Annals of Biology **38** (1) : 11-19, 2022

Impact Score : 0.32 (Scopus)

Estimation of Biochemical and Ultrastructural Alterations as Biomarkers in *Spodoptera littoralis* Exposed to a *Zygophyllum album* Fraction

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(Received : January 14, 2022; Accepted : March, 21, 2022)

ABSTRACT

One integrated pest management strategy to control *Spodoptera littoralis* (Lipedoptera : Noctudiae) includes the application of naturally originating insecticides. For the first time the mode of action of a fraction isolated from the aerial parts of *Zygophyllum album* plant as an ethanolic extract against *S. littoralis* has been reported. The fraction was separated using column chromatography and recognized with gas chromatography–Mass spectrometry (GC-MS). Scanning and transmission electron microscopes were used for further analysis, the structural and biochemical changes that occurred in *S. littoralis* larvae due to the fraction application were assessed as biomarkers. GC-MS technique showed that pinocarvone (13.67%) and E-15-heptadecenal (11.47%) were the principal compounds of the *Z. album* fraction. Also, such fraction had ovicidal and larvicidal activities against *S. littoralis*. Compared with control larvae, abnormalities in the head capsule, antennae, spiracles and integument were observed in the treated larvae. Moreover, the treated larvae showed significant inhibition of some biochemical activities e. g. amino acid transferases (AST and ALT), chitinase enzymes, and total soluble protein content. Additionally, the tested fraction affected some biological parameters such as the incubation period of eggs, larval mortality, duration of larvae and pupae and weight of pupae. The *Z. album* fraction proved to be a promising naturally derived agent for the control of *S. littoralis* larvae and eggs.

Key words : *Zygophyllum album, Spodoptera littoralis*, larvicide, ovicide, ultrastructure

INTRODUCTION

The insect pest species *Spodoptera littoralis* (Boisduval) (Lepidoptera : Noctuidae) has several common names, including the Egyptian cotton leafworm, tobacco cutworm, Mediterranean brocade moth and African cotton leafworm. The species appeared in Egypt and Africa and Asia's temperate zones and has moved eastwards into Turkey and northwards into eastern Spain, southern France and northern Italy. The range of hosts for *S. littoralis* is diverse and covers over 40 families containing at least 87 species of economically important crops. Therefore, EPPO (2015) listed *S. littoralis* as an A_2 quarantine pest, while organizations such as CPPC, NAPPO and OIRSA also consider it an important quarantine pest. In an attempt to control this pest, farmers have applied substantial amounts of synthetic chemical pesticide, which has led to the appearance of resistant strains and pollution of the environment that has influenced many non-target organisms. Recently, researchers have intensified their efforts to discover novel control agents from natural origins that could remove or minimize pesticide usage e. g. "green pesticides" extracted from natural plant sources. The effects of such pesticides have been examined in a broad range of insect species for their potential inclusion in integrated pest management programs (Khedr, 2016; Mead *et al.,* 2016).

In Egypt, especially in desert environments, there is a wealth of wild plant species with medicinal and pharmaceutical properties. The *Zygophyllum* genus contains such species, which are considered some of the most important desert vegetation components and widely used in traditional medicines. Many plants belonging to this genus have antioxidant, antitumor, antimicrobial, antidiabetic and anti-inflammatory activities (Mnafgui *et al.*, 2016; Barzegar *et al.*, 2018), which are in part due to their phytochemical constituents include flavonoids, phenols, terpenes and sterols (Abdel--Hamid *et al.*, 2016). *Zygophyllum album* L. (Zygophyllales : Zygophyllaceae) is one of nine *Zygophyllum* species recorded in Egypt. It is known to be toxic to the *Tuta absoluta* moth (Abderrahmene, 2020).

Recently, new microscopy analysis techniques, including the use of scanning or transmission electron microscopes, have helped researchers to understand several aspects of insect cuticle histology and morphology (Rajabi *et al.*, 2017) and subsequently explain the lethal effects of insecticides. However, to the best of our knowledge, the mode of action of *Z. album* toxicity has not been studied in *S. littoralis* in a similar manner. Therefore, in the present investigation, the chemical constituents of a *Z. album* fraction, obtained from the aerial parts of *Z. album* as an ethanolic extract, were identified. The effects of the fraction were assessed against the eggs and 4th instar larvae of *S. littoralis*. Specifically, the extract's larvicidal mechanisms were determined by evaluating biochemical and ultrastructural alterations as biomarkers.

