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Research Article
Pesticidal Effect of Leaves Extract of Different Plants on the Larvae of Cotton Leaf Worm, *Spodoptera littoralis*

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Abstract

Background and Objective: The cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), represents the worst lepidopterous pest on most vegetable crops. Moreover, its host range is extensive because it is a polyphagous and cosmopolitan pest that can cause an estimated loss of nearly 25.8 to 100% of crop production. Natural controls have replaced pesticides due to the environmental damage that chemical pesticides cause. Eggplant, tomato, okra and pepper are Egypt's most significant vegetable crops and the leaves of these plants are cheap and can act as botanical and nontoxic pesticides. **Materials and Methods:** *Spodoptera littoralis* larvae were reared in the laboratory and plant leaves of eggplant, tomato, okra and pepper were extracted in the laboratory with organic solvents. Then, these extracts were applied to the larvae. Therefore, the histological sectors in larvae bodies were prepared and the changes were observed compared with the control. **Results:** This study proved the efficiency of all plant leaf extracts, especially those of eggplant and tomato leaves (LC_{50} of 1191.9 and 1582.7 ppm, respectively). In contrast, okra and pepper leaf extracts had the highest LC_{50} (3904.8 and 5431.6 ppm, respectively). Also, the abnormalities in the midgut and internal tissues were evident in the larvae treated with eggplant leaf extracts, followed by tomato leaf extracts and okra leaf extracts. Larvae treated with pepper leaf extracts were as normal as the control. **Conclusion:** The current study opens horizons to new plant extracts that are cheap and unused plant parts and make them helpful as new and good insecticides.

Key words: *Spodoptera littoralis*, eggplant leaves, tomato leaves, pepper leaves, okra leaves, histological changes

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Spodoptera littoralis (Boisd.) (Lep., Noctuidae), sometimes known as the Egyptian cotton leafworm, is a significant pest of cotton and other cultivated crops in Egypt and other Middle Eastern nations[1]. More than 87 host plants from 40 different plant groups are infected by it[2].

In Egypt, this pest has been managed for years using pesticides. However, most pesticides have hazardous side effects on both plants and animals. In addition, various issues are found with insecticides. One of these is pest resistance to each of the principal classes of synthetic pesticides[3].

Research on botanical pesticides has been ongoing for a long time to synthesize alternatives to traditional pesticides. The phytochemical examination of eggplant reveals that it is an excellent source of many necessary substances, such as tryptophan, flavonoids and steroid alkaloids found in its leaves and fruits. These substances are valuable in treating several illnesses, including anti-platelet, hypolipidemia, cancer, hypotension and asthma[4]. The antioxidants, phenolic compounds and other nutrients found in tomatoes help to prevent oxidative cell damage by lowering the levels of free radicals[5]. In addition, okra plant extract consists mainly of triacylglycerols and fatty acids[6]. Furthermore, pepper leaves contain high enough terpenoid content[7]. This research aimed to evaluate the effects of eggplant, tomato, okra and pepper leaf extracts on larvae of the cotton leaf worm, *Spodoptera littoralis* and determine the histological changes in the body of treated larvae compared with control.

MATERIALS AND METHODS

Study area: The experiments were carried out in the Plant Protection Research Institute, Mansoura Branch, 2020 to 2021.

Rearing of the cotton leaf worm, *Spodoptera littoralis*: The Plant Protection Research Institute, Dokki, Egypt, provided a laboratory-sensitive strain of the cotton leafworm, *S. littoralis* (Lepidoptera: Noctuidae). Under conditions of 65±5% RH, 27±2°C, 10 hrs dark and 14 hrs light, on castor leaves that were provided daily, the larval stages were grown in a lab. On the 3rd day after emerging, the adults were kept apart and mated in clean jars (4 lb). Then, in order to increase egg production, they were fed a 10% honey solution and given fresh, green tafla leaves of *Nerium oleander* L.[8].

Plant sample preparation and extraction: After a month of room temperature drying, pepper, tomato, eggplant and okra plant leaves were ground into a fine powder. Each plant's powder was immersed for roughly a week in a solution of petroleum ether, ethanol and acetone (1:1:1). After that, the contents of the flasks were filtered after being shaken in a shaker. Under reduced pressure, the solvents were evaporated, after which the crude extracts were weighed and stored in a deep freezer.

