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THE POTENTIAL OF NATURAL VENOM OF *APIS MELLIFERA* FOR THE CONTROL OF GRAINS WEEVIL ADULTS (*SITOPHILUS GRANARIUS* - COLEOPTER-CURCULIONIDAE)

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A B S T R A C T

The natural venom of honey bees, *Apis mellifera* was applied for the control of *Sitophilus granaries* adults. Five dose levels of 1.1, 2.4, 3.7, 5.0, and 6.3µg/insect of bee venom were tested against the adult stage of *S. granarius*. The adult mortality increased gradually and this parameter correlated with an increase in the doses of bee venom. Higher and lower mortalities were 94.3 and 20.2% after 72 hr of adult treatment with the doses of 6.3 and 1.1µg/insect of bee venom, respectively. The LD50 value of the venom was 3.9 and 3.3µg/insect after 24 and 72 hr of adult treatment, respectively. Chemical survey of the active fraction number 10 of the venom indicated the presence of 5.47% carbon (C), 17.96% oxygen (O), 68% nitrogen (N) and 8% hydrogen (H) by using elementary analysis. Also spectrophotometer screening of the active venom fraction by using UV, IR, and MS spectrum proved the molecular weight of the active toxic components was 2847.7MW. The chemical components of the active fraction (no. 10) supported the presence of aromatic chain attached to the functional groups of amine (NH), carboxylic (COH), and carbonyl (CO). These analyses supported the empirical chemical formula of the biological active fraction of the bee venom, which is, $C_{31}H_{228}N_{38}O_{32}$.

Keywords: Natural toxins, bee venom, insect control, cereals weevils.

INTRODUCTION

Recent findings of natural insecticides such as botanical insecticides, insect growth regulators (IGR), fungal, algal and animal toxins are possibilities for use against stored product insects.

Bee venom had insecticidal and hemolytic activities against cricket insect nymphs (Jerome *et al.,* 2001). When social Hymenoptera bees sting, alarm pheromones is released, stimulating more bees to attack the target (Collins *et al.,* 1982; Evans 1985; and Kastberger *et al.,* 1998). On the other hand, the bee immune system depends on the defense reaction (Glinski and Buczek, 2003). Bee venom can challenge the larvae of *Senotainia tricupis, Mermis sp*. and the parasitic mite, *Acarapis Varroa jacobsoni* (Rose and Briggs, 1969; Blum, 1978; Hider, 1988; Hoffman, 1996; Glinski and Jarosz, 2001 and Charles, 2005). The toxicity reaction of bee venom was due to the biological active components (Hider, 1988 and Hoffman, 1996 and Charles, 2005). Golden, (1989) and Mazdak *et al*., (2004), reported the melittin compound responsible for toxicity in the venom. Infrared (IR) spectrum and chemical analysis of melittin suggested the presence of glycerol (Schneider *et al*., 1991; Kodaka, 1998; Taroni *et al*., 2000 and Wright *et al*., 2002). In addition, *Apis mellifera* venom contains several toxins of peptides and proteins (Edstrom, 1992 and Nakajima 1986). The venom sting can be fatal to many insect species (Nabil *et al*., 1998). While its injection causes hypersensitive effect (Kai and Jun, 2003, and Muller *et al*., 1981). Web MD. (2013) reported that bee venom poison is what makes bee stings painful. Bee venom is used to make medicine. Bee venom should not be confused with bee pollen, honey, or royal jelly. Other venoms are derived from related members of the insect order Hymenoptera. Schmidt, (1986) and Meier, (1995) reported on the toxicity of bee venom. LD50 for rate

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was (2.8mg/kg) and for human (500-1500 mg/kg) sting recorded the mammalian toxicity by the bee venom. Different peaks appeared by IR of bee venom (Palma *et al*., 1995). Potential medical use for bee venom, is for arthritic and rheumatic conditions (Banks and Shipolini, 1986 and Edstrom, 1992).

