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Assessment of Biorational Botanical Extracts on the Desert Locust Schistocerca Gregaria Forskal (Orthoptera: Acrididae)

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ABSTRACT

In the current study, the Biorational Botanical Extracts on the Desert Locust Schistocerca gregaria Forskal (Orthoptera: Acrididae) were assessed. The most effective plant extract (with) was Azadirachta indica. The recoded LC50 value was 1.39x 10^2 and 25.13 mg/ml (for normal hexane and methylene chloride solvents, respectively). Nymph's duration was prolonged about 7 days with formulated A. indica and Citrullus colocynthis. Also nymphs suffered from weight loss and died before reaching the 5th instar. A sub-lethal dose of A. indica (LC10) led to detachment of the hypodermis or displacement of the basement membrane of the cuticle. Increased dose (LC25) led to complete dissolution of the hypodermal walls. A. indica extract (LC10) also caused enlargement of the mid-gut epithelium and rupture of some cells within. These effects were manifested after 28 days' post treatment. A higher dose (LC25) led to destruction of the brush border and after 28 days, led to dissolution of the cytoplasm and appearance of vacuoles. C. colocynth extract (LC10) caused detachment of the nidii; after 28 days, nuclei lost their appearance and a higher dose (LC25) led to compete dissolution of the nuclei.

Keywords: Azadirachta Indica, Citrullus Colocynthis, Desert Locust, Biorational Bioassay, Histology

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INTRODUCTION

Desert locust is a sever pest in North Africa and sometimes invade Egypt especially in South (FAO). Control of this pest depends on the state of the swarms and how it can harm. In most cases, conventional pesticides are used and hazards of its accumulation are known to everybody. This perhaps strengthens the need of less hazardous pesticides. Plant extracts might offer this need through its safety in nature and the fact that they are biodegradable, *Azadirachta indica* extracts were the most used for research against many insects as well as many other extracts [1]. Results obtained had discussed many aspects of application of such extracts including histological studies on embryos, reproductive systems and cuticle [2] and El-Shazly *et al.*, (2008) [3] on the neuroendocrine cells of *Heteracris littoralis.*

In the present work, the authors aim to add more information about effects of some plant extracts on the desert locust and its effects on mortality and histopathology of the mid gut and cuticle.

MATERIALS AND METHODS

Insect's colony:
Were obtained the data from the Desert Locust Research Department, Ministry of Agriculture, Dokki, Giza, Egypt. They were reared at 30±1ºC in electrically heated wooden cages (60×60×40 cm^3) and uncontrolled relative humidity. They were fed clover (*Trifolium alexandrinum*) from October till June and then on maize (*Zea maize*). Tests were carried out on newly molted 2nd instar. The tested plant extracts used were: seeds of Neem (*Azadirachta indica*), leaves of bitter Cucumber (*Citrullus colocynthis*) and lace flower (*Ammimajus*) and fruits of Spearmint (*Menthami crophylla*).

Botanical Extracts:

The above plant parts were cleaned and dried under room temperature, then were grind in an electric mill. The solvents used were methylene chloride and normal hexane. Samples of 200 g powder were soaked in 800 ml of methylene chloride for 4 days and were shacked for 1 hour/day using an electric shaker. The extract was filtered over anhydrous sodium Sulphate. The solvent was then evaporated under reduced pressure using a rotatory evaporator at 30C°. The remained powder was soaked again in 800 ml of 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. Extracts of *A. indica, A. majus*and *M. microphylla* were carried out by Freedman *et al.*, (1979) method with some modification and the extract of *C. colocynthis* was carried out by using Soxhlet apparatus using the same order of solvents [4].

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

Considering the crude extract as 100%, a known weight of the crude was added to a similar volume of the solvent (acetone) to obtain stock solution. Stock solution of each extract was made prior to use. Successive dilutions were carried out to obtain the tested solutions. Five concentrations were prepared for each plant extract (20, 40, 60, 80 and 100 mg/ml).

Formulated extracts were prepared in the form of emulsifiable concentrate; simply the extract was weighed, mixed with a mixture of xylene and normal butanol in a small portion, then, an emulsifier was added (Polyethylene glycol) (for details see [5]).

Bioassay and statistical analysis:

The different concentrations of each extract were applied on insects using leaf dipping technique. Clover leaves were dipped in each tested concentration for 20 seconds and left to dry. Control leaves were dipped in acetone. The dried leaves were put singly in plastic cups. Ten insects were transferred to each cup and were allowed to feed on the leaves. Three replicates for each concentration were done. After 24 h surviving, they were transferred to clean cups and were supplied daily with fresh leaves until the end of experiment.

