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Full Length Research Paper

Biochemical and histopathological effect of crude extracts on *Spodoptera littoralis* larvae

Sayed M. Rawi[1]*, Fayey A. Bakry[2] and Mansour A. Al-Hazmi[1]

[1]Faculty of Science and Arts, Khulias, King Abdul- Aziz University, Jeddah, UAK.
[2]Medical Malacology Department, Theodor Bilharz ,Research Institute ,Giza, Fgypt,

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The present investigation aims to throw light on the effect of methylene chloride and normal hexane extracts of *Azadirachta indica, Citrullus colocynthis, Ammi majus* and *Mentha microphylla* on the 4th larval instar of the cotton leaf worm, *Spodoptera littoralis* under laboratory conditions. The results showed that the methylene chloride extract of *A. indica, C. colocynthis* are the most potent extracts against *S. littoralis* larval. The present study was also extended to conduct the insecticidal effect of the most potent extracts *A. indica, C. colocynthis* methylene chloride extract post formulation on *S. littoralis* larval. Marked biochemical changes however, being recognized in pest as marked decrease in total lipids, total protein and glucose contents. The activity of both ALAT and ASAT are also being highly affected. The tested dose levels also, showed highly histopathological disturbances in the midgut and body wall cells of this pest. Among the most recorded observations are vacuolation, destruction of the cells.

Key words: Cotton leaf worm (*Spodoptera littoralis*), *Azadirachta indica*, *Citrullus colocynthis*, *Ammi majus*, *Mentha microphylla* plant, biochemical and histopathological studies.

INTRODUCTION

The cotton leaf worm, *S. littoralis* (Boised) is a swarming polyphagous, foliage feeding insect that is distributed throughout the world. This insect is one of the major cotton pests that causes considerable damage to many impotent vegetables and crops (Shonouda and Osmam, 2000; Maged El-Din and El- Gengaihi, 2000; El-Khawas and Abd El-Gawad, 2002).

Many different countries search for less dangerous pesticides by using the naturally occurring herbs that can be applied effectively in habitats (Ebieri, 1992; Shoeb et al*.,* 1992; Rawi et al., 1995; 1996; Bruno et al*.,* 2003; Sadek, 2003).

Many workers reported that plants are considered as one of the richest sources that can be used as pest control agents (Hoste et al., 2000; Jannet et al*.,* 2000; Nakatani et al*.,* 2002; Schmidt and Assembe, 2002). In Egypt, attempts have been done to monitor insecticidal activity of different plants extracts against many insects (Farag, 2002; Sadek, 2003).

The aim of the present investigation is to determine insecticidal effects of two different organic solvents of *A. indica* and *C. colocynthis* against the 4th larval instar of *S. littoralis* larval. The present study is also extended to evaluate the most potent promising plant extracts post formulation on the biological and physiological activities of the studied pests.

MATERIALS AND METHODS

Experimental pests

Cotton leaf worm (Spodoptera littoralis)

A colony of the cotton leaf worm, *S. littoralis* (Family: Lepidoptera) is maintained in the laboratory for many generations under laboratory conditions at 25±2°C according to Ghoneim (1985). It was obtained from Central Laboratory of Insecticides, Agriculture Research Center, Dokki, Giza, Egypt. The tests were carried out on the 4th instar larvae.

*Corresponding author. E-mail: sayedrawi48@yahoo.com

Plants

Four plants are used for this study. *Azadirachta indica* (Neem, Family Meliaceae) was collected from Sudan. *Citrullus colocynthis* (Handal, Family Cucurbitaceae), *Ammi majus* (Khela,Umbelliferae) and *Mentha microphylla* (Habq El bahr, Family Labiatae) were collected from Egypt.

Plant extracts preparation

Leaves of *A. majus* and *M. microphylla*, fruits of *C. colocynthis* and seeds of *A. indica* were collected, cleaned from the dust and debris and then dried separately under room temperature. The plants were grounded with an electric mill. Two solvents were used for extraction: methylene chloride and normal hexane.

A sample of 200 g powder of each plant was soaked in 800 ml of the solvent methylene chloride for 4 days and shook for 1 h every day using an electric shaker. The extract was filtered over anhydrous sodium sulphate. The solvent was then evaporated under reduced pressure using a rotator evaporator at 30°C. After that, the remaining powder was soaked again in 800 ml of the solvent normal hexane and treated as methylene chloride by the same procedure. The extracted solutions were left away for complete dryness to obtain the crude extracts. The extracts of *A. indica, A. maius* and *M. microphvlla* were carried out by Freedman et al. (1979) method with some modifications, and the extract of *C. colocvnthis* was carried out by using Soxhlet apparatus in the same order of solvents.

In all cases, the crude extract was transferred quantitatively to a clean and dark flask, and then kept in the refrigerator until used for toxicological investigations.

Stock solution

A known weight of the crude extract was added to an appropriate volume of the solvent (acetone) to obtain the stock solution. Control stock solution was prepared by adding the same volume of acetone used in the test stock to the appropriate prepared one parallel to the tested ones being compared.

Bioessay test

The different concentrations of each plant extract were tested on the 4th instar larvae of *S. littoralis*. Leaf dipping technique was used. The same sizes of fresh castor bean leaves were dipped in each tested concentration of plant extracts and in the control for 20 s and left to dry. The dried leaves were put singly in plastic cups. Ten larvae were transferred to each cup and allowed to feed on the treated leaves for one day. Three replicates for each concentration were done. After 24 h surviving larvae were transferred to clean cups and supplied daily with untreated leaves until the end of experiment. For control experiments, plants leaves were dipped separately in different concentrations prepared from acetone. Mortality counts were recorded daily after 24 h from treatment until the end of experiment and corrected according to Abbott (1925). Also, development duration of instars was observed and calculated according to Dempster (1957). Malformation of different stages of pests was observed and the percentage of deformation was calculated. Mortality values of the 7th and 14th days after exposure were analyzed by probit analysis (LDP line) to obtain $LC_{50,}$ LC_{90} and slope for each extract according to a method adopted by Finney (1971). The most effective plant extracts were selected for further experiments.

