World Journal of Agricultural Research, 2018, Vol. 6, No. 1, 5-9
Available online at http://pubs.sciepub.com/wjar/6/1/2

DOI:10.12691/wjar-6-1-2

Evaluation the Toxicity of Honey Bee Venom on *Achroia grisella* Developmental Stages

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Abstract The common control method used to control the lesser wax moth *A. grisella* was fumigation with toxic gases; however, many insect pests of honey bees have developed resistance to the conventional control methods. This study aimed to study the toxicity of crude bee venom on developmental stages of *A. grisella* as safer alternative and replacement of these chemicals. The bee venom was collected by placing the electric bee venom collector device at the entrance of the beehive. Newly deposited eggs of *A. grisella* were assayed to evaluate the crude honey bee venom effect on the viability of eggs. Dried crude honey bee venom was diluted with pure acetone to concentrations of 50, 25, 12.5 and 6.25 µg/µl. Egg hatchability was significantly ($p<0.05$) affected by the treatment. The corrected mortality of the treated eggs was 50.54% in the higher concentration of 50µg/µl with average unhatched eggs of 17.5 eggs per total of 25 eggs with the median lethal concentration (LC_{50}) of 52.89 µg /µl. The topical application of crude honey bee venom was applied on 3rd instar larvae with concentrations of 0, 6.25, 12.5, 25, 50 µg. The calculated mortality percentages for all treatments were 8% at the lower concentration and 52% at the high concentration. The calculated lethal median concentration LC_{50} was 38.27 µg /µl.

Keywords: *bee venom, toxicity, wax moth*

Cite This Article: Montasir O. Mahgoub, Wei H. Lau, Dzolkhifli Bin Omar, and Ahmed M. El Naim, "Evaluation the Toxicity of Honey Bee Venom on *Achroia grisella* Developmental Stages." *World Journal of Agricultural Research*, vol. 6, no. 1 (2018): 5-9. doi: 10.12691/wjar-6-1-2.

1. Introduction

The common control method used to control *A. grisella* was fumigation with toxic gases; however, many insect pests of honey bees have developed resistance to the conventional control treatments in addition to the residual effect causing a serious limitation for the marketing value and consumption of bee products in recent decades [1]. Synthetic chemical pesticides have an acute environmental deleterious impact on honey bees when widely used, as well as residual effects [2].

The objective of recent research were to find and develop new and safer means for pest control; possibly, the new alternative and replacement of these chemicals could be natural toxins that have been produced and developed by arthropods over many years [3]. Arthropod toxins are promising compounds for producing a new pesticide control system [4]. Hymenopterous insects produce complex venoms that contain several types of material, such as proteins, peptides, and other low molecular components. The solitary hymenopteran insects, such as many parasitoids and predators use these kinds of venom to paralyze or kill their host, while the social insects use venoms as a tool for defence to protect their colonies from intruders and predators.

Honey bee workers produce the venom in a special long and thin branched gland at the end of their abdomen [5]. Chen *et al.* [6] characterized the major active components of honey bee venom as apamin, mast-cell degranulating peptide, phospholipase A_2 and melittin [7]. The content of fresh liquid honey bee venom compared to dry honey venom are slightly different, even though the biological activity of dry bee venom persists and can be used as an active toxin [8].

This study aimed to study the toxicity of crude bee venom on developmental stages of lesser wax moth *A. grisella.*