Mortality was recorded daily after 24 h from treatment until the end of experiment and was corrected according to Abbott (1925) [6]. Also duration of instars was observed and was calculated, malformation of different stages of insects was observed and the percentage of deformation was calculated. Survivors of *Sh. gregaria* were weighed every 5 days up to 20 days. The body weight reduction parentage of insects was calculated and recorded.

Mortality values 7 and 14 days after exposure were analyzed by Probit analysis (LDP line) to obtain LC_{50}, LC_{90} and slope for each extract according to method adopted by Finney (1971) [7]. Data obtained were analyzed by student (*t*) test.

Histopathological examination:

The LC_{10} and LC_{25} of the two formulated extracts were used. Samples of tested insects were dissected after 48 hours from exposure. Another sample was taken after 4 weeks. Body wall and mid gut were fixed separately in alcoholic Bouin's solution for 24hrs, washed in ethanol (70%) then dehydrated in an ascending series (70-100%) of alcohol, then infiltrated and embedded in Paraffin wax of melting point 60ºC. Sections were cut at 6 µm and were stained with Ehrlisch's haematoxylin and eosin.

RESULTS

Toxicity testing of non-formulated plant extracts on *Sh. gregaria*2^{nd} instar*:* 7 days' post treatment:

The results recorded in Table 1 and Fig. 1 showed that, from all the tested plants normal hexane extract, *A. indica* plant was the only one affected on 2^{nd} instars of *Sh. gregaria.* The recoded LC_{50} and LC_{90} values of such extract were $1.39 \times 10^2$and 3.70×10^2 mg/ml, respectively. On the other hand, the extracts of all other plants with methylene chloride showed a higher potency against the tested pests. In a descending order, the recorded LC_{50} values were that of *A. indica, C. colocynthis, A. majus,* and *M. microphylla,* respectively.

For the calculated toxicity indices, the maximum value was attained with the effect of *A. indica* (100)

followed by *C. colocynthis* (70.25), *A. majus* (58.05) and *M. microphylla* (49.21), in decreasing order, respectively.

14 days' post treatment:

Results recorded in Table 2 and Fig. 2 showed that the different plant extracts have been affected against 2nd instars of *Sh. gregaria* except *C. colocynthis* normal hexane extract.

For the recorded LC_{50} values of the tested plant extracts when using normal hexane as solvent, the LC_{50} of *A. majus* (1.82 x 10^2 mg/ml) was the most toxic. On the other hand, when using methylene chloride as solvent, the extract of *A. indica* was the most potent effective since the recorded LC_{50} was 25.13 ppm followed by *C. colocynthis, A. Majus* and *M. microphylla* in a decreasing order, respectively.

About the calculated value of toxicity indices, the highest value (100) was achieved with *A. indica* methylene chloride extract and the lowest value (10.09) was recorded with *M. micrpphylla* normal hexane extract.

Table 1. Toxicity of non-formulated extract of different plants on Sh. gregaria nymphs after 7 days of treatment

Toxicity test	Normal hexane extract			
	A. indica	*C. colocynthis*	*A. majus*	*M. microphylla*
LC_{50} (mg/ml)	1.39×10²	-	-	-
LC_{90} (mg/ml)	3.70×10²	-	-	-
Slope	3.01	-	-	-
Toxicity index	43.11	-	-	-
	Methylene chloride extract			
	A.indica	*C. colocynthis*	*A. majus*	*M. microphylla*
LC_{50} (mg/ml)	59.93	85.31	1.03×10²	1.22×10²
LC_{90} (mg/ml)	7.87×10²	1.35×10³	2.87xl0²	6.48×10²
Slope	1.15	1.07	2.89	1.76
Toxicity index	100	70.25	58.05	49.21

