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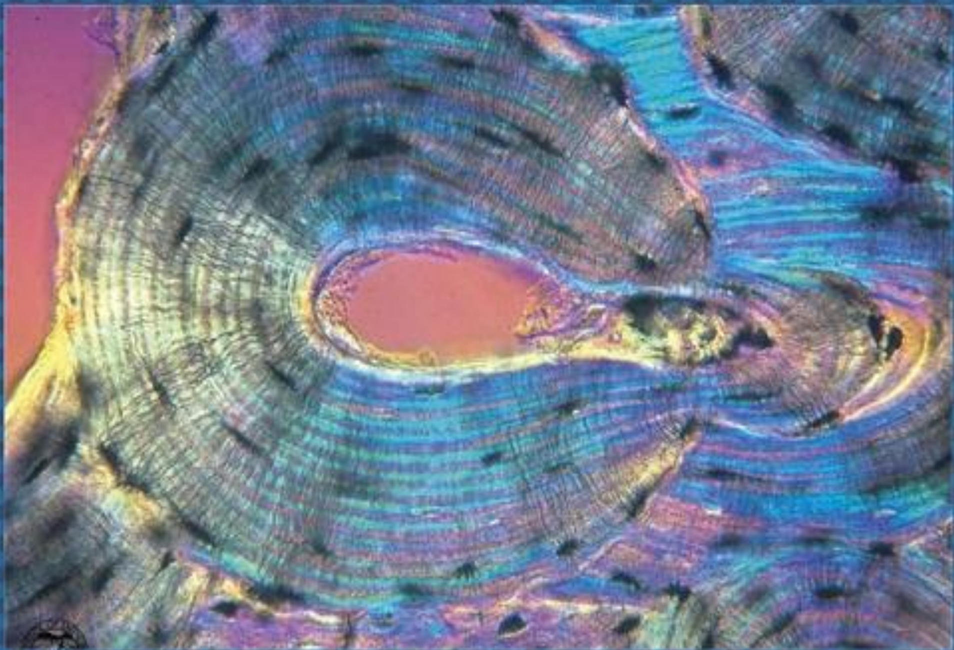

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Potentiality Assessment of The Activity of Scorpion Venom as An Insecticide Towards *Spodoptera frugiperda*: Histological and Electron Microscopy Studies

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ABSTRACT

There are thousands of arthropods around us in all environments, which are considered pests and cause many diseases in humans, animals, and plants. It also contributes to the damage to many agricultural crops around the world. Harmful arthropods are controlled using chemical pesticides, which cause great harm to human health and expose them to many risks. Modern science is now turning to the use of bio-insecticides, which use living organisms and their derivatives for control and are showing promising results. In this work, we used scorpion venom as an insecticide because scorpion venom contains many polypeptides that affect the prey and cause paralysis and death. We studied the outcome of scorpion venom on the larvae that infect corn sweet and cause many losses annually, and the venom as an insecticide showed promising results. Its composition is a rich source of small, highly effective insecticidal proteins that absorb or kill insects. Our results revealed that scorpion venom as an insecticide against *Spodoptera frugiperda* had a considerable and great effect. After four days of scorpion venom application on *S. frugiperda* larvae, macroscopical, histological, and scanning electron microscopy examinations revealed a significant reduction in insects until death. Conclusion: scorpion venom showed hopeful insecticidal activity and suitable toxic advantages as associated with other recognized insecticide sets.

INTRODUCTION

Arthropods have succeeded in invading all environments. Therefore, in the most diverse organisms, it represents more than 80% of animals (Abd El-Aziz, 2018, Ødegaard, 2000). Insects cause about fourteen percent of total crop losses and twenty percent of losses to grain food (Pimentel, 2009; Oerke& Dehne, 2004). *Spodoptera frugiperda* (J.E. Smith, 1797) (Order: Lepidoptera, Family: Noctuidae), or the Fall Armyworm, is a serious and critical pest of corn. For this purpose, corn-sweet production worldwide remains at risk. It has been considered a food security risk (Sagar *et al*., 2022).

