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Effect of *Hyptis brevipes* (Lamiaceae) Methanol Extract on *Spodoptera littoralis* (Lepidoptera: Noctuidae) Larvae

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ABSTRACT

The effect of *Hyptis brevipes* (Lamiaceae) methanol extract on *Spodoptera littoralis* (Noctuidae) larval growth and development was evaluated. The likely histological damage occurred to the vital organs (midgut and Malpighian tubules) following exposure to this extract was also determined. Castor bean leaves treated with different concentrations of extract were fed to 3rd larvae for two days. The LC_{50} value was 1.99% (95% F.L. = 1.19% - 2.79%; slope = 2.452± 0.737) four days following treatment. 2.5% of extract caused retardation in larval life up to 21 days; formation of deformed individuals; 90%larval mortality and 89.4% reduction in adult emergence. The histological changes in the midgut elicited after 7 days of treatment by 2.5% of extract were: detachment of the peritrophic membrane and degeneration of muscles and epithelial cells' boundaries (lesion). After 4 days of treatment with 5%, apoptotic configuration including cytoplasmic vacuolization and chromatin condensation in epithelial cells of midgut and Malpighian tubules were observed. As detected histochemically, the total protein content was decreased in the midgut epithelium of the treated larva comparing to control. From this study, the methanol extract of *H. brevipes* could be used effectively in the control program of *S. littoralis* and need further studies for isolation of their active constituents.

Keywords: *Spodoptera littoralis, Hyptis brevipes,* methanol extract, larval development, midgut, Malpighian tubules, histology

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INTRODUCTION

The Egyptian cotton leaveworm *Spodoptera littoralis* (Lepidoptera: Noctuidae) has a national concern with a wild host range [1]. Heavy infestation resulted in defoliation of the attacked plants causing sever loss of crop production [2-3]. Regarding to environmental consequences, it was shown that *S. littoralis* has a potential disruptive impact on native tri-trophic interactions involving *Brassica rapa* [4]. The midgut epithelial cells have the ability to release digestive enzymes and absorb the digestive food. Also, the gut enzymes play an important role in the insect resistance to some insecticides [5]. The importance of midgut to insect life leads to consider this organ as a target organ for controlling *S. littorali*. This insect has the ability to develop a quick resistance to the most conventional insecticides [6]. It is very important to discover novel ecofriendly compounds to replace the hazard synthetic insecticides.

Many plant species release secondary metabolites to enhance the defense mechanism of these plants against the attack of herbivorous insects. The mint family Lamiaceae is one of the largest families of flowering plants with great economic importance worldwide. The genus *Hyptis* (Lamiaceae) has more than 300 species distributed mainly at the Central States of Brazil. It is known mainly for the essential oils along with terpenoids, flavonoids, pyrones compounds [7-8]. The genus has great economic importance as they poses antimicrobial [9]; antiulceragenic [10] and insecticidal activity [11-13]. Recently, data presented in previous published works [8,14] showed that *H. brevipes* dichloromethane extract possess antifeeding activity and caused disruption to the growth and development of *S. littoralis* larvae. The activity of plant extract is depending on the following: solvent system (polarity of solvent) used for extraction, plant species, plant parts used, method of extraction, method of application and target insect [15-16]. Using different solvent system for *H. suaveolens* extraction gave different insecticidal results against *Culex quinquefasciatus* larvae. After 24h of treatment, the results showed that acetone extract elicited highest larval mortality followed by petroleum ether and chloroform with LC_{50} values of 485.6, 493.4 and 625.9 mg/L, respectively [17]. Dichloromethane extract of *Artemisia monosperma* (feeding method) surpass the methanolic ones against *S. littoralis* larvae [18].

The insecticidal activity of methanol extract of *H. brevipes* has not been investigated to date. The present study aims to evaluate the insecticidal activity of the aerial parts of *H. brevipes* methanol extract on *S. littoralis* larvae and determine the possible damage occurred to midgut and Malpighian tubules following exposure to this extract. Furthermore, mercury bromophenol blue stain technique was used to ascertain the effect of the extract on the total protein contents in midgut epithelial cells.

