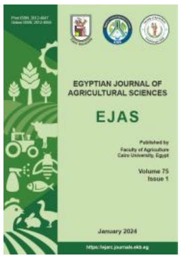
Egypt. J. Agric. Sci., 2024, 75 (Special Issue): 21-31.
Doi: 10.21608/ejarc.2024.340545

Egyptian Journal of Agricultural Sciences
Journal homepage: www.ejarc.journals.ekb.eg
Published by: Faculty of Agriculture, Cairo University, Egypt
Print ISSN 2812-4847 Online ISSN 2812-4855



14th International Conference of Arab Beekeepers Union, 13-15 November, 2023, Cairo-Egypt

Influence of Electric Amplitude and the Frequency Level combinations on Venom Productivity of Honey Bee Colonies in Egypt

By
Enas O. Nour El-Deen*[1] and Ahmed A. Shaheen[2]

1 Department of Beekeeping Research, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt
2 Department of Plant Protection, Faculty of Agriculture, Al Azhar University, Cairo, Egypt

ABSTRACT

The aim of this research was studying the effect of different combinations of electric amplitudes and frequency levels on venom production and foraging behavior during the season of clover nectar. The obtained results revealed that venom production significantly increased with increasing the electric amplitudes. The quantities of collected venom were 38.0±22.8, 76.4±17.7 and 83.3 ±19.8 mg / col. for amplitudes of 12, 24 and 36 Volts, respectively. However, the highest venom yield occurred with 50 Hz/s, (83.1±21.1 mg/ col.) and the least was recorded with 30 Hz/s, (49.1± 24.5 mg/ col.). The best combination between amplitude and frequency level was 24 V with 50 Hz/s regarding venom yield which was safe for bees, while using 36 V with 80 Hz/s caused a significant death reached 33.1± 23.6 workers/colony / 45 min. of application. The results indicated that the ratios of pollen collectors (PC) to the total returning foragers (TF) before venom collection were similar in treatment and control groups (29.7± 1.7 % and 29.6± 1.7 %, respectively). However, after one day of venom collection, the foraging force of treated colonies was significantly less (166.0± 6.0 bee/col.) than the control group (336.7± 24.5 bee/ col.). The PC remained similar to that recorded for control colonies (28.6% and 29.6%, respectively). The TF was recovered and significantly raised in their force than control colonies, from the second day of application until the end of experiment. The majority of these workers were converted to PC, especially during the second (68.9±1.0 %) and third (67.0±0.7 %) days after excitation. This may be to compensate the previously consumed beebread due to venom collection.

***Keywords**: Impulses frequency, Electric, Amplitudes, Venom, Honey bee.*

1. INTRODUTION

The products of honey bees, especially bee venom, attained a discriminate position in the last two decades among the other natural products in different fields of medicine, pharmacology, therapeutic activity, nutritional benefits and food technology (Cornara *et al.*, 2017; Ghosh *et al.*, 2020; Olas, 2022; Varol *et al.*, 2022; Lima *et al.*, 2020, and Asma *et al.*, 2022).

The bee's venom therapy is used, among all bee products, because it is a rich source of various pharmaceuticals, with at least 18 active components of polypeptides and enzymes. Thus, it has been utilized for a significant time as an "alternative therapy" to treat many scleroses, where the results were promising as anti-cancer bio-agent, which indicates that this apitherapeutic product will take place as a supplement in cancer treatment in the near future (El-Bassiony and Khalil, 2007; Abdela and Jilo 2016; Mahfouz *et al.*, 2020 and Sengul and Vatansev, 2021).

The demand for large quantities of uncontaminated venom increased with the advance in the bee venom therapy. Thus, the methods of collection were rapidly evaluated in different countries to achieve this purpose. The

*Corresponding author E. mail: drenasosman2014@gmail.com

Received: 26.11.2023 **Accepted: 06.02.2024** **Available online: 09.02.2024**

best method for quantitative production of bee venom was first reported by Markovic and Molnar (1954) by exposing workers to a mild electrical shock to stimulate stinging response. When the bee comes into contact with two wires connected to alternate electric charge, it stings through a piece of nylon sheet stretched over a glass plate that is fitted under the wires to receive the droplets of venom. However, the observations recorded during venom collection resulted in development of more collector devices (Palmer, 1961; Benton *et al.*, 1963; Morse and Benton, 1964a, b; Gunnison, 1966; Brandeburgo, 1992; Omar, 1994a; Simics; 1995, 1999 and Rybak and Skubida, 2007). Therefore, various modifications of power supply, type of current amplitude and the frequency of impulses, time of excitation, under different circumstances, etc., were investigated (Benton and Morse 1966; Malaiu *et al.*, 1981; Nobre, 1990; Omar, 1994b; Fakhimzadeh 1998; Muszynska and Rybak, 2002, Mohanny, 2005 and Abdulraouf and Mohamed, 2023). The impact of bee venom collection on numerous activities of the treated colonies was take in consideration by several researchers, (Khodairy and Omar 2003; Omar, 2011; Sanad and Mohanny, 2013; Nowar, 2016; Hussein *et al.*, 2019 and Aparna *et al.*, 2023) but the effect on foraging behavior from the point of pollen collection stile scarce.