MATERIALS AND METHODS

Aerial parts of *Z. album* were gathered from the Egyptian desert at North Sinai and identified taxonomically at the Herbarium of Flora and Phyto-Taxonomy Research (CAIM), Horticultural Research Institute, Giza, Egypt. All reagents or chemicals used in this research were purchased from Gomhoria Co., Egypt.

The aerial parts of *Z. album* were air-dried in shade at room temperature until they were fully dry. Subsequently, to produce the ethanolic extract of *Z. album*, 200 g of plant powder was soaked in one litre of 70% ethanol for three days at room temperature before being filtrated by filter paper (Whatman No. 1) then concentrated using a rotary evaporator (PV 05 Janke & Kunkel; IKA-Werke, Germany). Isolation of fractions containing the bioactive compounds of *Z. album* ethanolic extract was achieved by column chromatography (size : 100 × 2 cm). The column was filled with silica gel (60v-120 mesh; Alpha Chemika) and then hexane was added as a preelution step of the column. The crude ethanolic extracts of *Z. album* were dissolved in 5 ml acetone then vortexed in 100 mg silica until the acetone evaporated and a dry sample was obtained. This dry powder was poured into the top of the column through folded filter paper and covered with hexane solvent.

The fractions were clarified by increasing the polarity of the mobile phase as a combination of hexane and hexane : acetone (9 : 1, 8 : 2, 7 : 3, 6 : 4, 5 : 5, 4 : 6, 7 : 3, 2 : 8 and 1 : 9), ending with acetone alone. Coloured bands were collected individually. Fraction No. 9 was selected for the assay of toxicity against *S. littoralis*.

The bioactive constituents of fraction No. 9 were indentified using gas chromatography–Mass spectrometry (GC-MS) examination. The recognized compounds were represented as percentage of the relative peak zone, retention time and mass spectra based on National Institute Standards and Technology Willy library records for the GC-MS method.

S. littoralis laboratory strain, raised for more than 30 generations, was produced from egg masses and reared away from any pesticidal contamination at the Cotton Leafworm Department, Sharqia Governorate. These cotton leafworms were raised under stable circumstances of 27±2°C with 65±5% relative humidity to obtain insect cultures for use in the present study.

Ovicidal action : Four concentrations of the tested *Z. album* fraction were prepared by ethanol (70%) as a solvent (0.50, 1.00, 2.00 and 4.00% v/v). As the female moth beguan to lay eggs, egg masses three-day-old were dipped for 10 sec. in each tested concentration or the control (distilled water application only). The treated masses were left to dry in air then moved to Petri dishes (five masses per one dish). The eggs were inspected daily until two days after the control eggs hatched. The egg masses mortality percentages were monitored and then corrected.

To evaluate the larvicidal action of the *Z. album* fraction against freshly molted fourth instar larvae of *S. littoralis*, three serial concentrations of the fraction were prepared using 70% ethanol : 4, 7 and 10% v/v. Freshly collected castor bean leaves were bunched, and dipped in the chosen concentrations for 10 sec for each before being dried at air temperature (i. e. the leaf dip technique).

Newly molted fourth instar larvae were moved to these treated leaves. Control discs were dipped only in 70% ethanol. Each examined concentration as well as the control were replicated three times (30 larvae per replicate). Larvae were fed on the treated discs for two days and then on untreated discs. Mortality percentages were detected at 72 h post-application and adjusted to estimate lethal values.

After treating fourth instar larvae for 72 h with the LC_{50} of the *Z. album* fraction, ultrastructural observations were made using both scanning and transmission electron microscope techniques. At least five treated and untreated individual larvae were examined with each type of electron microscope. The whole body, head capsule and body segments of larvae were observed. For transmission electron microscopy, ultrastructural sections were produced and stained to observe the cuticle under a JEOL 1010 Transmission Electron Microscope. Both SEM and TEM observations were completed in Al-Azhar University (The Regional Center for Mycology and Biotechnology).

Sample preparation : After 72 h of treatment, samples of *S. littoralis* (fourth instar larvae) applied with the LC_{50} fraction of *Z. album* and so the control were choosen for biochemical tests until use. For analyses, 5 mg of treated or control larvae were homogenized in distilled water using a chilled Glass Teflon Tissue Homogenizer (MPW-309, Poland) surrounded by a crushed ice for 3 min. The homogenated larvae were then centrifuged at 8000 rpm and 5°C for 15 min in a refrigerated microcentrifuge (Hettich, Germany) to eliminate hemocytes. Afterwards, the resulted supernatants were moved to clean tubes then kept at -20°C until they were needed. All biochemical assays were replicated three times.

Total soluble protein was assessed agreeing to Bradford (1976) via bovine serum albumin (standard). Aspartate amino transferase (AST; EC : 2.6.1.1) and alanine aminotransferase (ALT; EC : 2.6.1.2) enzymes were measured colorimetrically.