MATERIALS AND METHODS

Plant material

The Ecuadorian plant *H. brevipes* was kindly identified by Dr. Felipe Ghia (Botany Department, Reversa Forestal Endesa, Provincia del Prichincha, Ecuador) and provided by Prof. Dr./ Hesham R. El-Seedi (Natural product Laboratory, Department of Chemistry, Faculty of Science, Menoufia University). Powder of the aerial parts of *H. brevipes* was extracted with methanol at room temperature with occasional stirring and then filtered. The extract was evaporated in vacuum and the crude extract was stored in fridge at 4C° till used.

Insect model

A laboratory susceptible strain of *S. littoralis* was initially obtained from Agricultural Research Center (Dokki, Giza, Egypt). Larvae were reared in glass jars (at 28±2°C; 65%R.H and 14:10 L: D cycle) and exclusively fed on castor bean leaves *Ricinus communis* (Euphorbiaceae) till pupation. Adults were supplied with 10% (w/v) sucrose solution [19].

Effect of *H. brevipes* methanol extract on *S. littoralis* larvae

The effect of the aerial parts of *H. brevipes* methanol extract on the survival and development of *S. littoralis* larvae was studied in the laboratory. Different concentrations (5%, 2.5% and 1.25%; w/v) of extract were assayed against the 3rd larval instar of *S. littoralis.* The extract was dissolved in a mixture of acetone: water (3:2, v/v). Thirty pieces of castor bean leaves (2 cm^2) were treated with 1 ml of each concentration of plant extract. Leaves treated with solvent used as positive control, while that treated with water was

considered as negative control. The treated and control leaves were left to stand (at room temperature) for solvent evaporation and then offered to the tested larvae. These larvae (20 larvae /concentration/replicate) were fed on the treated leaves for two consecutive days, while the control ones were fed on leaves treated with solvent. After that, all larvae were fed on un-treated fresh castor bean leaves till pupation. This experiment was done in triplicates. The LC_{50} values were calculated according to Finney [20]. The reduction in the adult emergence was calculated according to Khazanie [21], while the cumulative larval mortality was corrected using Abbot's formula [22].

Histological alteration induced by *H. brevipes* methanol extract

The possible damage occurred to midgut and Malpighian tubules of *S. littoralis* larvae following exposure to *H. brevipes* methanol extract were determined. The mid-guts of the treated *S. littoralis* larvae and control ones (as mentioned above) were removed 4 and 7days of treatment. Parts of the larval mid-gut were fixed for 24h in Boun's solution. The fixed specimens were dehydrated using graded series of ethanol, cleared in xylol, embedded in parablast and sectioned at 5 µm thick. Sections were stained with hematoxyline and eosin (H/e) for histological examination. Mercury bromophenol blue stain technique [23] was used for protein determination in the larval midgut epithelium. Stained sections were mounted on glass slides in DePeX-mounting medium under cover slips. Microscopic examination and photographs were carried out by Olympus microscope attached with Olympus digital camera (BX41, Department of Zoology, Faculty of Science, Menoufia University, Menoufia, Egypt).

RESULTS

Effects of *H. brevipes* methanol extract on *S. littoralis* larvae

Castor bean leaves treated with methanol extract of *H. brevipes* and fed to 3rd instar larvae of *S. littoralis* for two days were toxic to larvae. The potential effect of *H. brevipes* methanol extract against larvae was indicated by the LC_{50} value which was 1.99% (95% F.L. = 1.19%- 2.79%; slope= 2.452± 0.737) after four days of treatment. Larvae fed on leaves treated with 1.25% of extract sustained from the treatment and died causing reduction in pupation and adult emergence by 15% and 84.2%, respectively (Table 1).

Table 1: Effect of *H. brevipes* methanol extract on growth and development of *S. littorals* larvae fed treated castor bean leaves for two days

Concentration (%)	% Larval mortality on the					*% Terminated larvae	*% Pupation	*% Adult emergence		
	2nd day	3rd day	4th day	5th day	total period			total	**malformed	+% Reduction
0	5	5	5	5	5	0	95	95	0	0
1.25	0	10	35	55	85	15	15	15	0	84.2
2.5	5	35	60	65	90	20	10	10	20	89.5
5	5	45	85	100	100	0	0	0	0	100

* Relative to the number of larvae.
**Relative to the number of emerged adults.
+% Reduction=(C-T/C) X 100 (Khazanie, 1979); where C = number of emerged adults in control and T = number of emerged adults after treatment.
**Terminated larvae (the larval life of which was extended up to 12-21 days after treatment).

