped and uncapped honey was measured using an empty standard Langstroth frame divided into square inches before ten days of honey extraction. The honey area was transformed into weight using the following formula:

$$\text{Honey yield (kg)} = \frac{\text{Area of honey (inch}^2\text{)} \times 10.64}{1000}$$

where 10.64 = amount of honey (g) in one square inch, based on averages calculated from capped and uncapped honey from combs of different thickness (Shawer et al., 1986). By the ending of the banana and Egyptian clover flow seasons, the honey was extracted from the honeycomb using a honey extractor (extractors work by centrifugal force). The honey yield was weighed and recorded.

2.5. Statistical analysis

The differences between the free and caged queen colonies were tested by the one-way analysis of variance (ANOVA), which indicated significant differences between the free and caged queen colonies. The normality in data was tested by the Shapiro-Wilk normality test, which indicated the normal distribution of the data. Therefore, the original data was analyzed. The ANOVA was used to assess the differences between the free and caged queen colonies tested via the SPSS software (SPSS, 2006).

3. Results

Data in Table 1 showed that the brood rearing in the colonies of the free queen was relatively similar to the colonies of the caged queen during 26 August and 6 September. The colonies of the caged queen became without any brood during 30 September and 12 October, while the colonies of the free queen had 594.20 and 570.40 inch² brood areas during the previous dates, respectively. Generally, from 26 August until 12 October, the brood area in the free queen colonies was significantly ($P < 0.01$) higher than the caged queen colonies.

Data in Table 2 showed that from November to May, the free queen colonies reared more brood higher than the caged queen colonies without substantial differences. Except for November and March, the free queen colonies stored more pollen than the caged queen colonies during all periods without significant differences.

As illustrated in Fig. 1, the highest rate (35%) of the developed ovaries workers was found on 5 October in the caged queen colonies. The highest rate of the developed ovaries workers (15%) in the

Table 1
Influence of caging the queen on worker sealed brood area (inch²) during the banana flow season.

Colonies	26 August	6 September	18 September	30 September	12 October
Free queen	545.60 ± 2.80	570.45 ± 3.95	558.61 ± 5.56	594.24 ± 3.69	570.40 ± 3.82
Caged queen	550.63 ± 4.22	568.86 ± 3.99	525.48 ± 4.57	00.00	00.00
Significance	NS	NS	**	**	**

Values are the mean ± S.E. ** indicate P < 0.01 between treatments. NS indicates P > 0.05 between treatments.

Table 2
Areas (inch²) of stored pollen and worker sealed brood in the colonies after releasing the queen.

Months	Worker sealed brood		Sig.	Stored pollen		Sig.
	Caged queen	Free queen		Caged queen	Free queen	
Nov.	841.88 ± 22.51	992.00 ± 34.68	NS	130.00 ± 6.90	126.50 ± 11.02	NS
Dec.	683.12 ± 16.94	752.00 ± 16.21	NS	83.00 ± 5.42	87.50 ± 9.97	NS
Jan.	707.50 ± 8.55	743.12 ± 10.60	NS	66.25 ± 6.63	73.75 ± 9.31	NS
Feb.	855.00 ± 24.65	886.87 ± 20.14	NS	81.25 ± 6.63	87.50 ± 11.25	NS
Mar.	1055.00 ± 26.87	1100.25 ± 12.41	NS	102.25 ± 12.54	93.75 ± 11.39	NS
Apr.	1301.25 ± 18.22	1360.25 ± 17.58	NS	117.75 ± 15.01	125.00 ± 18.20	NS
May	1451.87 ± 28.01	1518.75 ± 15.49	NS	232.50 ± 15.97	241.25 ± 15.63	NS
Total	6895.62 ± 31.67	7353.24 ± 38.33	NS	813.00 ± 17.68	835.25 ± 21.18	NS

Values are the mean ± S.E. NS indicates P > 0.05 between treatments.