Major insect pest of stored grains is the grains weevils, *Sitophilus granarius*. It is one of the more dangerous cereal pests. It believed to have spread from the eastern Mediterranean area to cooler regions throughout the world. It may cause almost complete destruction of grain in grain elevators, farmer's bins, or ships. It has primarily become a pest of stored products and depends on people for transportation.

At present, use of chemical insecticides is the main answer to the damage caused by stored product pests, inducing ecotoxicity problems and the occurrence of resistance within insect populations. Investigations concerning the toxic action of different insecticides against stored product insects has been reported by several authors (Kashi and Bond, 1975; Ren *et al*., 1999, and Fredric *et al*., 2003).

Aim of this study is to evaluate the effect of Yemen honeybee venom on adult mortality of the grains weevil, *Sitophilus granarius*. The second objective is to conduct chemical analysis of the active fraction of this venom.

METHODS AND MATERIALS

Rearing insects: Grains weevil, *Sitophilus granarius* (Coleoptera-Curculionidae) were obtained from a colony at Agriculture Research center in Giza. The colony was reared on wheat seeds at 27.5±1.5ºC and 70% ± 5% (R.H.) according to the method of Frederic *et al*., (2003) with some modifications.

Bee venom: The bee venom was collected from active bee workers in honey bee colonies located at Faculty of Agriculture, Cairo University. The collected venom was dissolved in 5ml-distilled, water considering on each worker produces 0.3 mg venom (Aly 1994). Then five doses (1.1, 2.4, 3.7, 5.0, and 6.3µg/insect) were prepared.

Bioassay and statistical analysis: The topical treatment of *Sitophilus granarius* adult was done by using Transferpette – micropipette, 1-50µ, according to the protocol described by Delobel *et al*.(1998) as follows: Thirty insects divided into three replicates (10 adult/replicate) were topically treated with 5 doses (1.1, 2.4, 3.7, 5.0, and 6.3µg/insect), and mortality was then monitored daily up to 3 days after the beginning of the treatment.

Thirty adults in control experiment were used in three replicates without treatment. Treated and control insects were kept under 27.5±1.5ºC and 70% ± 5% relative humidity (RH). The adult mortality of *S. granarius* was calculated after 24 and 72hr of treatment and the corrected mortality was estimated according to Abbot (1925). Estimation of LD50 values was made using probit analysis (Finney, 1971).

Fractionation and identification the biological active components: Venom was chemically analyzed at the microanalytical Center, Faculty of Science Cairo University. Venom was fractionated using column chromatography with water solvent according to the method adopted by Watson *et al.,* (1979) with some modifications. The active fraction was subjected to spectrophotometer analysis by using Infrared (FTIR-460 Plus), Ultraviolet (UV-160IPC) and Mass spectrophotometer (Mass-Bruker method, 2005). Also the elementary analysis for calculating the percentage of Carbon (C), hydrogen (H), oxygen (O) and nitrogen (N) was determined.

RESULTS AND DISCUSSION

Effect of bee venom on adult weevils: The most effective dose of bee venom on *Sitophilus granarius* adults was the highest value (6.3µg/insect), which caused 94.3% of adult mortality after 72hr post-treatment compared to no mortality of control weevils (Table1).

Table (1): Effect of the bee venom on percent mortality of adult *S. granarius* weevils.

Doses µg/insect	Number of the tested insects	% Adult mortality	
		24hr post treatment	72hr post treatment
1.1	20	13.1	20.2
2.4	30	28.4	38.6
3.7	35	45.6	61.3
5.0	25	53.7	82.4
6.3	25	75.1	94.3
Control	25	00.0	00.0

The lowest mortality of *S. granarius* adult was observed at the lowest dose (1.1µg/insect). Mortality rates were 13.1 and 20.2% at 24 and 72hr post-treatment intervals respectively. No mortality of control beetles was observed. Similar to this result the bee venom had insecticide activity against the cricket nymph due to its hemolytic activities (Jerome *et al.,* 2001). Also survival of the bee depends on its toxic defense against predators and parasitic insects and mites (Glinski and Buczek, 2003).

