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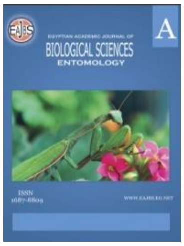

Anticipated Factors Affecting Extraction of Venom from Honey Bees colonies by Electrical Impulses.

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ABSTRACT

Some factors affecting bee venom extraction by electrical impulses directly from honey bee colonies were studied. Two experiments were conducted during the summer months to study the effect of defensive response degree, colony strength level, and season time of collection. Twenty-four carniolan hybrid honey bee colonies were used.

The results revealed that defensive response was depended on the colony strength and season time of collection. When the number of stings par colony was determined, the stings number was related to the number of frames covered with bees. Bee venom amount which weakly extracted significantly differed during summer months (July and August). From the results of the variability of extracted venom amounts, the main peak of production was recorded during July month. Negative correlation coefficients were recorded between defensive response and collected bee venom from three strength levels from honey bee colonies.

INTRODUCTION

Honey bee defensive behaviour is a complex trait that modified by many factors as honey bee race, weather factors, colony strength and health, time of the year and foraging activity (Collilns, 1979, 1981; Collins *et al*., 1984; Maritz *et al*., 1987; Woyke, 1992 and Hant *et al*., 1998). These factors together with solar radiation and wind speed can determine up to 92% of factors affecting the defensive reaction of honey bee colonies (Southwick and Mortiz, 1987). Also, the influence of the presence or absence of nectar in the field on colony defensive behaviour was studied by Downs and Ratineks (2000).

Nowadays, dry bee venom production has become one of the important bee colony products that are used for the cure of many human diseases. Dry bee venom is extracted by modern methods dependent on electrical shock stimulation and dry material can be obtained directly from honey bee colonies. Many authors improved electrical method techniques and devices in various parts of the world. In Egypt, Omar (1994) evaluated a new device for obtaining a high quantity of dry bee venom from honey bee colonies. Omar (1997) recorded that venom extraction from honey bee colonies after queen removal or after artificial crowding and after honey extraction with one day increased venom quantity. Omar (2010) reported that there was a relationship between colony defensive behaviour and the quantity of dry bee venom collected by the electrical impulses method.

The objective of the present work was to study the effect of aggressiveness response variability during summer months on venom quantities extracted by electrical impulses from honey bee colonies.

MATERIALS AND METHODS

The experimental work was carried out at the apiary of Agriculture College, Assiut Univ., during summer season (June – August 2019). Twenty-four local hybrid of carniolan honey bee colonies were chosen at three strength levels. The relative colony strength level was measured by a number of frames covered with bees. The colonies were divided into three levels:

L_1, strong colonies (9 frames).

L_2, medium colonies (7 frames).

L_1, weak colonies (5 frames)

Experimental Work:

The present study was carried out in two steps:

First Step: Primary Evaluation of Honey Bee Aggressiveness Response Degree:

Praimanry test was used to evaluate the aggressiveness response for every honey bee colony group. Two applications were carried out at two weeks interval during June month, 2019. This step was used to calculate the grade of defensive response distribution for each strength level.

The defensive response was classified into 5 grades. The number of stings in the first grade ranged from 1 to 5 stings/colony/1 min until the 5th one from 21 to 25 stings/colony/1 min. The distribution of the defensive response percentage of every colony group was determined. Relative Aggressiveness Rate (RAR) was calculated as a number of stings for one frame covered with bees after the following equation:

$$RAR = \frac{S}{F} x\ 100$$

Where:

RAR= Relative Aggressiveness Rate.

S = No. of stings/colony/1 min.

F = No. of frame/colony/1 min.

Second step:Determination of Venom Production Fluctuation in Relation to The Change of Defensive Response Degree:

After calculation of aggressiveness degree distribution of the eight honey bee colonies from each strength level, five colonies from each group were used in the second step. The colonies were selected with a similar defensive response rate. The second step was carried out during the period extended from July to August 2019 to study the influence of colony strength or aggressiveness rate on venom production in summer season. Seven extractions were weekly applied for venom collection.

Measurements:

Aggressiveness Degree Determination:

In order to measure the aggressiveness degree of each colony, the ball method described by Stort (1974) was used. The measurement was carried out at combs top position without smoking. The bees were permitted to sting for a period of one minute after the first sting. At the end of each test, every ball was sealed in plastic bags and the number of stings was counted. The defensive response test firstly was done before excitation by electrical impulses one hour before.

Extraction of Dry Bee Venom:

Honey bee colonies were excited by electrical impulses for venom production after aggressiveness degree measurement with one hour. An electronic impulses generator developed by Omar (1994) was used for colony excitation. One selected waveform belonged to the sin form group, amplitude 16 V and frequency 50 HZ were used. After a period of 30 minutes for venom extraction, the venom collection boards were collected and the glass plate boards were stored for 24 hours under condition of room temperature for dryness.

