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Some Factors Affecting Venom Productivity by Electrical Impulses From Honey Bee Colonies*

Mohamed M. Khodairy[1], Mostafa H. Hussein[1], Alaam A. Nafady[2] and Eslam M. O. [1]

Plant Protection Dept., Fac. of Agric. Assiut Univ[1]. Department of Pathology., Fac. of Vete. Medicine , Assiut Univ[2].

Key words: Honey bee colonies - bee venom – electrical – impulses – venom productivity.

Abstract:

To improve the amount of dry bee venom extracts from honey bee colonies by electrical impulses, some factors affecting venom extraction were tested in the apiary of Agriculture Faculty, Assiut University during 2008 and 2009 seasons. Results revealed that the scarcity of bee bread stored in honey bee colonies reduced significantly amount of dry bee venom extracted by 50.3% in comparison with the bee bread sufficiency. When honey bee colonies were provided by supplementary feeding, the venom production decreased only by 41.76%.Venom quantities collected from aggressiveness colonies in comparison with the calm one was relatively inferior. The venom production correlated negatively with the aggressiveness behavior of colony because the honey bee workers stingers were unstable on the collection boards at the high aggressive degree. Using venom collection boards over the colony frams produced 93.22 mg/colony with a significant increment (37.41%) of dry bee venom compared with the position at hive entrance.

Introduction

Today, bee venom therapy is a part from apitherapy which utilizes bee products for treatment. Bee venom become one of the important bee colony products that is used for the cure of many diseases. Nowadays, bee venom is collected by modern methods depend on electrical shock stimulation and dry bee venom material can obtained directly from honey bee colonies. Many authors as Palmer, 1961; Benton *et al*., 1963; Malaiu *et al*., 1981; Brandeburgo, 1992; Omar, 1994; Rybak and Muszyska, 1998; Rybak and Skubida, 2007 improved the electrical methods, techniques and devices in various parts of the world. Honey bee venom is produced by two glands associated with sting apparatus of worker bees, the acid gland and alkaline gland (Dade, 1962). Many factors affecting glands development and venom secretion were studied with many authors. Dietz (1975) reported

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that honey bees used protein of pollen mainly to provide structural elements of muscles, glands and other tissues. The production of a potent bee venom required good nectar, honey and pollen sources. Consequently, protein mainly is required for full production of venom. Bees raised without pollen have little and less effective venom in their venom sacs. Omar (1994) recorded that excitation by electric impulses for venom production decreased the areas of bee bread. It explained that the stressed bees by electric impulses need much more protein for developing venom glands. Honey bee defense behaviour is a complex trait affected by genetic composition and modified by external factors. Races of honey bee differ in their temper defensive behaviour and the type of stimulant. Once the sting is deposited, alarm pheromons is suddenly liberated from glands associated with their sting apparatus and their mandibles (Maschwitz, 1964). Also, the aggressiveness of defense behaviour may be affected by weather, time of year, bee health and factors affecting foraging activity (Collins, 1979; Collins *et al*., 1984 and Moritz *et al*., 1987). Queen removal from honey bee colonies, crowding and honey extraction increased defense behaviour and venom quantities extracted (Omar, 1997).

The collection venom boards position at honey bee hives differed from apparatus to an other. Some investigators as Benton *et al*. (1963) used it underneath the brood chamber of honey bee colonies for 5 minutes. The results were not satisfactory to obtain larger quantities of bee venom. Malaiu *et al*. (1981), Brandeburgo (1992) and Omar *et al*. (1993) used it at the hive interance to avoid any harmiful effect on honey bees activities inside the colony. Gholamian (2007) used two kind of venom collector apparatuses one used out side and an other inside the hive. The rate of venom production by the collector out of the hive was low and much trouble for user during venom collection.

The present work aimed to study some factors affecting honey bee venom productivity such as feeding supply, defense behaviour and method of collection for extracting more venom quantities.