Toxicity index

The equation of Sin (1950) was applied to evaluate the efficiencies as follows:

$$\text{Toxicity index} = \frac{|LC_{50} \text{ 0f the most effective compound}}{\text{LC50 of the compound used}} \times 100$$

In this equation the most toxic compound has been given 100 unit on the toxicity index scale. The selective toxicity ratio (SER) is compressed in terms of the mean lethal concentrations (MLC) in the following way;

$$\text{SER} = \frac{\text{MLC for species A}}{\text{MLC for species B}}$$

Biochemical studies and sampling

After feeding on treated leaves with LC_{10} of the two extracts for 24 h, the live pests (*S. littoralis)* were collected and allowed to feed on normal leaves after 1, 2 and 10 days. A specific number of each pest were taken and subjected directly to biochemical assays.

The collected pests were collected at intervals. The homogenates were centrifuged at 3500 r p m for 10 min and the supernatants were filtered through glass wool to remove fatty materials. They were kept in deep freezer at -20°C before being used for determination of glucose, total protein and lipid concentration, aspartate amino transferase (ASAT) and alanine amino transferase (ALAT) activities.

Aspartate amino transferase (ASAT) and alanine amino transferase (ALAT) activities were determined according to the method of Reitman and Frankel (1957). Total protein was determined according to the method of Weichselbaum (1946). Glucose concentration was determined according to the method of Trinder (1962) and total lipids were determined according to the method of Zollner and Kirsch (1962).

Histopathological examination

The LC_{10} and LC_{25} of the two extracts were used. Samples of tested individuals from both insect species were dissected after 48 h from exposure. Another sample was taken after 10 days using *S. littoralis*. Body wall and mid gut of both species were fixed separately in alcoholic Bouin' s solution for 24 h, washed in ethanol (70%) and then dehydrated in an according series (70-100%) of ethyl alcohol. Infiltration embedding of samples was carried out in Paraffin wax. Sections (5 µm) were stained in Ehrlisch´s haematoxylin and eosin.

Statistical analyses

Data obtained were analyzed by student (t) test according to the equation of Dixon and Massay (1957). Significant differences were established at $P<0.05$ and $P<00.01$ levels.

RESULTS

Toxicity testing and selective toxicity ratio of plant extracts

Effect on the 4th larval instar of the cotton leaf worm Spodoptera littoralis

7 days post treatment: From the data recorded in Table 1

Table 1. Toxicity of non-formulated extract of different plants on *Spodoptera littoralis* larvae after 7 days of treatment.

Toxicity testing \ Plant	Extract							
	Normal hexane extract				Methylene chloride extract			
	A. indica	***C. colocynthi***	***A. majus***	***M. microphylla***	***A. indica***	***C. colocynthis***	***A. majus***	***M. microphylla***
LC_{50}	2.29×10^2	8.44×10^2	2.06×10^2	2.53×10^2	90.15	117	300	217
LC_{90}	2.49×10^2	7.22×10^2	7.13 × 102	8.28×10^2	2.86×10^2	4.14×10^2	6.94×10^2	1.04×10^2
Slope	1.24	1.38	2.38	2.49	0.85	0.83	0.94	1.89
Toxicity index	39.36	10.68	43.76	35.63	100	76.85	30.01	41.51

LC_{50}: Median lethal concentration. LC_{90}: Acute lethal concentration.

Table 2. Toxicity of non-formulated extract of different plants on *Spodoptera littoralis* larvae after 14 days of treatment.

Toxicity testing \ Plant	Extract							
	Normal hexane extract				Methylene chloride extract			
	A. indica	***C. colocynthi***	***A. majus***	***M. microphylla***	***A. indica***	***C. colocynthis***	***A. majus***	***M. microphylla***
LC_{50}	2.06×10^2	*	3.8×10^2	6.19×10^2	33.02	68.65	1.41×10^2	2.73×10^2
LC_{90}	1.83×10^2	*	1.45×10^2	2.36×10^2	1.66×10^2	2.81×10^2	6.6×10^2	8.57×10^2
Slope	0.66	*	0.81	0.81	0.75	0.80	0.77	0.86
Toxicity index	16.03	*	8.69	5.33	100	48.11	23.48	12.11

LC_{50}: Median lethal concentration; LC90: Acute lethal concentration. All Larvae are are metamorphosed.

1, it was observed that, the different plant extracts had an effect on the cotton leaf worm, *S. littoralis*. Generally, the percentages of larval mortality were more by using methylene chloride as extract than normal hexane. The LC_{50} values by using methylene chloride were 90.15, 117.00, 300.00 and 217.6 mg/ml for *A. indica, C. colocynthis, A. majus* and *M. microphylla* extracts, respectively. Comparatively, by using normal hexane for the same plants, the half lethal toxicity values were $2.79x10^2$, $2.53x10^2$ and $8.44x10^2$ mg/ml for *A. majus*, *M. microphylla*, *A. indica* and finally *C. colocynthis* which showed the least toxicity.

About the determined LC_{90} values, *A. majus*, *M. microphylla* normal hexane extracts were the most potent effective against the 4th larval instar of *S. littoralis*. The determined values were 7.13×10^2 and 8.28×10^2 mg/ml, respectively. On the other hand, the least toxicity values were that of *C. colocynthis* (7.22×10^2) normal hexane extract and *A. majus* (6.98×10^2) methylene chloride extract.

In the present investigation, the assay of toxicity index is also very important for evaluating the toxicity of each plant extract when using either normal hexane or methylene chloride as extract. However, the least toxicity index value was attained with the effect of *C. colocynthis* normal hexane extract (10.68) and the higher value was given with the effect of *A. indica* methylene chloride extract (100).

14 days post treatment: The toxicity testing on the 4th larval instar of *S. littoralis* post 14 days of treatment with the tested plants extracts is given in Table 2. The obtained LC_{50} values revealed the higher potency of *A. indica*, *C. colocynthis* and *A. majus* methylene chloride extract. The LC_{50} values were 33.02, 68.65 and 1.41×10^2 mg/ml,

respectively. On the other hand, *M. microphylla* methylene chloride extract appears to be the less effective since the recorded LC_{50} value was 2.73×10^2 mg/ml.

Regarding the effect of normal hexane extracts of the same plants on the *S. littoralis* larvae, all the recorded values are less effective compared to those recorded with methylene chloride. The higher potency of such type of extract was attained on *A. indica* plant since the recorded value was 2.06×10^2 mg/ml. In *C. colocynthis* treatments, all larvae were metamorphosed into pupae, since no larval mortality was attained.

