The Efficacy of a New Modified Apparatus for Collecting Bee Venom in Relation to Some Biological Aspects of Honeybee Colonies

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Abstract: The experiments were conducted at the experimental apiary of the plant Protection Department, Faculty of Agriculture, South Valley University, Qenna, Egypt during the period of March 2012 to November 2012 to study the effect of gathering bee venom with the aid of a modified collecting device on the average of dead workers, and the scale of sealed brood, with references to the effect of the period of the day, months and seasons on the weights of collected bee venom. Results indicated that the modified device of gathering bee venom from hives was successfully gave adequate quantities of bee venom along the period of the experiment, March 2012 to November 2012. It could be concluded that the best period for collecting bee venom was between 4 pm to 6 pm at August month, as Summer season giving the highest weights of bee venom (0.0185 g / day). Furthermore, it could be concluded that the safety period for bee venom collecting process was 1-3 pm which gave the lowest numbers of dead workers as a side effect of gathering process (26.74 worker / day). Moreover, the least side effect of gathering process on the decreasing area of sealed brood was recorded at November month (11.3 %).
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1. Introduction

Apitoxin, or honey bee venom, is a bitter colorless liquid. The active portion of the venom is a complex mixture of proteins, which causes local inflammation and acts as an anticoagulant. The venom is produced in the abdomen of worker bees from a mixture of acidic and basic secretions. Apitoxin is acidic (pH 4.5 to 5.5). A honeybee can inject 0.1 mg of venom via its stinger and similar to nettle toxin. It is estimated that 1% of the population is allergic to bee stings. **Simics** (1994) describes devices and publications available from the publisher, and publications by other authors moreover, he briefly describes the characteristics of honey bee (*Apis mellifera*) venom and the various electrical collecting devices which have been developed to collect it, and discussed the technique of venom collection and the effects on the bees, furthermore, the author concerned with the quality and composition of bee venom, its use in medicine and venom-containing products, including homeopathic medicines, which are available commercially.

There are many studies on the chemical and medicinal properties of the honeybee venoms, at Egypt, **Zalat *et al.*(2002),** who analysed the venom composition of the Egyptian Carniolan honeybees *Apis mellifera lamarckii*, and *A. mellifera carnica*, in addition to a hybrid with unknown origin using electrophoresis (SDSPAGE). While, in France **David *et al.* (1997)** described the allergenic substances found in Hymenoptera venoms furthermore they described the enzymatic and cytotoxic properties of the phospholipase A2 of the honey bee *Apis mellifera* and the immune response mediated by T lymphocytes. Also, at Egypt **Khodairy and Omar (2003)** determined the relationship between bee venom produced by electrical impulses and certain characters of honey bee colonies (i.e. bee population, brood, stored pollen, stored honey areas and yield and foraging activity) and the variability of venom quantity collected from colonies at different periods of active season and found significant variations in the amounts of collected venom at different periods of active season, in addition they reported that the amount of venom was high in June compared with that collected in May and July, finally they found positive correlations between venom production and each of the bee population, bee brood, stored pollen, uncapped and capped honey areas and foraging activity. Moreover, **Malaiu *et al.* (1981). Schumacher *et al.* (1994)** conducted good experiments on the effect of bee venom collection on bee activity.

As for the collection of honeybee venoms, scientists were designed many apertures to collect it i.e., at Fuji, **Miao (1983), at USA, Malcon (1992),** at Portugal, **Nobre (1990)** described a device for the collection of honey bee (*Apis mellifera*) venom. The device consists of an electrified glass plate; when the bees sting it they do not lose their stings and are not