2. Materials and Methods

2.1. Crude Honey Bee Venom Source and Collection Process

Honey bee venom was collected from the active colonies of *Apis cerana* L. located in field number 10 of the University Putra Malaysia (UPM) apiary. Honey bee venom was collected using two types of electric venom collectors; namely, models C-J 201® Cheongjin Tech, Korea and IGK BV0805®, Bulgaria (Figure 1). The bee venom collection was carried out by placing the bee venom collector device at the entrance of the beehive. The

collector was connected to a battery to generate a mild electric impulse in the wire frame. A few seconds later, an offence was started by the worker bee guards that covered the frame of the device. The workers reacted to the electric impulse by stinging the surface of the glass, which was located under the electric wires where the venom accumulated in liquid form. The process continued for 30 minutes. After this period, the device was disconnected from the electricity supply. The glass was removed and allowed to dry for 20 minutes under shade. The collected bee venom was scraped with a sharp clean scraper. The collected crude honey bee venom samples were transferred to 14 ml falcon tubes and dissolved in 10 ml deionized water. The solution mixed well using a vortex mixture for 5 minutes. The sample was centrifuged for 10 minutes at 4°C. The final supernatant was filtered by 0.45µm filter and kept at 4°C until used [9].

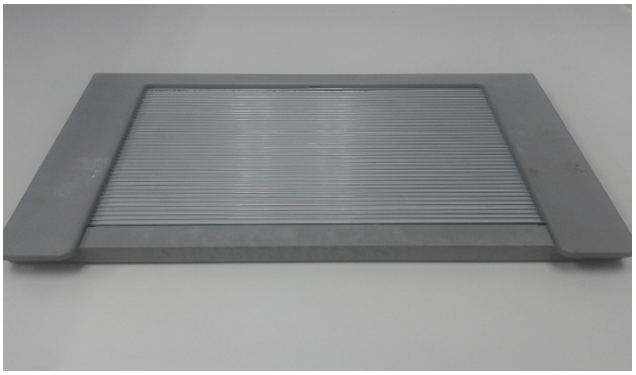

Figure 1. Venom electric collector device model C-J 201® Cheongjin Tech

2.2. Apparatus and Chromatographic Conditions of Crude Honeybee *Apis cerana* L. Venom

Melittin, apamin and phospholipase A_2 were purchased from Sigma Chemical Co. (USA). The HPLC water and acetonitrile and Trifluro acetic acid (TFA) were purchased from Fisher Scientific (USA), and all were of HPLC grade. The chemical composition analysis was carried out to separate the components of the honey bee venom. Dried bee venom collected from the active colonies of honey bee *Apis cerana.* High performance liquid chromatography (HPLC) was from Waters (USA) coupled with a diode array detector (2996). The analyses were carried out using a C18 silica column (250 mm × 4.6 mm, 5µm particle size) Kinetex® from Phenomenex (USA) and 30 °C was used as the column temperature. The flow rate was 1.0 ml/min. The instrument was controlled by Empower 2, from Waters (USA).

The analysis method was carried out according to Rybak-Chmielewska and Szczêsna [10] with a slight modification as follows; the solvents used for the mobile phase were 0.1% trifluoroacetic acid (TFA) in water for solvent A and 0.1% TFA in acetonitrile: water 90:10 for solvent B. The gradient elution was from 2% B to 65% B in 80 minutes the column temperature was 30°C, and the flow rate was 1.0 ml/minute. The injection volume was 30 µl per sample. The detection of venom compounds was performed at 220 nm wavelength.

Seven standard points were used by serial dilutions of 10, 50, 100, 300, 500, 750 and 1000 ppm respectively, were used to construct the standard calibration curve and get the standard equation and r^2 value. A sample of honey bee venom was dehydrated with double distilled water in a concentration of 1000 ppm and injected into the system (30 µl per sample) after filtration through a 0.45 µm filter.

Diode array detection was used for the identification of the peak retention times. The linearity was determined for the calibration curves obtained by HPLC analysis. Evaluation of each point was repeated six times and each calibration curve was fitted by linear regression. To study repeatability (within-day precision), a sample was analysed 6 times within 1 day, while the within-laboratory reproducibility (day-to-day precision) was studied by a sample injected 3 times on three different days during a period of 3 weeks.