Regarding the toxicity index, there can be very large differences between susceptibility and the toxic action of the tested plant extract. The maximum value was attained with *A. indica* methylene chloride extract (100) and the lower value (5.33) was given with the effect of *M. microphylla* normal hexane extract.

Biochemical effects

Effect of the tested extracts on ASAT activity

The activity of aspartate aminotransferase in the whole body of the homogenate of the 4th instar larvae of *S. littoralis* was determined after treating with LC_{10} extracts of *A. indica and C.colocynthis*. The result is given in Table 3.

The data recorded revealed increase in the level of the enzyme activity in all the test intervals after exposure to LC_{10} of the two tested extracts. As compared to the control level, the percentages of changes at the 1st, 5th and 10th days were 4.63, 12.18 and 11.70%, respectively after exposure to *A. indica* extract and 6.09, 8.76 and 17.25% respectively post exposure to *C. colocynthis*. On the other hand, the maximum percentage of increase was got after 5 and 10 days post exposure to *A. indica and C. colocynthis*, respectively.

Effect of the tested extracts on ALAT activity

The data recorded in Table 4 show that the effect of LC_{10} of both plants induced a marked decrease in ALAT activity at the 1st and 5th days. This was followed by an increase at the 10th day for the two tested extracts. The percentages of changes at 1, 5 and 10 days were -4.25, -4.84 and 4.57%, respectively in *C. colocynthis* extract, as compared with the control level.

Effect of the tested extracts on total protein

The data in Table 5 show the effect of LC_{10} extracts of *A. indica* and *C. colocynthis* on the concentration of total protein of the 4th instar larvae of *S. littoralis*. From the data recorded, it was shown that there was a highly significant decrease in the level of total protein of treated larvae post exposure to LC_{10} of *A. indica and C. colocynthis* extracts throughout the test intervals. The percentage of changes reached its maximal after 10 days (-13.5 and -11.77%) for both extracts of *A. indica* and *C. colocynthis*, respectively.

Effect of the tested extracts on glucose content

Data in Table 6 showed the effect of LC_{10} extracts of *A. indica* and *C. colocynthis* on glucose concentration of 4th Instar larvae of *S. littoralis*. The data recorded showed a significant decrease of glucose content throughout the test periods. The recorded values were, however, significantly decreased by -12.26, -12.35 and -30.79% post the 1st, 5th and 10th days of *A. indica* exposure. On the other hand, the post exposure to the dose level of LC_{10} *C. colocynthis* extract showed a more highly significant decrease of *S. littoralis* larvae glucose level on the 1st and 10th days. The recorded values were decreased by -9.63 and -17.88%, respectively compared to the control level.

Effect of the tested formulated extracts on total lipid extract

Table 7 showed the effect of LC_{10} *A. indica* and C. *colocynthis* formulated extracts on the concentration of total lipid of the 4th Instar larvae of *S. littoralis*. The data recorded revealed highly significant decrease in the level of total lipid post treatment with LC_{10} A*. indica* and *C. colocynthis* throughout the test intervals. The maximal percentage of reduction was reached after 10 days (-30.23 and -41.19%) for both *A. indica* and *C. colocynthis* formulated extracts, respectively.

Effect of the tested extracts on lipid content

Table 8 showed the effect of LC_{10} of *A. indica and C. colocynthis* extracts on the concentration of total lipid of the 4th Instar larvae of *S. littoralis*. The data recorded revealed highly significant decrease in the level of total lipid post treatment with LC_{10} of *A. indica* or *C. colocynthis* throughout the test intervals. The maximal percentage of reduction was reached after 10 days (-30.23 and -41.19%) for both *A. indica and C. colocynthis* formulated extracts.

Histopathological effects

Histopathological changes in the body wall of treated Spodoptera littoralis

The histopathological changes appearing in the integument of the 4th instar larvae treated with LC_{10} extract of *A. indica* 48 h post treatment were dissolution

Table 3. Mean activity of ASAT in *S. littoralis* larvae treated with the formulated extract of *A. indica* and *C. colocynthis.*

Formulated extract	Dose mg/ml	Exposure period (day)					
	LC_{25}	1	%Change	5	%Change	10	%Change
	Control	19.19 ± 0.29		16.99 ± 0.41		14.95 ± 0.23	
Azadirachta indica	6.64	20.08 ± 0.38	4.63	19.06 ± 0.37**	12.18	16.7 ± 0.5*	11.7
Citrullus colocynthis	7.80	20.36 ± 0.30*	6.09	18.48 ± 0.35*	8.76	17.53 ± 0.16**	17.25

Values are presented as Mean±SE. –Non-significant P> 0.05; *Significant P<0.05; ** high significant P<0.01.

Table 4. Mean activity of ALAT in *S. littoralis* larvae treated with the formulated extract of *A. indica* and *C. colocynthis.*

Formulated extract of	Dose mg/ml	Exposure period (day)					
	LC_{25}	1	%Change	5	%Change	10	%Change
	Control	15.27 ± 0.22		13.22 ± 0.17		11.14 ± 0.19	
Azadirachta indica	6.64	14.62 ± 0.12	-4.25	12.58 ± 0.15*	-4.84	11.65 ± 0.25	4.57
Citrullus colocynthis	7.80	13.44 ± 0.11**	-11.98	11.84 ± 0.16**	-10.43	11.49 ± 0.16	3.14

Values are presented as Mean±SE. –Non-significant P> 0.05; *Significant P<0.05; ** high significant P<0.01.

Table 5. Effect of formulated extract of *A. indica* and *C. colocynthis* on total protein concentration of *S. littoralis* larvae.

Formulated extract	Dose mg/ml	Exposure period (day)					
	LC_{25}	1	%Change	5	Change %	10	Change %
	Control	45.59 ± 0.20		48.35 ± 0.15		45.53 ± 0.21	
Azadirachta indica	6.64	42.35 ± 0.21**	-7.11	41.18 ± 0.20**	-14.83	39.35 ± 0.115**	-13.57
Citrullus colocynthis	7.80	43.17 ± 0.251**	-5.31	41.0 ± 0.326**	-15.20	40.17 ± 0.27**	-11.77

Values are presented as Mean±SE. –Non-significant P> 0.05; *Significant P<0.05; ** high significant P<0.01.