Chitinase (EC 3.2.1.14) activity was determined using 3,5-dinitrosalicylic acid reagent to identify the free aldehydic groups of hexoamines liberated following chitin digestion.

The LC_{50} of the *Z. album* fraction against fourth instar *S. littoralis* larvae was used to calculate specific some biological measurements that happened in the consecutive stages of treated larvae.

Three replicates were used to test the fraction and control (10 larvae per replicate) via the leaf dip technique (described earlier). Selected larvae were starved for 4 h and then transferred onto treated or untreated discs. The discs were replaced with fresh leaves after 48 h of treatment. Larvae were maintained under laboratory conditions and observed daily until pupation. Larval and pupal durations, larval mortality and pupal weight were assessed as parameters of the long-term bioactivity of the *Z. album* fraction.

To estimate LC values of the *Z. album* fraction, corrected mortality percentages (calculated via the calculated percentage of mortalities versus corresponding concentrations) were subjected to probit analysis. Biochemical and biological parameter data were statistically analyzed for significant differences between the untreated and treated groups using Student's t-test. All values were expressed as means ± standard error.

RESULTS AND DISCUSSION

The chemical constituents of the *Z. album* fraction are shown in Table 1 according to their retention times. GC-MS revealed 11 peaks corresponding to the main active ingredients (84.40%) of the fraction (Fig. 1). The major active components were pinocarvone (13.67%), E-15-heptadecenal (11.47%), cycloeicosane (10.96%), 14-hexadecenal (10.21%), 1-tetradecene (8.77%) and the phenol, 2-tert-butyl-4-isopropyl-5-methylphenol (8.43%).

In this study, 11 chemical compounds were recorded in the fraction of the aerial parts of the *Z. album* ethanolic extract, which represented 84.4% of the fraction. Nine of these compounds had C_{10} - C_{20} carbon atoms, and most were compounds previously reported as being responsible for toxicity and various activities against *S. littoralis*. For instance, in the range C_{10} - C_{20}, alkanes are absorbed in the small intestine.

Ovicidal activity : The data presented in Table 2 show that the tested *Z. album* fraction had ovicidal effects on three day old eggs of *S. littoralis*. The LC_{50} and LC_{90} values were 1.199 and 4.171%, respectively.

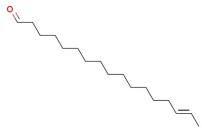
Fig. 2. illustrates the retarded incubation period of *S. littoralis* eggs after treatment with all tested concentrations of the *Z. album* fraction and relative to the incubation period in the control. The incubation period was three days for the control but up to five days for the 4% concentration of the fraction.

As shown in Table 3, the *Z. album* fraction

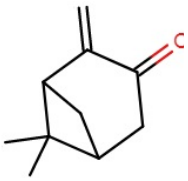
Table 1. Chemical constituents of the fraction of the aerial parts of *Zygophyllum album* obtained by ethanolic extract

S. No.	Rt (min)	MW	MF	Area (%)	Identified compound	Library CAS. No.
1.	9:30	136	C10H16	6.19	D-limonene	Wiley 9 5989-27-5
2.	15.46	150	C10H14O	13.67	Pinocarvone	Wiley 9 30460-92-5
3.	19.20	196	C14H28	8.77	1-tetradecene	Wiley 9 1120-36-1
4.	22.41	206	C14H22O	8.43	2-tert-butyl-4-isopropyl-5-methylphenol	Wiley 9 30061-94-0
5.	23.97	238	C16H30O	10.21	E-14-hexadecenal	Mainlib 330207-53-9
6.	24.14	226	C16H34	2.05	Hexadecane	Wiley 9 544-76-3
7.	26.32	240	C17H36	4.14	Heptadecane	Wiley 9 629-78-7
8.	28.27	252	C17H32O	11.47	E-15-heptadecenal	Mainlib NA
9.	32.18	280	C20H40	10.96	Cycloeicosane	Mainlib 296-56-0
10.	35.75	310	C22H46	5.68	Docosane	Wiley 9 629-97-0
11.	39.04	336	C24H48	2.43	Cyclotetracosane	Mainlib 297-03-0

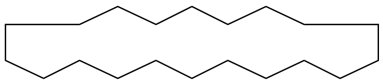
Rt : Retention time, MW : Molecular weight and MF : Molecular formula.
The structure of the major components