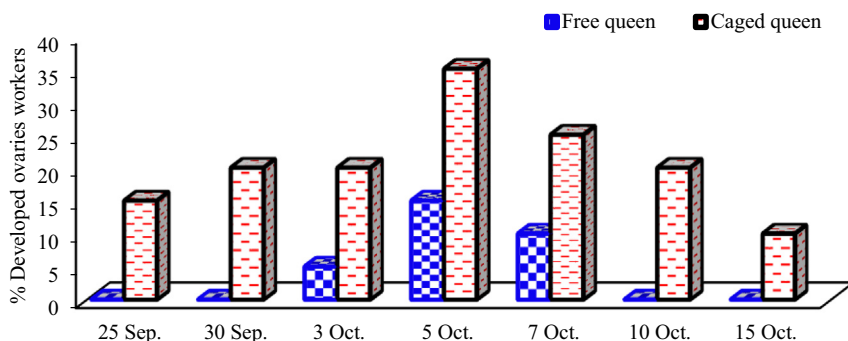


Fig. 1. Percentage of the developed ovaries workers in the free and caged queen colonies during the banana flow season.

free queen colonies was found on 5 October. Generally, the rate of the developed ovaries workers was higher (P < 0.01) in the caged queen colonies compared to the free queen colonies, but the workers in both groups did not reach the egg-laying stage.

Data in Table 3 showed that honey yields (kg) before ten days of extraction were 2.23 and 3.34.00 kg/colony vs. 3.77 and 3.25 kg/colony for banana and Egyptian clover flow seasons in the free and caged queen colonies, respectively. The data showed that the free queen colonies produced less honey than caged queen colonies (2.55 kg/colony vs. 4.62 kg/colony) in the banana flow season. In comparison, free queen colonies surpassed the caged queen colonies in the flow season of Egyptian clover (4.72 kg/colony vs. 4.26 kg/colony). The total honey yield of both seasons in the caged queen colonies was significantly (P < 0.01) higher than the free queen colonies (8.88 kg/colony vs. 7.27 kg/colony).

4. Discussion

Caging the queen negatively affected the brood rearing during the banana flow season. The brood area in the free queen colonies was significantly larger than that in the caged queen colonies during the queen-caging period. Because caging the queen prevents laying eggs, resulting in the absence of brood. Adgaba et al. (2013) and Holmes et al. (2014) found relatively similar results. Simultaneously, none significant differences were detected in the brood areas between the caged and free queen colonies after releasing the caged queen. Because after releasing the queen, they directly started to lay eggs to make up for the deficiency of brood.

The caged queens were released after extraction of the banana honey, and the experiment continued until the end of the next flow season to find if there is a negative effect of caging the queen on

Table 3
Influence of caging the queen on honey yield (kg) through the banana and Egyptian clover flow seasons.

Flow season	Ten days before extract			Extract day		
	Caged queen	Free queen	Sig.	Caged queen	Free queen	Sig.
Banana	3.77 ± 0.14	2.23 ± 0.11	**	4.62 ± 0.17	2.55 ± 0.05	**
Egyptian clover	3.25 ± 0.16	3.34 ± 0.16	**	4.26 ± 0.10	4.72 ± 0.10	**
Total	7.02 ± 0.18	5.57 ± 0.24	**	8.88 ± 0.17	7.27 ± 0.15	**

Values are the mean ± S.E. ** indicate P < 0.01 between treatments.

the activity and growth of the colony. The free queen colonies surpassed the caged queen ones in storing pollen from November to May except for November and March without substantial differences. These variations due to the superiority of the colony population in the free queen colonies resulted from brood rearing during the banana flow season. A significant positive correlation has reportedly been found between storing pollen and the colony size (Jevtic et al., 2009; Taha and AL-Kahtani, 2013; Taha, 2014; Taha and AL-Kahtani, 2020).

