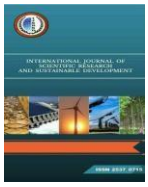

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الاتحاد العربي للتنمية المستدامة والبيئة

Studies on effectiveness of bee venom as an insecticide against cotton leafworm, Spodoptera littoralis

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Abstract

The study mainly aimed at the effectiveness of bee venom which is a natural product and overcame antibiotic resistance and side effects on the second instar of cotton leaf worm *Spodoptera littoralis* (lepidoptera: Noctuidea) that is one of the most common insect pests. Four different doses of bee venom were treated (0.125gm,0.25gm, 0.5gm and 1gm) to evaluate total morality of second and forth instars larvae of *S. littoralis*. The results showed that 16.67%, 30%, 53.33%, 76.66% were total morality percentage, respectively. In addition, the 4th instar larvae of *S. littoralis* were treated by LC50 to study the histological changes in the mid gut after 3 and 5 days. The changes were completely separation or destruction of basement membrane and sever proliferation of epithelial cells after the two days, but the midgut in control was identical.

دراسات على فعالية سم النحل كمبيد حشري ضد دودة ورقة القطن

أمنيه فيصل إبراهيم، شرين فتحي الاتربي، غادة السيد عبد الله

معهد بحوث وقاية النباتات ،مركز البحوث الزراعيه، الدقي ،الجيزة ، مصر

تهدف الدراسة الى معرفة كفاءة سم النحل فى التغلب على كل من العمر اليرقى الثانى و العمر اليرقى الرابع لدودة ورق القطن ،حيث أنه منتج طبيعى يعمل كمضاد حيوى، وتعد دودة ورق القطن أحد أهم الآفات الحشرية . وقد تمت المعاملة بأربع تركيزات من سم النحل وهم (0.125 جم، 0.25 جم، 0.5 جم و 1 جم) لتقدير نسب الموت على العمر اليرقى الثانى لدودة ورق القطن. وقد أظهرت النتائج نسب موت كالآتى :16.67%، 30%، 53.33% و 76.66 % للأربع تركيزات ،على التوالى. وبالاضافة الى ذلك تم معاملة العمر اليرقى الرابع بالتركيز النصف مميت LC50 لدراسة التأثيرات الهستولوجية داخل المعي الوسطى لجسم اليرقات بعد اليوم الثالث والخامس من المعاملة. وأوضحت النتائج تدمير كامل للغشاء القاعدي وانتشار شديد للخلايا الطلائية فى كلا اليومين لكن التشوه كان أكثر بعد اليوم الخامس، بينما فى الكنترول كانت الخلايا مثالية بدون تشوه.

معلومات عن البحث:

الكلمات الدالة:

سم النحل، دودة ورق القطن، هستولوجى

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List of abbreviations

S. littoralis: *Spodoptera littoralis.*

LC_{50}: 50% Lethal Concentration

BV: Bee Venom

B. venom: Bee venom

LC_{90}: 90% Lethal Concentration

Conc: Concentration

1. INTRODUCTION

The cotton leaf worm, *Spodoptera littoralis* (lepidoptera: Noctuidea) is one of the most insect pests that attacks huge economic crops for example cotton, tomato and potato (Senrung et al.,2014). The adult lays between 300 to 500 eggs so accordingly chemical control is applied and it is considered that the common methods for this insect pest, it develops resistance to many pesticides (Niranjankumar and Regupathy, 2001**;** Marouf et al.,2021).

Recently, researchers try to find new and safer ways for pest control; for instance, the new alternative and replacement of these chemicals could be natural toxins that have been produced by arthropods over many years (Manzoli et al., 2003). One of these arthropods produced is bee venom, that is produced by the glands of *Apis mellifera* (Banks and Shipolini, 1986; Palma and Brochetto-Braga, 1993).