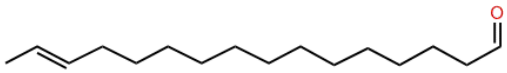
E-15-heptadecenal



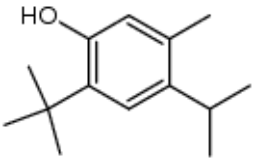
Pinocarvone



Cycloeicosane



E-14-hexadecenal



2-tert-butyl-4-isopropyl-5-methyl-phenol

1-tetradecene

Table 2. Ovicidal action of the *Z. album* fraction on three-day-old eggs of *S. littoralis*

Treatment	LC_{50} (%) (LCL-UCL)	LC_{90} (%) (LCL-UCL)	Slope
Z. album fraction	1.199 (1.041-1.371)	4.171 (3.348-5.653)	2.367±0.226

LCL : Lower confidence limit, UCL : Upper confidence limit, LC_{50} : Concentration that kills 50% of insects and LC_{90} : Concentration that kills 90% of insects.

Table 3. Larvicidal action of the *Z. album* fraction against fourth instar larvae of *S. littoralis*

Treatment	LC_{50} (%) (LCL-UCL)	LC_{90} (%) (LCL-UCL)	Slope
Z. album fraction	15.746 (8.600-29.709)	74.198 (40.530-135.937)	1.930±0.941

LCL : Lower confidence limit, UCL : Upper confidence limit, LC_{50} : Concentration that kills 50% of insects and LC_{90} : Concentration that kills 90% of insects.

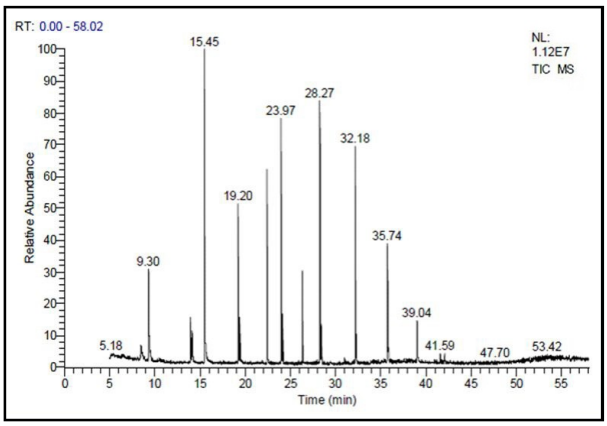


Fig. 1. Gas chromatography profile of the *Zygophyllum album* fraction.

produced larvicidal action against the fourth instar *S. littoralis* after 72 h of treatment. The LC_{50} and LC_{90} were 15.743 and 74.198%, respectively.

Generally, the resemblances in the metabolic/ toxicokinetic profiles of C_{10} - C_{20} aliphatic hydrocarbons show that these ingredients are possible to have similar toxic characters. Toxicity in the ethanolic fraction could be due to the presence of the phenole (2-tert-butyl-4-isopropyl-5-methylphenol). Various *Z. album* extracts' antioxidant actions were due to their phenolic compounds and could be attributable to the existence of sterols, flavonoids and triterpenes in the plant (Ramesh and Mohanraju, 2021). Pinocarvone was the major compound of the *Z. album* fraction, which was in harmony with Stappen *et al.* (2015), who mentioned that pinocarvone was the basic component in the essential oil obtained from the aerial parts of *Hyssopus officinalis*. Pinocarvone is a monoterpenoid compound characterized by its many exploitable biological properties, including antifungal, antibacterial, anticancer and insecticidal properties.

Based on LC_{50} values from the toxicity experiments, the *Z. album* fraction had ovicidal

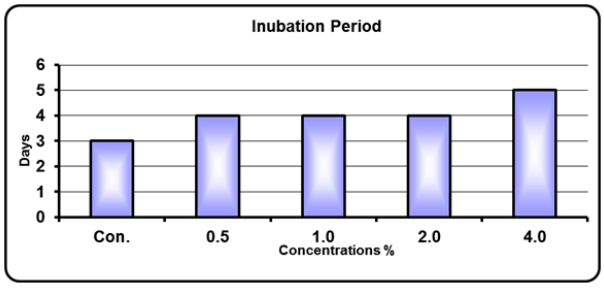


Fig. 2. Effects of the *Zygophyllum album* fraction on the incubation period of *Spodoptera littoralis*.

and insecticidal activities against *S. littoralis*. Abderrahmane *et al.* (2020) drew the same conclusion; an aqueous extract of *Z. album* had larvicidal and ovicidal effects against *Tuta absoluta*.