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It can cause losses of 8.3 to 20.6 million tons in corn yield annually, which represents 21:53% of all production (Day *et al.,* 2020). *S. frugiperda* was first discovered in 2016 on the African continent, starting in West and Central Africa (Goergen *et al.,* 2016; Ahissou *et al*., 2021), Rwanda (Uzayisenga et al., 2018), Senegal (Brevault *et al.,* 2018), Sudan (Ebadi, 2022), and Egypt (Youssif, 2021; Rashed *et al.,* 2022). *S. frugiperda* has been recorded as potentially causing maize yield losses of 8–20 tons per year, equivalent to US$13 billion per year, in 12 African countries (Day *et al.,* 2020). Presently, arthropod venoms are documented and considered the greatest important resources of bioactive molecules, while most of this arsenal is still unknown. (Pimenta & Lima, 2005). Peptides and acylpolyamines represent the chief neurotoxins, and they have been utilized as material, to expand information about the mechanisms of molecular neurotransmission (Mortari *et al.,* 2007). The research on venom neurotoxins has focused on the innovation of original bioinsecticides; furthermore, the progress of tools for the investigate of therapies prospect suitable for the cure of neurological disorders (Esperanza *et al.,* 2022). Over thousands of years of evolution, they have developed toxic evolutions of biological organic complexes that paralyze and/or kill other organisms, with a vital role in providing nutrition and defense against predators. Arthropod toxins are made by a variety of biologically organic mixtures, such as phytoncides, peptides, acylpolyamine toxins, and biogenic compounds (Rash & Hodgson, 2002). A number of arthropod pests represent vectors for transmitting infections to humans, animals, and plants (Nauen, 2007). Spiders and scorpions contain venoms that contain a mixture of peptides that they release from predators, select prey, and ingest during their nights. They are units against bacteria, fungi, viruses, and protozoa (Wang and Wang, 2016). Another possible function is defense against pathogens, which is often ignored in the animal toxicology literature.

Hence, we imagine that microbes reaping their adaptive properties is only a byproduct. However, there are very few studies that have worked on the role of ecological scorpion venom effects in interactions between guests and pathogens. (van der Meijden *et al.,* 2017; Schendel *et al.,* 2019). Scorpion was the first animal to arise from the marine toward soil life about 350 million years ago (Froy &Gurevitz, 2003). Since that time, its protein venoms have varied greatly in sequence as they affect high-voltage sodium channels. However, these Peptides keep a certain linked by 4 disulfide bonds owing to evolutionary leads to a high permanence and an aptitude to tolerate mutations in the amino acid sequence (Gurevitz *et al.,* 2001). In this era of increasing wants to increased agricultural production and the inevitable use of insecticides and herbicides, the low doses required for insect repellent and insect repellent poisons have increased their potential for controlling insect pests. These toxic peptides typically reach their target receptors via voltage-gated sodium channels when a scorpion stings its prey or enemy (Bosmans *et al.,* 2005). Thus, any attempt to practice these toxins scientifically needs advantaged resources to transport them to the circulatory system of the target animal. Simple formulations cannot meet this necessity, as toxic polypeptides do not spear the insect's gut and withstand the harsh conditions of this environment. For this aim, it has become clear that we must adjust their toxin's overall bioavailability (Trung *et al.,* 2006). Now the world and modern science are turning to using bio-insecticides that use living organisms and their derivatives in control to protect against harm to humans and their animals, and studies have shown promising results. Abdel Aziz *et al.* (2023) worked on the hexane extract of

Sargassum latifolium as a new source of pesticides and a biologically active insecticide. In this work, we used scorpion venom as an insecticide because scorpion venom contains many peptides that it wants to get rid of and cause paralysis and death. We performed the excrement on *Spodoptera frugiperda* larvae, which caused a deleterious impact on the crops (Fig. 1).

