Larvicidal Efficacy and Histopathological Alterations in the Midgut of Saw-Toothed Grain Beetle Larvae, Oryzaephilus surinamensis (L.) (Coleoptera: Silvanidae), Induced by Melia azedarach Extract and Deltamethrin

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Abstract

There were three distinct concentrations of the chemical herbicide that were utilized: 0.2, 1.0, and 0.5 milliliters per liter. An example of a control was water that had been distilled. There was a treatment period of 24, 48, and 72 hours for each dosage. Moreover, the plant extract Melia azedarach was used in three distinct concentrations: two, one, and half a milliliter per liter). For each of the three strengths, distilled water served as the control, and the treatments were carried out for a duration of 24, 48, and 72 hours, respectively. For each treatment, there were three more larval samples that were utilized. Ten larvae were placed in each of the further samples that were taken. Using a dosage of 2 milliliters per liter of the chemical pesticide Deltamethrin, both the insecticide and the plant extract were highly effective in eliminating the bug over a period of 72 hours. After 72 hours, the plant extract performed more effectively when it was at a concentration of 2 milliliters per liter.

Due to the environmental hazards of chemical insecticides and the growing interest in Integrated Pest Management (IPM), the histopathological effects of both the synthetic insecticide deltamethrin (2.5%) and the ethanolic extract of Melia azedarach leaves were evaluated against the third-instar larvae of the saw-toothed grain beetle. Histological sections were prepared at a thickness of 4 \mum and stained with hematoxylin and eosin (H&E). Microscopic examination revealed that larvae treatment with the chemical insecticide led to body wall degradation, acute necrosis, and structural disintegration of the midgut wall, along with cellular debris accumulation within its lumen and muscular atrophy. Similarly, the M. azedarach extract induced severe structural destruction characterized by digestive tract deformation, cytoplasmic vacuolation in epithelial cells, and degeneration and rupture of the adjacent Malpighian tubules. These findings demonstrate the high efficacy of both agents in causing a fatal structural and functional collapse of the pest, thereby offering promising prospects for reducing the reliance on synthetic insecticides to ensure environmental safety.

The study results confirmed a direct relationship between increased concentration, exposure time, and mortality rate, and that *O. surinamensis* larvae were more sensitive to all the treatments under study.

Keywords: *Melia azedarach*, larva, chemical pesticide Deltamethrin, *O. surinamensis*, Histopathology, Midgut alterations

Introduction

O. surinamensis is a global food storage pest, prominent in tropical and subtropical regions, as well as being widespread in temperate climates due to the insect's ability to exploit various storage environments and the interconnectedness of international food supply chains. Recent analyses indicate that the species is now recorded in Tens of countries across Asia, Africa, Europe, North America, and Oceania, making it a cosmopolitan pest adapted to diverse crops and grains (1). Immediately following the head is a broad body segment bearing six sharp, tooth-like projections on either side, the most distinctive feature used by researchers to differentiate this beetle species. The head bears vertical antennae, and the three terminal nodes are relatively long and enlarged, composed of several segments that help the insect detect odors and locate food sources. It also possesses relatively small compound eyes, indicating a greater reliance on chemical senses rather than vision. *O. surinamensis* is characterized by a small body, typically 2.5–3.5 mm long, which is flattened and narrow, allowing it to squeeze into cracks and tight spaces within grains and stored products. The adult insect is predominantly dark brown or reddish-brown in color (2). Chemical control has proven ineffective due to the wide host range and the development of resistance to tens of insecticides (3). In addition to the emergence of many health and environmental problems and their negative side effects on living organisms and their danger to human health, it should be noted that 75% of the pesticides produced in the world are carcinogenic substances. Some researchers have resorted to using plant extracts as alternatives to chemical pesticides or complementary to them, as they are safe natural substances that are broken down by microorganisms and environmental factors in a short time, in addition to their high efficiency against insects and low toxicity to humans, mammals and beneficial insects (4).