The external morphologies of untreated *S. littoralis* larvae and those treated with the LC_{50} of the *Z. album* fraction were compared with microscopy. Generally, the outer body surface of the untreated larva was slender, smooth and differentiated into the head, thorax and abdomen (Fig. 3a). Scanning electron microscopy revealed many types of deformity in treated larvae e. g. an extremely elongated and destructed body wall (Fig. 3b), issues in the intersegmental area resulting in cuticle degradation along the whole body, and the formation of fissures in the cuticle (Fig. 3c), as well as the entire body being inwardly compressed, dried and shrunken with constriction to the thorax or abdomen (Fig. 3d). Untreated larvae were characterized by a normal, sclerotized, rounded head with the posterior edge retracted into the prothorax. Y-

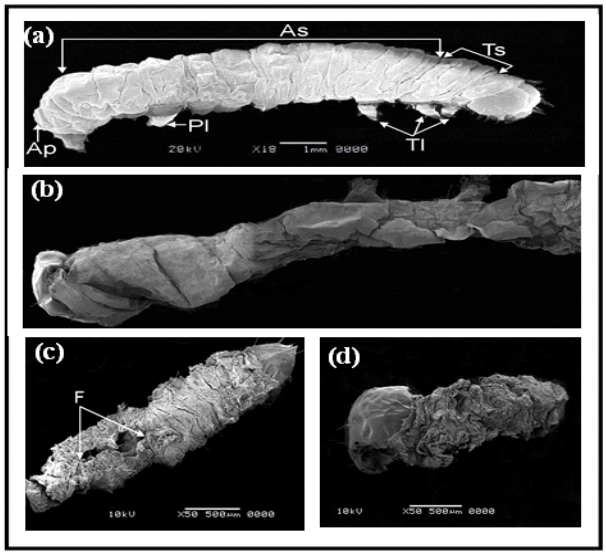


Fig. 3. General morphological features of the external body of the fourth instar *Spodoptera littoralis* larvae observed under a scanning electron microscope. (a) Untreated larva and (b-d) larva treated with the LC_{50} of the *Zygophyllum album* fraction. (b) Extremely elongated and destructed body wall, (c) cuticle degradation fissures, and (d) compressed, dried, and shrunken body with thorax or abdomen constriction.Ap : anal plate; As : abdominal segments; F : fissure; Pl : prolegs; Tl : thoracic legs; and Ts : thoracic segments.

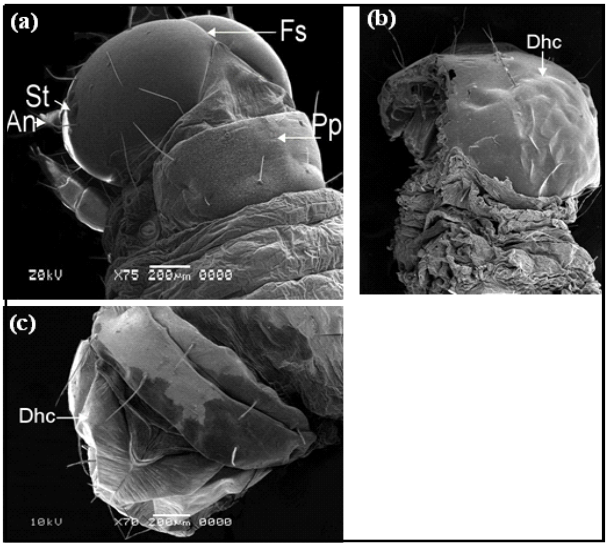


Fig. 4. Scanning electron micrographs of *Spodoptera littoralis* fourth instar larvae heads. (a) Untreated larva with a normal rounded and sclerotized head. (b and c) Larva treated with the LC_{50} of the *Zygophyllum album* fraction. (b) Damaged head capsule and destructed antennae. (c) Alliterated prothoracic plate and frons. An : antenna; Dhc : deformed head capsule; Fs : frontal suture; and Pp : prothoracic plate.

shape structure divided the head into three major parts : Two lateral regions and one frons (central). Six lateral vision spots (stemmata) arranged on each epicranium "C shape". The antennae were tiny, consisted of three segments. Additionally, adfrontal area laterally surrounded the frons (Fig. 4a). In contrast, treated larvae had damaged head capsules and complete destruction of the larval antennae (Fig. 4b). There was differentiation between the epicranial regions as they became fused. Further alterations in the prothoracic plate and frons area were recorded (Fig. 4c).

The abdomen of untreated larvae consisted of 10 segments that became broader than the head. The first seven segments closely resembled each other; however, the abdominal segment entirely differed from the preceding segments and bore the spiracles (external openings of the respiratory system). These abdominal spiracles were circular with a symmetrically swollen peritreme and located at the lateral edge of the body and were surrounding their opening (Fig. 5a). On the other hand, the body segments of treated larvae were undifferentiated, more likely to be fused and shrunken and contained deformed spiracles (Fig. 5b).