Materials and Methods

The experimental work was carried out in apiary of Agriculture College, Assiut University during active seasons of 2008 and 2009. Local hybrid of Carniolan honey bee colonies and electrical shock technique designed by Omar *et al*. (1993) were used (Fig. 1). All experiments were carried out under one selected waveform belonged to Sin form group, amplitude 16 V and frequency 50 HZ.

After a period of $^{1}/_{4}$ hrs of venom extraction, the boards were collected and the glass of

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boards were stored for dryness 24 hours at room temperature. The total amount of dry venom extracted was determined by scrapting the surface of glass plate by slide glass. The amount of venom was weighed by electrical balance.

Experiments:

Three experiments were carried out for developing venom extraction from honey bee colonies as follows:

1. **Relationship between the stored areas of bee bread and venom productivity:**

Twelve honeybee colonies from local hybrid Carniolan bees equal in strength (8 frams covered with bees) were selected and divided in three groups as follows:

G1- Honeybee colonies were provided by access from bee bread areas (2 frams) which considered as sufficient proteinic food supply.

G2- Honeybee colonies free from bee bread frams were provided by pollen traps at the hive entrance to keep the colonies free from proteinic food source.

G3- Honeybee colonies free from bee bread frams were provided by pollen traps at the hives entrance. Traditional pollen substitute (200 gm) was added

(A)

(B)

Fig. (1) : (A) Electronic impulses generator designed in Assiut University.
(B) Venom collection board attached at the hive entrance.

weekly for every colony during the experimental period.

After 18 days from the beginning of experiment, the colonies were excited by electrical impulses for 15 minutes and the amount of dry venom was measured. The extraction was repeated three times during cotton honey flow (August 2009).

Relationship between the aggressivety of colony and venom productivity:

Ten honeybee colonies at equal strength (8 combs covered with bees and contain sufficient stored honey and pollen areas were selected for this experiment from the apiary colonies to obtain two levels of aggressivety. The aggressiveness degree is measured by ball method described by Stort, 1974. The defensive behaviour test was carried out during July 2008 and 2009. The measurement was carried out at the comb tops position without smoking. The bees were permitted to stings for a period of one minute after the first sting. At the end of each test every ball was sealed in plastic bags and stings were counted. The colonies were divited to two groups (each 5 colonies), after the number of stings on the ball as follows:

1- Aggressive colonies (more than 30 stings/minutes).
2- Calm colonies (less than 15 stings/minutes).

The colonies were excited by electrical impulses for 15 minutes and the amount of dry venom was measured.

Relationship between position of venom collection boards and venom productivity:

Fifteen honeybee colonies from local hybrid Carniolan bees equal in strength (8 frams covered with bees) were divided into three groups as follows:

G1- Colonies were excited and the venom collection boards were located at the hive entrance.

G2- Colonies were excited and the venom collection board were located at lateral position inside the hive and beside the last fram.

G3- Colonies were excited and the venom collection board were located over the colony frams under the outer cover of the hive. All the colonies groups were excited by electrical impulses for 15 minutes and the amount of dry venom was determined. The extraction was applicated three times during cotton honey flow (August, 2008).

Statistical analysis:

Data obtained were statistically analysed using a randomized complete design. Appropriate comparisons of means were carried out by the method of least significant differences (L.S.D.). The t test was used to compare specific pairs of means. Regression between aggressive and calm colonies was estimated.

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Results and Discussion

Data presented in table (1) and illustrated in fig. (2) shows the amounts of dry venom obtained from local hybrid Carniolan bees under different level of proteinic food presence. Results indicated that excitation by electrical impulses produced 84.3 mg dry venom/colony within15 minutes from honey bee colonies contained sufficient areas from bee bread. The bee venom production decreased to 41.9 mg dry venom/colony within 15 minutes when honey bee colonies

Table (1):Effect of proteinic food abundant inside honey bee colonies on venom productivity.