The extract caused impairment of larval growth and development, in which the larval developmental period was retarded (Fig. 1). At a concentration of 2.5%, the growth and development of 20% of the treated larvae was extended up to 21 days (terminated larvae). After that, some of these larvae (10%) could not complete their development and died (Fig. 2 b), others (10%) transformed into pupae. Some of these pupae (7.1%) developed into an intermediate stage (pupa-adult intermediate) and died (Figs. 2f-g), while others emerged into adults (Table 1). Twenty percentages of these adults was malformed (Fig. 2d).

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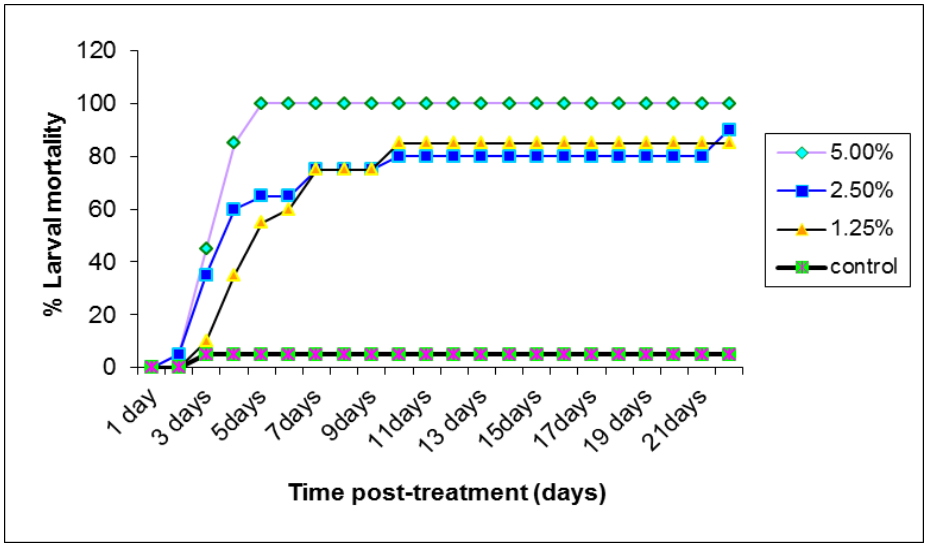
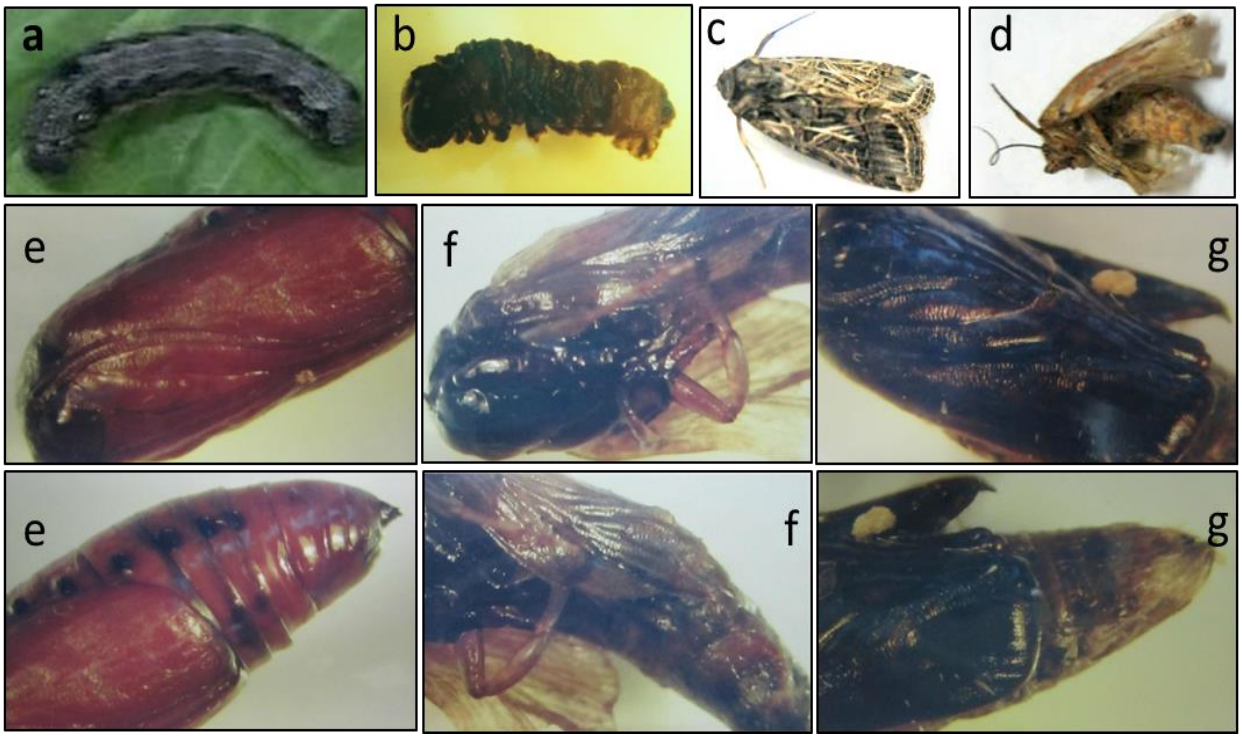