The total amounts of dry venom were determined by scraping the surface of the glass plate by slide glass. The amount of dry venom produced from every board's glass was weighed separately by electrical balance.

Statistical Analysis:

Data of all treatments were statistically analyzed using Statistix 8.1 software (Analytical software, 2003) and subjected to the analysis of variance under a complete randomized design. Means were separated by the Least significant Difference test (LSD test) at a significant level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Praimanry Evaluation of Honey Bee Colonies Defensive Response Degree:

Data presented in tables (1&2) showed the means of defensive response rate of the three strength levels of honey bee colonies after two applications during June, 2019. Data cleared that number of stings par colony related with the number of hive frames covered with bees. The general means of defensive response degree (stings/colony/1 min) ranged between 12.32 to 20.00 stings per colony for different strength levels used. The values of defensive response degree differed significantly between the strongest level (9 frames) and the lowest one (5 frames).

From another hand, after calculation the Relative Aggressiveness Rate (RAR) (number of stings par one frame) calculation for each colony strength level, the number of stings/frames changed significantly from strongest colonies (9 frames) toward the weak colonies (5 frames). RAR decreased from 2.46 (sting/frame) in weak colonies to 2.22 (sting/frame) in strong colonies (Table 1).

Table 1: Preiminary evaluation of the defensive response of honey bee groups (June, 2019).

Colony strength level (L)	No. of stings/colony/min			Stings/frame (RAR)
	1st app.	2nd app.	General mean	
L_1 (9 frames)	20.25 a	19.75 a	20.00 A	2.22
L_2 (7 frames)	15.38 b	16.25 ab	15.82 B	2.26
L_3 (5 frames)	11.63 c	13.00 b	12.32 B	2.46

Means followed by the same letter are not significantly different P= 0.05.

Table 2: Frequency distribution percentages of the defensive response of colony strength levels.

Colony strength level	Grades of defensive response % (stings/colony/ min)				
	I (0-5)	II (6-10)	III (11-15)	IV (16-20)	V (21-25)
L_1 (9 frames)	0	25%	62.5%	12.5%	0
L_2 (7 frames)	0	12.5%	25.0%	62.5%	0
L_3 (5 frames)	0	0	12.5%	25%	62.5%

The present results indicated that the defensive behaviour of the honey bee was depended on the colony strength level under the same environmental conditions. Additionally, the strongest colony had 1.6 times more frequent stinging than the weak ones from the present results, when the colony of each group receives the same stimulus. The defensive response of honey bee colonies is initiated by guard bees (Moore *et al*., 1987). The guards and stingers play different roles during the defensive response of honey bee colonies (Breed *et al*., 1990).

The defensive response of different honey bee colony groups was classified into 5 grades as described in the methodology. Frequency distributions percentage of defensive response have been determined in all strength groups of honey bee colonies. The percentages of these distributions are illustrated in Fig. (1). The obtained data showed that the highest percentage (62.5%) from aggressive honey bee colonies was recorded in grates III, IV and V for L_1, L_2 and L_3 levels, respectively.

From the present results, it may be conducted that the difference among colony strength levels affecting defensive response grade.

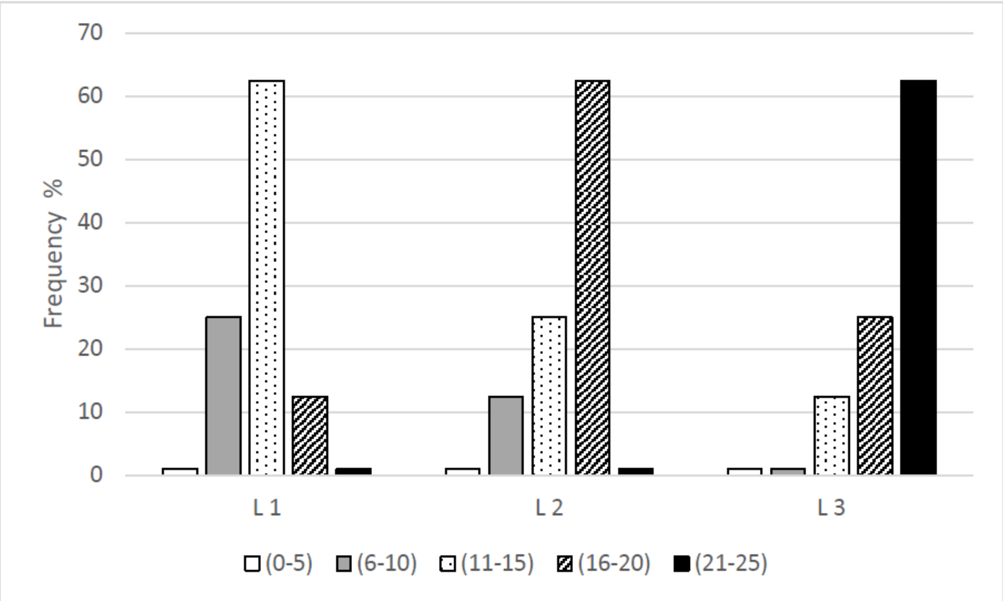

Fig. 1: Frequency distribution percentage of defensive response grades of different colony strength levels.