Preparing the stock solution of the tested plant leaf extracts: Stock quantities of each extract were made using distilled water and the weight of the examined plant (w/v), together with Tween 80 (0.1%) as an emulsifier. Glass bottles with glass stoppers were used to store the stock concentrations, which were then refrigerated. The LC-P lines were drawn using four diluted concentrations of each plant leaf extract from the stock solutions, which were generated regularly. For each concentration, three replicates were made.

Plant's active ingredients: The active ingredients and symbols of tested plants were shown in Table 1.

Method of application

Leaf dipping method: Castor leaves were dipped in the concentration under test and allowed to dry in a lab setting. Larvae in their second instar were permitted to eat the leaves. Three replicates of each concentration were made. The living recipients of the treatment were observed daily until final mortality, which was estimated and corrected using Abbott's formula[13]. After treatment, mortality was monitored every day for 7 days. The emulsifier served as a control and water alone

Table 1: Active ingredients and symbols of tested plants

Plant	Active ingredient	Chemical symbol
Eggplant (*Solanum melongena*)	Nasunin[9]	$C_{42}H_{47}CLO_{23}$
Tomato (*Lycopersicon* spp.)	Lycopene[10]	$C_{40}H_{56}$
Okra (*Abelmoschus esculentus* L)	Hexanedioic acid[11]	$C_{22}H_{42}O_4$
Pepper (*Capsicum annuum* group)	Capsaicin[12]	$C_{18}H_{27}NO_3$

was employed similarly. The following equation was used to determine the LC_{50} index[14]:

$$\text{Toxicity index for } LC_{50} = \frac{LC_{50} \text{ of the most effective compound}}{LC_{50} \text{ of the least effective compound}} \times 100$$

Statistical analysis: LC_{50} values were calculated using the statistical method of probitanalysis[15].

Histopathological studies: The leaf dipping method was used to apply the LC_{50} of the investigated substances to the larvae in their fourth instar. The tissue processing, sectioning and staining were carried out according to Corzo *et al.*[16] and Wick[17].

Larvae were removed 96 hrs following treatment, transferred to an alcoholic version of Bouin's solution, used as a fixative to dehydrate them and remove the yellow color and then rinsed in a series of ethanol solutions. The larvae were first immersed for 2 hrs at 40°C in 50% ethyl alcohol before being left for 24 hrs. The larvae, however, underwent a series of alcoholic treatments, each lasting two hours at room temperature, starting with 80% alcohol, followed by 90%, 96% alcohol and finally 100% alcohol. The larvae were placed in an amyl acetate solution and a soft paraffin wax solution after being dehydrated and they were left there at 50°C for 24 hrs. At intervals of 24 hrs, the larvae were added to soft paraffin wax three times at 50°C. Then, a mixture of one part of hard paraffin wax was added to the larvae and in the final step, the larvae were immersed in the wax mixture. Serial sections measuring 6 microns thick were cut using a microtome, mounted on clean slides using Mayer's albumin, stained with hematoxylin, counter stained with alcohol and then prepared for analysis and photomicroscopy.

RESULTS

Toxicity effect

Efficiency of plant leaf extracts on larvae of *Spodoptera littoralis*: Table 2 showed that eggplant leaf extracts had the highest mortality proportion, followed by tomato leaf extracts, okra leaf extracts and pepper leaf extracts. At 10.000 ppm, the mortality rate was 90, 83.33, 73.33 and 66.67 ppm for eggplant extracts, tomato extracts, okra extracts and pepper extracts.

Determination of the median LC_{50} of some plant extracts against 2nd instar larvae of the cotton leafworm, *Spodoptera littoralis*.

Table 3 demonstrated the most efficient plant extract against larvae of *S. littoralis*. Eggplant extract was the most effective extract with a sub-lethal concentration of LC_{50} 1191.9 ppm, followed by tomato extract with LC_{50} 1582.7 ppm, then okra extract with LC_{50} 3904.8 ppm and pepper extract with LC_{50} 5431.6 ppm. Also, the LC_{90} of eggplant extract was 13012 ppm, followed by tomato extract with an LC_{90} of 17597.9 ppm, okra extract with an LC_{90} of 28531.81 ppm and pepper extract with an LC_{90} of 37097.09 ppm.