Deltamethrin is a synthetic pyrethroid insecticide with a very high level of activity against a wide range of insects, including Lepidoptera, Hemiptera, Diptera, and Coeoptera., It works by direct contact and ingestion. It is mostly used to protect crops (85% of total production), of which 45% is used for cotton, 25% for fruit and vegetable crops, 20% for cereals, corn, and soybeans, and the remaining 10% for miscellaneous crops, such as coffee, corn, and hops (5).
The study of histopathological changes is a vital tool for evaluating the insecticidal efficiency of plant extracts and insect growth regulators (IGRs) by accurately determining their mode of action within the pest's body. The midgut stands out as one of the most critical target vital tissues, given its primary role in secreting digestive enzymes and absorbing nutrients. Exposure to these bioactive compounds induces severe damage to its epithelial cells, manifested by the destruction of the brush border, detachment of cells from their basement membrane, and disruption of the peritrophic membrane. Consequently, this results in complete functional impairment and total paralysis of the insect's digestive and physiological processes.(6)

Aims of the study:
1- Investigating the histopathological alterations induced by the alcoholic extract of Melia azedarach leaves and the chemical insecticide deltamethrin on the internal tissue architecture (midgut) of the saw-toothed grain beetle larvae, and comparing them with the normal condition
2- Investigating the compatibility of the chemical pesticide Deltamethrin and the plant extract of *Melia azedarach* for killing *O. surinamensis* larvae in vitro.

Materials and Methods

Study Location and Dates:
The current study was conducted in the Graduate Studies Laboratory, University of Samarra, College of Education, Department of Biology, from October 2025 to April 2026.

Laboratory Breeding of *O. surinamensis*
Sites in Salah al-Din Governorate/Samarra District were selected due to the presence of large numbers of *O. surinamensis*. This was attributed to the availability of stored grains that are infested by *O. surinamensis* during the winter months due to the insect's activity. These grains were chosen to be untreated with insecticides to ensure accurate results in the experiments conducted during our study in June, July, and August.
O. surinamensis was classified at the University of Baghdad / Research Center / Natural History Museum in document No. (10) dated (8/11/2025) Issue 67. *O. surinamensis* was collected in plastic containers prepared for this purpose with a capacity of 1 liter of grains infected with the insect and transferred to the incubator under laboratory conditions at a temperature of 26 ± 2 degrees Celsius and a relative humidity of 65 ± 5% and a light period of 10-14 hours. After that, it was placed in a Breeding cage of *O. surinamensis*, whose dimensions were 85 cm in length × 50 cm in width × 85 cm in height (7).

Preparation of the *Melia azedarach* plant extract for the control of *O. surinamensis*:
The extraction method used an organic solvent, namely petroleum ether, and a Soxhlet vacuum rotary evaporator (voltage 7).
The plant extracts were prepared in the laboratory of the College of Education for Pure Sciences - Samarra University. The seeds of the plants used in the current study were ground using an electric mill. 100 grams of *Melia azedarach* powder were taken separately and placed in the thimble (tube filter paper) of the extraction apparatus, which had a capacity of 500 ml. 250 ml of petroleum ether at a concentration of 95% were placed in the thimble, and the extraction was carried out for 48 hours at a temperature of 55-60°C.
After the extraction was completed, the extract was concentrated in the Vacuum rotary evaporator and the Scarlet device of the Central Laboratory for Graduate Studies at Samarra University, under a temperature of 55-60°C to get rid of the residue of the solvent used and until a thick gel-like liquid was obtained, i.e., after the alcohol evaporation stopped. The process was repeated more than once until sufficient quantities of the concentrated extract were obtained. The extract was then placed in dark-colored glass bottles and kept in the refrigerator until use (8).

Preparation of the required concentrations for the plant extract experiment:
To obtain the required concentrations or stock solution, 1 gram of seed extract from the aforementioned plants was taken and placed in a 100 ml glass container. 99 ml of distilled water was added, and then Twin 80 polysorbate was added dropwise to the mixture (distilled water and extract) until it formed a suspension that was easy to mix and spray. This resulted in a 1% solution, equivalent to 10,000 ppm. According to Dalton's dilution equation C1V1 = C2V2, the other required concentrations were prepared: 1000, 500, 100, and 10,000 ppm for the plant extracts. The control was made using distilled water only.

Preparation of Deltamethrin chemical concentrations for controlling *O. surinamensis* larvae
Different concentrations of the chemical pesticide Deltamethrin, namely 2, 1, and 0.5 ml/L, were prepared according to the recommendations of the pesticide manufacturer. 1000 ml of distilled water was added to each of the experimental concentrations. In the control laboratory, only distilled water was used

without the addition of the pesticide. For each dose, three copies were used, with 10 larvae of *O. surinamensis* for each replicate after 24, 48, and 72 hours. The results were recorded, and it was found that the highest percentage of *O. surinamensis* killing was after 72 hours at a concentration of 1 ml/L. The insecticide works by paralyzing the insect and preventing feeding, thus killing it. It also works by damaging the insect's digestive system through food contamination, causing the digestive tract of the targeted insects to rupture.

