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Effect of Rearing Queen Bees, *Apis mellifera* L. Raised from Grafted Larvae Under Magnetic Field Conditions on Its Characteristics

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ABSTRACT

This work was carried out at Economic Entomology & Agricultural Zoology Department Faculty of Agriculture, Menoufia University. In order to study the impact of honey bee queens raised from grafted larvae exposed to the magnetic field and the unexposed in some characteristics. Carniolan honey bee hybrid was prepared to be reared from and others to represent the queens. The obtained results in the two seasons indicated that the effect of exposure of grafted queen cups to different times of magnetic field on the acceptance percentages of grafted larvae in beeswax cell cups and differed significantly and ranged from 72.22 to 82.33% for the first year 2017 and from 70, 82.33 % for the second year 2018. The acceptance in 3 days exposure times was significantly higher (82.33%) than the one-day exposure time compared with control. Finally, it could be concluded from the obtained results that exposing grafted queen cell in mass rearing production in honey bee colonies may affect the morphometric characters differed from one day to three days compared with non-exposed colonies, therefore, it could be recommended that beekeeper must be trained to expose his rearing queens in the breeding time to the magnetic field to increase the ability of queens and workers to avoid the bad effects of these radiations.

INTRODUCTION

Honeybees are one of the foremost important economic pollinators within the worldwide, which declines as a neighborhood of worldwide problem in pollinators disappearing (Gallai *et al.*, 2009 and Hallmann *et al.*, 2017).

Queen is the mother of the hive and therefore the responsibility for controlling the egg lying both fertile and sterile, releases pheromones to stop both workers from developing a new queen and developing their ovaries and overlapping generations (Winston, 1987 and Delaney *et al.*, 2010).

The standard of queens being reared in honey bee colonies depends on many factors like age of used larvae, time of the season, biological status, and the number of queens being reared (Tarpy *et al.*, 2000, Koc and Karacaoğlu 2004, Skowronek *et al.*, 2004, Mohammad *et al.*, 2011). Consistent with Eskov (1990) good-quality queens develop from queen cells being reared in good conditions. The Honey bee workers collect

food from a distance of up to 12 km far away from the hive (Dyer and Sharbowski 2002). To seek out their back to home, workers remind memories around the hive.

The workers determined the directional information from the sun. Even when the sun is sheltered from any obstacles, the bees can still estimate the sun's position by the polarization pattern within the sky. The space information is measured by optic flow perceived by the bee while on the wing. This information is integrated then used for navigation; honey bees used geomagnetic fields for orientation (Chao-Hung *et al.*, 2016). Honey bees species are exposed to low-frequency electromagnetic fields as an abiotic environmental factor that is emitted from a spread of anthropogenic sources, including power lines, and have recently been shown to possess a big impact on the cognitive abilities and behavior of honey bees. The effects of levels of magnetic on aversive learning and aggression levels, which are critical factors for bees to take care of colony strength. (Hayes *et al.*, 2008 and Potts *et al.*, 2010).

Electromagnetic pollution is emerging as an abiotic environmental factor that has the potential to affect insect biology and thus may contribute to the environmental stress load that insects currently experience in global ecosystems (Shepherd *et al.*, 2018 and Wyszowska *et al.*, 2016). Pollution of the environment with magnetic waves has increased within the last century, with a serious source of power transmission lines (WHO, 2007). A spread of various effects on insects including changes in developmental biology, locomotors behavior, and immune reaction (Dimitrijević *et al.*, 2014, Zmejkoski *et al.*, 2017, Maliszewska *et al.*, 2018, and Valadez *et al.*, 2017).