Histological studies of larvae of *Spodoptera littoralis*: The histological changes between larvae treated with eggplant extract and non-treated larvae (control) were illustrated in Fig. 1. The histological changes revealed that the eggplant extract was the most effective and caused many changes in the internal tissues of the treated larvae. The sections in the midgut of treated larvae with eggplant leaf extract showed complete separation of the basement membrane of the midgut. Also, destruction of the peritrophic membrane was

Table 2: Mortality % of 2nd instar larvae of the cotton leaf worm, *S. littoralis*, treated with some plant extracts under laboratory conditions

		Mortality after treatments (%)				
Treatment	Concentration (ppm)	1st day	3rd day	5th day	7th day	Total mortality
Tomato extract	1000	-	6.67%	13.33%	20%	40
	2500	3.33%	10%	20%	26.67%	60
	5000	6.67%	13.33%	26.67%	26.67%	73.33
	10000	6.67%	20%	26.67%	30%	83.33
Eggplant extract	1000	-	10%	20%	20%	50
	2500	3.33%	13.33%	20%	23.33%	60
	5000	6.67%	20%	23.33%	26.67%	76.67
	10000	10%	26.67%	30%	23.33%	90
Pepper extract	1000	-	-	3.33%	10%	13.33
	2500	-	3.33%	10%	16.67%	30
	5000	3.33%	10%	16.67%	16.67%	46.67
	10000	3.33%	16.67%	26.67%	20%	66.67
Okra extract	1000	-	3.33%	6.67%	10%	20
	2500	-	6.67%	10%	20%	36.67
	5000	3.33%	13.33%	23.33%	16.67%	56.67
	10000	6.67%	13.33%	26.67%	26.67%	73.33

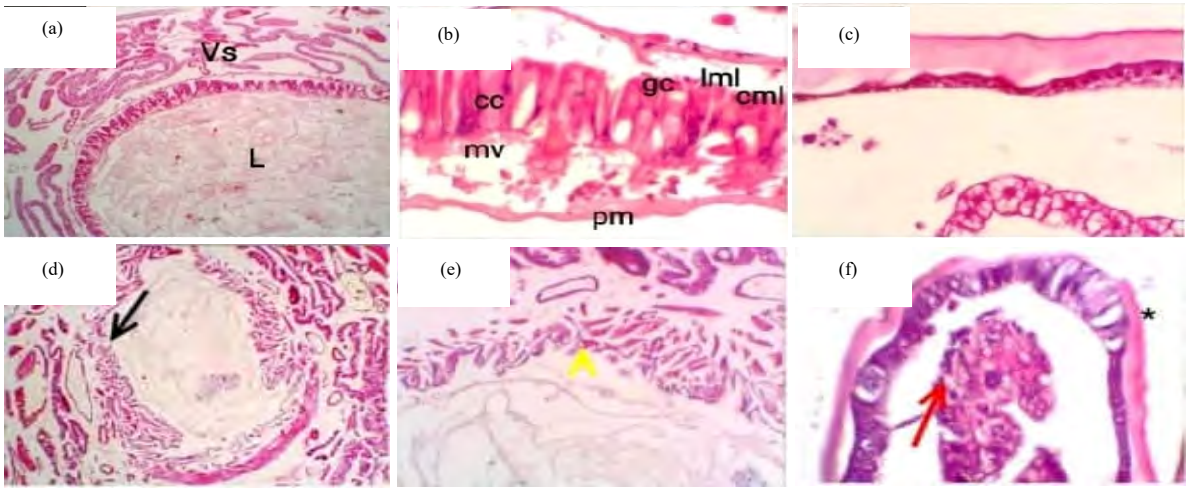

Fig. 1(a-f): Histological changes in larvae treated with eggplant extract and control, (a-c) Microscopic pictures of H&E stained *S. littoralis* sections of different regions, as (a, b = midgut) and (c = cuticle) showing normal and continuous structure and (d-f) Microscopic pictures of H&E stained midgut of *S. littoralis* treated with eggplant extract

cc: Columnar cell, mv: Microvilli, cml: Circular muscle layer, lml: Longitudinal muscle layer, gc: Goblet cell, pm: Peritrophic membrane, L: Lumen, vs: Vesicles, Black arrow: Complete separation for the basement membrane, Arrow head: Complete destruction in the peritrophic membrane, *Disappearing of basement membrane and increase of vacuoles and decreased the cuticle thickness with abnormal deposited and Red arrow: Necrosis fo the some cells

Table 3: Efficacy of some plant extracts against 2nd instar larvae of the cotton leafworm, *S. littoralis*

Treatment	Concentration	Corrected mortality (%)	LC_{50}	LC_{90}	Slope±SD	Toxicity index LC_{50}	LC_{90}/LC_{50}
Tomato extract	1000	40	1582.7	17597.9	1.23±0.18	75.31	11.1
	2500	60					
	5000	73.33					
	10000	83.33					
Eggplant extract	1000	50	1191.9	13012.4	1.23±0.19	100.00	10.9
	2500	60					
	5000	76.67					
	10000	90					
Pepper extract	1000	13.33	5431.6	37097.1	1.54±0.19	21.94	6.8
	2500	30					
	5000	46.67					
	10000	66.67					
Okra extract	1000	20	3904.8	28531.8	1.48±0.19	30.52	7.3
	2500	36.67					
	5000	56.67					
	10000	73.33					

observed. The disappearance of the basement membrane and the increase of vacuoles decreased the cuticle thickness, with abnormal deposits and necrosis of some cells. In contrast, the control showed a normal and continuous structure.