Relationship Between Variability of Defensive Response and Amounts of Dry Bee Venom Extracted:

From data presented in table (3) and illustrated in fig. (2), high variations were recorded in a number of stings/colonies as an indication for defensive response behavior. The defensive response of honey bee colonies differed significantly during summer months (July-August). In general, two peaks of aggressiveness behaviour were recorded during the defensive response test. The highest peak was recorded in 15 July (22 stings/colony) and the second one on 12 August (24 stings/colony). Some authors explained the reason for defensive behaviour variability. They documented that environmental factors such as temperature (Rothenbuhler, 1974) and humidity (Collins *et al.,* 1982) strongly affected defensive behaviour in the field. The presence or absence of nectar and pollen in the field (Breed and Rogers, 1991), and also colony genotypes (Downs and Ratineks, 2000) were recorded as factors affecting the defensive behavior of honey bee colonies during the active season.

Table 3: The fluctuation of the defensive response of three strength levels of honey bee colonies during summer season (July–August, 2019).

Date	No. of stings/colony/1 min.			
	L_1 (9 frames)	L_2 (7 frames)	L_3 (5 frames)	G. mean
15 July	24.20 a	23.80 a	20.60 b	22.87 B
22 July	14.80 fg	13.20 gh	10.00 i	12.67 G
29 July	20.20 b	19.00 c	15.40 f	18.20 D
5 Aug.	20.80 b	20.20 b	18.80 cd	19.93 C
12 Aug.	25.00 a	24.40 a	23.80 a	24.40 A
19 Aug.	17.40 de	15.80 ef	15.40 f	16.20 E
26 Aug.	15.40 f	12.60 h	11.80 h	13.27 F
G. mean	**19.69 (A)**	**18.43 (B)**	**16.54 (C)**	

Means followed by the same letter are not significantly different P= 0.05.

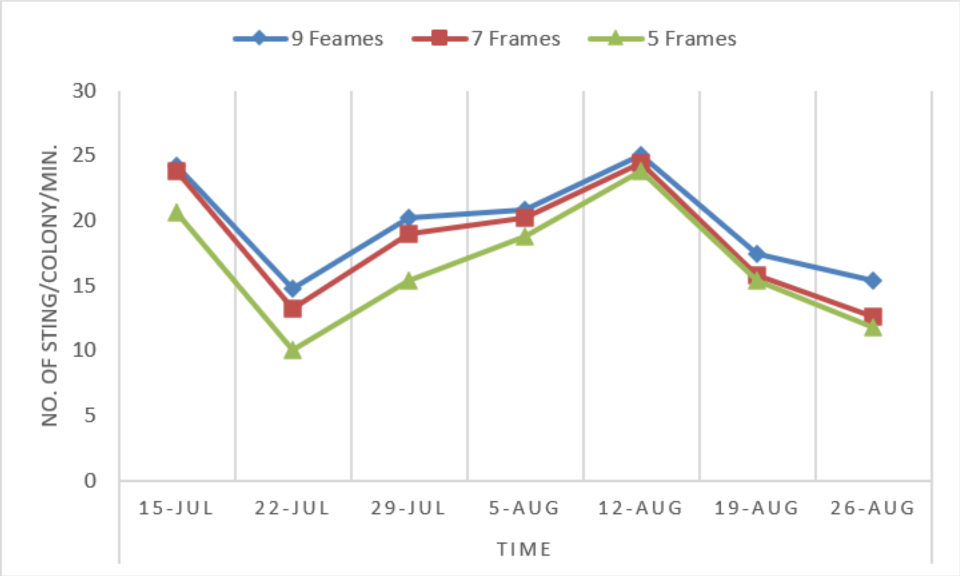

Fig. 2: The fluctuation of the defensive response of three strength levels of honey bee colonies during summer season.

Variability of dry venom amounts extracted from different colony strength levels during summer season was presented in table (4) and illustrated in figure (3). The results showed that means of bee venom amounts collected weekly during summer months (July-August) significantly differed. General means of dry bee venom amounts extracted from different colony strength levels significantly differed. The maximal dry venom amounts were extracted during 22-July compared with the following collection dates.