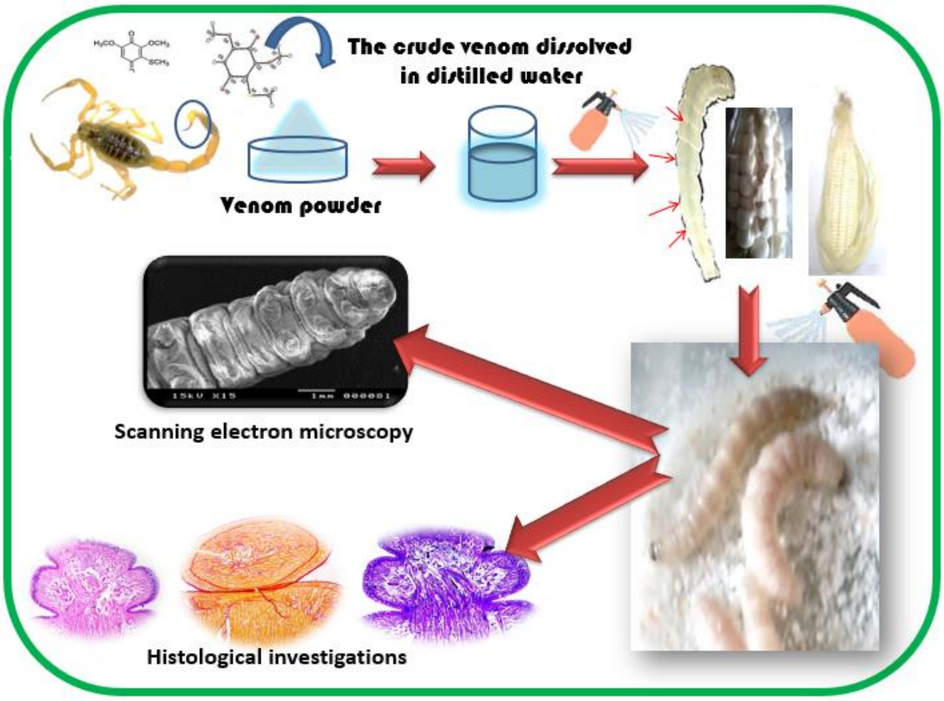

Fig. 1: An illustration of the observational research.

MATERIALS AND METHODS

1-Experiment Design and Insect Larvae Source:

Scorpion venom powder was obtained from company Egyptian animals (Abu Rawash), while the fall armyworm (*Spodoptera frugiperda*) in this study was collected from Assiut Farm. The abovementioned insects were developed under typical survival situations as surveys (25 to 28 °C) with 12 successive hours of dim and sunny, and all groups were fed corn powder. For 15 days.

2-Estimate of the Spray Insecticidal Activity of Scorpion Venom:

The samples (insects) were placed in a Petri dish. In laboratory conditions, insects are surrounded by small pieces of wet filter paper to ensure good environmental humidity. So that the larvae do not dehydrate and die. We put in two grams of corn powder to feed the insects. From scorpion crude venom 100 µg dissolved in 200 µL distilled water (0.5 % concentration) was sprayed on insects to penetrate body pores twice daily for four days (60 ± 3 mg of the body weight by a spray of 1 µL of the sample solution in distilled water on insects). Fifty insect larvae were used for each group (Group 1: control without exposure to insecticide, and Group 2: treatment with exposure to insecticide).

3-Morphological Examination:

The impacts of scorpion venom on *Spodoptera frugiperda* were detected.

4-Histological Investigations:

The fresh circulatory hemoglobin of *S. frugiperda* larvae was taken from the tip of the end. Spread on slides and dry quickly as usual. It was then fixed in absolute methyl alcohol for 2-3 minutes, after which it was allowed to dry in a slanting position and then stained with H&E stain. Five µm of tissue from separate groups and scorpion The venom apparatus was put on slides and let to dry overnight. at 37°C. After being put in xylene for dewax and hydrated in precise alcohol sequences, they were stained with Hematoxylin and Eosin (Drury & Wallington, 1980), Masson's trichrome (Kiernan, 2008), and Sirius Red (Sweat *et al.*, 1964).

5-Scanning Electron Microscopy (SEM):

For 1.5 hours, samples of untreated and treated insects and larvae were located in phosphate buffer solution (50 mM, pH 7.2) and 5% glutaraldehyde in sodium cacodylate solution (for fixation it puts on cover slides). Dry in ethanol after rinsing in distilled water. Critical point drying was carried out already; they were then fixed in an osmium tetroxide solution of 1% (w/v) in a similar buffer for 48 hours at 4 °C. The Joel JSM 35 Scanning Electron Microscope was used to analyze the samples after mounting them on carbon or gold-coated stubs.

6-Mortality:

Mortality of *Spodoptera frugiperda* exposure to scorpion venom after exposure to organophosphate insecticide, the insects were gently transferred to a petri dish covered with wet filter paper for moisture at 25°C. When the mouth revealed no reaction, the larvae became paralyzed and unable to move. The insect was documented as having died, and the time of death was recorded.

7-Statistical Analysis:

Investigations were conducted in triplicate. Using Prism 8 statistical software and a one-way ANOVA, the significance of variances among the control group and the other three treated groups was evaluated. Significant or very significant data were defined as $P \leq 0.05$ or ≤ 0.01 accordingly.

8-Ethical Statements:

The Ethics Committee of the Assiut University Faculty of Veterinary Medicine, authorized this study (06-2023-0131). ARRIVE criteria were adhered to in all operations carried out during the study that included animals.