Bee venom is colourless viscous liquid, strong aromatic bitter tast. B.V. is off-white or yellowish white powder in dry state. It's composed of 40 substances (Zhu et al., 2007). Melittin is amagor compound; about 50%of the dry B. venom (BV) consists of amino acids, phospholipids, polypeptides, mineral substances, volatile organic acid, formic acid and some antibiotics (Amany Hagag et al., 2015). B.v. use to treat different diseases (Önemi and Hegazi, 2012).

The aim of this present paper was to evaluate the effectiveness of bee venom against cotton leaf worm *S. littoralis* and then to note the effectiveness of the cells by histological study.

2. MATERIAL AND METHODS

2.1.Tested cotton leaf worm

Laboratory strain of *S. littoralis* was reared under constant conditions of 27±2°C, photoperiod of 14 h light and 10 h dark and 65±5% R.H. Larvae were fed on castor leaves that were used at second instar for laboratory experiments.

2.2. Venom collection

Venom was collected from workers of honey bee (*Apis mellifera carnica*) (Benton et al., 1963; Brandeburgo, 1992) with 4-weeks intervals. An electric BV gathering method under various conditions; Voltage from 24 to 30V, Impulse span: 2-3 seconds Pauses: 3 to 6 seconds: motivation recurrence from 50 to 1000 Hz. The bee venom was collected by the imported electric shock device

(VC-6F model from Apitronic Services, 9611 No. 4 Road, Richmond, B.C., Canada), according to the method reported by Markovic and Molnar (1954). It comprises a bee venom collection frame with wire electrodes . Using electrical impulses to stimulate the bee workers to sting through latex placed on a glass plate to prevent venom pollution and collected the dry venom using sharp scraper Fakhim zadeh, 1998). Bees that come into contact with the wires received a mild electrical shock and stung onto the glass sheet. The alarm odor, which evaporated from the venom, mobilized and irritated the other bees and they also started to sting. The frames with the fresh dried bee venom on them are carefully packed into a special container for transportation to the laboratory. After that, bee venom is packed up in the dark glass jars and stored in a cool and dry place.

2.3.Tested bee venom on larva of cotton leaf worm:

The Toxicity action of bee venom was evaluated by using 2nd instar larvae, ten larvae for each replicate were put on each leaf discs. Castro bean leaves were applied by dipping leaves into the treatment for 20 sec. and left for the air to dry. The treatment concentrations were 0.125 gm, 025 gm, 0.5gm and 1 gm. Water was untreated check and the same number of leaf discs was applied after 1, 3, 5 and 7 days. The percentage of mortality was recorded and data was corrected relativity to control mortality (Abbott, 1925). LC_{50} and LC_{90} values were recorded by using probit analysis statical method of (Finney,1971. Sun equation 1950) used to determine LC_{50} index

$$\textbf{Toxicity index for } \mathbf{LC_{50}} = \frac{\text{LC50 of the most of effective compund}}{\text{LC50 of the least of effective compund}} \times 100$$

2.4. Histological studies

LC_{50} of all bee venom concentrations were applied to 4th instar larvae. Tissue processing sectioning and staining carried out according to (Lilli, 1965 and lynch, 1969) and post-treatment after 96 hours.

4th instar larvae were taken and transferred into alcoholic Bouin's solution used as a fixative, larvae were hydrated and removed the yellow color of Bouin's solution by rinsing in a series of ethanol solutions. Larvae were transferred first into 50% ethyl alcohol for 2hrs. at 40̊ C (two change) then they left for 24hrs., then, larvae were passed through a series of alcoholic treatments each for two hrs., at room temperature starting with 80% followed by 90%, 96% and ending with alcohol. After dehydration, the larvae were placed in solution of amylacetate solution and soft paraffin wax and leaving them for 24 hrs. at 50 C̊ and repeated this process for three time. A mixture of one part of hard paraffin wax was added to the larvae which were embedded in wax mixture used in the last step. Serial sections at 6 microns were made by microtome and mounted to clean slides using Mayer's albumin. Units were mounted on glass slides and stained with heamatoxylene and counterstained in alcoholic solution and prepared for examination and photo-microscopy.