Transmission electron microscopy : In the normal untreated larvae, the integument consisted of an outer non-cellular part (i. e. the cuticle) and an inner cellular layer (i. e. the epidermis). The normal cuticle was composed of parallel-running chitin microfilaments associated with protein-forming sheets (i. e. laminae) to afford elasticity to the exoskeleton. The epicuticle often had no laminae and was separated into oblique sections comprising three sub-layers : the epicuticle was the thin outermost layer, the outer exocuticle layer was a relatively thick layer characterized by a rigid structure and dark colour, and the inner endocuticle was the thickest layer, characterized as lacking colour but soft and flexible (Fig. 6a). In contrast to untreated larvae, those treated with the LC_{50} of the *Z. album* could not shed the old cuticle. Therefore, the epicuticle could not be

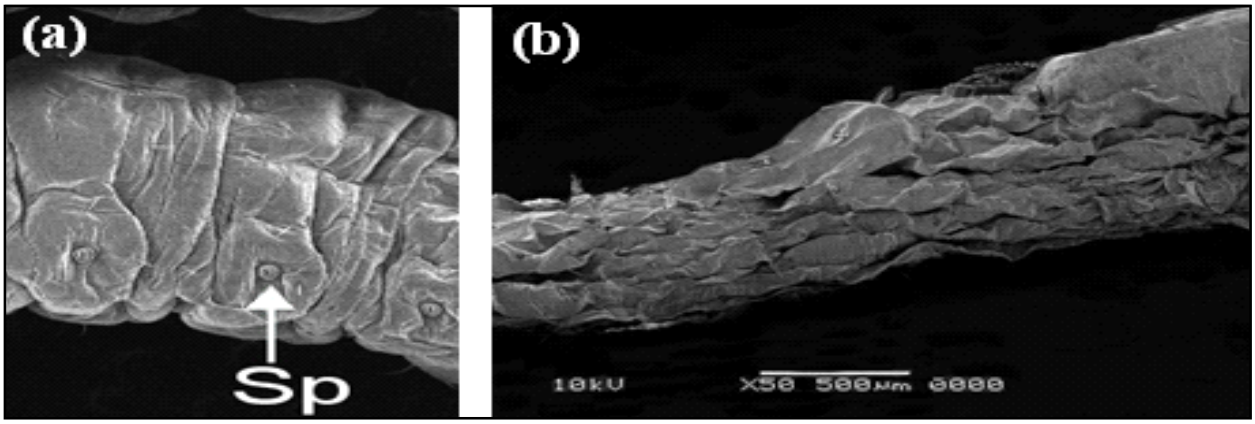


Fig. 5. Scanning electron micrographs of *Spodoptera littoralis* fourth instar larvae abdominal segments. (a) Untreated larva with normal spiracles (Sp). (b) Larva treated with the LC_{50} of the *Zygophyllum album* fraction exhibiting fused and shrunken abdominal segments with deformed spiracles.