electrocuted. The plate can be powered by either a 12 V battery or a transformer of 220 V, alternating the current into 12 V continuous current at 3 amps/h. A standard 12 V ignition coil is used to produce high voltage pulses. The primary winding of the coil is interrupted by a contact breaker, the secondary winding producing high voltage (~ 10 V) high frequency energy. The current from the ignition coil passes by a flexible cable to a metal plate which is placed, by pressure, against the glass plate. Wires connected to earth are arranged in parallel on top of the glass plate, so establishing a negative terminal and delivering the required shock to the bees. The system is able to produce enough current to supply more than 20 plates. The venom quickly dries onto the plate and can be scraped off using a flexible blade. The powdered venom should not be left exposed to sunlight for any length of time and must be stored at 0 degrees C or in a domestic freezer. It is stressed that when handling the powdered venom, precautions should be taken to protect the eyes and lungs. The system is able to provide enough current to supply more than 20 plates. The glass plate may either be in the form of a platform, put inside the hive, or it may be placed at the entrance of the hive to form a landing board for returning bees. The venom deposited on the plate soon dries and can be scraped off using a flexible blade. At Pulawy, Poland, **Rybak *et al.* (1995),** described an apparatus consists of an electro stimulator (generator) which passes current through electodes mounted every 5 mm in venom-collecting frames fitted in one of the hive bodies. The frames include a glass screen on which the venom is deposited. The results showed that the optimal electrical parameters are impuls frequency, 1 KHz; voltage, 25V; impulse duration, 1s; interval between impulses, 2s. The best results were obtained when venom collection was carried out every 14 days, for 1 h (early morning, before bee flight) or 2 h (when foraging was occurring), with the collection frames in the upper body. Mid-July was the best period for venom collection. At Stara Zagora, Poland, **Nenchev and Stoichev (1997),** used electro stimulation to collect bee venom in 36 similar bee colonies. They found that bee venom extraction at the tested technological regimes varied from 39.00 to 222.83 mg. The bees release from 60.85 to 65.57% of the total venom quantity on side of the collector (facing the beehive's entrance). The increased duration of the electro stimulation (from 60 to 90 min) did not bring about more venom. They added that technological regime of production, including work (30 min), rest (60 min), work (30 min), applied every other fortnight was reliable in relation top the quantity of bee venom, is less disturbing for the bees and is convenient for application. At Helsinki, Finland, **Fakhim (1998)**, described a device which like a container of the size of a honey super, the sides of which consist of copper wires strung vertically 3.63 mm apart, alternately live and grounded. The bottom (removable) is also wired, whereas the lid is made of a plastic net (1.5 mm mesh). A central rod provides a resting place for bees. When in use glass plates covered with plastic sheet (through which the bees sting) are attached to the sides and button of the device, and alternating electric current (22 v, 50 Hz) is passed through the wires. The total 5 min operating period consists of alternating 3s and 7s periods, when the current is on and off, respectively. Each collection uses 2-4 combs of honey bees/colony; a device is given of selecting and handling the bees. In testes of the device, 8 colonies, yielded an average of 0.21 g venom (0.026 g/colony). Bees from several colonies can be mixed and exposed to electric current simultaneously if desired. At Karaj, Iran, **Bahreini *et al.* (2000),** described a venom-collecting apparatus. The device was a 42 x 50 x 58 cm cage like box, with inner walls that were equipped with electric wires, which would be sequentially charged and discharged. Bees that would come in contact with two adjacent wires would receive an electric shock of 21 volts for 3 seconds. After a lapse of 7 seconds, the wire is recharged and ready for the next electric shock. This 10 second cycle continues for a duration of 5 minutes, during which bees are made to sting on the plastic covering of a glass plate. Venom deposited on the glass plates is scraped off by a sharp lancet in the laboratory. In a 6 month experiment, venom was extracted from 16 colonies of bees (8 treatment and 8 control animals) every 15 days. Throughout the experiment, 838 mg of venom per colony was obtained. No diverse effect was observed in the production of honey. At China, Fuzhou, **Zhou *et al.* (2003)** reported that the collection of venom by electric shocking was not beneficial to honey bee (*Apis mellifera ligustica*) population and production, when the venom was collected continuously once every 3 days and up to 10 times, the output of honey dropped significantly by 45.64-49.90%, that of royal jelly by 46.17% and that of larval acceptance rate by 31.05%. However, the venom collection had no significant effect on the average output of royal jelly of individual cup, effectiveness of queens oviposition and volume of sugar consumption. There were no apparent differences in the honey bee population after collecting venom continuously for 11 days. However, the population reduced by 12.87 and 28.81% after 23 and 35 days, respectively.