The effect of tomato leaf extract on the histological and internal tissues of the larvae of *S. littoralis* was shown in Fig. 2. Also, there was a partial separation in the midgut's basement membrane and some necrotic cells' appearance. Besides, there was a partial separation of the peritrophic membrane, decreased cuticle thickness and increased vacuoles.

Furthermore, Fig. 3 showed that the larvae of *Spodoptera littoralis* treated with okra leaf extract showed a scattering of the nuclear content of the ill-defined epithelial cells of the midgut with disorganized, shortened and confluent microvilli membranes. A minor vacuolization caused the cells to start swelling. This abnormality in tissues was less than the abnormalities in tissues in larvae that were treated with eggplant and tomato leaf extracts. Furthermore, the untreated larvae of *S. littoralis* showed a normal and continuous structure of midgut tissues.

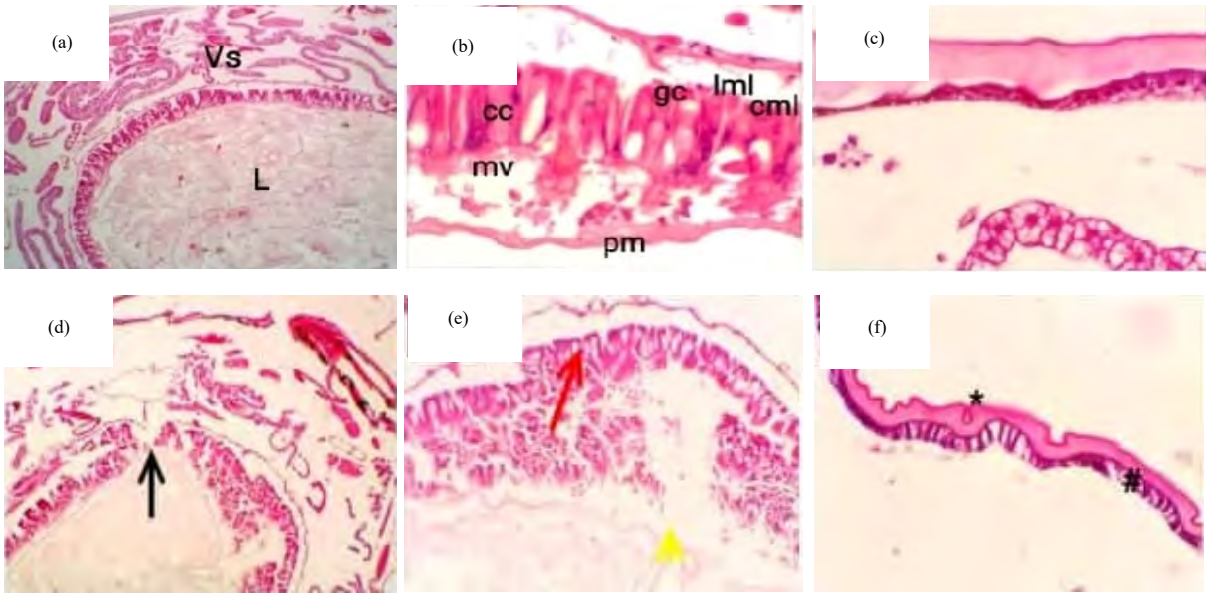

Fig. 2(a-f): Histological alterations in control and tomato extract-treated larvae, (a-c) Microscopic pictures of H&E stained *S. littoralis* sections of different regions, as (a, b = midgut) and (c = cuticle) showing normal and continuous structure and (d-f) Microscopic pictures of H&E stained midgut of *S. littoralis* treated with tomato extract

cc: Columnar cell, mv: Microvilli, cml: Circular muscle layer, lml: Longitudinal muscle layer, gc: Goblet cell, pm: Peritrophic membrane, L: Lumen, vs: Vesicles, Black arrow: Partially separation in basement membrane of midgut, Red arrow: Appear of some necrotic cells, Arrow head: Partially separation of the peritrophic membrane, *Decreased the cuticle thickness and #Increase of vacuoles