Electromagnetic fields are a selected sort of electromagnetic wave within the frequency range 3–300 Hz that is emitted from anthropogenic devices. However, to date, there's no evidence that honey bee magnetoreceptors use the magnetite-based magnetoreception system that the animals can sense the sector through the ferromagnetic crystals of magnetite Fe₃O₄ located in their bodies (Hsu, *et al.*, 2007). Iron granule-containing cells are present within the abdomen of the honey bee and these have had been suggested to function as a magnetoreceptor. Also found within the honey bee brain. Although no evidence exists of cryptochrome involvement in honey bee magnetoreception thus far, the cytochrome remains a candidate effector of magnetoreception within the honey bee. (Velarde, et al 2005).

Therefore, the current research aimed to study the effect of rearing queens under magnetic field conditions on some characteristics of queens.

MATERIALS AND METHODS

The study was conducted in summer (June–July) for successive years 2017 and 2018 at the apiary and laboratory of Economic Entomology and Agricultural Zoology Department, Faculty of Agriculture, Menoufia University. To review the effect of rearing queens under magnetic conditions on some characteristics of queens.

The Honey Bee Races:

The study used honey bee colonies from the Carniolan strain *Apis mellifera carnica*, the queen fitted in queen rearing apparatus with a fully raised comb to egg-laying. The comb containing the apparatus was kept within the brood chamber of the breeder colony.

Grafting Method:

Doolittle grafting method was used, beeswax cell cups (9 mm diameter) Larval grafting was done by priming queen cell cup with a speck of dilution (Royal jelly: sterilized water- 1:1) with the assistance of a camel hairbrush. Young larvae of about 24h age taken from selected breeder colonies were used for grafting. 45 cell cups for every treatment got per cell builder colony. Doolittle, (1915). McKinley (1963).

The Treatments:

Twelve colonies of a queenless were prepared to receive the cell cups of one-day grafted larvae (45 cup /colony). All the cell builder colonies utilized in the experiment were equalized in their bee strength, brood area, and pollen, honey stores. Acceptance of larvae within the cell cups was recorded 24h after the transfer of larvae. The accepted queen cells were recorded 72h after larval transfer. Successful sealing of queen cells was recorded five days after larval grafting. Percent raising and sealing of queen cells recorded.

Twelve hives were used as mating nuclei. Each nucleus was given two frames of bees with brood and an adequate population of nurse bees to attend the queen after its emergence. Sealed cells were taken from the bars of the queen rearing frame and fixed into the brood combs of the nuclei.

The emergence of queen bees from sealed cells was recorded on the 12th day after larval transfer. The emergence percentage was calculated.

The Effects of Changes Within the Magnetic Azimuth on Bee Resting Behaviour.

Grafted larvae exposed to 3 times of magnetic waves compared with control. To generate uniform magnetic fields by using electric coils to get electromagnetic exposure to high frequency. Actually, Merritt and Ruben coil systems were used to generate volumes of uniform magnetic flux in several frequencies. With these coil systems, we could generate a homogeneous magnetic flux during a considerable volume surrounding the middle of the coil system. Four sets of thin enamelled wire ($O = 0.5$ mm, $\rho = 1.7 \times 10^{-8} \Omega \cdot m$) were wound into four coils connected to a DC power supply to get a consistent magnetic flux at the central area of the coils. The coil was placed vertical to the geomagnetic field (approximately 40 μT) and generated a 65 μT field, which induced the horizontal component to rotate 60° clockwise. The square coils were turned on/off to vary the magnetic azimuth every 5 minutes during the stimulus period. For one day. Two days, three days after acceptance compared with control (De Souza *et al.*, 2019, Mahir Murat *et al.*, 2019).

Queen bee emergence weight is often used as a top-quality think about the evaluation of queen bees because high emergence queen bees have a bigger spermathecal diameter and higher ovariole number. On the opposite hand, it's stated that queen body size or emergence weight is often used as a reliable index for determining the standard of the queen.

Statistical Analysis:

Statistical analyses were done using Analysis of Variance (ANOVA) for Completely Randomized Design (CRD), following various transformations wherever necessary. The least significant difference (L.S.D.) values were figured out to work out the importance of differences among the mean values at a 5 percent level of significance.