RESULTS

1-Morphological and Histological Examinations:

a)-The scorpion's venom apparatus:

Based on the results, we obtained the morphology and histology of the scorpion's venom apparatus (*Leiurus quinquestriatus*). The venom apparatus consists of a bulbus vesicle-equipped stinger. The yellowish vesicle and globose. The vesicle has a sharp point, and is shallow, arched, and shorter than the brown aculeus (Fig. 2A). Histological investigation of the venom apparatus showed that two entirely distinct, two-sided venom glands, one on each side of the midline, were found in the telson's vesicle. The thick cuticle covering the telson is composed of two layers: the endocuticle is located within and the exocuticle is located outside. (Fig. 2, B, and C).

b)-Larvae of *Spodoptera frugiperda*:

To determine the effect of scorpion venom on the biological efficacy of the larvae of *S. frugiperda*. Based on our observation, the feeding behavior of all insect' larvae was feeble in all treatments after insecticide exposure in comparison with control. Morphological symptoms appear suddenly, including the inability to move, yellowish-white color, and swelling of the larval body due to obstructive body structures in the affected skin, with no tearing of the skin after death. Larvae exposure to insecticides rapidly developed a brownish color, a waxy appearance, and liquefaction of the larvae (Fig. 3). We further investigated the impacts of histopathological observation of scorpion venom on *S. frugiperda* larvae using wax histology. The sections demonstrated any clear indications of damage or different morphology of the tissue (Fig. 4, A, C, and E) among those under control. We saw a full basement membrane, a layer of visceral or striated muscle, and consistent patterns of epithelial and goblet cells. Grade I measurements were taken in the typical control area. Different degrees of tissue damage emerged following treatment with scorpion venom as an insecticide. These included sloughed epithelial cells, enlarged lumen, partial or whole endothelium displacement, and ulceration with endothelium bleeding. (Fig. 4, B, D, and F). A comparative photomicrograph represents *S. frugiperda's* hemocyte cells; both live and dead, are exposed to scorpion venom. The viability of the hemocyte cells was observed by an H&E staining analyze (Fig. 5, A, B).

2-Scanning Electron Microscopy Examination:

In this work, scanning electron microscopic examination has shown that viable cells prevent stain diffusion, although dead cells stain dark because they penetrate the stain through the fragmented cell membrane (Fig. 5, C, and D). Electron microscopic examination revealed S. frugiperda on corn plants in more detail. We noticed the external appearance of the control group is intact, and there is no damage. The mouth parts are intact (Fig. 6, A, B), but in group 2 which was exposed to the insecticide, there are clear deformities in the mouth parts and complete closure of the mouth and in the body as a whole with the presence of deformities. Ulcers in the back of the body under SEM (Fig. 6, C, D).

3-Mortality:

S. frugiperda larvae were highly susceptible to scorpion venom insecticide, with 14% mortality on the first day (<0.0001), 23.255%, on the second day (<0.0001), 45.45% and on the third day (0.0213) and 100%, full mortality after four days only (<0.0001) (Fig. 7).