Mortality of *S. granaries* adults by the venom may be due to the action of its toxic protein and peptide components. Rose and Briggs (1969) observed hypopharyngeal gland secretions of young honeybee workers, which contain proteins. Secretions considered as bacteriostatic and bactericidal to a wide range of bacterial species. The venom can challenge many saprophytic and pathogenic organisms (bacteria and viruses), protozoan and metazoan parasites (larvae of *Senotainia tricupis, Mermis sp*.) and parasitic mites, *Acarapis Varroa jacobsoni* (Glinski and Jarosz, 2001).

The LD50 value of the bee venom was 3.9 and 3.3µg/insect after 24 and 72hr, respectively. There was a lack of correlation between adult weevil mortality and venom properties. This correlation suggests the presence of biological active components in this venom that may be responsible for weevil mortality. The toxicity action of the venom may be due to its effect on the sensory organs. Hider (1988), Hoffman (1996) and Charles, 2005 suggested, the toxicity effects of venom on many different organisms are due to (a)-neurotoxin (paralysis of the nervous system), (b)-Hemorrhagic (increase the permeability of the blood capillaries), and (c)-hemolytic effects. On the other hand, LD50 value of bee venom for rats is 2.8mg/kg and for human it has been estimated to be 500-1500 mg/kg (Schmidt, 1986 and Meier, 1995).

Effect of bee venom active fractions on adult grain weevils: Data recorded in Table (2), clearly indicates that fractions number 8, 9 and 10 were the most active fractions particularly number 10 against the adult weevils. These fractions induced 42.3, 66.7, and 89.6% of adult *S. granaries* mortality respectively compared to 0% of the control group. On the other hand, the venom effect decreased at fractions 5-6 and 11-15 and had no lethal effect on adult weevils at fractions numbered 1-4 (Table 2). This may be due to a decrease in the active components of the venom of these fractions.

Table (2) Effect of active venom fractions on the percent mortality of *Sitophilus granarius* adults.

Fraction numbers	% Mortality of *S. granarius*
1	00.0
2	00.0
3	00.0
4	00.0
5	2.40
6	6.10
7	18.4
8	42.3
9	66.7
10	89.6
11	23.8
12	11.2
13	2.30

Fractionation and Identification of the biological active components of bee venom

Elementary analysis: The elementary analysis of the active components revealed the percentage of Carbon(C) (5.47%), Hydrogen (H) (8%), Nitrogen (N) (68%) and Oxygen (O) (17.96%).

Infrared (IR) spectrophotometer analysis: Infrared absorption spectra (Fig. 1) of the active fraction (10) showing the different maxima wavenumber to which different assignments are introduced (Table 3).