The objective of this work is to determine the influence of different current amplitudes in combination with various frequencies on the obtained yield of venom, besides the effect of venom collection on the foraging workers distinct toward pollen collection.

2. MATERIALS AND METHODS

This study was carried out at privet apiary in El-Bagour district, Menoufia governorate, during the nectar flow of the Egyptian clover, *Trifolium alexandrinum* L. (May and June) of year 2023.

2.1. The principal components of a bee venom collector device

The bee venom was collected by locally designed electric shock device, which consists of three major units. The first unit is a power supply representing by a dry battery to input 11.5 – 12.5V direct current (DC). The second is a converter unit, which converts the input direct current into alternative current (AC). The third one is a collector wooden frame (48.5 x 33 x 2.5cm) which holding a parallel wire grid at a distance of 5 mm from each other and a glass plate of 3 mm thickness covered with a thin sheet of nylon was inserted 2 mm under the wires. The nylon sheet enables the workers to pull out their stings during the off time of electrical shock besides protect the extracted venom from contamination (Simics, 1999).

2.2. Determination of venom quantity

After the collection process stopped by about half hour the glass plate covered with venom of each colony removed and carefully transported in a special container to the laboratory and placed in a dark well-ventilated site to dry. In the following day, the dried venom is scraped with a sharp scraper and gathered in a dark glass vial after weighing on 0.001 g digital balance.

2.3. Position of collecting board and time of application

The collecting boards are placed in an upside-down position on top bars of each colony after maintain 12 mm distance between the frames and the glass plate to ensure the proper movement of bees. The collecting boards were connected with wires to each other and finally to the collector device. The current of impulses in the wires were automatically controlled the current release for 2 sec. at 3 sec. intervals. The timing of application in this research was one hour before sunset (6–7 pm) according to studies of El-Feel (2017).

2.4. Honey bee colonies

For all the following experiments colonies of the local hybrid Carniolan, *Apis mellifera carnica*, race was selected. Each colony contained 6–7 brood combs, 2-3 honey and pollen combs and all the nine combs were completely covered with healthy workers.

2.5. Effect of different combinations of voltages and frequencies on the harvested yield of dried bee venom

This investigation was undertaken to evaluate the influence of combination between three amplitudes (12, 24 and 36 Volt) with three frequencies (30, 50 and 80 Hz) for harvested the highest amount of dried bee venom and their impact on workers survival during 45 min of excitation. The wave form of sine type was applied during all applications. It was controlled the above factors by using integrated circuits in the device. The bees killed on or under the wires of collecting board and on the hive, front was separately gathered and counted. For this test, twenty-seven honey bee colonies of the mentioned above were used during the period from 2–25 of May, 2023 where they divided into three groups, each contained nine colonies of different combinations. Each group was examined throughout one week of the mentioned period.

2.6. Effect of venom collection by electrical shocks on foraging workers behavior

Eighteen honey bee colonies were prepared as previously mentioned, and divided into two equal sets. Starting from May, 28 the total numbers of foraging workers (TF) and pollen collectors, (PC) returning to each colony between 11–12am were, separately, daily counted for 3 min using a handle counter and stop watch. The period of this observation continued 3 days (until May, 30th where it considered as before treatment period). After the third counts on May, 30th, the first set of colonies exposed to application of venom collection by electrical shocks before sun set for 90 min with amplitude 24 Volt and frequency 50 Hz/s. The collecting plates were left within treated colonies over-night and removed in the next morning two hours before registration of forager's activity (May, 31th) which was normally continued to June, for the treated and untreated colonies (these days considered as after treatment period). The percentages of pollen collectors to the total returning foragers (PC/TF) were determined.

2.7. Statistical analysis

Data of all experiments were analyzed in a randomized complete block design (Two Way Anova table) by MSTAT-C version 1.41 (Snedecor and Cochran, 1980). All means were compared by Duncan's multiple range test at level 0.05.

3. RESULTS AND DISCUSSION

3.1. Effect of different combinations of voltages and frequencies on the harvested yield of dried bee venom

Data revealed in Table (1) and Figure (1) show the impact of several current amplitudes, (12 V, 24 V and 36 V) combined with various frequencies (30, 50 and 80 Hz/s) on the amount of collected dry bee venom by electrical shocks. These results appear that the potent of dried bee venom extraction. In general, it increased with the increasing in amplitude used where the significant highest yield of venom (83.3 mg /col. / 45min) was recorded for 36 volt and the lowest amount was 38.0 mg/col./45min for 12 volts. These results agreed with Omar (1994a) who found that the change in the amplitudes from 12V to 24V increased venom extraction at all frequency levels.