Table 6. Effect of formulated extract of *A. indica* and *C. colocynthis* on glucose concentration of *S. littoralis* larvae.

Formulated extract	Dose mg/ml	Exposure period (day)					
	LC_{25}	1	%Change	5	%Change	10	%Change
	Control	66.4 ± 0.87		53.4 ± 0.98		60.40 ± 0.68	
Azadirachta indica	6.64	55.60 ± 0.75**	-16.26	46.8 ± 1.02**	-12.35	41.8 ± 0.73**	-30.79
Citrullus colocynthis	7.80	60.0 ± 0.55**	-9.63	53.8 ± 0.73**	-0.73	49.6 ± 0.68**	-17.88

Values are presented as Mean±SE. –Non-significant P> 0.05; *Significant P<0.05; ** high significant P<0.01

Table 7. Effect of formulated extract of *A. indica* and *C. colocynthis* on total lipid concentration of *S. littoralis* larvae.

Formulated extract	Dose mg/ml	Exposure period (day)					
	LC_{25}	1	%Change	5	%Change	10	%Change
	Control	8.36 ± 0.11		7.09 ± 0.08		6.72 ±0.07	
Azadirachta indica	6.64	7.23 ± 0.09**	-13.51	6.44 ± 0.09**	-9.16	5.71 ± 0.14**	-15.02
Citrullus colocynthis	7.80	6.35 ± 0.12**	-24.04	5.80 ± 0.14**	-18.19	4.83 ± 0.11**	-28.12

Values are presented as Mean±SE. –Non-significant P> 0.05; *Significant P<0.05; ** high significant P<0.01.

of cytoplasmic contents and cell boundaries. Also, the nuclear contents are homogenously distributed (Figure 1) in *A. indica* compared to control (Figure 2). After 10 days, the integument of the 4th instar larvae treated with *S. littoralis* was high and irregular thickness of body wall was also observed (Figure 3).

The treatment with LC_{10} and LC_{25} extract of *C. locynthis* showed cytoplasm granulation (Figure 4), completely destroyed epidermal cells, fat accumulation (Figure 5) and detaching and folding of epithelium cells. Separation of epidermis from cuticle and distortion of muscle fibers is shown in Figure 6.

Histopathological changes in the midgut of treated S. littoralis

Extract of *A. indica* at LC_{10} and LC_{25} dose induced histological damage in the larval midgut, as some of the epithelial cells were vacuolated and destruction of nuclear content also occurred (Figure 7). This is in contrast with control (Figure 8). The extract of *C. colocynthis* at LC_{10} dose level caused degeneration of columnar epithelial cells and vacuolation (Figure 9). The treatment with LC_{25} of formulated extract of *C. colocynthis* caused vacuolation, and detachment of the columnar epithelium cells, after 48 h treatment (Figure 10). This became more apparent and severe after ten days as shown in Figures 11 and 12. The severe detachment of cells from their basement membrane is well seen in Figure 12, whereas in Figure 13 the degeneration of nuclear contents, granulation of cytoplasmic contents and vacuolation were clearly observed.

DISCUSSION

Since the mortality potency of the investigated plant extracts is not the same with that caused by conventional insecticides and because no initial change was observed, the different mortality percentages and the hazard effects of the tested extracts were recorded post 7 and 14 days from treatment. According to Stak and Rangus (1994), neem acts so slowly and one week may be a short interval for its evaluation against aphids. Also, as reported by Schauer (1987), Schmutterer (1990) and Lowery et al. (1993), extracts of neem had no direct contact toxicity. On the basis of the LC_{50} values of the tested plant extracts post 7 and 14 days on the 4th larval instar of *S. littoralis*, it was found that the methylene chloride extracts of the tested plants were more superior to that of normal hexane. The maximum effect was given with *A. indica* followed by *C. colocynthis, M. microphylla and A. majus*. This is in accordance with the findings of other investigators using different plants insecticides including *A. indica* (Mansour et al., 1987; Ismail et al., 1995; Badr, et al., 2000; El-Khawas and Abd El-Gawad, 2002).

The insecticidal potency of *A. indica and C. colocynthis* being more than the extracts of other tested plants can be attributed to several factors including plant specific differences of the extracted active ingredients, types of the extracted products, differences in their mode of action, method of penetrations and the behavioral characteristics of the studied pests (Schmutterer, 1990; Roger et al., 1995). It is now well established that in many plants including the tested plants especially *A. indica* and *C. colocynthis,* the activity is due to the presence of saponin (Marston and Hostettmann, 1985; El-Gengaihi et al., 1988; Rawi et al.,1996), triterpenoid (Schmutterer, 1988) and alkaloids components (Kogan, 1986). Tannins compounds (Klock and Chan, 1982) effect seems to be very specific dependent.

The present study was also extended to evaluate the effect of *A. indica and C. colocynthis* methylene chloride extracts which have shown most plants insecticidal effects post formulation. On the basis of LC_{50} value and in comparison to the values recorded previously, the detected values showed a considerable decrease. They were decreased by -171, -200, -108 and -301% for *S. littoralis* larvae post 7 and 14 days treatment with *A. indica and C. colocynthis*, respectively. This means increased extracts toxicity by formulation.