3. RESULTS AND DISCUSSION

3.1. Mortality of bee venom against 2nd instarof the cotton leaf worm *S. littoralis*

To show the effect of bee venom on 2nd instar of S. littoralis, data in Table (1) presented that, the

concentration 1gm of bee venom was the highest one which caused 76.66 % percentage of mortality rate. The lowest concentration of bee venom was .0125 gm which caused 16.67 % of mortality rate, while the rest of bee venom concentrations were 0.25gm, 0.5 gm which caused 30%, 53.33 % of mortality rate.

Our results were agreed with (Nassar, 2013) who tested bee venom against the adult of grains weevil, *Sitophilus granarius* (Coleoptera: Curculionidae). Five dose levels of bee venoum were tested 1.1, 2.4, 3.7, 5.0, and 6.3 µg. 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 hrs. of adult treatment with the doses of 6.3 and 1.1µg/insect of bee venom, respectively (Sadek et al., 2022) tested bee venom on *S. littoralis* and mentioned that, bee venom had an increased response with increasing the dose.

Table (1): mortality percentage of 2nd instar larvae of the cotton leaf worm *S. littoralis* treated with bee venom under laboratory conditions.

Concentration of bee venom	Mortality after treatments %				Total mortality %
	1st day	3rd day	5th day	7th day	
0.125 gm/L	----	6.67	6.67	3.33	16.67
0.25 gm/L	6.67	10	10	3.33	30
0.5 gm/L	10	23.33	16.67	3.33	53.33
1 gm/L	53.33	20	----	3.33	76.66

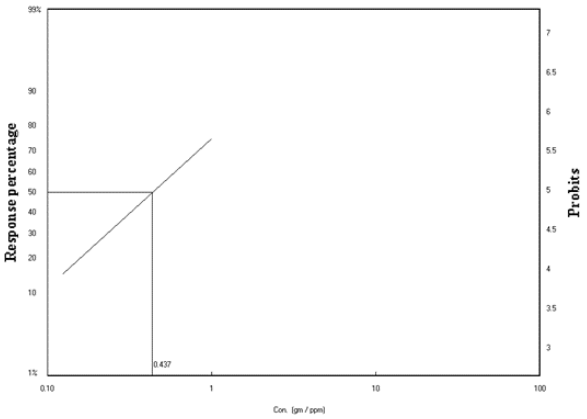
3.2. Effective of bee venom and its toxicity index against 2nd instar larvae of S. littioralis:

Table (2) and Fig. (1) illustrated that, LC_{50} for bee venom was 0.437 while LC_{90} was 2.065, the slope value was 1.9. Moreover, LC_{90}/ LC_{50} was 4.725; from this point the lowest slop value or the highest LC_{90}/LC_{50} indicate that, the steepest toxicity line. (Mahgoub et al., 2018) mentioned that, the median lethal concentration (LC_{50}) of 52.89 µg /µl applied on unhatched egg of *Achroia grisella* and the calculated lethal median concentration LC_{50} was 38.27 µg /µl instar of bee venom against the 3rd instar of *A. grisella*.

Table (2) effective of bee venom against 2nd instar larvae of *S. littioralis*

Treatments	Concentration (gm/ L)	Corrected mortality	C_{50}	C_{90}	Slope ± S.D.	LC_{90}/LC_{50}	R	P
Bee venom	0.125	16.67	0.437	0.065	1.9± 0.21	4.725	0.997	0.747
	0.250	30						
	0.5	53.33						
	1	76.67						

R: Correlation P: Probability



Fig(1) LC-P line foe bee venom against 2nd instar of *S. littioralis*

3.3. Histological study on the effectiveness of bee venom on the 4th instar of cotton leaf worm

To illustrate the effectiveness of bee venom on the 4th instar of *S. littoralis*, it has been studied the histological change of the 4th instar, the report was done at the Animal Health Research Institute, Agriculture Research Center. Histological examination of the cut section of control during 3 days and 5 days treatment without bee venom Fig (2) showed that; muscular layer of mid gut followed internally by intact thin basement membrane where the epithelium rested. The epithelial layer formed of one layer of columnar cells with dark round nucleus in the middle part of each cell, the lumen surrounded by peritrophic membrane which envelops the food materials.