From the previous points of view the aim of this study was to evaluate the effect of a new modified aperture for collecting bee venom, on some

biological aspects of honeybee colonies, at Qenna Governorate, Egypt.

2. Material and Methods

The experiments were conducted at the experimental apiary of the plant protection Department, Faculty of Agriculture, South Valley University, Qenna, Egypt during the period of March 2012 to November 2012.

Twenty honey bee colonies equal in both of strength and population were chosen for the experiments, each treatment consists of four colonies, in addition to check treatment which was represented by four colonies.

The purpose of this study was to know the effect of the artificially collecting of honeybee venom with the aid of a device designed by Mohanny, (2005) & (2007) which consists of an equipment produces electrical impulses to stimulate the bees to sting. The device is a small box measuring 24x25 cm with a height of 13 cm. It has a front panel which is placed for visual indicator of the on/ off function, a stranger output ensuing a maximum of the collector frame, electric button is fixed on the visual indicator to on / off the direct current, electric button to charger the dry battery and button have an output to measure the seven levels of impulse frequency and seven levels of impulse duration, in addition, the collector plate (Mohanny, 2005) is a frame made of aluminum holding wire grids, these grids consists of parallel copper wires some spaced 0.5 cm in between, fixed to the aluminum frame (35 cm X 36 cm). Underneath these wire grids, there is a glass plate (35 cm x 26 cm), which is covered with a plastic polythene sheet stretched plastic (Commercially available, e.g., it is a cling film, used for covering food) to avoid contaminating of the venom. A space is not needed between the glass and the plastic as the stings can penetrate the plastic sheet and deposit the venom on the glass. By using this process, the bees are able to withdraw their stings safely.

Time of collecting bee venom and its weights:

Venom was collected every three days from the period of March to November 2012, four times a day (4-6 am, 9-11am, 1-3 pm, and 4-6 pm).

Collected bee venom was gathered, weighted using an electrical balance, and store in the refrigerator.

The effect of venom collection on dead workers:

The effect of bee venom collection process (Mohanny, 2005) on dead workers of each colony were recorded by counting dead bee workers, every three days, at each hive on the wire of collecting device, four times a day (4-6 am, 9-11am, 1-3 pm, and 4-6 pm) from the period of March to November 2012.

The effect of venom collection on the brood area:

Sealed worker brood bee area (cm^2) per frame were measured with the aid of plastic scale at the hives under investigation, every 13 days, from the period of March to November 2012.

Statistical analysis:

Data were analyzed by the computer, using ANOVA test with LSD at 5% level (**SAS Institute. 2003**), in addition to **Little and Hills (1978)**.

3. Results and Discussion

The effect of collecting time and season of bee venom on its weights:

Data presented in Table (1) indicated that there were significant differences in the weights of collecting bee venom per day among the four times of collection (LSD 5% = 0.011), where the highest weights of bee venom was recorded with the time of 4-6 pm (0.166 g / day), followed by the time of 4-6 am giving grand mean of 0.118 g/ day, while the treatments of 9-11 am and 1-3 pm gave 0.099 and 0.080 g / day, respectively.

Regarding to the effect of collecting season of bee venom on the obtained bee venom, statistical analysis of data in Table (1) indicated that, there were significant differences (LSD 5% = 0.007) among the months and seasons of the period of study. Results revealed that the highest amounts of bee venom were recorded at August month (0.185 g / day),and the least amount was recorded at March month (0.031 g / day), while the rest months occupied intermediate states in this respect. As for seasons results in Table (1) indicated that there were significant differences among the three seasons, where Summer season occupied the first rank giving the highest amount of bee venom recording 0.161 g / day, followed by Autumn season giving an average of 0.116 g /day, while Spring season gave the lowest amounts of bee venom registering 0.040 g / day.

The effect of venom collection process on dead workers:

Regarding to the effect of collecting bee venom process on the numbers of dead workers as a side effect result of using the new collecting device of bee venom at the period of March to November 2012, statistical analysis of data in Table (2) indicated that, there were significant differences (LSD 5% = 8.14) among the times of collection. Results revealed that the highest numbers of dead workers was recorded with the times of 4-6 am and 4-6 pm (51.24 and 49.32 worker / day), while the treatments of 9-11 am and 1-3 pm gave only 33.49 and 26.74 worker / day, respectively.