RESULTS AND DISCUSSION

This work was conducted at the apiary and laboratory of Economic Entomology and Agricultural Zoology Department, Faculty of Agriculture, Menoufia University, during the summer (June–July) for successive years 2017 and 2018. To study the effect of rearing queens under magnetic conditions on some characteristics of queens.

Data recorded in the Tables 1 &2 and statistically analyzed by (ANOVA test), to know the effect of magnetic condition on some honeybee characteristics of queens.

Data presented in Table (1) show the effect of exposure of grafted queen cups to different times of magnetic field on the acceptance percentages of grafted larvae in

beeswax cell cups and differed significantly and ranged from 72.22 to 82.33% for the first year 2017 and from 70, 82.33 % for the second year 2018. The acceptance in 3 days exposure times was significantly higher (82.33%) than the one-day exposure time compared with control. The percentages of raising cells during the first year 2017 breeding season ranged from 90.76, 95.52%, which are higher as compared to the second year 2018, which were 93.93, 97.33% during the present study.

The length of sealed queen cells differed from 24.20, 29.40 mm for the year 2017 and 23.10, 29.34mm for the year 2018.

These data agreed with Bognoczky (1967), Chang (1979), El-din and Elsamni (1990). Dodoglu and Emsen (2007)., Cengiz *et al.* (2009), Abdulaziz *et al.* (2013), Ahmed and Dar SA. (2013), Heikh and Shahnawaz (2013). Bo'zena and Jerzy (2015), and Chao-Hung et al (2016), Dhaliwal NK, Singh J, and Chhuneja PK. (2017). Samet and Ethem (2018). Wu, *et al.* (2018).

Table 1. The mean number of grafted larvae, Acceptance percentage, % rising of cells, mean length of sealed queen cells (mm) during (June, July) of 2017 and 2018.

Exposure time	Mean values for the successive year 2017				Mean values for the successive year 2018			
	Number of grafted larvae	Acceptance %	Raising of cells %	Sealed queen cell Length(mm)	Number of grafted larvae	Acceptance %	Raising of cells %	Sealed queen cell Length(mm)
1day	90	72.22b	90.76b	27.52b	90	70.00d	93.65c	26.82b
2 days	90	81.11a	94.52a	24.20b	90	82.33a	97.33a	23.10c
3 days	90	82.33a	94.66a	29.40a	90	73.33c	93.93b	29.34a
Control	90	73.44b	95.52a	25.30b	90	80.00b	97.22a	26.64b
LSD	0.00	8.90	3.77	1.89	0.00	2.34	0.12	2.53

The mean values with different letters in the same column are significantly different ($P \leq 0.05$).

Data presented in Table (2) show the effect of exposure of grafted queen cups to different times of magnetic field on some morphometric characters of virgin queens.

Data showed that the body weight (mg) were 168.05, 161.69, 172.59 and 166.33 for 1, 2, 3 days of exposure compared with control respectively during 2017 and were 166.02, 159.49, 171.44 and 169.33 for 1, 2, 3 days of exposure compared with control respectively during 2018. the data showed significant differences between all treatments.

Also, data showed significant differences between treatments in the body length (mm), which was 16.70, 15.98, 16.05, and 16.41 mm for 1, 2, 3 days of exposure compared with control respectively during 2017 and were 16.20, 16.10, 15.96 and 16.38 mm for 1, 2, 3 days of exposure compared with control respectively during 2018.

On the other hand, when comparing the differences in both head length (HL) and head width (HW) mm, the results showed that (HL) 2.49 mm, (HW) 2.72mm for 1-day exposure, (HL) 2.51 mm, (HW)2.79 mm for 2-day exposure, (HL) 2.52 mm, (HW) 2.72 mm for 3-day exposure compared with control which was (HL)2.49 mm and (HW) 2.74 mm for the first year 2017. But for the second year, 2018 were (HL) 2.44 mm, (HW) 2.69 mm for 1-day exposure, (HL) 2.51 mm, (HW)2.77 mm for 2-day exposure, (HL) 2.57 mm, (HW) 2.74 mm for 3-day exposure compared with control which was (HL)2.42 mm and (HW) 2.69 mm.