The recommended dosage for the growth regulator is 0.8 ml/liter. The insecticide should be diluted with water. The safety period is 3 days, and it can be diluted with water according to the manufacturer's recommendations on the insecticide container.

Statistical Analysis:

The results were statistically analyzed using MINITAB software (version 17) according to the analysis of variance (ANOVA) test. The arithmetic means were compared using Duncan's multiple range test, at a significance level of ≥ 0.05 ($p \geq 9$).

Results and Discussion

Table (1) shows the effect of the alcoholic extract of *Melia azedarach* on the larvae of *O. surinamensis*

Average concentration	hours 72	hours 48	hours 24	Concentration
12.2 c	20.0 d	13.3 d	3.3 d	0.5
52.2 b	73.3 b	50.0 b	33.3 c	1
84.4 a	100.0 a	86.7 a	66.7 b	2
	64.4 a	50.6 a	34.4 b	Average time
0.0 d	0.0 c	0.0 c	0.0 c	Control

The variation in the killing rates was primarily attributed to the synergistic effect of the concentration against the duration of the exposure. The result was an initial killing rate of 3.3% after 24 hours of treatment and a final killing rate of 100% after 72 hours of treatment or exposure to the chemical (anti-midge). The average killing rate at the highest killing concentration of2 was 84.4% while the average killing rate produced by concentration one was 12.2 % , thus concentration two produced the greatest average overall killing rate. The average killing rate corresponding to the 72 hours and 24 hours of exposure to the chemicals were 64.4% and 34.4%, respectively. These results indicate that the larvae killed increased as the concentrations and lengths of exposure increased..

The results of our current study were similar to what (10) found when mixing powders of *Azedirachta indica*, *Zanthoxylum zanthexyloides* and comparing them to the compound Pirimiphosomethyl with cowpea seeds and feeding them to the southern cowpea beetle. The first plant was more effective in reducing the female's ability to lay eggs, and the powders used had a toxic effect on the seeds for 5 months.

The results of the current study contradicted the findings of (11) that the use of *Melia azedarach* fruit powder at a concentration of 2 to protect stores from the red flour beetle *Tribolium castaneum* led to positive results similar to the effect of the comparable lindane powder, and that treating these insects with this compound caused them to slow down in growth compared to untreated insects.

The study's findings aligned with what (12) had concluded regarding the toxicity of *Melia azedarach* plant extract in adult predators, *Coccinella septempunctata*, by feeding the predator on castor leaves after covering them in the alcoholic extract of *Melia azedarach* plant at a concentration of 1.5%, where the mortality rate for the predator was 3.3% compared to the mortality rate for the predator of 0.1%, which was 93.3%.

Table (2) shows the effect of the insecticide Deltamethrin on the larvae of *O. surinamensis*

Average concentration	hours 72	hours 48	hours 24	Concentration
11.1 c	16.7 d	13.3 d	3.3 d	0.5
38.9 b	53.3 b	40.0 b	23.3 c	1
72.2 a	86.7 a	73.3 a	56.7 b	2
	52.2 a	42.2 a	27.8 b	Average time
0.0 d	0.0 c	0.0 c	0.0 c	Control

Because of the interaction between dose and exposure period, Table 2's fatality rates varied greatly. After 72 hours of therapy, the chance of mortality was 86.7%, and after 24 hours, it was just 3.3%. Death rates were most significantly impacted by concentration, with 72.2% of deaths occurring at concentration 2 and just 11.1% at concentration 1. The average fatality rate from contact time was 52.2% after 72 hours. It was just 27.8% after a day. According to the findings, O. surinamensis mortality increased with both amount and contact time.The findings of study (13), which used dichlorvos insecticide to control adult sand flies, showed that the insecticide achieved the highest mortality rate, ranging from 46.77% to 50.55% after 48 hours.

The results of the current study are consistent with those of study (14), conducted in Sudan to determine the sensitivity of sand flies (*Phlebotomus papatasi*) collected from three regions of Sudan to various insecticides, including permethrin, DDT, malathion, and propoxur. This study demonstrated the insect's inability to resist the toxic effects of malathion in two regions, while exhibiting resistance in the third region, respectively, after 24 and 48 hours of larval treatment.
The results of the current study are consistent with those reported by (15) when cypermethrin was used on the larvae of the watermelon fly,