Regarding to the effect of collecting month of bee venom on the numbers of dead workers, statistical analysis of data in Table (2) indicated that,

there were significant differences (LSD 5% = 5.42) among the months of the period of study. Results revealed that the highest average numbers of dead workers were recorded at June month (55.0 worker / day),and the least amount was recorded at November month (14.1 worker / day), while the rest months occupied intermediate states in this respect. As for seasons results in Table (2) indicated that there were significant differences among the three seasons, where Summer season occupied the first rank giving the highest average numbers of dead workers recording 50.3 worker / day, followed by Spring season giving an average of 40.9 worker /day, while Autumn season gave the lowest means of dead workers registering 31.7 worker / day.

The effect of venom collection on the sealed brood area of bee colony:

As for the effect of using a modified device (Mohanny, 2005) in the collecting bee venom process on the sealed brood area as a positive or negative side effect result of using the new collecting device of bee venom at the period of March to November 2012, statistical analysis of data in Table (3) indicated that, the highest decrease in the area of sealed brood at the treated hives, in comparison with untreated ones, was recorded at July month giving 18.1 %, and the lowest decrease percentages was registered with the November month (11.3 %), while the least months recorded intermediate percentages (14.4 and 16.0 %).

Regarding to the effect of using new device in collecting process of bee venom, data in Table (3) indicated that the highest decrease in the area of sealed brood at the treated hives, in comparison with untreated ones, was recorded at Summer month giving 16.9 %, followed by Spring season giving 15.8 % decrease in the area of sealed brood, while Autumn season gave the least decrease percentage as a side effect of bee venom collection process giving only 13.8 % decrease. The average scale of sealed brood (cm2) / colony was 1387.2 cm2 at treated hives in comparison with 1578.8 cm2 at untreated ones.

These results indicated that the modified device of gathering bee venom (**Mohanny, 2005**) from hives was successfully gave adequate quantities of bee venom along the period of the experiment, March 2012 to November 2012. It could be concluded that the best period for collecting bee venom was at the period of 4 – 6 pm at August month, as Summer season giving the highest weights of bee venom (0.0185 g / day). Furthermore, it could be concluded that the safety period for bee venom collecting process was 1-3 pm which gave the lowest numbers of dead workers as a side effect of gathering process (26.74 worker / day). Moreover, the least side effect of gathering process on the decreasing area of sealed brood was recorded at November month (11.3 %).

These results are in harmony with those obtained by **Kaviani *et al.* (1995),** In Iran, indicated that electrical shock device was designed based on a new method of milking *Apis mellifera* for venom, consisting of 2 min intervals between each period of shock. A total of 9203 mg of dried honey bee venom (HBV) was collected during spring and summer of 1992 and 1993 from 8 hives. **Simics (1995)**, in Abberta, Canada, found that colonies were relatively unaffected by the procedure; and observation during the collection period showed that, on average, 68 bees died per colony after collectors use. **Skubida *et al.* (1995),** in Pulawy, Poland, compared different bee venom collection methods for the amount of venom collected and for their effect on the status of honey bee colonies and their wintering as well as on their general productivity (honey, pollen, beeswax). Venom-collecting frames were inserted (I) in the lower hive body, or (2) in the upper hive body, or (3) in an empty body placed between the upper and lower bodies. A fourth technique involved a super with a fixed set of 6 venom-collecting frames (incorporating removable glass plates for scraping off the venom). **Funari *et al.* (2001),** in Botucatu, Brazil, who used 15 beehives: 5 with Africanized queens sisters *Apis mellifera*, 5 with Italian queens sisters *Apis mellifera ligustica*, and 5 with Carniolan queens sisters *Apis mellifera carnica*. The queens were fertilized naturally. The following data were obtained from the foraging bees: venom quantity in reservoir, 0.117±0.015, 0.139±0.020, and 0.147±0.024 (mg); venom quantity liberated in extraction apparatus, 0.73±0.012, 0.057±0.011 and 0.059±0.013 (mg); and sting electro stimulus threshold (volts), 10.75±1.37, 15.11±2.00, and 15.01±1.63 for Africanized, Italian x Africanized and Carniolan x Africanized, respectively the Africanized honeybees possess less venom in reservoir than the European hybrids (Carniolan and Italian). However, they liberated a larger quantity of venom in the extraction apparatus and required lower electro stimulus threshold to promote stinging. In contrast, **Zhou *et al.* (2003),** in Fuzhou, China, reported that the collection of bee venom by electric shocking was not beneficial to honeybee population and production.