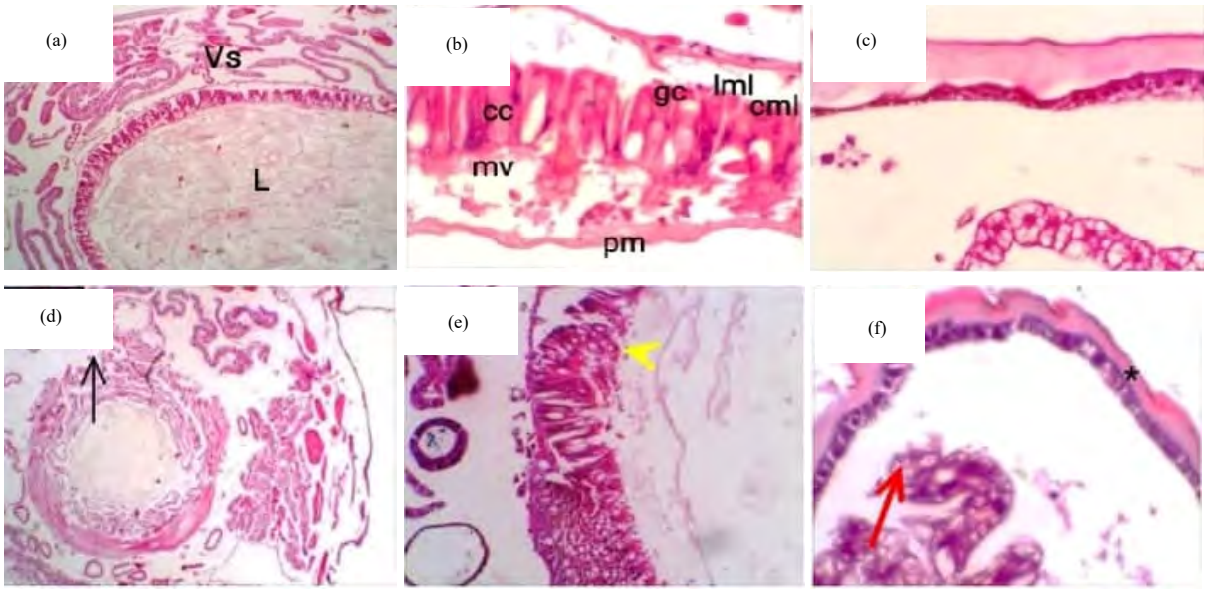

Fig. 3(a-f): Histological alterations in control and okra extract-treated larvae, (a-c) Microscopic pictures of H&E stained *S. littoralis* sections of different regions, as (a, b = midgut) and (c = cuticle) showing normal and continuous structure and (d-f) Microscopic pictures of H&E stained midgut of *S. littoralis* treated with okra extract

cc: Columnar cell, mv: Microvilli, cml: Circular muscle layer, lml: Longitudinal muscle layer, gc: Goblet cell, pm: Peritrophic membrane, L: Lumen, vs: Vesicles, Black arrow: Scattering of the nuclear content of the ill-defined epithelial cell of midgut, arrow head: Disorganized, shortened and confluent microvilli membranes, *Decreased the cuticle thickness and Red arrow: Cells began to swell via a slight vacuolization