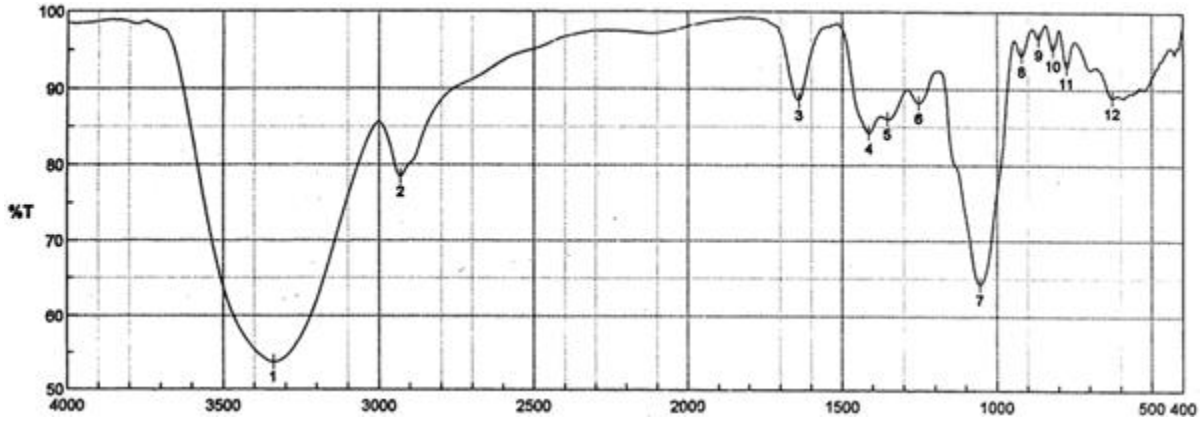

Figure 1. Infrared (IR) spectrophotometry of bee venom.

Table 3. Infrared (IR) maxima (wave number) with its transition.

Peaks no.	Maxima Cm-1	% Transition	Peaks no.	Maxima Cm-1	% Transition
1	3338.18	53.7303	7	1053.91	64.2488
2	2931.27	78.5415	8	920.843	94.4015
3	1641.13	88.5232	9	865.882	96.5969
4	1413.57	84.1679	10	819.598	95.1972
5	1353.78	86.0324	11	775.244	92.9522
6	1253.5	88.1592	12	628.68	88.9303

Ultraviolet (UV) spectrophotometer analysis: The Ultraviolet (UV) absorption spectrum of the highest effective fraction (10) is graphically illustrated in Fig. (2). It is evident that the active fraction of the bee venom had four bands with wavelength of 284.50, 286.00, 287.50 and 292.00nm at the absorption of 0.418, 0.406, 0.360, and 0.298 absorbance respectively. This indicated the presence of aromatic which tend to partition in the center of cyclodextrins and which connect to ethyl or acetone group.

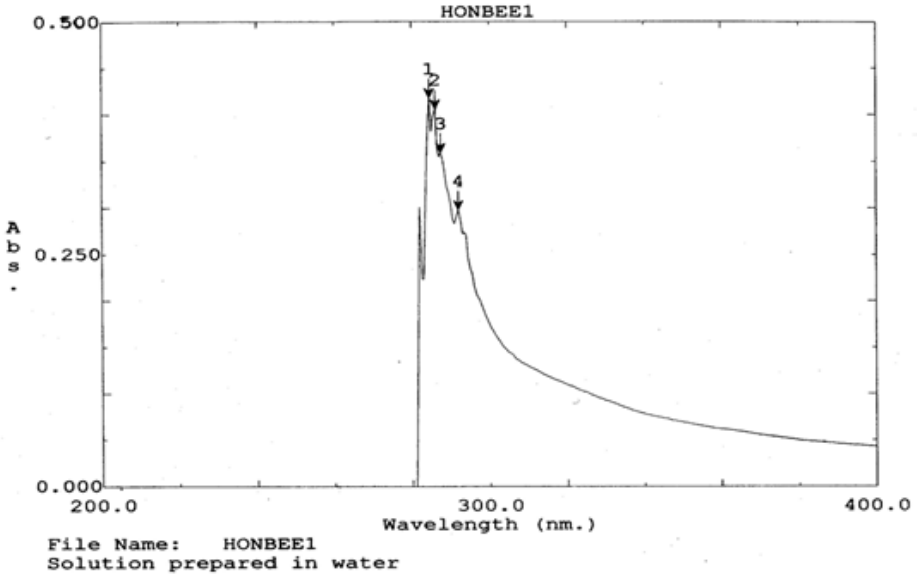

Figure 2. Ultraviolet (UV) spectrophotometry of bee venom.

According to Omar, (1971) and Silverstein and Bassler, (1976) different assignment of active chemical components of bee venom was as follows:

1-A short bending at 3338.18cm-1 to 2931.27cm-1 assigned to the possible presence of primary amine – NH stretching vibration.