Fig. 1: Mortality response of *S. littoralis* larvae fed castor bean leaves treated with different concentrations of methanol extract of *H. brevipes* for two days



Figs. 2a-g. Effect of *H. brevipes* methanol extract on *S. littoralis* treated as 3rd larval instar. a: normal larva; b: larva failed to pupate (terminated larva); c-normal adult; d: malformed adult; e: upper and lower part of normal pupa; f-g: Pupae-adult intermediate in which the upper part resemble pupal stage while the lower part resemble the adult stage.

Histological alteration induced by *H. brevipes* methanol extract

The normal structure of the midgut and Malpighian tubules of the 5th larval instar of *S. littoralis* is illustrated in Figures 3 and 4. Castor bean leaves treated with *H. brevipes* methanol extract and fed to *S. littoralis* larvae caused many histological changes in the larval midgut and Malpighian tubules (Figs. 5-7). After 7 days of treatment, the

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concentration of 2.5% of extract caused detachment of the peritrophic membrane, degeneration (lesion) of epithelial cells' boundaries and detachment of the muscle bundles (Fig. 5). Typical morphological changes consistent with apoptosis were observed in larvae treated with 5% of methanol extract after 4 days of treatment. These changes include cytoplasmic vacuolization and chromatin condensation in the epithelial cells of midgut (Fig.6) and Malpighian tubules (Fig. 7a) of the treated larvae. Degeneration in the epithelium (epithelial lesion) of Malpighian tubules (Fig. 7b) was observed as well.

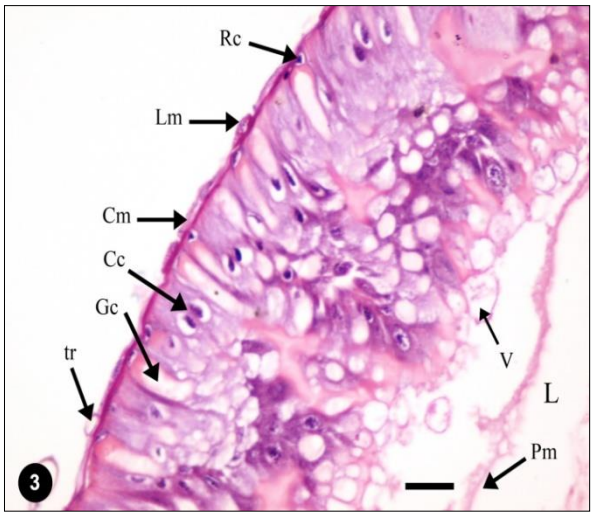

Fig.3: Transverse section of midgut of *S. littoralis* 5th instar larva, fed treated castor bean leaves with acetone (solvent) and stained with H/E. Scale bar =25µm. Cm=circular muscle; Gc= goblet cell; L=lumen; Lm = longitudinal muscle; Pm= peritrophic membrane; Rc= regenerative cell; v= vesicles.