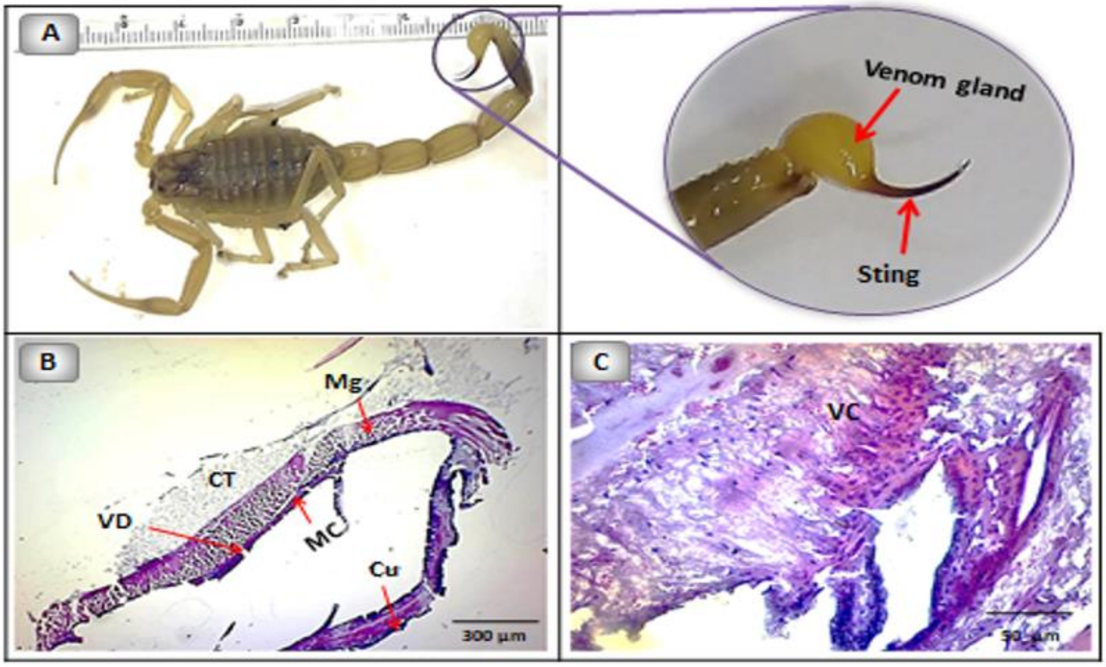

Fig. 2: Photograph of scorpions (*Leiurus quinquestriatus*) and venom apparatus of scorpion showing, :(A) Light micrographs of venom glands of (*L. quinquestriatus*) showing their histological structures, (B) T.S. of venom glands showing their histological structure and (C) T.S. of scorpion´s venom gland showing venom-producing cells with different types of granules. (**Cu:** Cuticle, **MC:** mucous cells, **VD:** Venom duct, Mg**:** mucous granules, **VC:** venom-producing cells, **CT:** Connective tissue) (Stained H&E).

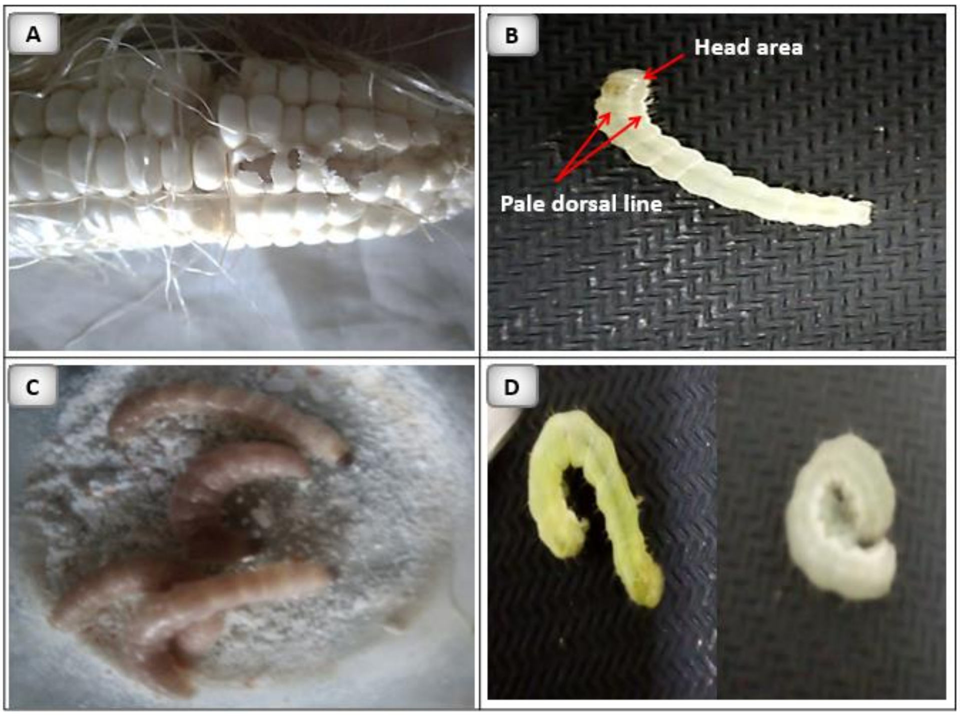

Fig. 3: Photograph showing (A):fall armyworm (*Spodoptera frugiperda*) ; larva on maize cob,(B): fall armyworm (*Spodoptera frugiperda*) larva ,(C): scorpion venom as an insecticide towards *Spodoptera frugiperda* and (D): *Spodoptera frugiperda* larva after exposure to scorpion venom.