Regarding to the effect of frequency levels, the amount of collected venom was significantly higher at frequency of 50 Hz/s (83.1 mg/col./45min) than both of 80 Hz/s (65.4 mg/col.) and 30 Hz/s (49.1 mg/col.) as shown in Table (1). However, the combinations between the tested amplitudes and frequency levels, cleared that the highest venom quantity was significantly achieved at frequency of 50 Hz/s and 36 volt (105.8 ± 7.0 mg/col.). The current of 24 V and 80 Hz/s came next with significant increase (92.3 ± 6.8 mg/ col.) than the rest combinations as illustrated in Fig. (1). The amplitude of 24 V with 50 Hz/s attained significant increase in venom collection (79.5 ± 6.9 mg/col.) than the residual combinations except the treatment of 36 V with 80 Hz/s (75.5 ± 12.1 mg / col.). The lowest venom production was recorded for 12 V with 30 Hz/s, (21.6 ±11.8 mg/col.). In this respect, Malaiu *et al.* (1981) obtained a good yield of venom by electrical stimulation using a generator produces series of alternating impulses consisting of a positive amplitude to 45 V for 1.5 µs, followed by a negative amplitude of 60 V for 7 µs and the frequency of the impulses was 58 Hz/s.

As the safety of workers is very important to survive and continue of ordinary activities of honey bee colonies, the effect of the tested combinations between electric amplitudes and frequency levels on the workers life was take in consideration as recorded in Table (2). It is obvious, in general, that the highest electric amplitude and frequency level caused significant harmful for workers life than other categories. The amplitude of 36 V killed 35.0± 39.2 bee/ col./45min while the frequency of 80 Hz/s killed 33.1 ± 23.6 bee/col./45min.Colonies exposed to 24 Volt lost significant numbers of bees per colony than those treated by 12 V (7.9 ± 7.1 and 2.8 ± 0.8, respectively).

On the other hand, the highest rate of dead bees occurred due to application of 36 V

Table (1): Effect of various amplitudes and frequencies on the mean* amounts of dry venom (mg) harvested by electrical shock device during May, 2 – 25, 2023.

Amplitude	No. of frequencies (Hz) / S						Mean / amplitude ±sd	
	30 Hz/s		50 Hz/s		80 Hz/s			
	Range	Mean ± sd	Range	Mean ± sd	Range	Mean ± sd		
12 Volt	5.4 - 34	21.6 ±11.8 g	53 - 76	64.0 ± 9.5 ef	16 - 37	28.5 ± 6.5 g	38.0 C	± 22.8
24 Volt	52 - 65	57.3 ± 5.6 f	73 - 88	79.5 ± 6.9 c	84 - 100	92.3 ± 6.8 b	76.4 B	± 17.7
36 Volt	63 - 74	68.5 ± 6.8 de	98 - 105	105.8 ± 7 a	65 - 86	75.5 ± 12.1 cd	83.3 A	± 19.8
Mean/ frequency	**49.1 C**		**83.1 A**		**65.4 B**		**65.9**	±17.0
± Sd	± 24.5		± 21.1		± 33.1		± 24.4	

*Means with the same small or capital letters in the same column or row do not significantly differ according to DMRT at 0.05 of probabilities.

Table (2): Effect of various amplitudes and frequencies on the mean* numbers of dead workers of electrical shocks for venom collection during May, 2 - 25, 2023.

Amplitude	No. of frequencies (Hz) / S						Mean / amplitude ±sd	
	30 Hz/s		50 Hz/s		80 Hz/s			
	Range	Mean ± Sd	Range	Mean ± Sd	Range	Mean ± Sd		
12 Volt	1 - 4	**2.3** ± 1.5 d	0 - 5	**2.3** ± 2.5 d	1 - 7	**3.7** ± 3.1 cd	**2.8 C**	± 0.8
24 Volt	0 - 7	**3.0** ± 3.6 d	0 - 10	**4.7** ± 5.0 cd	8 - 24	**16.0** ± 8.0 b	**7.9 B**	± 7.1
36 Volt	2 - 10	**6.3** ± 4.0 c	5 - 34	**19.0** ± 14.5 b	37 - 115	**79.7** ± 39.5 a	**35.0 A**	± 39.2
Mean/ frequency	**3.9 C**		**8.7 B**		**33.1 A**		**15.2**	± 15.7
± Sd	± 2.1		± 9.0		± 40.8		± 17.3	

*Means with the same small or capital letters in the same column or row do not significantly differ according to DMRT at 0.05 of probabilities

Table (3): Mean* numbers of total foragers, numbers of pollen collectors (3 min) and percentages of pollen collectors / total foragers before and after venom collection from honeybee colonies during clover nectar flow in El-Bagour district, Menoufia governorate, Egypt, 2023.