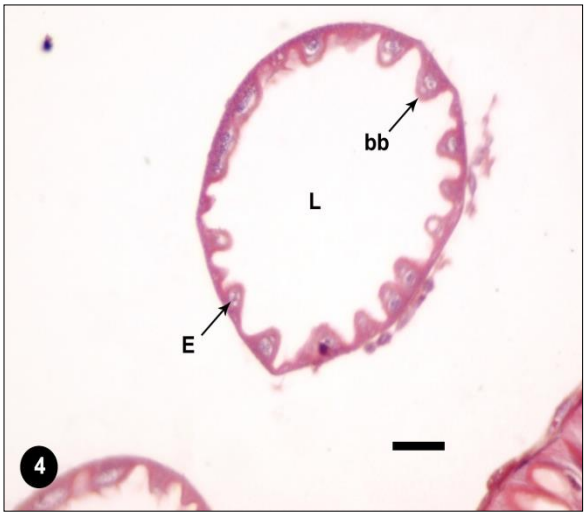

Fig.4: Transverse section of Malpighian tubules of *S. littoralis* 5th instar larva, fed treated castor bean leaves with acetone (solvent) and stained with H/E. Scale bar =25µm. bb=brush border, E= epithelial cell, L= lumen.

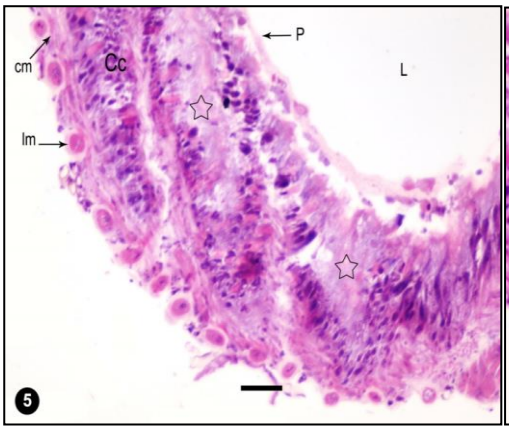

Fig. 5: Transverse section of midgut of *S. littoralis* larva fed treated castor bean leaves with 2.5% of *H. brevipes* methanol extract and stained with H/E. Scale bar =25µm. Degeneration of cells' boundaries (point star) and detachment of the muscle bundles was observed. Cc= columnar cells. Cm=circular muscle; L=lumen; Lm = longitudinal muscle; P= peritrophic membrane.

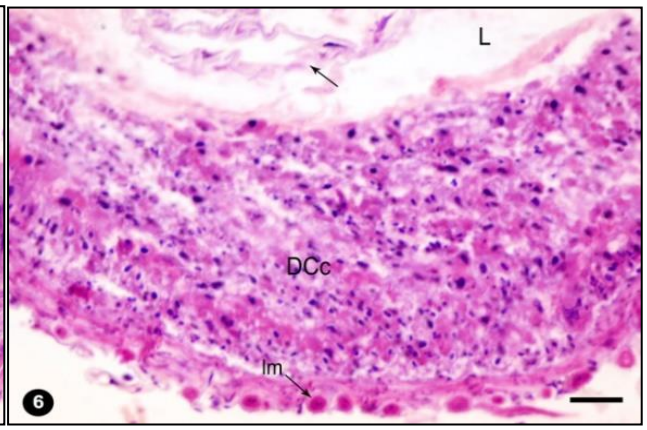

Fig. 6: Transverse section of midgut of *S. littoralis* larva fed treated castor bean leaves with 5% of *H. brevipes* methanol extract and stained with H/E. Scale bar =25µm. Destruction of epithelium with apoptotic epithelial cells was observed in the midgut section. DCc= destruction columnar cells; L=lumen; Lm = longitudinal muscle.

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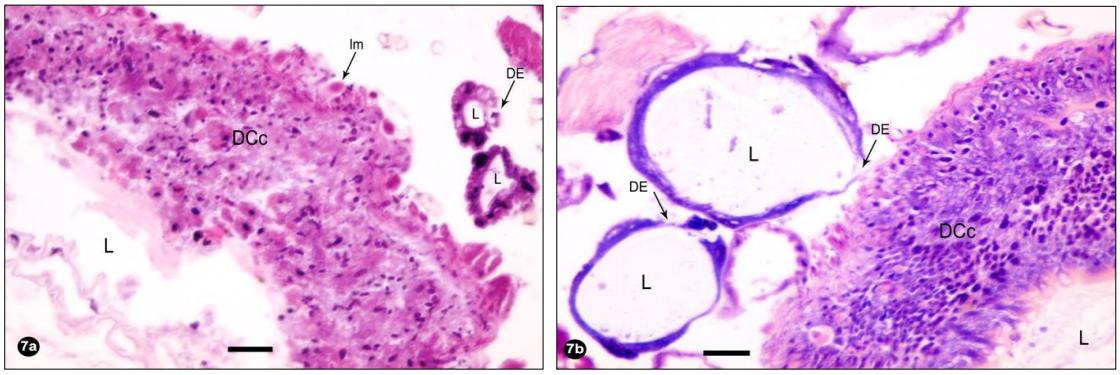