2.3. Lesser Wax Moth Collection and Rearing Conditions

This study was conducted at the Toxicology laboratory, Faculty of Agriculture, University Putra Malaysia. The developmental stages of *A. grisella* were collected from infested honey beehives near Batu Pahat; Johor, Malaysia during October 2008. Larvae were reared on an artificial diet containing wheat flour, glycerine, yeast, honey and water, kept under 12:12 light: dark photoperiod at 25±1°C, 66±2 RH.

2.4. Bioassay of Crude Honey Bee Venom on Eggs of *A. grisella* by Topical Application

Newly deposited eggs (24 hours) by females of laboratory-reared culture of *A. grisella* were assayed to evaluate the crude honey bee venom effect on the viability of eggs. Dried crude honey bee venom was prepared to test its potency on the *A. grisella* eggs; a diluted concentration of CHBV dissolved in acetone [11]. Twenty-five eggs (experimental unit) were treated with five treatments s of CHBV of 50, 25, 12.5 and 6.25 µg/µl; each treatment replicated four times. The control group was treated with pure acetone. The treatments applied topically as a droplet of 5 µl on the eggs mass.

2.5. Bioassay of Crude Honey Bee Venom on 3rd Larval Instar of *A. grisella* by Topical Application

Dried crude honey bee venom was prepared to assess its potency on *A. grisella* 3rd larval stage, different concentrations of CHBV were dissolved in acetone [11] and applied topically as a droplet of 1µl on the dorsal site of the larvae. Acetone was used as carrier of crude honey bee venom in the topical application test, which involved five treatments applied by 1 µl as a drop on the larval dorsal surface of the thorax of the larvae. Twenty-five larvae were treated in each replicate with different concentrations, i.e. 50, 25, 12.5 and 6.25 µg /µl; the control group received 1 µl of pure acetone [12]. The treatment was carried out using a 10 µl micro syringe (Agilent micro syringe®).

2.6. Bioassay of Crude Honey Bee Venom on 3rd Larval Instar of *A. grisella* by Injection Application

Third larval instar was assayed by four doses of 3.25, 1.7, 0.6 and 0.3 µg /µl CHBV. Each dose replicated four times. The dry CHBV collected was maintained at -20°C, the powder dissolved at a high concentration of 100µg/µl, and then serial dilution was carried out. The injection solutions were prepared in a sterile phosphate buffer I0 mM, pH 7.2, 0.9% NaCI containing bovine serum albumin (BSA, 1% w/v) and filtered through 4.5 µm a membrane filter [13]. The injection application was applied by a calibrated micro syringe (Agilent micro syringe®) with a 27 gauge needle; the needle was inserted the thorax region as 1 µl in the haemocoel of treated larvae of *A. grisella* (Figure 2) [14].

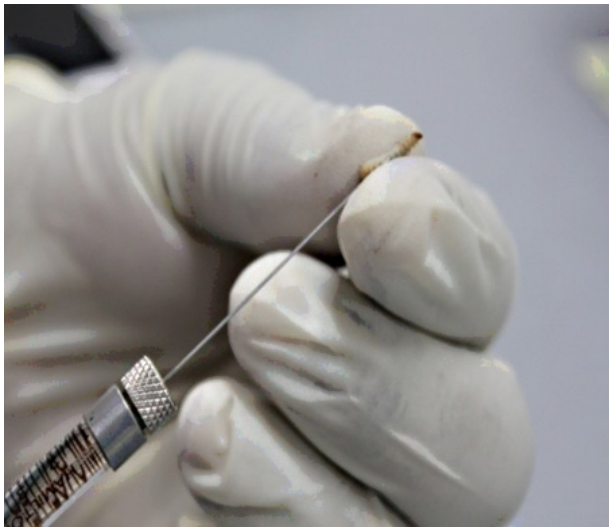

Figure 2. Injection treatment using micro syringe with a 27 gauge needle on the A. grisella larva

2.7. Statistical Analysis

A completely randomized design CRD applied in all experiments. For the assessment of toxicity, regression lines, LC_{50} values, 95% fiducial limits (FL) were determined using a log-probit analysis (Polo plus® V.2 software, LeOra). Mortality data of bioassays were analysed using Statistical software JMP®9. The statistical significance of differences between treatments was determined using analysis of variance (ANOVA) and the Tukey Kramer used for the mean comparison. The corrected mortality for all experiments was calculated using the Abbot Formula. The median lethal concentration values (LC_{50}) were calculated by probit analysis using statistical software (Polo plus® V. 2 LeOra).