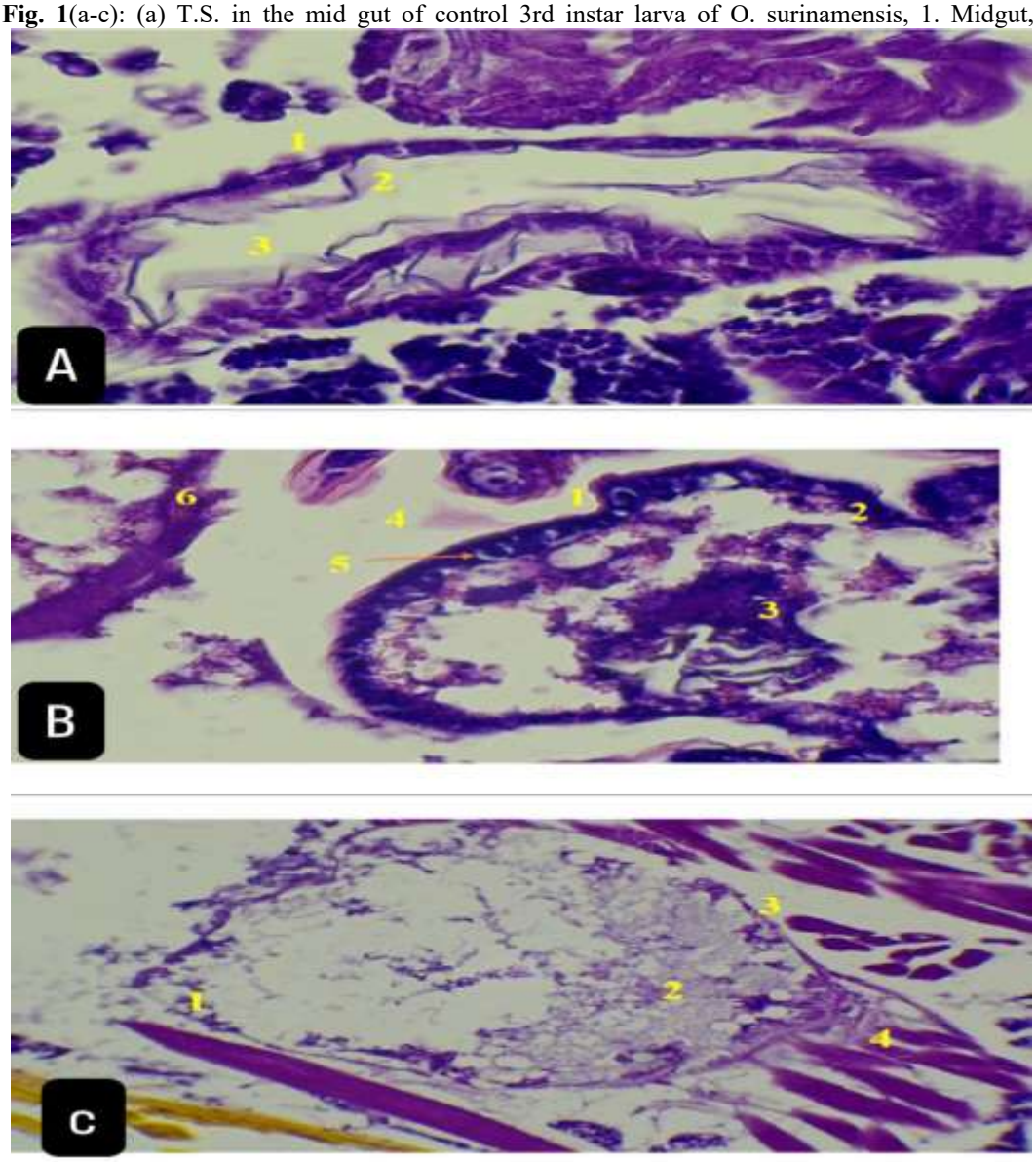
B.diversa. It was observed that the mortality rate increased with the duration of exposure to the toxin, reaching 50% when the exposure period was extended to 24 hours.
The results of the current study also agree with those of (16), whose study demonstrated significant differences in pupal mortality rates after 24 and 48 hours of treatment with malathion.
The results indicate a clear and significant difference in mortality rates compared to the control treatment, in which no deaths were recorded (0.0%). This suggests a strong positive correlation between increasing the concentration used and the duration of exposure, leading to a significant increase in the mortality rate of third-instar larvae of *O. surinamensis*.
The results also indicated that the second larval stage of the insect was more sensitive to the insecticide's effect on increasing cumulative mortality. The effect of the insect growth regulator Match on increasing mortality in immature stages may be attributed to its role as a larval growth inhibitor, as the mortality rate of hairy grain beetle (Khabra) larvae hatched from eggs treated with the insect growth regulator Match at a concentration of 0.75 ml/L and the insect growth regulator *Trigard Cyromazine* at a concentration of 0.30 g/L was 100%. However, prolonged larval development and deformities were observed with treatments using Match at a concentration of 0.25 ml/L and *Trigard* at a concentration of 0.70 ml/L.