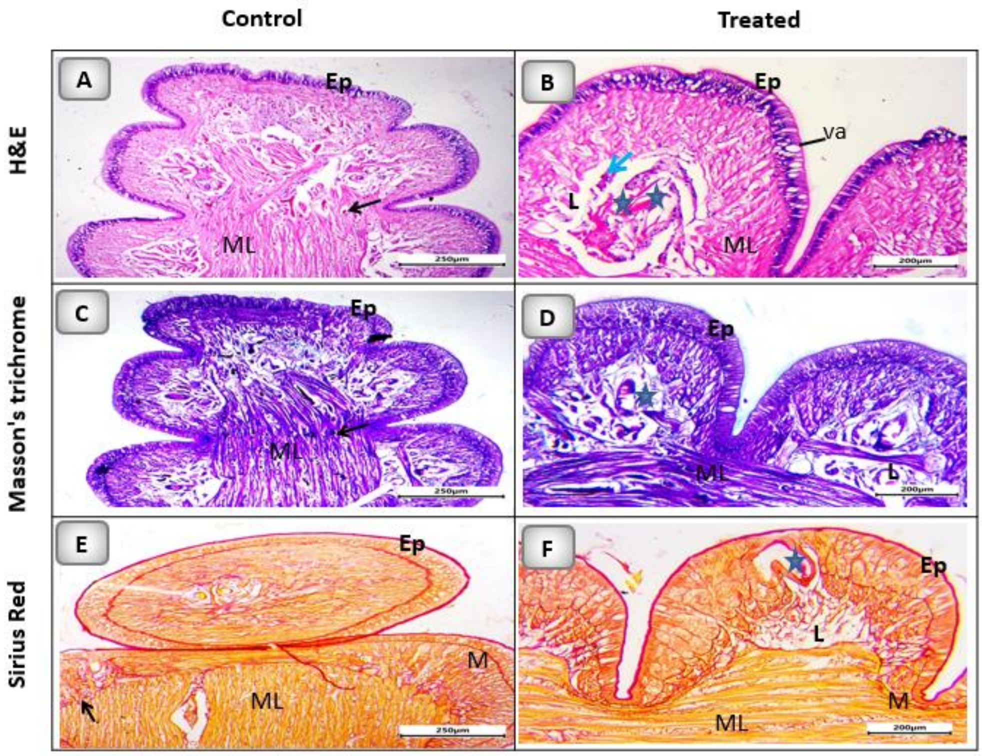

Fig. 4: Histological longitudinal section of *Spodoptera frugiperda* larva (A, C, E): control without exposure to insecticide and (B,D,F): treatment with exposure to insecticide. Stained with hematoxylin and eosin (H&E), Masson's trichrome and Sirius Red.

(**Ep:** epithelium, **L:** lumen, **M:** membrane, **Ml:** muscle layer, **Va:** vacuolation, An asterisk (*) denotescellular damage and displace-ment into the lumen, bluearrows point to haemocytes or immune cells within the body cavity (haemocoel) and ablackarrows point to granules).

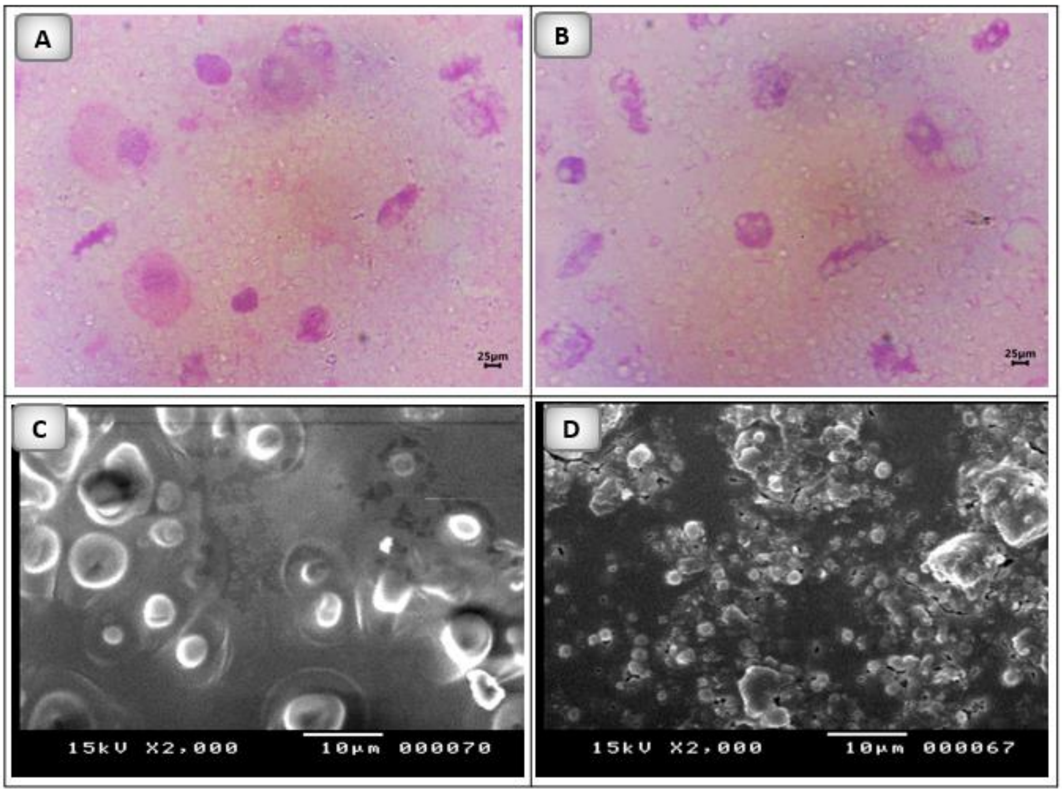

Fig. 5: Effect of Scorpion venoum toxin on the total circulatory hemocytes of *S. frugiperda* larvae, Comparative photomicrographs showing(A): live cells and (B) dead cells, Stained with hematoxylin and eosin (H&E), Comparative Scanning electron photomicrograph showing (C): live cells and (D) dead cells.