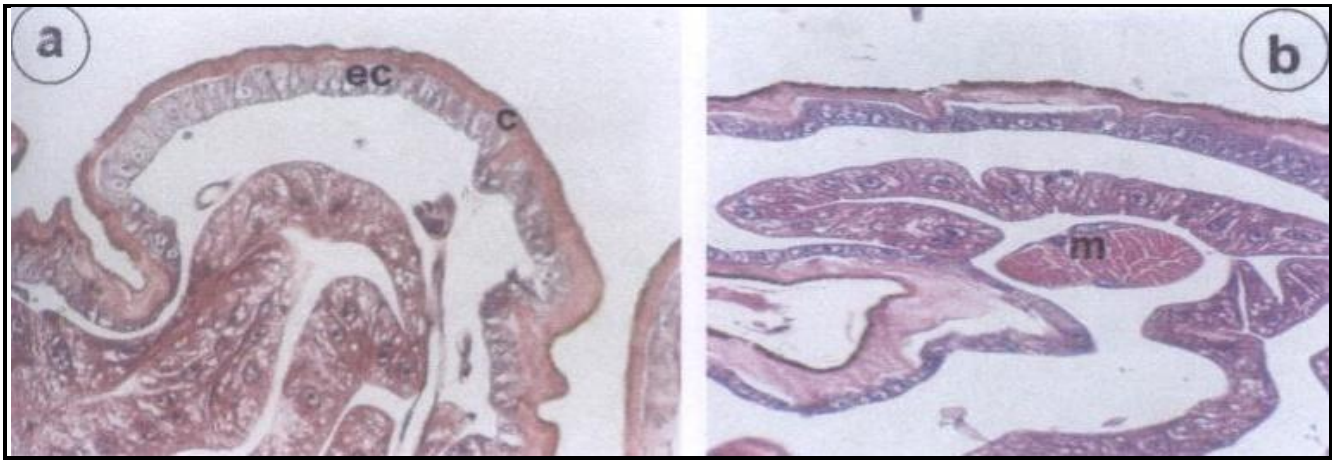

Figure 1. Cross section in the body wall of untreated *S. littoralis* larvae with showing epithelial cell (ec).cuticle (c) (a) after 48 h from the beginning of the experiment and muscle (m) (b) after 10 days from the beginning of the experiment (×400 H&E).

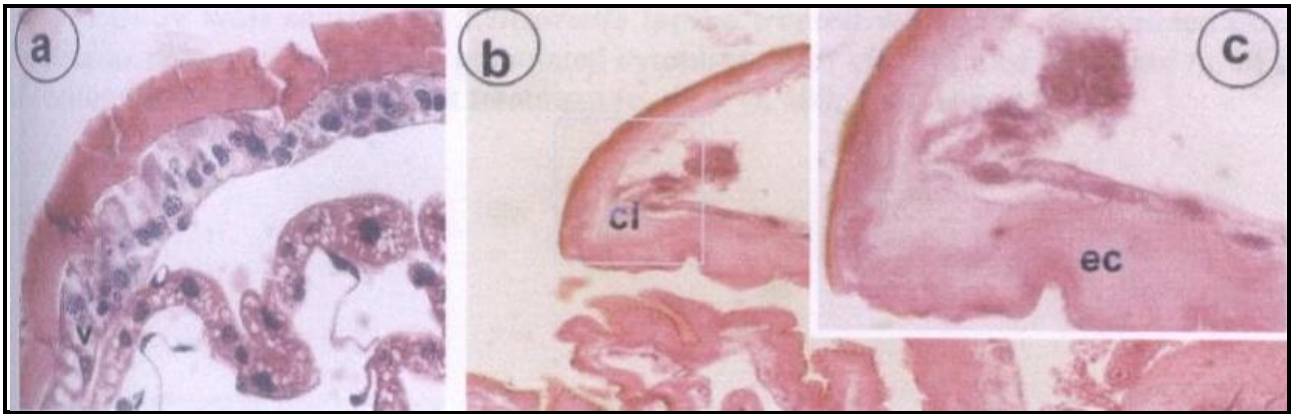

Figure 2. Body wall sections of *S. littoralis* larvae treated with LC_{10} formulated extract of *A. indica* showing vacuolation(v) after 48 h of treatment (a) (400×) and cuticular laver (cl) and epithelial cells (ec) 10 days post treatment (b.c). (×400.1000 H&E).

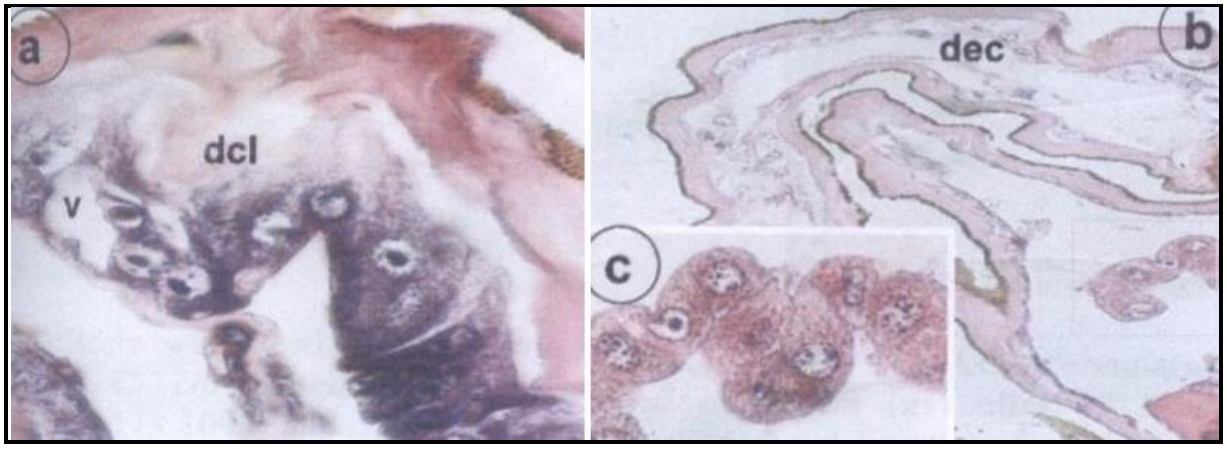

Figure 3. Body wall sections of *Spodoptera littoralis* larvae treated with LC_{25} formulated extract of *A. indica* showing detachement cuticular lamellae (del). vacuolation(v) after 48 h of treatment (a) (400×) and detached epithelial cell (dec) 10 days post treatment (b.c). (×400.1000 H&E).