2-Sharp peaks at 1641.13cm-1 and 1413.57cm-1 indicating that aromatic attached to the function groups of C=N, C=C, C=O, and NH stretching by multiple combination.

3-A peak at 1353.78cm-1 into 1253.5cm-1 indicated to the possible presence of ethyl-ester stretched to primary aromatic group and attached with chain of single bond groups of C-C, C-O, and C-N.

4-Band of 1053.91cm-1 to 920.843cm-1 supported the possible presence of chlorine (CL) connected to mono-substituted aromatic group.

5- A band of 865.882cm-1 to 628.68cm-1 supported the presence of aromatic assigned.

Mass Spectroscopy: Mass spectroscopy (Fig. 3) determines the sample molecular weight of the active fraction (10), which is 2847.7MW depending on the calculation of Bruker (2005).

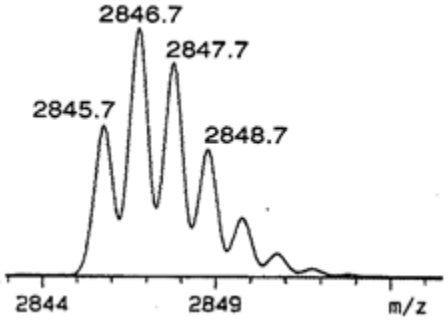

Figure 3. Mass (MS) spectroscopy of bee venom (Bruker 2005).

The obtained results of UV and IR spectrum were insured the active component contained form of aromatic structure. This structure was attached to some function groups such as NH, CO, CH and CL. The mass spectrum revealed that the venom molecular weight is 2847.7MW, which suggests that combined with elementary analysis; the proposed chemical formula is $C_{31}H_{228}N_{38}O_{32}$. This formula may suggest the active component of the venom in the form of aromatic tryptophane attached to different functional groups and this suggests the presence of indole tryptophan ring (Schneider *et al*., 1991; Kodaka, 1998, and Taroni *et al*., 2000). Edstrom, (1992) and Nakajima, (1986) came to the conclusion that bee venom contains biogenic amine compounds, such as histamine, serotonin, dopamine, noradrenaline, and polyamines as well as several toxins, peptides, and proteins. On the other hand Golden (1989) reported that Melittin is the main compound responsible for most of the toxic reactions. Similar to this result, Mazdak *et al*., (2004) concluded that melittin is a residue peptide obtained as aromatic tryptophan and act as to inhibit peptide activity inside insects and animal bodies. The chromatography of bee venoms revealed unique peaks, which might be used to identify these populations (Palma *et al*., 1995). On the other hand, the infrared (IR) spectrum suggested the presence of glycerol in the form of aromatic tryptophan (Wright *et al*., 2002). The aromatic tryptophan was supported in this study by the finding that aromatic tend to partition in the center of cyclodextrins (Schneider *et al*., 1991; Kodaka, 1998, and Taroni *et al*., 2000). The empirical chemical formula of the active components of the bee venom was suggested as **C31H228N38O32** with its molecular weight of 2847.7MW of the aromatic tryptophan ring.

CONCLUSION

Environmental and human health problems associated with the use of synthetic pesticides have prompted the demand for non-polluting, biologically specific insecticides. The current study tested the use and action of biological active compounds of bee venom against grain weevil, *Sitophilus granaries*. Weevils died within 24hrs. Exposure at doses of 1.1, 2.4, 3.7,5.0 and 6.3 µg/insect of bee venom. Weevil's mortalities were increased as increasing doses. The active ingredients of venom was subjected to UV analysis had four bands with wavelength of 284.50, 286.00, 287.50 and 292.00nm. Also IR absorbance supported the presence of 865.882cm^{-1} to 628.68cm^{-1} bands of aromatic assigned. Mass spectrum revealed that the venom molecular weight is 2847.7MW, which suggests that combined with elementary analysis; the proposed chemical formula of the active components of bee venom is $C_{31}H_{228}N_{38}O_{32}$.

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