3. Results and Discussion

3.1. Chromatographic Analysis of Crude Honey Bee Venom

Concentrated crude bee venom was used to produce the pure melittin peptide after the separation process. This process was carried out using high performance liquid chromatography (HPLC). Figure 3 shows the analysis of the C18 HPLC chromatogram of crude honey bee venom. The major components of honey bee venom are displayed in the chromatogram. Melittin peptide appeared in high quantity compared to the other components. This finding agrees with the analysis of honey bee venom carried out by many workers: Lowy *et al.* [15], Hider [16], Rybak-Chmielewska and Szczêsna [10]; Wang *et al.* [17] and Jo *et al.* [6] who confirmed that the major compound of honey bee venom is the melittin peptide.

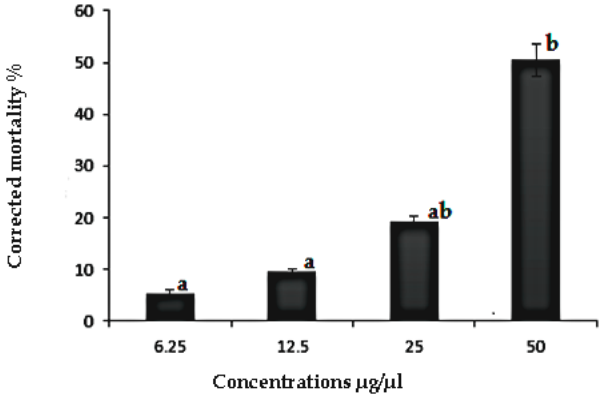

Figure 3. HPLC Chromatogram of crude honey bee venom from *A. cerana* showed the major components melittin, phospholipase A_2 and apamin

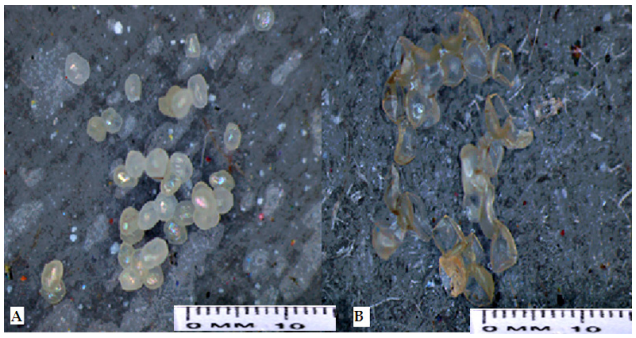

Figure 4. Eggs of *A. grisella*, (A) control treatment, (B) treated eggs by crude honey bee venom

3.2. Bioassay of Crude Honey Bee Venom by Topical Application on the Egg Stage of *A. grisella*

In this study, the crude honey bee venom (CHBV) from honey bee *Apis cerana* was assayed topically on the egg stage of the lesser wax moth, *A. grisella*, as a base for the application of the biopesticide on the first stage of this pest (Figure 4). Egg hatchability was significantly ($p<0.05$) affected by the treatment. The corrected mortality using the Abbot Formula [18] of the treated eggs was 50.54% in the higher concentration of 50µg/µl with average unhatched eggs of 17.5 eggs per total of 25 eggs (Figure 4) with the median lethal concentration (LC_{50}) of 52.89 µg /µl (Table 1).

When the egg masses were treated with low concentrations of 6.25 and 12.50 µg/µl the results showed egg mortality of 12% and 16%, which were statistically similar and not different from the control 7%. The highest doses 50 µg/µl resulted in a high mortality of 54%. Figure 5.