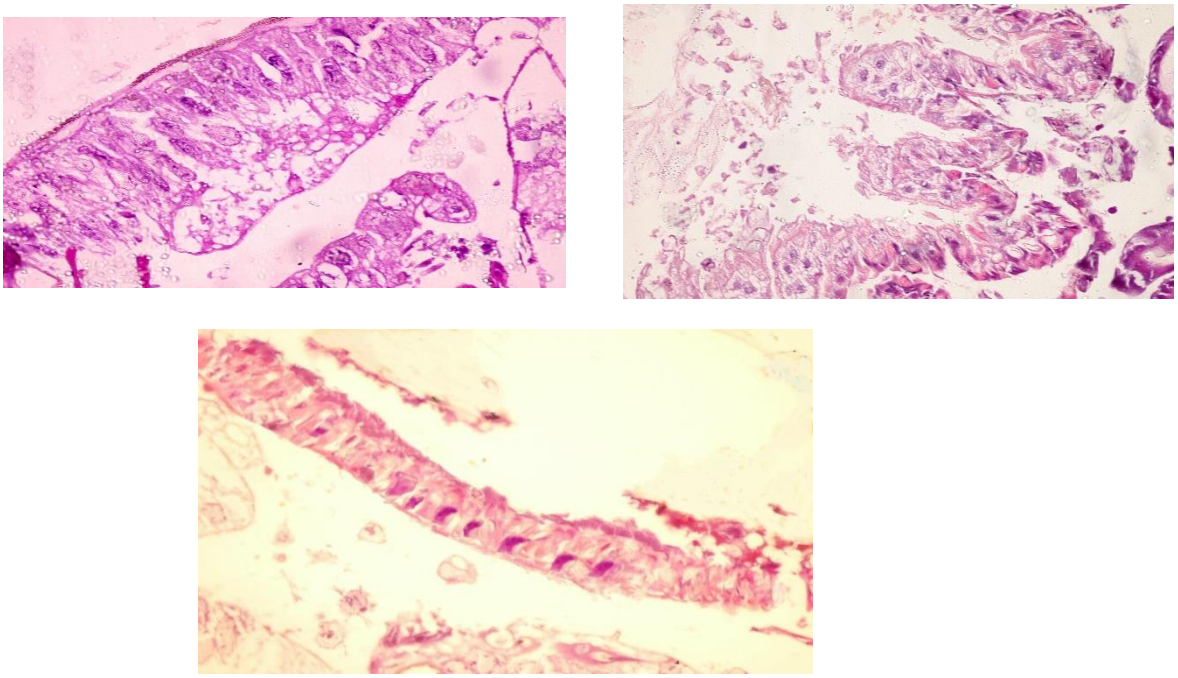


Fig (2) histological structure of the normal mid gut of the 4th instar of spodoptera littoralis

Otherwise, the effect of bee venom on 4th instar of *S. littoralis* after 3days of treatment conformed that the cut section of midgut either completely separation or destruction of basement vacuolar degeneration, increase goblet cells and sever proliferation of epithelial cells membrane. Other epithelial cells appeared detached and necrosis with lysis in peritrophic membrane.