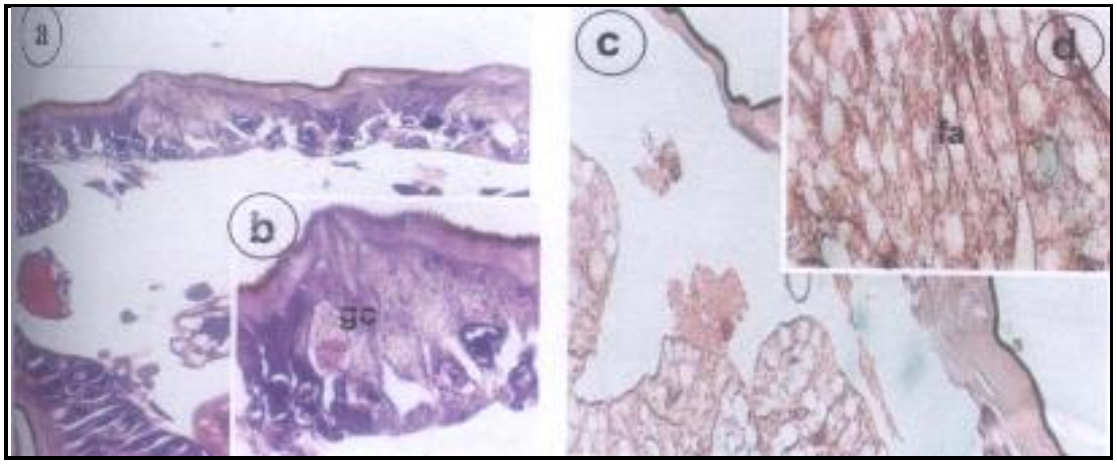

Figure 4. Body wall section of *S. littoralis* larvae with LC_{10} formulated extract of *Carallux colocvnthis* showing granulated cytoplasm (gc) after 48 h of treatment (a,b) and at accumulated (fa) 10 days post treatment (c.d). (×400.1000H&E)

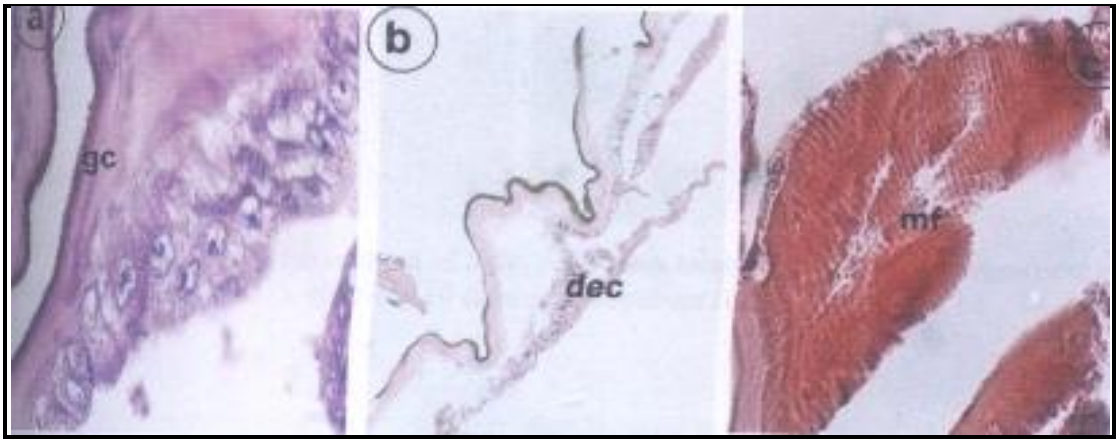

Figure 5. Body wall section of *S. littoralis* larvae treated with LC_{25} formulated extract of *Carallux colocvnthis* showing granulated cytoplasm (gc) after 48 h of treatment (a0 (× 1000) and detachement epithelial cell (dec) and distortion of muscle (ml) 10 days post treatment (b.c).(×400 H&E).

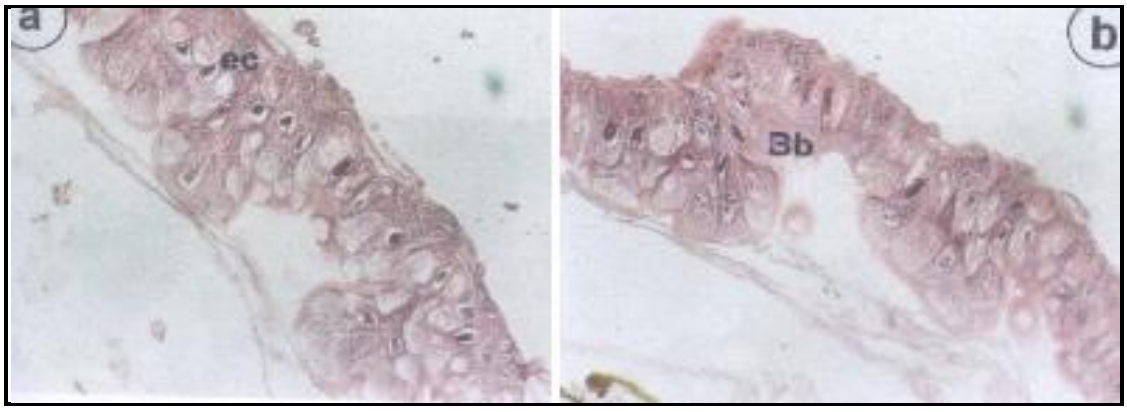

Figure 6. Cross sections in the midgut of untreated *Spodoptera littoralis* larva showing epithelial cells(ee) (a) after 48 h from the beginning of the experiment and brush (Bb) after 10 days from the beginning of the experiment (b).(×400 H&E).

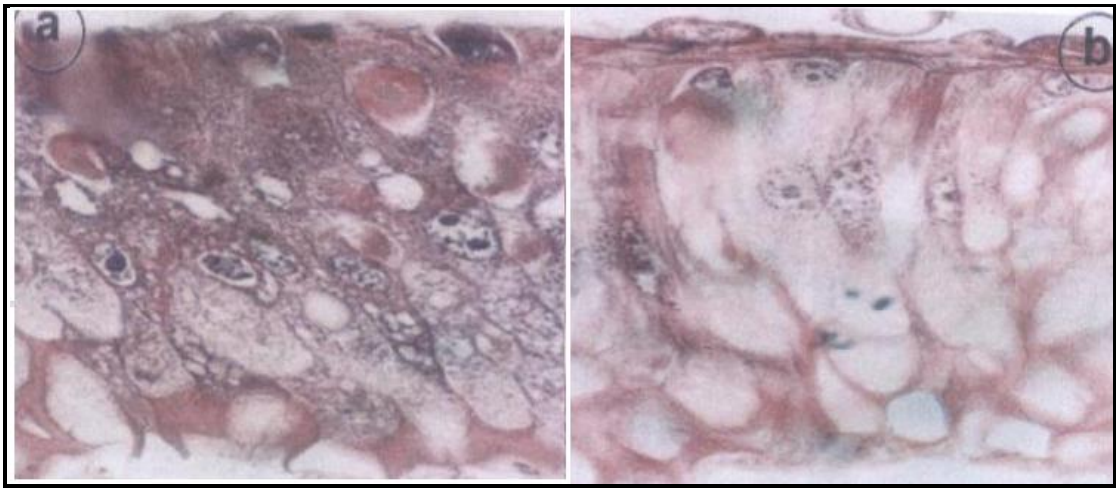

Figure 7. Cross sections in the midgut of *S. littoralis* larva treated with LC_{10} formulated extract of *A. indica* after 48 h(a) (×400) and 10 days post treatment (b). (×400 H&E).