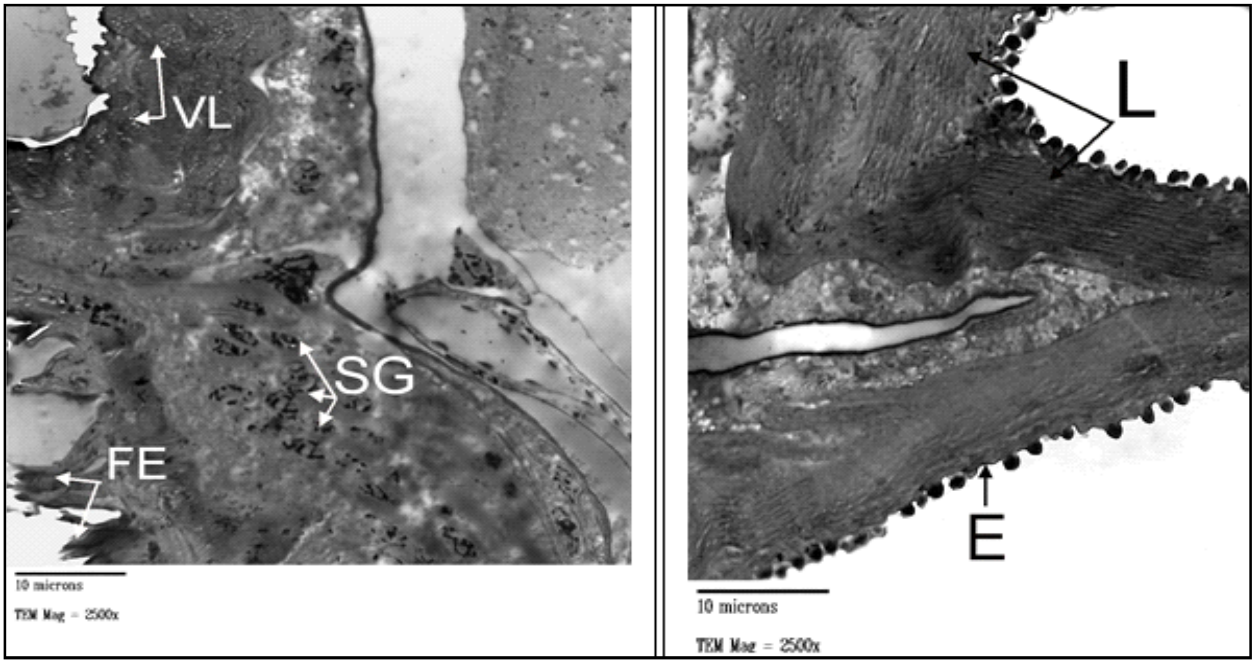


Fig. 6. Transmission electron micrographs of *Spodoptera littoralis* fourth instar larvae showing the transverse section of the integument or body wall. (a) Normal integument. (b) Abnormal integument in a larva treated with the LC_{50} of the *Zygophyllum album* fraction.
E : epicuticle; FE : fragmented epicuticle; L : lamina; SG : secretory granules; and VL : vaculated lamina.

differentiated from the endocuticle. The microfilament bands were undifferentiated and had lost their layered organization. Electron dense resides within the procuticle, which was devoid of oriented microfilaments and had lost contact with the underlying vacuolated epidermal cells (Fig. 6a). Moreover, the sub-chitinous layers were completely disintegrated in treated larvae (Fig. 6b).

The ultrastructural observations (via scanning and transmission electron microscopy) and biochemical assessments in the present study showed why mortality occurred in *S. littoralis* treated with the *Z. album* fraction. For example, abnormalities in the head capsule, antennae, spiracles and integument were recorded in treated larvae. Issues with the integument (i. e. the cuticle) of insects, which offers protection and is involved in defense against pathogenic and predator agents Also, any deformations in the major sense organs (e. g. the eyes and antennae) on the sclerotized head capsule can impair performance and inevitably lead to death. Moreover, deformed spiracles could cause respiratory impairments affecting larval metabolism and other processes, resulting in death.

Thus, larval mortality may ultimately be due to the deformations detected in the microscopic observations. Using scanning electron microscopy in *Culex pipiens,* Osman *et al.* (2020) showed prominent deformations in siphons, larval antennae and adult mouth parts caused by methanol extracts of *Ocimum basilicum* and *Eucalyptus globulus*. Similar disruption to the external morphology (i. e. the cuticle and head capsule) of *S. littoralis* larvae due to an ethanolic extract of *Taxodium distichum* was observed by Sabry (2018) via scanning electron microscopy. Using transmission electron microscopy, Khedr *et al.* (2015) detected ultrastructural changes, such as separation of the endocuticle from the epicuticle, in the cuticle of *S. littoralis* larvae treated with *Ipomea carnea*.

Larvae treated with the LC_{50} of the *Z. album* fraction recorded significant reduction in TSP (23.88±1.75 mg protein/g.b.wt.) when compared with the total soluble protein of control larvae (34.21±0.35). Comparing to control larvae, treated larvae had significantly reduced both enzymes activities (especially ALT). For AST and ALT, treated larvae had 0.88±0.03 and 8.75±0.38 µg pyruvate/g.b. wt/min, respectively; comparatively, the control showed 1.30±0.10 and 48.40±0.58, respectively (Table 4). Chitinase activity was significantly inhibited by treatment with the LC_{50} value of

the *Z. album* fraction (0.043±0.003 µg N-acetylglucoseamine/g.b.wt/min) relative to chitinase activity in the control (0.10 ± 0.005). The biochemical changes detected in the present study were likely to be the main mechanisms of *Z. album* toxicity toward fourth instar *S. littoralis* larvae. Specifically, the tested fraction disturbed the protein metabolism of *S. littoralis* larvae as shown by a significant reduction in the activities of two key protein enzymes, AST and ALT. These two amino transferases played important roles in linking amino acids and carbohydrate metabolism.