Condition		No. of Foragers / 3 min.				No. of Pollen Collectors / 3 min				Pollen Collectors %			
Day		Treatment ±Sd		Control ± Sd		Treatment ±Sd		Control ± Sd		Treatment ±Sd		Control ± Sd	
Before treatment													
May, 28		216.0 a	35.1	232.0 a	33.3	60.0 a	4.7	72.0 a	8.6	27.8 a	1.0	31.0 a	0.9
May,29		210.0 a	31.0	227.7 a	29.5	66.0 a	10.5	68.0 a	5.0	31.1 a	0.4	30.2 a	1.7
May, 30		275.7 a	48.5	237.0 a	48.0	83.0 a	14.0	65.7 a	20.0	30.2 a	0.2	27.7 a	2.8
Mean	± Sd	233.9Ba	1.7	232.2Aa	1.7	69.7Ba	11.9	68.6Aa	3.2	29.7Ba	1.7	29.6a	1.7
After treatment													
May,31		166.0 b	6.0	336.7 a	24.5	47.7 b	4.5	99.7 a	4.5	28.6 a	1.7	29.6 a	0.8
June, 1		320.0 a	14.0	280.0 a	78.0	220.7 a	6.5	92.0 b	25.0	68.9 a	1.0	32.9 b	0.2
June, 2		342.7 a	4.5	207.0 b	10.0	229.7 a	5.5	61.0 b	6.0	67.0 a	0.7	29.7 b	4.3
June, 3		335.0 a	23.0	209.7 b	7.5	106.0 a	11.0	64.3 b	15.5	31.6 a	1.1	32.7 a	1.8
June, 4		287.0 a	13.5	246.0 a	24.7	90.0 a	6.4	79.0 a	6.9	32.4 a	1.7	32.1 a	0.5
June, 5		305.0 a	9.3	289.0 a	19.9	87.0 a	5.3	84.0 a	8.8	28.5 a	0.9	29.1 a	2.1
Mean	**± Sd**	**292.6 Aa**	**65.2**	**261.4 Aa**	**50.3**	**130.2 Aa**	**76.1**	**80.0 Ab**	**15.2**	**42.8 Aa**	**19.6**	**31.0 Ab**	**1.7**

* Means with the same small or capital letter in the same row or column (for each parameter) do not significantly differ according to DMRT at 0.05 of probabilities

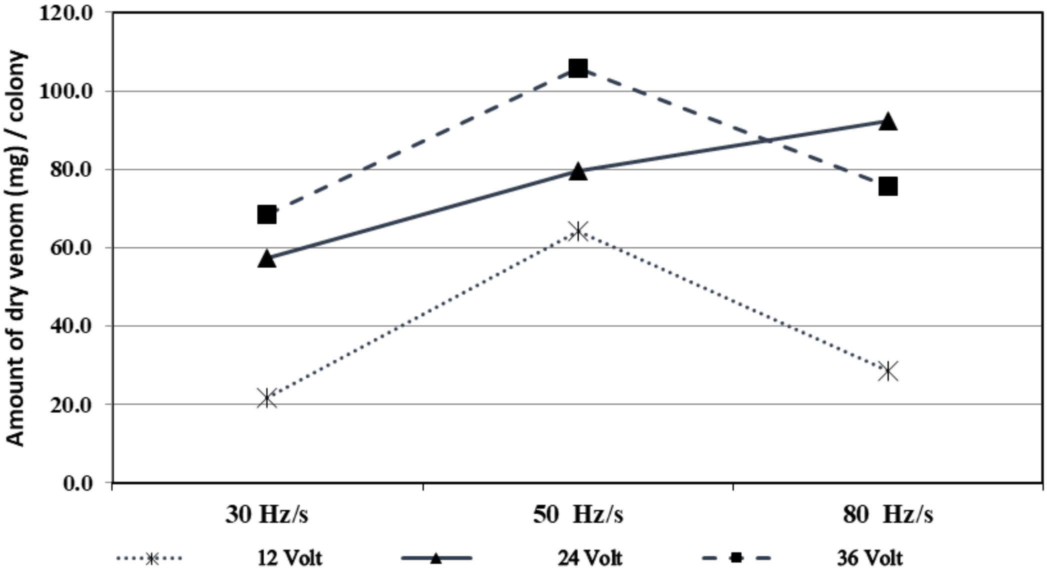


Fig. (1): Effect of various amplitudes and frequencies on mean amounts of dry venom (mg) harvested by electrical shocks during May, 15 - June, 7, 2023.

with 80 Hz/s (79.7 ± 39.5 bee/ col./45min), followed by 36 V and50 Hz/s (19.0± 14.5 bee/ col./45min) and 24 V and 80Hz/s (16.0± 8.0± bee/ col./45min). The other combinations had a neglect impact on workers survival as shown in Fig. (2).