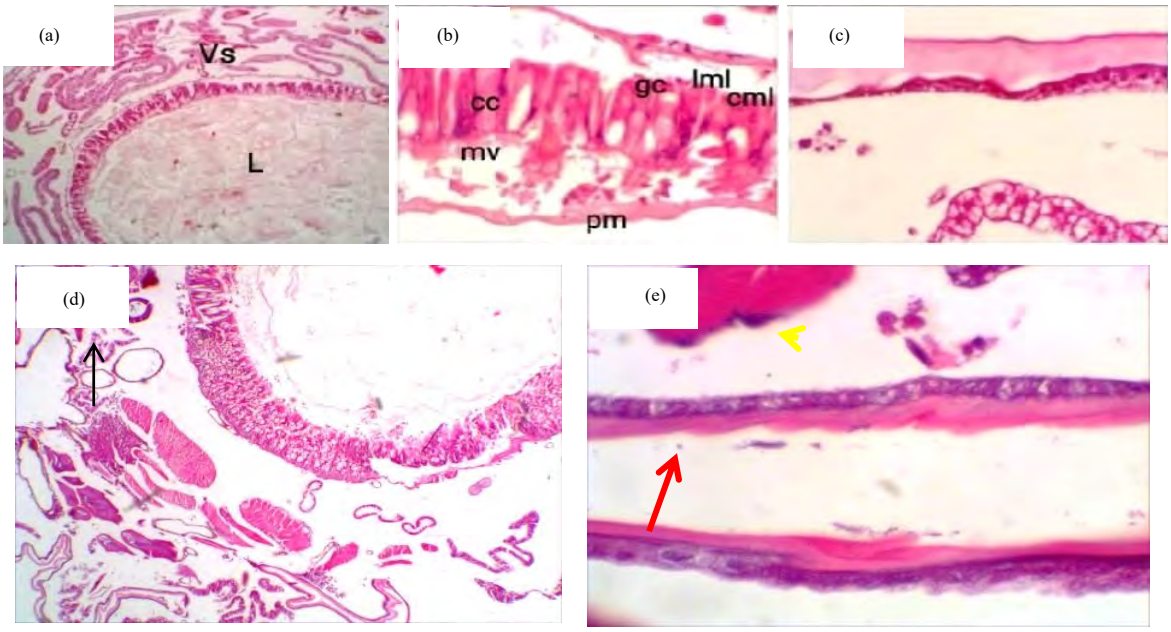

Fig. 4(a-e): Histological changes of larvae treated with pepper extract and control, (a-c): Microscopic pictures of H&E stained *S. littoralis* sections of different regions, as (a, b = midgut) and (c = cuticle) showing normal and continuous structure and (d-e) Microscopic pictures of H&E stained midgut of *S. littoralis* treated with pepper extract. This section shows the structure of the midgut is almost normal

cc: Columnar cell, mv: Microvilli, cml: Circular muscle layer, lml: Longitudinal muscle layer, gc: Goblet cell, pm: Peritrophic membrane, L: Lumen and vs: Vesicles

Likewise, the histological changes in the structure of *S. littoralis* larvae treated with pepper leaf extract was demonstrated in Fig. 4. The treated larvae showed that the structure of the midgut was almost intact.

DISCUSSION

Current results showed that the eggplant extract had a strong effect on the larvae of *S. littoralis* followed by tomato extract, okra and pepper extracts. Also, the treated larvae with eggplant extract showed high histological changes inside the tissues of the cuticle and midgut of the treated larvae. Still, these changes slightly decreased with larvae treated with tomato extract, then okra extract showed moderate changes but larvae treated with pepper extracts had slight changes. The highest effect of eggplant extract may be due to solanine which is found inside eggplant, this material is toxic against pests.

These results agreed with Sitap *et al.*[18] that eggplant leaf extracts had an effective substance valuable for humans. Ghada *et al.*[19] assessed the impact of an extract from tomato leaves against *Aphis gossypii* and proved its high efficiency. Also, Abd-Allah *et al.*[20] confirmed the effectiveness of tomato leaf extracts on larvae of *Tuta absoluta* and *Spodoptera littoralis*. El-Wahab *et al.*[21] observed that camphor and menthol extracts highly affected the unhatched eggs of *S. littoralis*. Also, Ahmed *et al.*[22] illustrated that onion leaf extract was highly effective with *S. littoralis*.

Current findings confirmed those of El-Din *et al.*[23], who discovered the effect of plant extracts of Jojoba oil and flaxseed oil on the larvae of pink bollworm and spiny bollworm. They observed the changes in the cuticle and mid-gut tissues of the 4th instar larvae that showed vacuolization with hypertrophied lining mucosal epithelial. Ahmed *et al.*[24] also reported that *A. ipsilon* had histological alterations in the midgut as a result of treatment of larvae with neem products.

CONCLUSION

Larva of *S. littoralis* is a destructive pest for all crops, so this study introduces eggplant extract as a new pesticide that is cheap and effective in causing mortality and malformation in the bodies of larvae. However, farmers burn the leaves of eggplant, causing pollution to the environment. We could use these leaves to control pests such as cotton leafworms.

SIGNIFICANCE STATEMENT

The fundamental concept of this manuscript is to make use of the insignificant parts of plants, particularly leaves, since they are useless when using chemical pesticides, which is very expensive. Additionally, growers burn these plant parts, increasing pollution. Therefore, by evaluating plant sections that contain harmful and beneficial substances for managing pests, such as alkaloids, triterpenes and others, the trials showed that these substances are effective at both controlling pests and causing malformation in their internal bodies.

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