From data illustrated in figure (3) two peaks for venom production were recorded during the test period. The main peak (74.16 mg/colony) was recorded on 22 July, 2019. The present results indicated that dry bee venom amounts depended on many factors as the date of extraction, colony strength level and defensive response degree.

Table 4: The fluctuation of venom production of three strength levels of honey bee colonies during summer season (July-August, 2019).

Date	No. of stings/colony/1 min.			General mean
	L_1 (9 frames)	L_2 (7 frames)	L_3 (5 frames)	
15 July	24.500 fghij	21.080 k	15.580 l	20.387 F
22 July	92.280 a	74.960 b	55.220 d	74.153 A
29 July	70.560 c	36.660 e	25.200 fgh	44.140 B
5 Aug.	34.100 e	26.420 f	22.880 hijk	27.800 C
12 Aug.	20.980 k	22.240 fg	15.600 l	19.607 F
19 Aug.	24.720 fghi	26.320 ijk	25.720 fg	25.587 D
26 Aug.	21.940 jk	23.760 ghij	22.100 ijk	22.600 E
G. mean	**41.297 (A)**	**33.063 (B)**	**26.043 (C)**	

Means followed by the same letter are not significantly different P= 0.05.

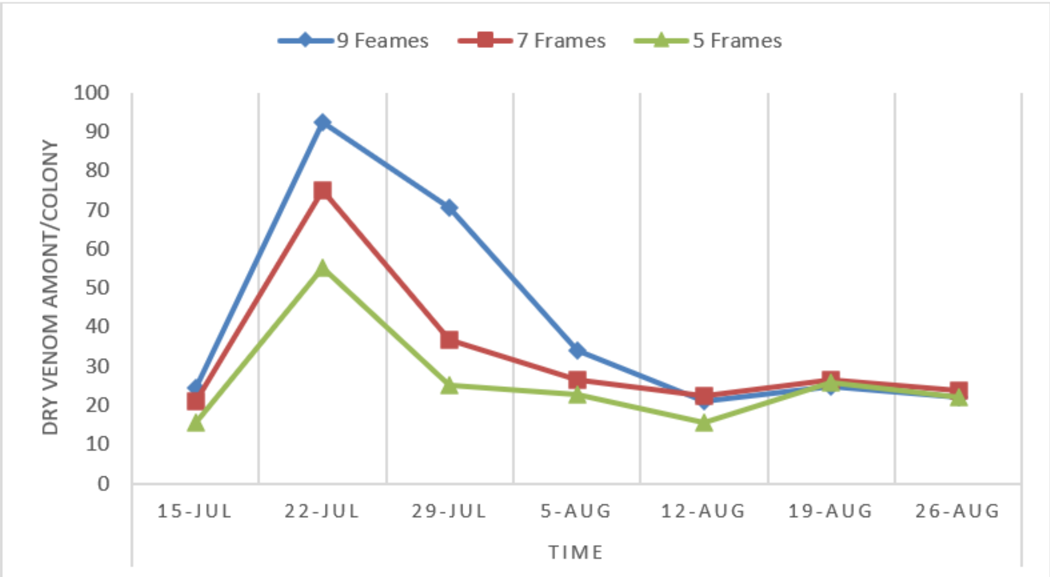

Fig. 3: The fluctuation of venom production of three strength levels of honey bee colonies during summer season.

These results may be due to several combinations of these factors which together affecting venom production by electrical impulses method.

As described by Hider (1988) venom production from honey bee colonies was higher during summer months in which there was a peak of bee activity. Also, Khodairy and Omar (2003) recorded that positive correlations were calculated between dry venom amount extracted from honey bee colonies by electrical impulses methods and each of bee population, bee brood, stored pollen, honey areas and bees foraging activity.

From data presented in tables (3 and 4), amounts of dry bee venom extracted during summer period decreased with increment of honey bee aggressiveness rate.

After using the simple correlation test, the relationships between defensive response and dry venom amount extracted by electrical impulses during summer months were recorded. Values of correlation coefficients are summarized in Table (5). Data show a negative correlation between defensive behavior and extracted bee venom amounts in all honey bee colony groups. The present results reflected the same observation maintained by Omar (2010) who reported that during experimental work for venom extraction, the venom collection boards during excitation time and stinging action were completely done. Whereas, the individuals of aggressive colonies were unstable on collection boards and run away. This behaviour causes decreasing in the amount of dry bee venom extracted under the same strength level.

The present result would be useful in the prediction time of summer season that might affect the potency of venom production.

Table 5: Correlation coefficient values between defensive response and venom amount for three strength levels.

Comparisons	r- values
L_1 (9 frames)	-0.4225
L_2 (7 frames)	-0.5040
L_3 (5 frames)	-0.6824

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