Treatments	Amount of dry bee venom (mg/colony within 15 minutes)	Deviation %
	Mean	
Bee bread sufficiency	84.3 A	-
Bee bread scarcity	41.9 C	-50.30%
Scarcity + supplementary feeding	49.1 BC	-41.76%
L.S.D. 5%	8.811	
1%	13.346	

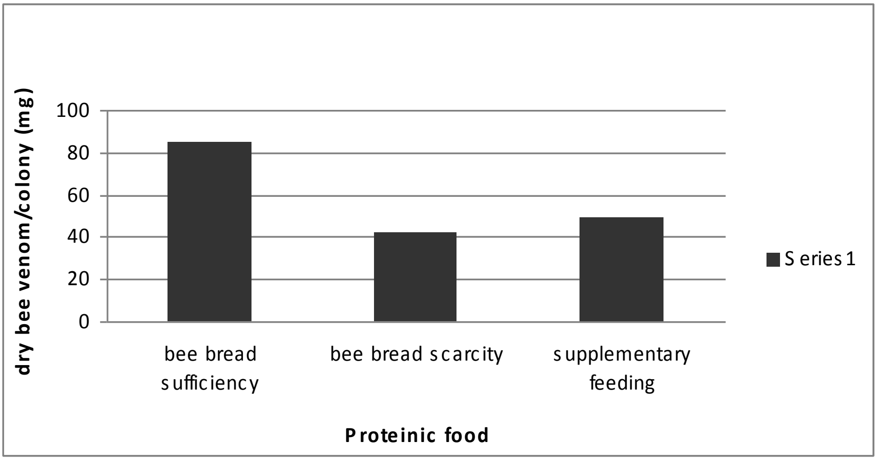

Fig. (2) Means of dry venom production under effect of different proteinic food deprived from bee bread. When supplementary feeding was administrated to the deprival colonies, the amount of dry venom produced increased slightly to 49.1 mg/colony. The results showed that scarcity of bee bread in honey bee colonies reduced venom production by 50.3%. When honey bee colonies provided with supplementary feeding, the venom production decreased only by 41.76%.

Honey bee workers used protein of pollen mainly to provide structural elements of muscles, glands and other tissues. Under normal conditions, pollen consumption diminishes when the bees are of 8 to 10 days old (Dietz, 1975). Omar, 1994 recorded that the stored pollen areas in excited honey bee colonies by electrical impulses for venom production decreased. This results can be explained that stressed bees by electrical impulses need much more protein for developing venom glands. While, Szymas and Przybyl (1996) in their study on the efficacy of feeding protein substitutes to honey bee found slightly poorer development of honey bee glands against that fed bee bread. Also, Hanna and Schmidt (2004) reported that the biotic effect of different pollen substitutes was poor in comparison with bee bread stored in honey bee colonies.

Data presented in table (2) and illustrated in fig. (3) shows that the amounts of dry venom obtained from two groups of local hybrid Carniolan colonies. During 2008 season, 32.34 mg venom/colony was extracted from calm group the same time. whereas only 25.90 mg venom/colony was produced from aggressive group in the same time . During 2009 season, the same trend was recorded. Calm and aggressive group produced 67.98 and 47.24 mg venom/colony respectively. In general the results indicated that the aggressevity of defense behaviour decreased the amount of dry bee venom extracted by electrical impulses significantly during the two seasons of study.

Table (2):Effect of aggressevity degree of honey bee colonies on bee venom productivity during cotton flow off 2008, 2009 seasons.