Table 1. Toxicity of crude honey bee venom applied to egg of *A. grisella.*

Stage	Time of mortality	N	Slope± SE	LC_{50} µg/µl	FL 95%
Egg	7 days	400	3.26± 0.75	52.89	42.44-86.63

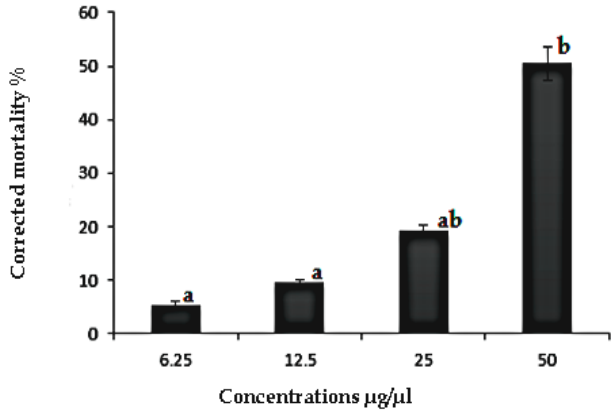

Figure 5. Corrected *A. grisella* eggs mortality caused by different concentrations of crude honey bee venom

These results agree with the results obtained by Bonfanti-Almeida *et al.* [19] who found that *Pyralidae Diatraea saccharalis* eggs were sensitive to the application of CHBV from *A. millefera* with LC_{50} 21.08 mg/ml. He observed that the newly laid eggs were more tolerable to CHBV than the three days eggs due to the adhesive material covering the surface of the newly laid eggs, which facilitates the egg attachment on the laying site and prevents the diffusion of venom inside them.

3.3. Toxicity of Crude Honey Bee Venom against the 3rd Instar Larvae of *A. grisella* by Topical Application

The topical application of crude honey bee venom was applied on 3rd instar larvae. The experiential unit contained five larvae, for each concentration of 0, 6.25, 12.5, 25, 50 µg/µl. Each treatment replicated five times and the mortality was observed after 10 days. No mortality was observed in the control group, which indicated no harmful effect from the acetone, which was used to facilitate the venom penetration through the larval skin cuticle. Analysis showed there was significant difference among treatments (Table 2). The calculated mortality percentages for all treatments were 8% at the lower concentration and 52% at the high concentration. The calculated lethal median concentration LC_{50} was 38.27 µg /µl (Table 3).

Table 2. Mortality caused by different concentrations of crude honey bee venom applied topically on 3rd larval instar of *A. grisella*

Concentrations µg/µl	N	Mortality %	F value
Control	25	0 a	12.03*
6.25	25	8 ab	
12.5	25	28 b	
25	25	44 c	
50	25	52 c	

Values within column connected by same letter are not significantly different.

Table 3. Toxicity of crude honey bee venom applied topically on 3rd larval instar of *A. grisella*

Stage	Time of mortality	N	Slope± SE	LC50 µg/µl	FL 95%
3rd larval instar	10 days	125	3.49±0.43	38.28	24.95-104.64

Many spider venoms cause serious toxicity to the insect larvae when applied topically, such as *Diabrotica undecimpunctata undecimpunctata* spider venom, which was applied topically on *Manduca sexta* larvae [20] (Quistad *et al.*, 1992). The larval mortality was assessed after CHBV was applied topically on the 3rd instar of *A. grisella.* Significant mortality was observed for the concentration of 50 µg /µl, which caused 52% mortality.

3.4. Toxicity of Crude Honey Bee Venom against the 3rd Instar Larvae of *A. grisella* by Injection Application

The CHBV was applied by haemocoel injection into 3rd instar larvae of *A. grisella* in concentrations of 0, 0.3, 0.6, 1.7 and 3.25 µg/µl of crude honey bee venom, as reported in Table 4. Significant mortality percentages were found for all CHBV treatments ($p<0.05$) used, except for the concentration of 0.3 mg/ml, which showed no significant differences in mortality compared to the control group. The higher concentration 3.25 µg/µl showed a high mortality percentage of 88.