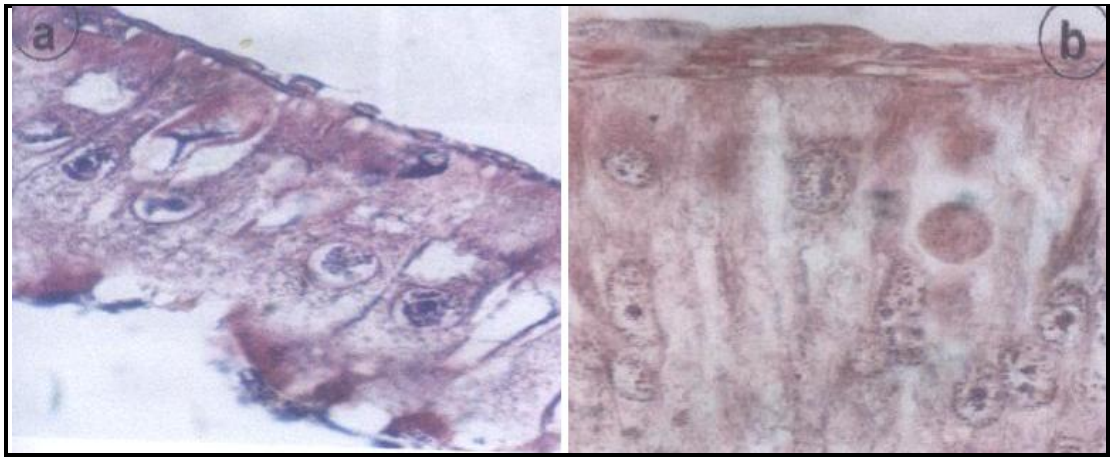

Figure 8. Cross sections in the midgut of *S. littoralis* larva treated with LC_{25} formulated extract of *A. indica* after 48 h(a) (x400) and 10 days post treatment (b). (×400 H&E).

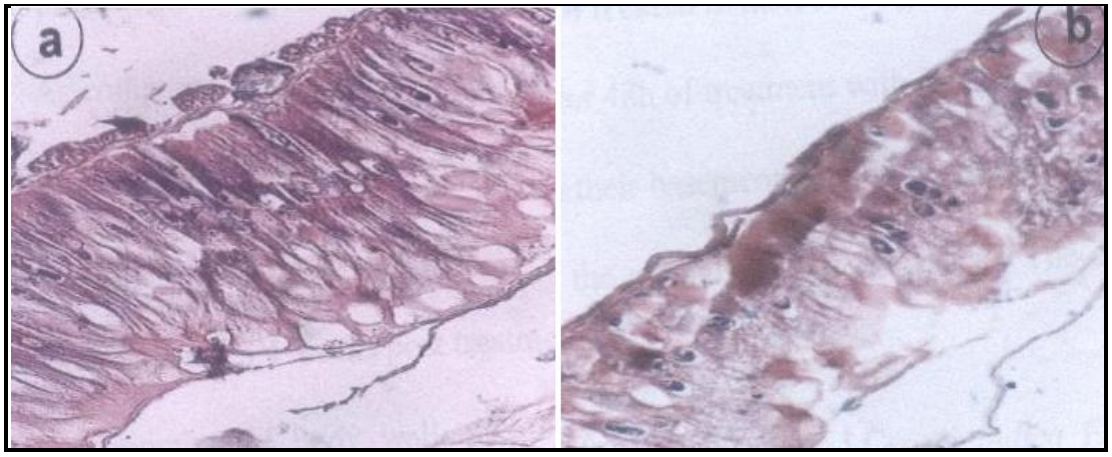

Figure 9. Cross sections in the mid gut of *S. littoralis* larva treated with LC_{10} formulated extract of *C. colocvnthis* after 48 h(a) and 10 days post treatment (b). (×400 H&E).

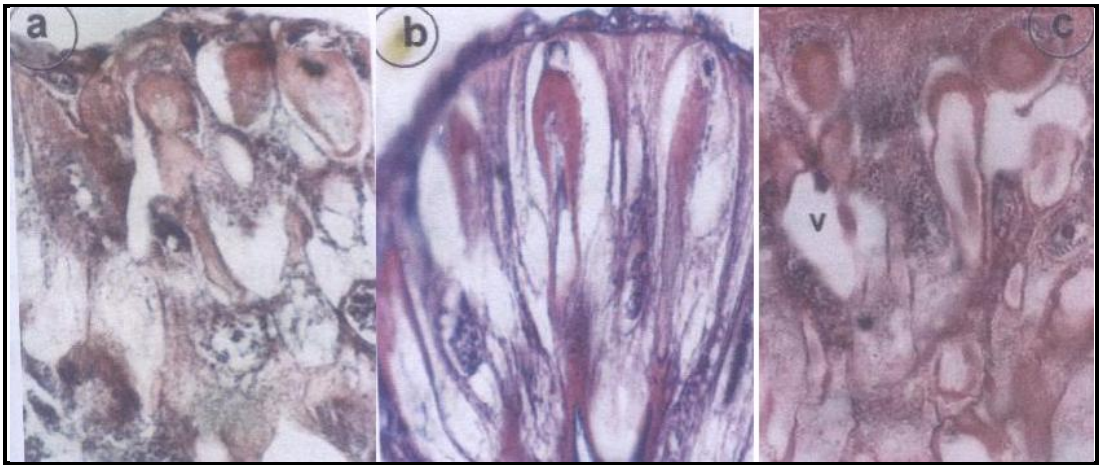

Figure 10. Cross sections in the mid gut of *S. littoralis* larva treated with LC_{25} formulated extract of *C. colocvnthis* after 48 h(a) and 10 days post treatment (b). (x 400 H&E).

The contrasting results obtained by using the tested formulated extracts can be explained on the basis of physic-chemical changes that contributed to methylene chloride formulated extract.