Fig. 7a-b:Transverse section of Malpighian tubules of *S. littoralis* larva fed treated castor bean leaves with *H. brevipes* methanol extract at concentration of 5% and stained with H/E. Scale bar =25µm.a- the epithelial cells with pycknotic nuclei. b- Degenerated of epithelium (epithelial lesion). DCc= Destruction columnar cells; DE= Degenaration of epithelium; L=lumen; Lm = longitudinal muscle.

Effects of *H. brevipes* methanol extract on total protein content

The effect of *H. brevipes* methanol extract on the total protein contents in the epithelial cells of midgut was determined by the mercury bromophenol blue stain technique. In control larvae, all structures in the epithelial cells exhibited positive stain ability with varying degrees of bluish colouration reaching its maximum in the nucleus (Fig. 8). Castor bean leaves treated with 5% *H. brevipes* methanol extract and fed to *S. littoralis* larvae for two days caused obvious decreased the total protein content in the cytoplasm of their midgut cells (Fig. 9) compared to control.

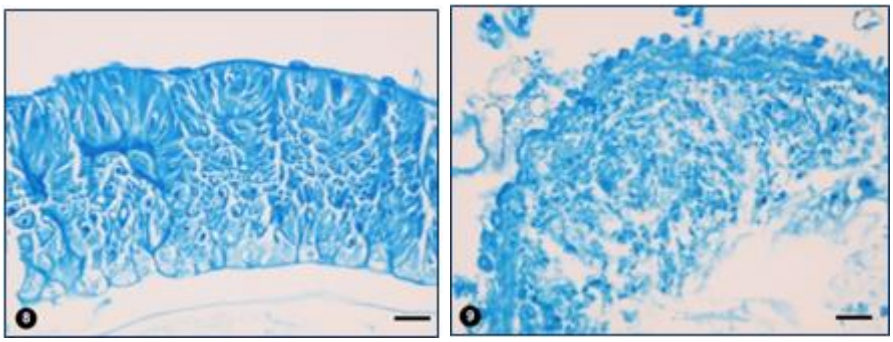

Fig.8: Transverse section of midgut of *S. littoralis* larva fed treated castor bean leaves with acetone (solvent) and stained with mercury bromophenol blue. Scale bar =25µm. Positive stain reaction in all cell structure was observed.

Fig.9: Transverse section of midgut of *S. littoralis* larva fed treated castor bean leaves with5% of *H. brevipes* methanol extract and stained with mercury bromophenol blue. Scale bar =25µm. An obvious decrease in the total protein especially in in the cytoplasm was observed.

DISCUSSION

The current study clearly demonstrates the potential insecticidal action of the aerial parts of *H. brevipes* methanol extract toward *S. littoralis* larvae. The extract disrupted the normal growth and development of the treated larvae with LC_{50} value of 1.99% (95% F.L. = 1.19%- 2.79%; slope= 2.452± 0.737) after four days of treatment. The developmental period of the treated larvae was retarded. These effects toward *S. littoralis* larvae may be due to the secondary metabolites persist in this extract. This plant is known to produce a range of terpinoids, flavonoids and pyrons [7-8]. The present results clearly indicated that the methanol extract of *H. brevipes* is potent to *S. littoralis* larvae. These effects are consistent with data reported by [11, 8, 24, 25, 26, 27, 28, 29]. The inhibitory effect of trypsin and chymotrypsin extracted from some varieties of maize and sorghum against *S. littoralis* larvae was determined by [24]. He stated that the purified proteins caused larval mortality and decreased the larval and pupal weight and percentage of pupation. A