Table 4. Mortality caused by different concentration after 24 hours of crude honey bee venom injected in 3rd larval instar of *A. grisella*

Concentrations mg/ml	N	Mortality%	F value
0 (Control)	25	20a	14.37
0.3	25	20a	
0.6	25	28ab	
1.7	25	60bc	
3.25	25	88c	

Values within column connected by same letter are not significantly different.

A similar effect for crude honey bee venom treatment was reported by Ross *et al*. [13] after haemocoel injection into lepidopteron larvae of *Manduca sexta.* The injected third instar larvae with different concentrations of CHBV showed many toxicity symptoms. These symptoms varied between losing body weight and feeding appetites to acute toxicity and larval mortality [13,21]. The larvicidal action of crude honey bee venom usually resulted from the synergic interaction action of the components of venom mainly apamin, melittin and phospholipase A_2 [21].

4. Conclusion

Melittin peptide was found in high quantities (56%) in the analysed samples. The crude honey bee venom displayed high activity in the developmental stages of the A. grisella tested by different types of applications, i.e. haemolymph injection and topical application. The injection of CHBV median lethal concentration LC50 was 1.62 µg/µl. The toxicity of crude honey bee venom

applied to the larvae of lesser wax moth, A. grisella, would be a motivation to farmers and beekeepers to control the early stage of this noxious insect pest inside active honey beehives because it is readily available and is easy to collect from the beehive. Moreover, it is a safe and strong alternative to chemical and other pesticides, which cannot be used during the active bee season inside the hives. As well as the stored comb between seasons can also be treated to prevent future infestation.

Acknowledgements

The authors are grateful to the Department of Agriculture Batu Pahat, Johor Malaysia for their support in providing the lesser wax moth samples used in this research. The gratitude also goes to the Department of Plant Protection, Faculty of Agriculture and University Putra Malaysia (UPM) for their financial support.

References

[1] Lodesani, M., and Costa, M. Limits of chemotherapy in beekeeping: development of resistance and the problem of residues. *Journal of Bee World*, 86(4), 102-109, 2005.

[2] Lu, C. S., Warchol, K. M. and Callahan, R. A. "In situ replication of honey bee colony collapse disorder". *Bulletin of insectology*, 65(1): 99-106, 2012.

[3] Manzoli, M., Gobbi, N., and Palma, M. "Insects as biological models to assay spider and scorpion venom toxicity". *Journal of Venomous Animals and Toxins Including Tropical Diseases*, 9(2), 174-185, 2003.

[4] Kirschbaum, J. "Potential implication of genetic engineering and other biotechnologies to insect control". *Annual Review of Entomology*, 30(1), 51-70, 1985.

[5] Peiren, N.,de Graaf, D. C.,Vanrobaeys, F., Danneels, E. L., Devreese, B., Van Beeumen, J.,and Jacobs, F. J. "Proteomic analysis of the honey bee worker venom gland focusing on the mechanisms of protection against tissue damage". *Toxicon*, 52(1), 72-83. 2008.

[6] Chen, Y. N.,Li, K. C.,Li, Z.,Shang, G. W.,Liu, D.,Lu, Z.,Zhang, J. W.,Ji, Y. H.,Gao, G. D.,and Chen, J. "Effects of bee venom peptidergic components on rat pain-related behaviors and inflammation". *Neuroscience*, 138(2), 631-640, 2006.

[7] Lima, P. R.,and Brochetto B., M. R. "Hymenoptera venom review focusing on *Apis mellifera*". *Journal of Venomous Animals and Toxins Including Tropical Diseases*, 9(2), 149-162, 2003.