Histological Preparation and Staining:
Histological sections (4 \mum thick) were prepared according to the method of Al-Haj [14]. Briefly, tissue specimens were fixed in 10% formalin for 2–4 hours, washed with running tap water, and dehydrated through a graded series of ethanol (70%, 80%, 90%, and 100% absolute). The specimens were then cleared in xylene, embedded in paraffin wax at 65°C, sectioned using a microtome, and mounted onto glass slides after flattening in a water bath at 40°C. Subsequently, sections were dewaxed in an oven. The tissue sections were then stained with Hematoxylin and Eosin (H&E) according to the protocol of Bancroft and Stevens [17]. This involved complete dewaxing in xylene, rehydration through a descending series of ethanol, and staining with hematoxylin for 5 minutes. After differentiation in acid alcohol, the sections were counterstained with eosin for 15 seconds. Finally, the slides were dehydrated through an ascending series of ethanol, cleared in xylene, mounted using DPX medium, and examined under a light microscope, revealing blue-stained nuclei and pink-stained cytoplasm.

Results

Histological sections of the third-instar larvae of the saw-toothed grain beetle (control treatment) demonstrate a normal, healthy, and well-integrated midgut architecture; where Figure (1, A) shows the integrity of the intestinal cells, Malpighian tubules, adipose tissue, and muscular bundles, with the brush border remaining continuous, dense, and undamaged, which completely aligns with the standard descriptions previously documented [18].
In contrast, the alcoholic extract of Melia azedarach leaves induced structural destruction and acute pathological alterations; as Figure (1, B) reveals a deformed digestive tract wall with a loss of its regular boundaries, accompanied by necrosis, cellular damage, and accumulation of debris within the lumen. This is associated with the atrophy of smooth muscles, cytoplasmic vacuolization around the nuclei, and degeneration of the adjacent Malpighian tubules, and these damages agree with what has been scientifically recorded [19] regarding effects that cause a fatal structural and functional collapse.
Concurrently, treatment with 2.5% deltamethrin insecticide resulted in a more severe illness and destruction; where Figure (1, C) exhibits acute necrosis and complete cell death of the epithelial lining, disintegration of the midgut wall, and accumulation of necrotic debris within the lumen, accompanied by atrophy of the intestinal muscles and advanced degeneration of the skeletal muscles and body wall, which impairs movement and feeding. These indicators correspond with previous findings [20] showing that the insecticide causes rupture of the protective cuticle, degeneration of muscle fibers, and complete erosion and destruction of the epidermis.

Fig. 1(a-c): (a) T.S. in the mid gut of control 3rd instar larva of O. surinamensis, 1. Midgut, 2. Malpighian tubule, 3. Adipose tissue, 4. Skeletal muscles, (b) T.S. in the mid gut of 3rd larva of O. surinamensis treated with alcoholic extract of Melia azedarach leaves, 1. Digestive tract with irregular boundaries, 2. Necrosis of a part of the digestive tract wall, 3. Accumulation of necrotic debris within the lumen, 4. Atrophy of the smooth muscles forming the digestive tract wall, 5. Cytoplasmic vacuolization around the nuclei of degenerated epithelial cells, 6. Degeneration of Malpighian tubule walls and (c) T.S. in the mid gut of 3rd instar larva of O. surinamensis treated with 2.5% deltamethrin, 1. Necrosis in the midgut wall, 2. Accumulation of necrotic debris (cellular debris) within the lumen, 3. Atrophy of smooth muscle bundles forming the digestive tract wall, 4. Clear degeneration in skeletal muscle bundles near the digestive tract.



Conclusions

1- The chemical insecticide Deltamethrin demonstrated high efficacy in killing both larvae and adults of the insect, resulting in the death of *O. surinamensis* three days after treatment, with a direct relationship between concentration and mortality rates, It caused acute necrosis and complete cell death of the intestinal epithelial lining, along with the disintegration of the midgut wall and atrophy of its muscles.

2- The alcoholic extract of *Melia azedarach* was effective against *O. surinamensis* larvae in vitro three days after treatment, with a direct relationship between concentration and mortality rates, It induced structural destruction and deformation of the digestive tract wall, along with cellular necrosis, vacuolization, atrophy of intestinal muscles, and degeneration of Malpighian tubules.

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