Decrease in PH and surface tension and increase of conductivity and viscosity are the main causes. As reported by El-Sisi et al. (1989), the reduced values of PH of formulated solutions lead to more attraction between the extracted particles and surface of treated plants. On the other hand, there is a particular concern about the intrinsic effect of the formulated extracts on PH and water contents of the target insects. Such change, however, causes a particular change on the respiratory rate (Simkiss and Mason, 1983). This causes passive loss of ions that leads to more bioavailability and finally mortality increase dramatically.

Initially before death, the tested pest failed completely to feed or grow normally and was unable to move, suggesting the previous explanations. El-Hariry and El-Sisi (1990) recorded that the decrease in the surface tension of the extracted particles increases their spreading and deposition on the surface of treated plants. In another experiment, Bode et al. (1976) postulated that the increase of viscosity significantly affected the efficiency of plant extracts thereby decreasing the longevity of the target pests. Also, according to Tawfik and El-Sisi (1987), increased electric conductivity of the extract coupled with increased mortality rate is due to increased deposition and penetration of the extracted particles. Further work is necessary to understand better the mechanisms of increasing toxicity by extract formulation, since a large number of authors have postulated the effect of different plant extract on the feeling and behavioral pattern of different insects (Bruno et al., 2003).

Data obtained from the biochemical effect of the tested extracts of *A. indica and C. colocynthis* at sublethal dose also confirm different degrees of action on total protein content, total lipid content, glucose content and transaminases activity of *S. littoralis* 4th larval instar.

In the present study, the activity of ALAT of both insects was decreased and the activity of ASAT was increased throughout the test periods. Elevation of ASAT after exposure to different toxic agents in invertebrate and vertebrate animals has been investigated by Reddy and Venugopal (1990) and Zidan et al. (2000).

In the present study, the greater and continuous release of ASAT might be due to the necessity of enhancing domination of aspartic acid for the process of gluconeogensis especially under conditions of impaired carbohydrate metabolism and/or a potential induced damage to parenchymal cells as reported by Rawi et al. (1996). On the other hand, the higher decrease in ALAT activity compared to that of ASAT suggests that with the use of formulated extract of both plants, the reaction involving oxaloacetate seems to gain more importance than others involving pyruvate (Rawi et al., 1996).

Data obtained also showed different pattern effect on total protein contents of the studied pests. At the tested dose level of *A. indica and C. colocynthis* the recorded values in both pests showed marked decrease of the total protein content. Extensive work has been carried out in order to determine how various toxic agents affect protein synthesis. A diminution in the rate of ATP synthesis and inhibition of RNA synthesis are also the main causes of decreased total protein content (Dianzani, 1976; El-Beih, 1988; Nabih et al., 1989). Also, Amer (1986), Ahmed et al. (1993) and Rawi et al. (1995) have reported that protein leakage during intoxication may arise from reduced body weight, conversion of protein to amino acids, degradation of protein to release energy or the direct effect of the tested extracts on the amino acids transport of the cell.

The behavioral pattern changed glucose level post treatment and showed significant decreases in both pests. The recorded effect was more pronounced with the

effect of *A. indica* extracts than *C. colocynthis*. At the end of the test period, the recorded concentrations were decreased by -30.79 and -42.40% in *S. littoralis*, post treatment with the extract of *A. indica*. These findings coincide with those of Abdo et al. (1995). Chitra and Reddy (2000) showed reduction in carbohydrate content of different instar larvae when treated with *Ammi majus, Apium graveolens, Melia azedarach* and *Vince rosea* extracts. Also, as reported by another investigator (Hashem et.al*.,* 1993), amylase is the most sensitive enzyme to the action of several molluscicddes; otherwise, the inhibition of the enzyme activity will in turn reduce glucose level in both pests through decreasing the hydrolytic rate of glycogen.

Regarding the total lipid content, a number of toxic agents have been found to cause disturbances of fats in different body organs of both vertebrate and invertebrate animals (Deboyses et al., 1989; Rawi et al., 1995). Data obtained in the present work disclosed a significant reduction in the lipid contents of the two pests throughout the test periods. These findings are in agreement with those obtained by Taha et al. (1989) and Mostafa (1993), that *Melia azedarach and Vince rosea* have significant inhibition in the lipid content in the 3rd nymphal instar of *S. gregaria* and 2ndlarval instar of fruit fly *C. capitata.* Anitha et.al. (1999) indicated that histopathological changes are one of the most definitive indicators of fat changes. In the present study, vacuolation, necrosis and destruction of epithelial cells and their boundaries are highly recognized in both epidermal and mid gut cells of both insects. So, these findings may give a good explanation for the recorded change in the lipid contents. In addition, the disturbances in the function of the internal organs as a consequence of structural damage may lead to inhibition of lipid synthesis.

The histopathological changes which occurred in the larval midgut of *S. littoralis* treated with *A. indica and C. colocynthis* extracts were vacuolation and necrosis of the epithelial cells and destruction of epithelial cells and their boundaries. Vacuoles may occur as a result of cell elongation or a result of excessive fat droplets which dissolve during fixation and dehydration process (Salkeld, 1951). Similar observations were also obtained by many authors for neem extract and other plant extracts against *S. littoralis and S. gregaria* and various insect species belonging to different order. Against *S. littoralis*, Schmidt et al. (1997) and Salam and Ahmed (1997) found that the *Melia azedarach* ex-tract caused destruction of epithelial cells. Also Younes et al. (1999) observed the degeneration of the epithelial cells and decay of its boundaries when *S. littoralis* larvae with the extracts of both *Clerodendro inerme* and *Conyza dioscoridis* caused slight and severe disinte-gration of the epithelium, fading of the boundaries of epithelial cells and detachment of epithelial cells(Emara and Assar, 2001). In contrast to these observations Schluter and Schulz (1983) found no effect of azadirachtin on the midgut epithelium of *Epilachna varivestis* larvae. This might reflect the variable susceptibility of different insect species.

The present histopathological destruction caused by the investigated plant insecticides may suggest that any of these extracts are capable of causing death of an insect when entering into tissues in adequate amounts. In conclusion, the results of the present study show that the emulsifiable concentrate of *A. indica and C. colocynthis* has high toxicity on *S. littoralis* and *S. gregaria.*

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