In this situation, Omar (1994a) found that combination of 24V and 50Hz/s killed 20.75 bee /col. during colony excitation for 30 min to collect bee venom. Rose (1994) and Simics (1995) agreed that the well-adjusted collector device should not kill more than5-15 bees/ colony during 30 min of excited colony to save the life span of workers and maintain colony population. Although Hussein (2013) used 18 V amplitude in his device and gained 81.1 mg/col., it caused a loss of 102 bees/ col. during 15 min. of collection. In contrast to our results, Sanad and Mohanny (2013) found significant differences among the death of workers within the months of BV collection. They recorded a higher number of dead bees in summer (50.3 workers/day) and lower in autumn (31.7 workers/day). Death of the bees probably resulted from the stress imposed on the bees from the collecting device, the electric currents, and fights between bees that occasionally occur in colonies during collection (Khodairy *et al.*, 2010). However, El-Feel (2017) noticed low scarce numbers of dead bees during gathering venom by electrical shocks. Therefore, it could be concluded from the above results that the design of electrical shock should be strong enough to make the bees sting, but not high enough to kill the workers. So, the different constructed devices based on exciting the defensive instinct of workers by electric impulses consisting of series complex waves. These wave forms belonged to four wave groups (sine, square, triangular and exponential) and the amount of extracted venom by electrical shocks seemed to depend on the form of the wave but not on its effective value as proved by Omar (1994a) and Omar (2020).

In consequence, the best results were obtained by combined 36 V, and 50Hz/s followed by 24 V with 80 or 50 Hz/s without negative effect on colony population. Accordingly, it could be gained a one gram of dry bee venom from 10-13 colonies ones a time at 45 min during clover nectar flow under study circumstances.

3.2. Effect of venom collection by electrical shocks on foraging workers behavior

Data presented in Table (3) revealed the mean numbers of total returning foragers (TF) and numbers of pollen collectors (PC) within three minutes before and after venom collection during the period from May, 28 - June, 5, 2023. The percentages of PC /TF for treated and control colonies were taken into consideration. It is clear from the results that no significant differences were appeared between treated and control colonies for three successive days before treatment concerning TF, PC and PC/TF ratio as shown in Figures (3-5).

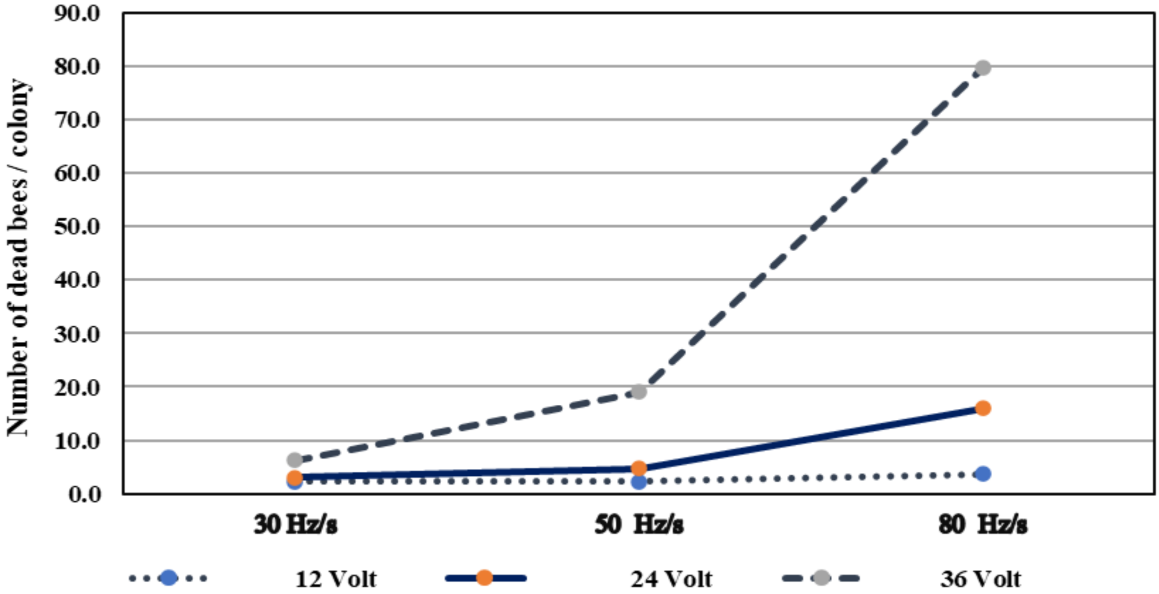


Fig. (2): Effect of various amplitudes and frequencies on the numbers of dead / colony during venom extraction by electrical shocks through May, 15 - June, 7, 2023.