Season	Amount of dry bee venom (mg/colony within 15 minutes)		Deviation %	t value	P
	Calm group	Aggressive group			
2008	32.34 ±0.79	25.90 ±0.82	-19.92%	5.66	0.01**
2009	67.98 ±6.45	47.24 ±1.98	-30.51%	3.07	0.05*

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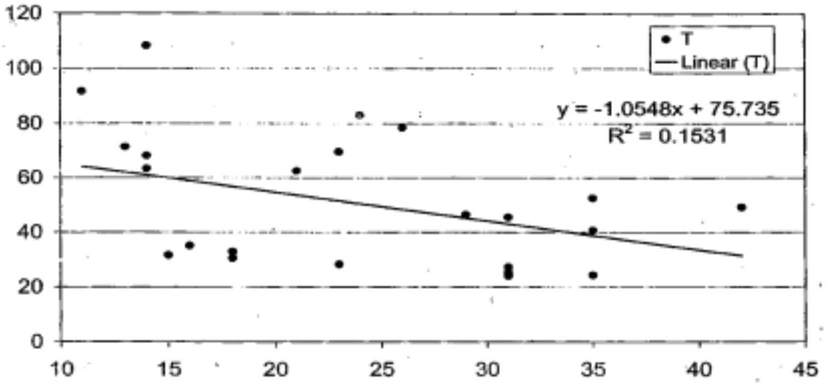

Fig. (3):Regression line of the relationship between defense behaviour rate and venom productivity.

Tompkins and Griffith (1979) reported that there are many factors affecting temper of bees including the genetic or inheritance as well as the environment of the hive and manipulation of the colony by beekeepers. Crewe and Hasting (1976) explained that the strains of honey bees that sting more in defensive assays, generally respond more quickly in large numbers to alarm pheromone, but perhaps different levels of isopentyl acetate or other alarm pheromone components could account for this higher defensive behaviour. Eliminating the genetic variance, environmental factors such as temperature, humidity with solar radiation and wind speed can determine up to 92% of the defense reaction of the colony (Southwick and Moritz, 1987).

The regression line indicated that there was a negative relation between the amount of dry bee venom extracted by electrical impulses and the aggressevity degree as a number of stings/ball/minute. The results showed that aggressevity behaviour can affected venom productivity with 15.31%. The calm colonies were more appropriated for venom extraction in comparison with aggressive one. From our observation during the experimental work, the honey bee workers of calm colonies were more stable on venom collection boards during extraction time and stinging action, whereas the individuals of aggressive colonies were unstable and runaway. This behaviour caused decreasing in amount of dry bee venom extracted.

Data presented in table (3) and illustrated in fig. (4) shows the effect of three positions of collection boards attachment to honey bee hive on amount of venom extracted during cotton flow of 2009 season. The normal position of collection boards when the device designed by Omar *et al*. (1993) was at the hive entrance. Dry venom amount extracted by using collection boards at the hive entrance was 67.84 mg/colony within 15 minutes. The second position was inside the hive near the last from covered with bees. The mean of dry venom decreased slightly to 59.02 mg/colony within 15 minutes (-13%). The third position was attachment the collection board over the top of colony frams

Table (3):Effect of collection board position on amount of dry bee venom extracted during 2009 season.

Position of collection board	Dry bee venom (mg/colony within 15 minutes)			% deviation from hive entrance
	Rang.		Mean S.E. ±	
	Min.	Max.		
Hive entrance	66.5	76.2	67.84BC ± 0.62	-
Inside the hive	51.1	63.7	59.02 C ± 3.46	-13.00%
Over frams top	77.3	111.0	93.22 A ± 4.33	+37.41%
L.S.D. 5% 1%			11.89 17.30	