The developed ovaries workers appeared after 20 days of caging the queen with a rate of 15% of the total tested workers, then increased to 20% on both 30 September and 3 October, then increased and reached the highest percentage (35%) after 30 days of caging the queen. Gradually decrease was occurred after releasing the queen on 5 October, recording 25% on 7 October and 10% on 15 October. On the other hand, the developed ovaries workers appeared in the free queen colonies later after 28 days with a lower rate (5%), then increased and reached the highest proportion (15%) after 30 days, then gradually decreased until disappeared after 35 days on 10 October. The early appearing of the developed ovaries workers in the caged queen colonies due to the reduction of the queen pheromones, which inhibit the ovaries development of honeybee worker (Free, 1987; Oldroyd et al., 2001; Slessor et al., 2005; Wanner et al., 2007; Trhlin and Rajchard, 2011; Taha, 2013). Caging the queen decreases the workers' exposure and distribution of pheromones on the honeybee hive (Holmes et al., 2014). Also, the absence of brood especially unsealed brood resulted in a high decrease of brood pheromones that inhibits ovary development in worker bees similar to the queen's pheromone (Mohammedi et al., 1998; Le Conte et al., 2001; Holmes et al., 2014).

A low portion of workers' ovaries developed in the free queen colonies due to reduced unsealed brood during the flow season. The brood pheromones inhibit ovary development in worker bees similarly to the queen's pheromone (Ratnieks, 1993; Mohammedi et al., 1996; Pettis et al., 1997; Le Conte et al., 2001; El-Enany, 2006) and they did not lay eggs. After releasing the queens on 5 October in the tested colonies, they started to lay eggs, and the presence of the unsealed brood with the queen inhibited the activation of workers' ovaries (Mohammedi et al., 1998; Strauss et al., 2008) and started to cure the laying workers. These results agree with El-Enany (2006), who used brood and mated queens to cure the laying workers in the queenless colonies. Also, Holmes et al. (2014) showed that the ability of *A. cerana* workers to develop their ovaries is determined by the distribution of pheromones of queens and brood on the honeybee hives.

The colonies with caged queens produced 81.18% banana honey yield more than the free queen colonies. These results agree with Adgaba et al. (2013) and Zaghoul et al. (2017). A high amount of honey was stored on honeycombs of the caged queen colonies because the queen stopped laying eggs, and the colony saved the honey consumed for rearing the brood during the flow season. According to Harbo (1993), 163 mg of honey is required to rear one honeybee worker, and about 6.5 kg of honey is needed to rear 40,000 workers. A negative correlation between the brood area and honey yield at the honey harvest time indicates that a large brood area at the peak of the honey flow has reduced the honey yield (Szabo and Lefkovitch, 1989; Adgaba et al., 2013; Taha and AL-Kahtani, 2013). Zaghoul et al. (2017) harvested high honey yield when using the queen exclusion system to enhance honey yield during citrus, Egyptian clover, and cotton flow seasons.

The Egyptian clover honey yield in the free queen colonies was higher than that in the caged queen colonies by 10.80%. The absence of worker brood in the caged queen colonies during the period from 18 September to 12 October because of caging the queen, which resulted in smaller colony population size, can

explain the superiority of the free queen colonies in honey yield in comparison with the caged queen colonies in the Egyptian clover season. A significant positive correlation between the honey yield and workers sealed brood area, and colony population size has been found (Jevtic et al., 2009; Taha and AL-Kahtani, 2013; Taha, 2014, 2015; Brar et al., 2018; Taha and AL-Kahtani, 2020).

The total honey yield in both banana and Egyptian clover flow seasons in the caged queen colonies was surpassed the total honey yield in the free queen colonies by 22.15%. This confirms the benefit of caging the queen during the flow season for increase the honey yield as reported by Adgaba et al. (2013) and Zaghoul et al. (2017).

5. Conclusion

It was concluded that caging the queen during the banana flow season helped the colonies to produce more honey yield. Also, caging the queen did not affect the colony strength after releasing the queen despite the ovaries development of some honeybee workers without egg-laying.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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