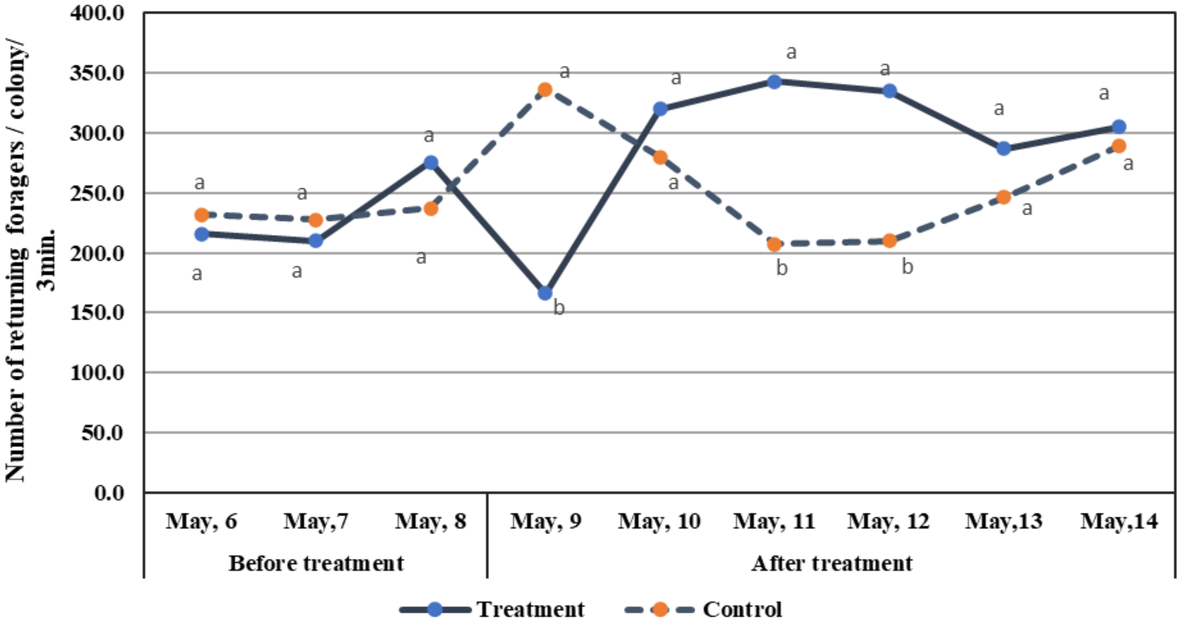


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Fig. (3): Mean numbers of returning workers before and after venom collection during clover nectar flow at El-Bagour, Menoufia governorate, Egypt. Similar ietter in each date do not significantly differ between control and treated colonies.

The values of before treatment parameters were 233.9± 1.7 and 232.2± 1.7; 69.7±11.9 and 68.6± 3.2 and 29.7± 1.7% and 29.6± 1.7 % for TF, PC and PC /TF ratio, respectively. In the after-treatment period, (May, 31– June, 5), there were a noticeable change in the behavior of all foraging rhythms as reported in Table (3).

The means of TF and PC were significantly and sharply decreased in the next day (May, 31) of venom collection in the treated colonies (166.0± 6.0 and 47.7± 4.5) than those counted in control ones (336.7±24.5 and 99.7± 4.5), respectively. In spite of, the ratios of PC/TF of the two tested groups were similar, (29.7± 1.7 and 29.6± 1.7, respectively).