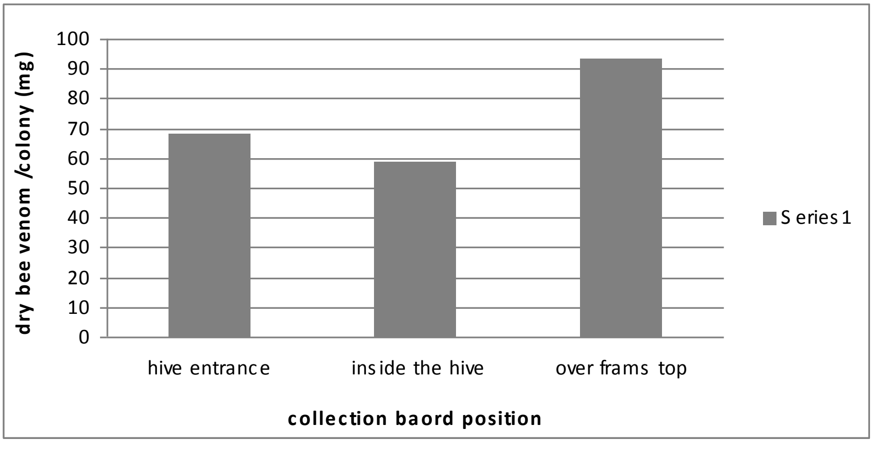

Fig. (4):Dry venom amount extracted under different collection board positions.

under the hive outer cover. The venom extraction achieved the highest amount (93.22 mg/colony/15 minutes). It increased significantly with 37.41% when compared by the position at hive entrance.

The observation during the experimental work showed that the honey bee workers were exited and crowded in a defense case to sting the glass of the collection board, then they retarned to the hive after 15-30 minutes and gave up the defense behaviour.

Maschwitz (1964) reported that when guards honey bee workers are standing at the entrance of the hive and disturbing, they released alarm pheromone to recuit other bees from the interior of the hive that are ready to sting. Moore *et al*. (1987) described that a small proportion of a colony worker population serves as guards; only about 10% of the workers perform guarding activities. Hunt *et al*. (2003) recorded that there are usually middle-aged workers (13 to 16 days old). Workers guard briefly for one to three days in the majority of cases and some workers may guard for as log as six days.

Exitation of honey bee workers happened directly when the collection boards were used over the top frams of honey bee colonies. The majgority of colony population were ready in defense case to sting. All the area of collection board was exposed to bees stinging.

Using electrical impulses methods for venom collection will be increased the income of beekeepers beside to other products and open the door to use it for human health at large scale.

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بعض العوامل المؤثرة على إنتاجية السم بواسطة استخدام النبضات الكهربية من طوائف نحل العسل

محمد محمد خضيرى[1] ، مصطفى حسن حسين[1] ، علام عبد الحميد نفادى[2] ، اسلام محمد عمر[1]

[1]قسم وقاية النبات – كلية الزراعة – جامعة أسيوط ، [2]قسم الباثولوجيا– كليه الطب البيطري– جامعة اسيوط

تم اختبار بعض العوامل المؤثرة على استخلاص سم النحل الجاف من طوائف نحل العسل باستخدام النبضات الكهربية وذلك بمنحل كلية الزراعة جامعة أسيوط خلال موسمى 2008 ، 2009 .

أوضحت النتائج أن ندرة حبوب اللقاح المخزنة فى الطوائف أدت إلى انخفاض كمية السم المستخلصة بما يعادل 50.30% مقارنة بالطوائف التى احتوت على مساحات وافرة من حبوب اللقاح المخزنة وعندما زودت الطوائف بالتغذية التعويضية ببديل صناعى لحبوب اللقاح نقصت إنتاجية السم بمقدار 40.76% فقط . وسجل من إختبار تأثير سلوك الشراسة فى طوائف نحل العسل أن كميات السم المستخلصة من الطوائف ذات الشراسة كانت أقل نسبياً من الكميات المستخلصة من الطوائف الهادئة. كما ظهرت علاقة إرتباط سالب بين سلوك الشراسة للطوائف وإنتاجية السم وربما يفسر ذلك لأن شغالات نحل العسل المفرزة للعسل لا تظهر استقرار على لوحات جمع السم عند إرتفاع معدل سلوك الشراسة بها. كما أظهرت نتائج اختبار تركيب لوحات جمع السم فى أوضاع مختلفة على طوائف نحل العسل أن وضع اللوحات أعلى قمة الإطارات قد أعطت زيادة معنوية تمثل 37.41% مقارنة بوضع اللوحات العادى على مداخل الخلايا .