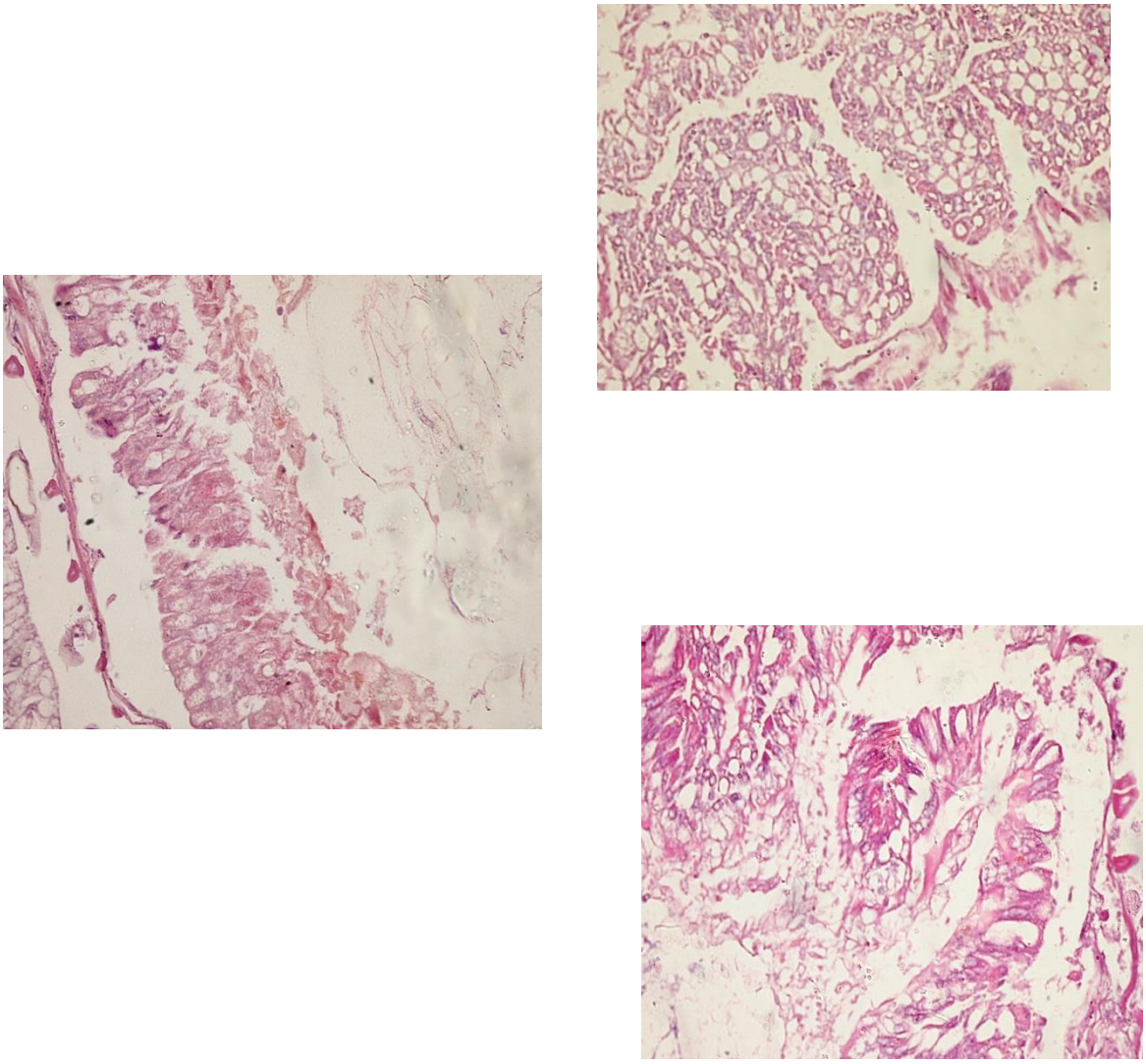


Fig (3) Histological a change of mid gut of 4th instar of *S. littoralis* that was treated by bee venom

The effectiveness of bee venom on the 4th instar in treatment after 5 days showed the same lesions as in treatment after 3 days but more sever.

The effectiveness of bee venom on 4th instar of *S. littoralis* was agreed with (Bodláková et al., 2022), histological changes were similar to bee venom treatment on American cockroach, Periplaneta americana, induced large destruction of muscle cell ultrastructure.