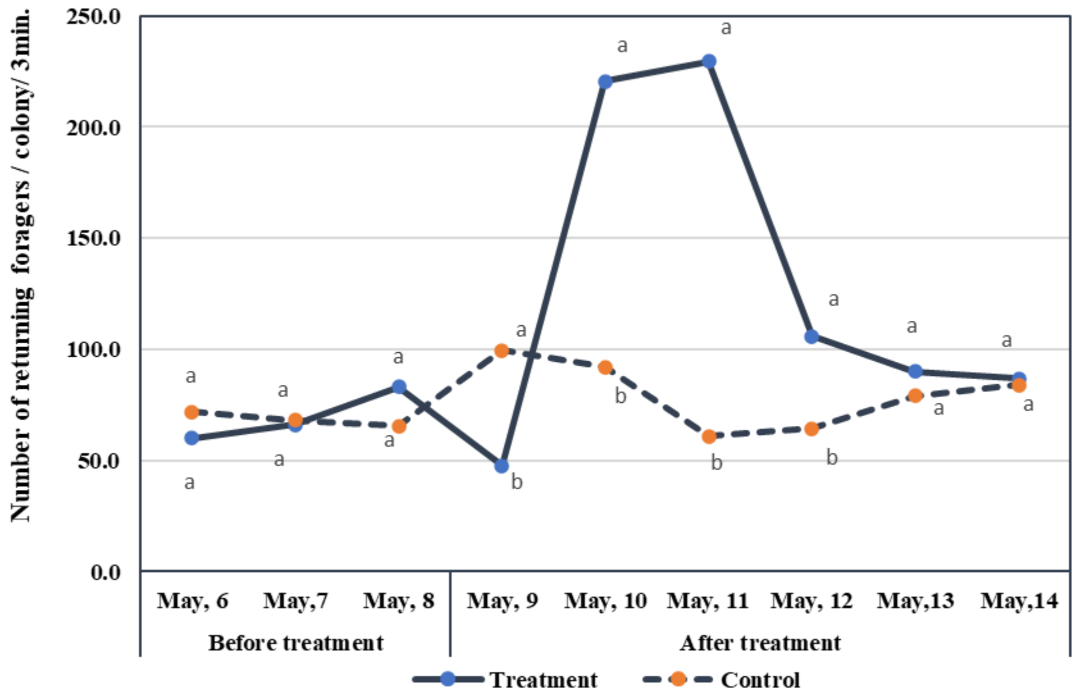

Fig. (4). Mean numbers of pollen collectors before and after venom collection during clover nectar flow at El-Bagour, Menoufia Governorate, Egypt. Similar ietter in each date do not significantly differ between control and treatments.

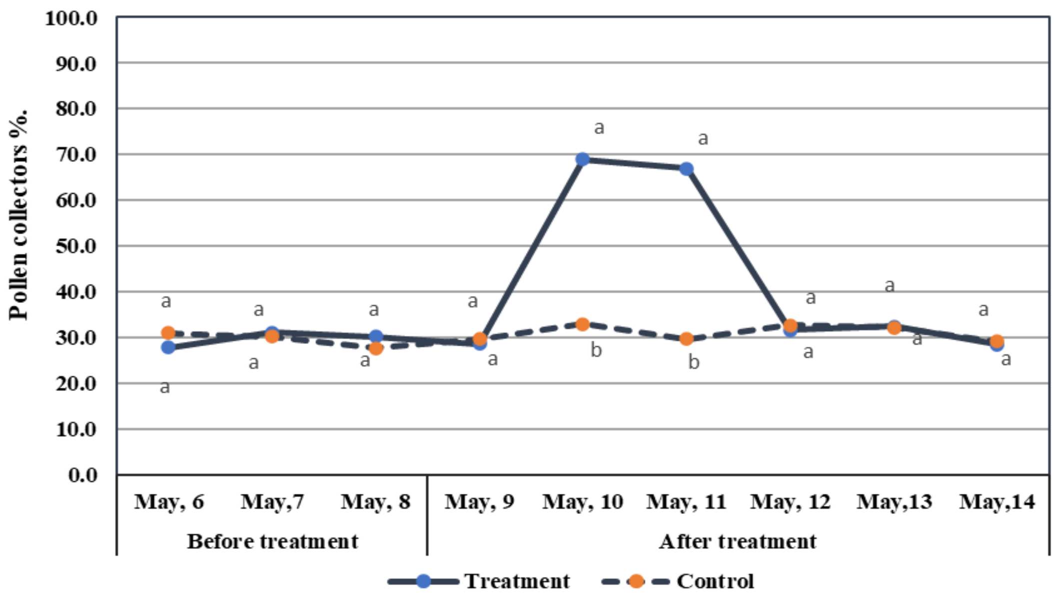

Fig. (5): The percentages of pollen collectors before and after venom collection during clover nectar flow at El-Bagour, Menoufia Governorate, Egypt. Similar ietter in each date do not significantly differ between control and treated colonies.

However, in the beginning of the second day after venom collection (June, 1), a sudden increase in numbers of both TF and PC in colonies exposed to electrical shocks than control ones were occurred. This changes in foraging behavior in treated group was followed by significant raises in the ratio of PC/TF in treated group (68.9±1.0 %) by about 2.1 time than those occurred in control colonies (32.9 ± 0.2 %) as shown in Fig. (5). The same trend was noticed during the third day after application.
Although the TF and PC are continuously increasing for treated than control colonies during the rest days of study (June, 3 – 5), the ratio of PC / TF returned to the normal rate from the fourth day after venom collection which recorded 31.6± 1.1% against 32.7± 1.8 % for control group as shown in Table (3) and Figs. (3-5).