[8] Bogdanov, S. "Bee Venom: Composition, Health, Medicine: A Review". *Journal of Peptides*, (1), 1-20, 2012.

[9] Zhou, J., Zhao, J., Zhang, S., Shen, J., Qi, Y., Xue, X., Li, Y.,Wu, L.,Zhang, J., Chen, F.,and Chen, L. "Quantification of melittin and apamin in bee venom lyophilized powder from *Apis mellifera* by liquid chromatography diode array detector tandem mass spectrometry". *Journal of Analytical Biochemistry*, 404(2), 171-8, 2010.

[10] Rybak-Chmielewska, H., and Szczêsna, T. "HPLC study of chemical composition of honeybee (*Apis mellifera* L.) venom". *Journal of Apicultural Science*, 48(2), 103-109, 2004.

[11] Moyses, E. W.,and Gfeller, F. J. Topical application as a method for comparing the effectiveness of insecticides against cat flea (Siphonaptera: Pulicidae). *Journal of Medical Entomology*, 38(2), 193-196, 2001.

[12] Rogers, M., Cole, T., Ramaswamy, S.,and Potter, D. "Behavioral changes in Japanese beetle and masked chafer grubs (Coleoptera: Scarabaeidae) after parasitism by Tiphiid wasps (Hymenoptera: Tiphiidae)". *Journal of Environmental Entomology*, 32(3), 618-625, 2003.

[13] Ross, D.C., Crim, J.W., Brown, M.R., Herzog, G.A., lea, A.O. "Toxic and antifeeding actions of melittin in the corn earworm, Heliothis zea (Boddie): Comparisons to bee venom and insecticides chlorpyriphos and cyromazine". *Toxicon*, 25(3): 307-313. 1987.

[14] West, E. J.,and Briggs, J. D. "In vitro toxin production by the fungus *Beauveria bassiana* and Bioassay in greater wax moth larvae". *Journal of Economic Entomology*, 61(3), 684-687, 1968.

[15] Lowy, P. H., Sarmiento, L.,and Mitchell, H. K. "Polypeptides minimine and melittin from bee venom: Effects on Drosophila". *Archives of Biochemistry and Biophysics*, 145(1), 338-343, 1971.

[16] Hider, R. C. "Honeybee venom: A rich source of pharmacologically active peptides". *Endeavour Journal*, 12(2), 60-65, 1988.

[17] Wang, C., Chen, T., Zhang, N., Yang, M., Li, B., Lü, X., Cao, X.,and Ling, C. "Melittin, a major component of bee venom, sensitizes human hepatocellular carcinoma cells to tumor necrosis factor related apoptosis-inducing ligand (TRAIL) induced apoptosis by activating CaMKII-TAK1-JNK/p38 and inhibiting IκBα kinase-NFκB". *Journal of Biological Chemistry*, 284(6), 3804-3813, 2009.

[18] Abbott, W. "A method of computing the effectiveness of an insecticide". *Journal of Economic Entomology*, 18(2), 265-267, 1925.

[19] Bonfanti-Almeida, J.,Gobbi, N.,andPalma, M. "Bioinsecticide action of the venom of Africanized bees *Apis mellifera* L. 1758 (Hymenoptera: Apidae): the most susceptible age of *Diatraea saccharalis* Fabricius 1794 (Lepeletier: Pyralidae) eggs to the venom action". *Journal of Venomous Animals and Toxins*, 2(1), 46-51, 1996.

[20] Quistad, G. B.,Dennis, P. A.,and Skinner, W. S. "Insecticidal activity of spider (Araneae), Centipede (Chilopoda), Scorpion (Scorpionida), and snake (Serpentes) Venoms". *Journal of Economic Entomology*, 85(1), 33-39, 1992.

[21] Quistad, G. B., Skinner, W. S., and Schooley, D. A. "Venoms of social hymenoptera—toxicity to the lepidopteran, *Manduca sexta*". *Journal of Insect Biochemistry*, 18(6), 511-514, 1988.