Table 2. Mean values of morphometric characters of virgin queens exposed to a magnetic field during (June, July) of 2017 and 2018.

Exposure time	body weight (mg)	Body Length (mm)	Head length (mm)	Head width (mm)	Thorax Length (mm)	Thorax width (mm)	Ovariole number	Spermathecal diameter (mm)
Mean values for the successive year 2017								
1day	168.05b	16.70a	2.49b	2.72c	3.02c	2.79a	149.6c	1.04c
2 days	161.69d	15.98d	2.51a	2.79a	3.10a	2.69b	156.2b	1.12b
3 days	172.59a	16.05c	2.52a	2.72c	3.11a	2.71b	161.2a	1.21a
Control	166.33c	16.41b	2.49b	2.74b	3.04b	2.77a	146.42d	1.18a
LSD	1.73	0.37	0.01	0.03	0.03	0.02	3.21	0.03
Mean values for successive year 2018								
1day	166.02c	16.20b	2.44c	2.69c	3.01c	2.68c	147.3c	1.06b
2 days	159.49d	16.10c	2.51b	2.77a	3.88a	2.71b	155.23b	1.09b
3 days	171.44a	15.96d	2.57a	2.74b	3.09b	2.79a	160.90a	1.19a
Control	169.33b	16.38a	2.42d	2.69c	3.01c	2.71b	145.22c	1.08b
LSD	1.73	0.37	0.01	0.03	0.03	0.02	3.21	0.03

The mean values with different letters in the same column are significantly different ($P \leq 0.05$).

Data showed significant differences between treatments in thorax length (TL) and thorax width (TW) mm, the results showed that (TL) 3.02 mm, (TW) 2.79mm for 1-day exposure, (TL) 3.10mm, (TW) 2.69 mm for 2-day exposure, (TL) 3.11mm, (TW) 2.71 mm for 3-day exposure compared with control which was (TL) 3.04mm and (TW) 2.77 mm for the first year 2017. But for the second year 2018 were (TL) 3.01 mm, (TW) 2.68 mm for 1-day exposure, (TL) 3.88 mm, (TW) 2.71 mm for 2-day exposure, (TL) 3.09mm, (TW) 2.79 mm for 3-day exposure compared with control which was (TL) 3.01 mm and (TW) 2.71 mm.

Data collected from dissection of queens exposed to magnetic field showed significant differences between treatments in a number of ovariole (NO) and spermathecal diameter (SD) mm, the results showed that (NO) 149.6, (SD) 1.04 mm for 1-day exposure, (NO) 156.2, (SD) 1.12 mm for 2-day exposure, (NO) 161.2, (SD) 1.21 mm for 3-day exposure compared with control which was (NO) 146.42 and (SD) 1.18 mm for the first year 2017. But for the second year 2018 were (NO) 147.3, (SD) 1.06 mm for 1-day exposure, (NO) 155.23, (SD) 1.09 mm for 2-day exposure, (NO) 160.90, (SD) 1.19 mm for 3-day exposure compared with control which was (NO) 145.22 and (SD) 1.08 mm.

These data agreed with FCC, (1999), Hsu, and Li (1994), Harst, *et al.* (2006), Stever, *et al.* (2007), Kumar, *et al.* (2010), Sharma and Kumar (2010), Sainudeen (2011), Kumar *et al.* (2013) and Shepherd *et al.* (2019)

Conclusion:

It could be concluded from the obtained results that exposing grafted queen cell in mass rearing production in honey bee colonies may affect the morphometric characters differed from one day to three days compared with non-exposed colonies, therefore, it could be recommended that beekeeper must be trained to expose his rearing queens in the breeding time to a magnetic field to increase the ability of queens and workers to avoid the bad effects of these radiations.

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