The present results indicated that exposing the workers to electric impulses with 24 V and 50 Hz for 90 min caused a disturbance in the foraging activity for a period of one day after removing the plate of venom collection. This disturbance period was less than that recorded by Morse and Benton (1964), where their colonies remained difficult to work for about 6-7 days after milking the venom. However, this finding is in accordance with those of Omar (1994c) who found that when colonies excited by 20 V for 30 min the behavior of workers returned to the normal activity after three hours of removing the board of collection. The increase in TF after stimulation by electrical shock agreed with those found by Omar (1994c) who reported that the mean number of foragers was positively correlated with increasing the used voltage (from 12 V to 20 V) and time of collection (from 15 to 30 min.). In addition, Khodairy and Omar (2003) showed positive correlations between the amount of venom production and different activities of colonies among them foraging rate of workers.

Dietz (1975) reported that honey bees used protein of pollen mainly to provide structural elements of muscles, glands and other tissues. He also found that bees raised without pollen have little and less effective venom in their venom sacs. Therefore, it was recently accepted that venom collection from honey bee colonies using electrical shocks causes a significant decrease in the stored bee bread due to the high consumption of protein within those colonies (Omar, 1994 c; *et al.*, 2006; El-Saeady, *et al.*, 2016; Nowar, 2016; El-Feel 2017; Argena *et al.*, 2021 and Badawy *et al*., 2022). This reduction in the areas of stored pollen within the colony stimulates a portion of foraging workers to alter their field task and collect pollen or nectar with pollen instead of nectar alone to compensate the reduction. In recent work, this alteration in the field forager task continued for two colonies.
According to the present findings, it could be preferable to collect venom when the colonies contain accesses of bee bread (such as the flowering season of clover during May and June) or providing supplementary protein feeding.

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تأثير توافقات من شدة التيار الكهربي و مستوى التردد على إنتاجية السم لطوائف نحل العسل فى مصر

إيناس عثمان نور الدين[1] و أحمد على شاهين[2]

1- *قسم بحوث النحل, معهد بحوث وقاية النباتات , مركز البحوث الزراعية, الجيزة- مصر*

2 – قسم وقاية النبات, كلية الزراعة, جامعة الازهر, القاهرة- مصر

ملخص

يهدف هذا البحث الى دراسة تأثير توافقات مختلفة من شدة التيار الكهربي مع مستويات مختلفة من الترددات على انتاجية السم من طوائف نحل العسل خلال موسم فيض رحيق عسل البرسيم المصرى. وقد أظهرت النتائج أن كمية السم الناتجة تزداد طردياً مع الزيادة فى شدة التيار المختبرة، حيث كانت 38, 0 , 76, 4 و83, 3 مج/ 45 ق / طائفة لشدة تيار 12 و 24 و 36 فولت على الترتيب. كما تم الحصول على أعلى محصول من السم عند استخدام درجة تردد 50 ذبذبة/ ثانية (83, 1 مج) بينما سجلت أقل كمية عند (49, 1 مج) مع درجة تردد 30 ذبذبة/ ثانية. أيضاً كان أعلى تعداد للشغالات الميتة (79, 7شغالة/ طائفة) نتجة التعرض إلى 36 فولت مع 80 ذبذبة/ثانية. أما بالنسبة لتأثير التعرض للصدمة الكهربية على سلوك النحل السارح، فقد لوحظ أن سلوك ونشاط الشغالات السارحة لم يختلف عن بعضه معنوياً بين المجموعتين فى فترة ما قبل المعاملة. ووجد أن نشاط السروح في طوائف المجموعة التى تعرضت للصدمة الكهربية لمدة 90 ق، انخفض فيها تعداد النحل السارح بدرجة معنوية عنه في طوائف المقارنة و ذلك فى اليوم التالى مباشرة لجمع . و مع ذلك لم تختلف نسبة جامعات حبوب اللقاح معنوياً بين المجموعتين (29, 7 % 29, 6%). كما ازداد تعداد النحل السارح وجامعات حبوب اللقاح ابتداءً من اليوم الثاني (10 مايو) بعد المعاملة وحتى نهاية التجربة عن طوائف المقارنة. بينما بلغت نسبة جامعات حبوب اللقاح 68, 9 % و 67, 0 % مقابل 32, 9 % و 29, 7 % في طوائف المقارنة خلال اليوم الثاني والثالث بعد المعاملة (10 و 11 مايو)، ثم تشابهت النسبة مع الكنترول بعد ذلك الى نهاية التجربة. ربما يكون ذلك لتعويض الفقد فى مخزون خبز النحل نتيجة إفراز السم وحاجة النحل إلى تعويض البروتين المفقود من أجسامها أثناء ذلك.

المؤتمر الدولي الرابع عشر لإتحاد النحالين العرب 13-15 نوفمبر 2023.

المجلة المصرية للعلوم الزراعية المجلد75 (إصدار خاص): 20-31.