novel of 8.7kDa protease inhibitor was isolated by [11] from *Hyptis suaveolens* seeds. This protein inhibits all trypsin-like proteases extracted from the gut of *Prostephanus truncates* (Coleoptera: Bostricidae) and *Manduca sexta* (Lepidoptera: Sphingidae). The *Acacia nilotica* (L) extracts were tested against *S. litura* larvae by [27].They found that the extract caused prolongation to larval and pupal periods with several pupal and adult deformities. The larvicidal activity of *Cabralea canjerana* (Meliaceae) extracts was evaluated against *Spodoptera frugiperda* by [28]. They stated that the extracts caused high larvicidal activity and induced various larval and pupal abnormalities. The activity of this extracts due to the triterpenes and mainly to the isolated new compound, dammarane [28]. The inhibitory effects of some essential oils extracted from medicinal plants against *Pseudaletia unipuncta* (Noctuidae) was evaluated by [29]. They reported that the essential oils of *Anethum graveolens,* significantly extended the larval duration of the treated larvae from 1.9 to 6.5 days.

The effect of *H. brevipes* methanol extract on the histological structure of the two vital organs (midgut and Malpighian tubules) of *S. littoralis* larvae was evaluated in the current study. Morphological changes consistent with apoptosis were observed in larvae treated with *H. brevipes* methanol extract. These changes include cytoplasmic vacuolization and chromatin condensation in the epithelial cells. The extract caused destruction to the epithelium of these two vital organs through apoptosis-induced death in epithelial cells. The midgut-epithelium-damaged could be responsible for the low consumed leaves by *S. littoralis* larvae observed in the present study. These results indicated that the primary target organ for *H. brevipes* methanol extract is the epithelial cells of midgut and Malpighian tubules. The present findings are similar to data reported by [30, 31, 32]. *Bacillus thuringiensis aizawai* (HD133 strain) produces many insecticidal proteins. Cry1Da fed to *S. littoralis* larvae caused histological changes in the midgut of the treated larvae which were: vesicle formation of the apical region, cellular vacuolation and destruction of the epithelial cells and their boundaries. The Cry 1Da protein of about 65kDa binds on *S. littoralis* brush border membrane [30]. The *Sclerotium rolfsii* lectin is resistance to the proteolysis of gut enzymes proteases of *Spodoptera litura* larva. The lectin binds to specific membrane glycoproteins on epithelial cells of the midgut. The activation of caspase-3-like activity and the DNA fragmentation observed in the midgut epithelium supported the mechanism of apoptosis-induced death by this lectin [31]. The effect of *Artemisia annua* methanol extract at a concentration of 2% (feeding method) on the histological structure of *Helicoverpa armigera* larvae was studied by [32]. They reported that the extract caused disruption of peritrophic membrane and destruction in the midgut tissue.

The architecture destruction of Malpighian tubules detected in the current study in *S. littoralis* larvae treated with *H. brevipes* methanol extract is in agreement with that of [33, 34, 14, 35]. The nucleopolyhedrovirus caused apoptosis in the Malpghian tubules of *Anticarsia gemmatalis* (Noctuidae) larvae. These may be activated by depletion of energy reserves and accumulation of marker proteins, respectively [33]. Selenium caused histological changes in the Malpighian tubules of silkworm expressed as degeneration of the cells along with their nuclei [34]. The dichloromethane extract of *H. brevipes* at concentration of 4% caused architecture destruction of Malpighian tubules of treated *S. littoralis* larvae after 4 days of treatment [14]. The extract of *Colocasia esculenta* at 100 ppm caused dramatic pathological lesion especially Malpighian tubules of the treated *Culex pipiens* larvae [35].

The methanol extract of *H. brevipes* in the present study caused marked decrease in the protein content in the larval midgut cells comparing to control. This effect may be due to the structural damage of the epithelial cells of the treated individuals. This result is confirmed by [5, 36, 37, 38, 14].

CONCLUSION

The methanol extract of *H. brevipes* in the current study caused dramatic effects on the development of *S. littoralis* larvae and elicited histological alteration in the two vital organs (midgut and Malpighian tubules). The total protein in the treated larvae was also decreased comparing to control. The architecture destruction in the tested organs and the low protein content in the midgut cells could be responsible for starvation of *S. littoralis* larvae which followed by retardation in their growth and finally death. These effects collectively may be due to the secondary metabolic compounds persist in the *H. brevipes* methanol extract. The methanol extract of this plant could serve as potential natural insecticide for controlling *S. littoralis* larvae and need further studies for isolation of their active constituents.

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