On the other hand, these results with agreement with (Abd-El-Aziz et al., 2020) who detected the histological examinations as a result of effect of (Coragen, runner and Spinosad) on the 4th instar of *S. littoralis*. They observed destruction of both goblet and columnar cells of midgut. Epithelial layer showed detached of the basement membrane, separation of both basement and peritrophic membrane.

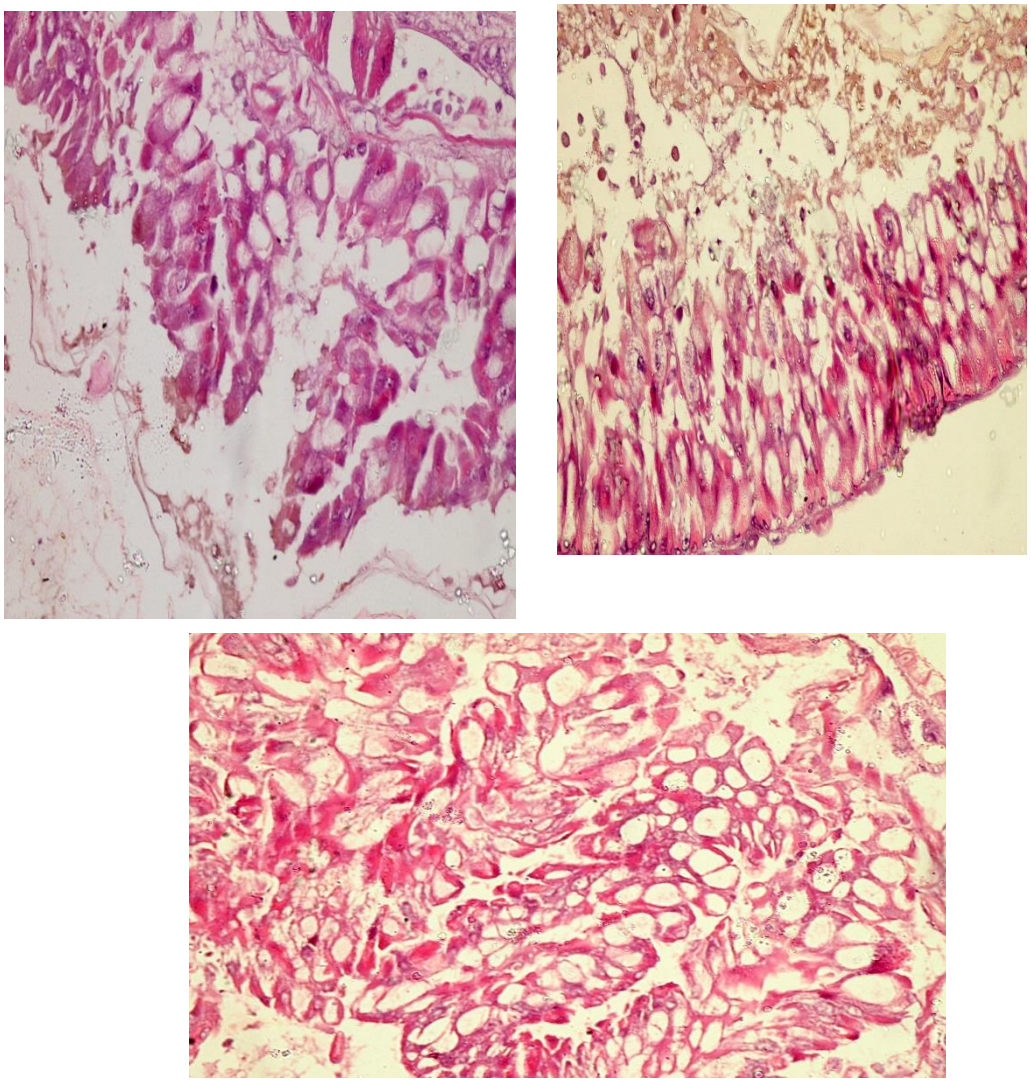


Fig (4) histological a change of mid gut of 4th instar of *S. littoralis* that was treated by bee venom

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