The word protein was initiated from the Greek word "proteins" which meaning first or more important. Proteins are vital for all lives. Many structures e. g. (muscles, cuticle and egg yolk) and (enzymes and hormones) are synthesized by proteins. In a previous study, Khedr *et al.* (2020) indicated that *Cinnamomum zeylanicum* essential oil reduced the total soluble protein in *S. littoralis* eggs. Similarly, El-lakwah (2018) found that ALT and AST activities decreased in the second and fourth instar larvae of *S. littoralis* treated with an extract of *Ocimum sanctum* leaves. The observed depletion of these two transferases was likely associated with the decreased total soluble protein level. Chitin has a polysaccharide structure and forms the basis of insect exoskeletons. Chitinase is very princable in degrading old chitin during molting process. Thus, any increase or decrease in chitinase can fail to molt and lead to larval mortality. In our study, treatment with the fraction of *Z. album* caused a significant reduction in chitinase enzyme activity in *S. littoralis*. Similarly, Sabry (2018) revealed a chitinase reduction in the fourth instar larvae of *S. littoralis* treated with an ethanolic extract of *Taxodium distichum*.

As shown in Table 5, the LC_{50} of the *Z. album* fraction caused significant lengthening of the larval duration from 10.00±0.50 days in the control to 13.16±0.33 days. The pupal duration of pupae from untreated larvae was 9.00±0.00 days, whereas that of pupae from treated larvae was significantly lengthened at 11.33±0.66 days. The *Z. album* fraction significantly reduced the average weight of pupae developed from treated larvae (0.312±0.005 g) relative to the weight of control pupae (0.340±0.006 g). There was no mortality in the control pupae, however, *Z. album* fraction-treated pupae showed a mortality of 8.00±0.57%.

In the current work, the *Z. album* fraction significantly affected specific biological activities of *S. littoralis*, i. e. the incubation period of treated eggs was retarded, larval mortality increased, both larval and pupal durations increased and pupal weight was reduced. Weight loss was associated with disturbed liver functions (linked to ALT and AST). In other studies, *Z. album* produced other biological activities such as antioxidant/ antiacetylcholinesterrase activities (Kchaou *et al.*, 2016) and anti-inflammatory/ antihypertensive activities (Mnafgui *et al.*, 2016).

Overall, the current study suggested that the toxicity of the *Z. album* fraction in fourth instar *S. littoralis* larvae occured due to the depletion of key enzymes involved in protein metabolism and chitinase, which failed the molting process and caused various deformed structures, ultimately leading to mortality. Indeed, the observed biochemical changes perhaps explained the distortions in larval ultrastructure. In other words, the relationship between the biochemical and ultrastructural outcomes could explain the various effects of

Table 4. Changes in specific biochemical parameters of *S. littoralis* larvae treated with the *Z. album* fraction

Treatments	Total soluble protein (mg protein/g.b. wt.)	Amino transferase enzymes		Chitinase enzyme (µg N-acetylglucoseamine /g.b.wt./min)
		AST (µg pyruvate/ g.b. wt./min)	ALT (µg pyruvate/ g.b. wt./min)	
Control	34.21±0.346	1.30±0.010	48.40±0.584	0.100±0.005
Z. album fraction	23.88±1.745	0.88±0.034	8.75±0.376	0.043±0.003
t	5.802	4.029	57.067	8.50
p	0.0044	0.0157	0.0001	0.011

Notes : Each datum represents the mean of three replicates. Data expressed as mean±standard error. Significant difference : $P < 0.05$. Larvae were treated at the LC_{50} level of the fraction of the aerial parts of *Z. album* ethanolic extract.

Table 5. Changes in specific biological parameters of *S. littoralis* after treatment with the *Z. album* fraction

Treatment	Larval duration (days)	Pupal duration (days)	Pupal weight (g)	Pupal mortality (%)
Control	10.00±0.50	9.00±0.00	0.340±0.006	0
Z. album fraction	13.16±0.33	11.33±0.66	0.312±0.005	8.00±0.57
t	5.269	3.500	3.325	15.856
p	0.0062	0.024	0.029	0.0001

Notes : Each datum represents the mean of three replicates. Data expressed as mean±standard error. Significant difference : P < 0.05. Larvae were treated at the LC_{50} level of the fraction of *Z. album* ethanolic extract's aerial parts.

the tested fraction of *Z. album* on *S. littoralis* larvae and pupae.

CONCLUSION

This research is the first to report the toxicity and possible mechanisms of an ethanolic extract fraction from the aerial parts of *Z. album* when the fraction is used against *S. littoralis* larvae. Data included measurements of the biochemical and ultrastructural alterations induced by this fraction. Further studies are necessary to test this fraction under field conditions and ensure it can be used as an eco-friendly control technique against the serious economic pest *S. littoralis*.

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