Finally, it could be concluded that the used aperture can be used for bee venom collection without any side effects or decrease in both of bee worker activity and the colony yields of honey and sealed brood.

Table 1: Average weights of collected bee venom with the aid of a modified aperture at four times per day during the period of March 2012 to November 2012

Months	Average weight of bee venom (g) / honey bee colony at four times of a day per week				
	4-6 am	9-11am	1-3 pm	4-6 pm	Grand Mean
Mar.	0.032	0.024	0.028	0.041	0.031 g
Apr.	0.049	0.026	0.029	0.060	0.041 fg
May	0.047	0.032	0.037	0.078	0.048 ef
Spring	0.042	0.027	0.031	0.059	0.040 fg
Jun.	0.164	0.079	0.157	0.179	0.145 c
Jul.	0.167	0.120	0.136	0.192	0.154 bc
Aug.	0.195	0.138	0.164	0.245	0.185 a
Summer	0.175	0.112	0.152	0.205	0.161 b
Sept.	0.167	0.062	0.084	0.219	0.133 d
Oct.	0.184	0.031	0.043	0.383	0.160 b
Nov.	0.060	0.027	0.038	0.100	0.056 d
Autumn	0.137	0.04	0.055	0.234	0.116 d
Grand Mean	0.118 b	0.099 d	0.080 c	0.166 a	

Values in column or row followed by different letter(s) are significantly different at 5% level. LSD 5% among months = 0.007; LSD 5% among times = 0.011

Table 2: Average numbers of dead bee workers at four times per day during the period of March 2012 to November 2012 at honeybee colonies provided with a modified aperture for venom collection

Months	Average numbers of dead bees / honey bee colony at four times per day				
	4-6 am	9-11am	1-3 pm	4-6 pm	Grand Mean
Mar.	28.9	29.0	22.7	31.1	27.9 c
Apr.	40.8	30.6	28.9	40.6	35.2 c
May	62.0	46.3	34.9	66.8	52.5 ab
Spring	43.9	35.3	28.8	46.1	40.9 b
Jun.	75.5	41.6	29.1	74.0	55.0 a
Jul.	68.4	36.9	30.2	56.1	48.0 ab
Aug.	69.5	32.9	29.2	60.5	48.1 ab
Summer	71.1	37.1	29.5	63.5	50.3 ab
Sept.	57.7	41.2	29.5	55.0	45.9 b
Oct.	43.1	29.5	24.1	44.2	35.2 c
Nov.	15.3	13.4	12.1	15.6	14.1 d
Autumn	38.7	28.0	21.9	38.2	31.7 c
Grand Mean	51.24 a	33.49 b	26.74 c	49.32 a	

Values in column or row followed by different letter(s) are significantly different at 5% level. LSD 5% among months = 5.42; LSD 5% among times = 8.14

Table 3: Effect of the use of a modified aperture for venom collection on the scales of bee sealed brood along the period of March 2012 to November 2012 in comparison with untreated colonies.

Months	Average scale of sealed brood (cm^2) / colony		Decrease %
	Untreated	Treated	
Mar.	2233.1	1945.1	14.8
Apr.	1480.0	1290.0	14.7
May	1739.0	1498.9	16.0
Spring	1817.4	1570.0	15.8
Jun.	2266.0	1955.4	15.9
Jul.	2397.3	2029.7	18.1
Aug.	2094.8	1795.7	16.7
Summer	2252.7	1926.9	16.9
Sept.	1832.2	1602.3	14.4
Oct.	1704.8	1481.9	15.0
Nov.	1199.4	1077.5	11.3
Autumn	1578.8	1387.2	13.8

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