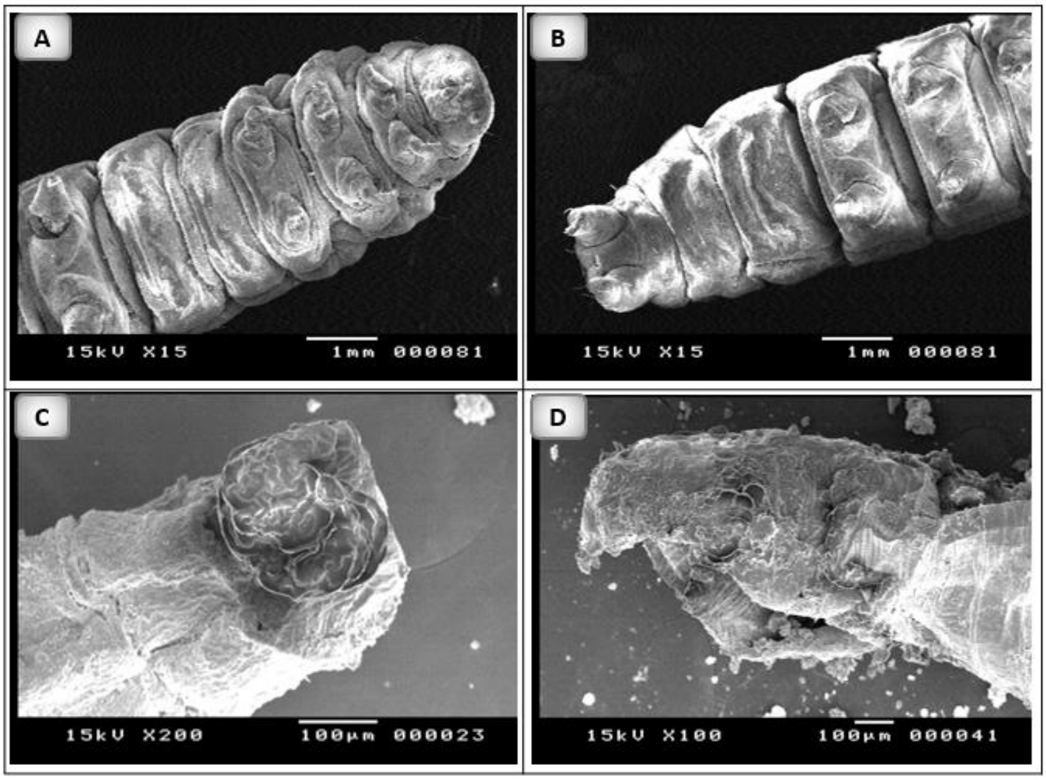

Fig. 6: Scanning electron photomicrograph of *Spodoptera frugiperda* larva, control without exposure to insecticide (A): anterior part, (B) posterior part and treatment with exposure to insecticide (C): anterior part, (D) posterior part.

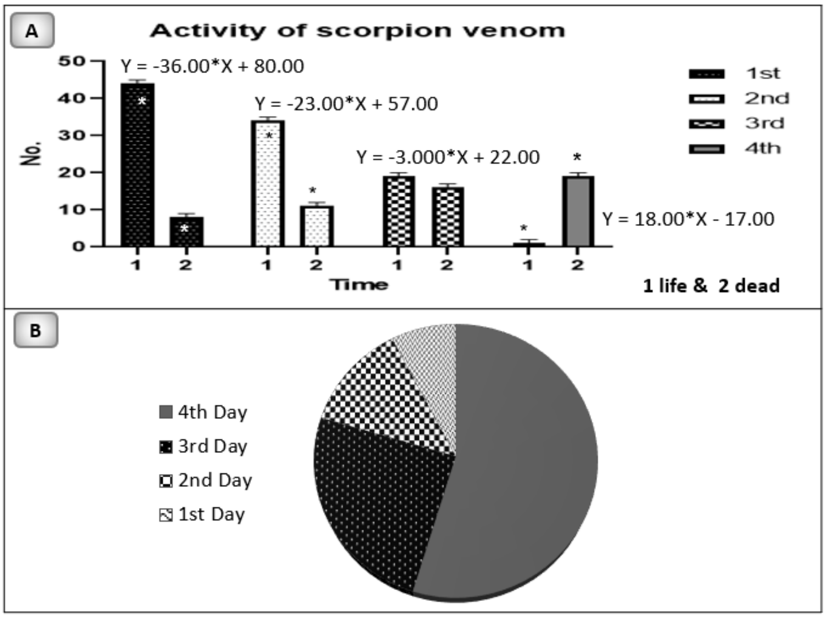

Fig. 7: (A): Response curves depict the number survival of *S. frugiperda* after exposure to scorpion venom insecticide and (B) Percentage (%) of insect mortality during the four days. Significant increased (N=50, P<0.001).

DISCUSSION

Although the sting of scorpion species found in many places in the world represents a medical problem, many types of external characteristics have been described. About one thousand five hundred species of scorpions have been described. There are only about twenty-five species worldwide that are considered dangerous to humans. In the present study, we described scorpions (Behçet *et al.,* 2009), and the venom apparatus of scorpions (*Leiurus quinquestriatus*) by morphological and histological observations. These results are in agreement with previous studies by Abd El-Aziz (2019), who recorded two species of scorpions (*Leiurus quinquestriatus* and *Androctonus crassicauda*) in Luxor, Egypt, and Abd El-Aziz (2022), who worked on scorpions (*Leiurus quinquestriatus*). Characterizing scorpion venoms may open up a new world of chemicals that have potential uses in biomedicine. Scorpion venom involves a compound mix of biologically active peptides that are used to capture prey or defend against predators. Toxic peptides are divided into two main groups: non-disulfide-bound peptides and disulfide-bound peptides (Rodriguez de la Vega and Possani, 2005). According to Bosmans and Tytgat (2007), alpha toxins strongly depolarize the cell membrane at low dosages, which is followed by a reduction in excitability. They prolong excitatory cells' action potentials at greater concentrations, which results in paralysis and arrhythmia. When we find myoclonic or spastic muscle responses, this indicates the action of β-toxins (Chippaux, 2012). The severity of scorpion poisoning is connected to the presence of neurotoxins in the venom (Torres-Larios et al., 2000). In the present work, the insects were sprayed on their bodies from the outside with scorpion venom dissolved in distilled water (in order to obtain a group of proteins), so that the venom extends to the larvae through the body openings and reaches the circulatory hemocytes and nervous system of *S. frugiperda* larvae. According to observation by macroscopic investigations, when the larvae stop moving and are paralyzed and abstain from movement and food, this means that they have died, and the number of deaths was calculated during the four days. On the fourth day, the death rate was almost 100%, and this indicates the ability of scorpion venom as an insecticide. Similarly, Ortiz *et al.* (2014) in recent years, fall armyworm has recently begun to pose a serious pest to corn crops, especially with global climate change (Day *et al.* 2017). The growth of broad-spectrum pesticide resistance has complicated their chemical control. Though present control of *S. frugiperda* depends mostly on the use of a variety of insecticides, for this reason, the choice of active insecticides to control these larvae is essential, and the sensitivity of *S. frugiperda* populations to insecticides needs to be monitored (Thirawut *et al.,* 2023). Bioinsecticides as organic pest control methods for insects as more effective and secure substitutes for chemical insecticides are being researched. Ordinary organisms or their metabolic byproducts can be employed as biopesticides to manage insect infestations. Creating bioinsecticides is one way to address environmental issues related to long-lasting, broad-spectrum chemical insecticides and to offer new ways to deal with insect pests that are resistant to insecticides (Nauen, 2002). In this study, the histopathology of cross-sections of body tissues showed widespread damage and deformities with bleeding and gaps in some parts when compared to the normal control group. This result is in agreement with Bravo et *al.* (2011), Fabrick & Wu (2015), Liu *et al.* (2021), and Dutta *et al*. (2023).

Conclusion

Scorpion venom works by causing paralysis or destroying cells. The main goal of this research was to determine how to apply venom as an insecticide and how to benefit from it by killing insects without any effect on humans or other mammals. Natural toxins, such as scorpion venom, are of interest to researchers and are biologically degradable, so they do not accumulate in the ground or in drinking water, remain on the skins of vegetables, or endanger our health as modern chemical pesticides do. However, in future research, we will separate specific proteins and use them as insecticides in Nps or MOF form.

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