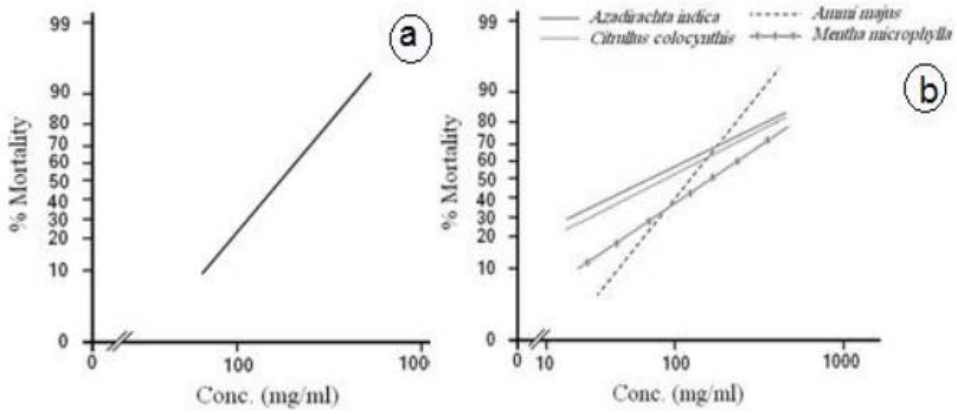

Fig. 1. Mortality percentage of Sh. gregaria nymph due to the effect of normal hexane extracts of A. indica (a) and methylene chloride extracts of different plants (b) after 7 days from treatment

Table 2. Toxicity of non formulated extract of different plants on *Sh. gregaria* nymphs after 14 days of treatment

Toxicity test	Normal hexane extract			
	A. indica	*C. colocynthis*	*A. majus*	*M. microphylla*
LC_{50} (mg/ml)	1.20×10²	-	1.82×10²	2.49×10²
LC_{90} (mg/ml)	1.64×10³	-	1.32×10³	1.32×10²
Slope	1.13	-	1.46	1.77
Toxicity index	20.93	-	1.80	10.09
	Methylene chloride extract			
	A. indica	*C. colocynthis*	*A. majus*	*M. microphylla*
LC_{50} (mg/ml)	25.13	39.92	66.78	70.35
LC_{90} (mg/ml)	2.96×10²	5.84×10²	5.34×10²	1.5×10³
Slope	1.20	1.10	1.42	1.09
Toxicity index	100	62.94	37.62	35.72

Toxicity of formulated plant extracts:

The formulated plant extracts of *A. indica* and *C. colocynthis* were more effective on the 2nd instar after 7 and 14 days of treatment (Table 3, Fig. 3). *A. indica* extract was more toxic than *C. colocynthis* as LC_{50} was 31.33 and 12.11 mg/ml (7 and 14 days) for *A. indica* compared with 42.3 and 15.36 for *C. colocynthis*, respectively. Toxicity index was considered to be 74.22 and 78.82 for *C. colocynthis* as compared with *A. indica* (100).

Effect on nymph duration:

Data presented in Table 4 showed that formulated *A. indica* and *C. colocynthis* extracts had prolonged significantly ($p<0.05$) the nymph duration by about 7-8 days more at concentration of 60 mg/ml. This increase was dose dependant with *A. indica* was a little pit potent than *C. colocynthis* ($p>0.05$). All nymphs died before reaching the 5th instar.

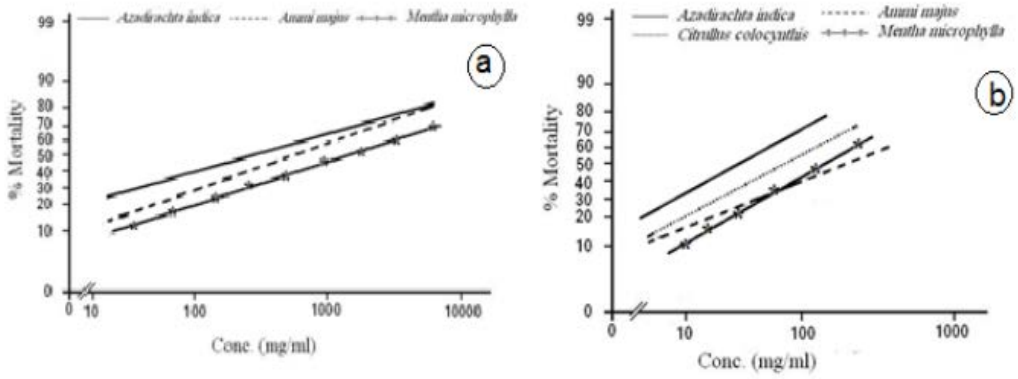

Fig. 2. Mortality percentage of *Sh. gregaria* nymph due to the effect of normal hexane (a) and methylene chloride extracts of different plants (b) after 14 days from treatment

Table 3. Toxicity of formulated extracts on *Sh. gregaria* 2nd instar after 7 and 14 days of treatment

Toxicity test	7 days		14 days	
	A. indica	*C. colocynthis*	*A. indica*	*C. colocynthis*
LC_{50} (mg/ml)	31.39	42.30	12.11	15.36
LC_{90} (mg/ml)	1.04×10^3	4.71×10^2	1.46×10^2	2.51×10^2
Slope	0.84	1.22	1.19	1.06
Toxicity index	100	74.22	100	78.82

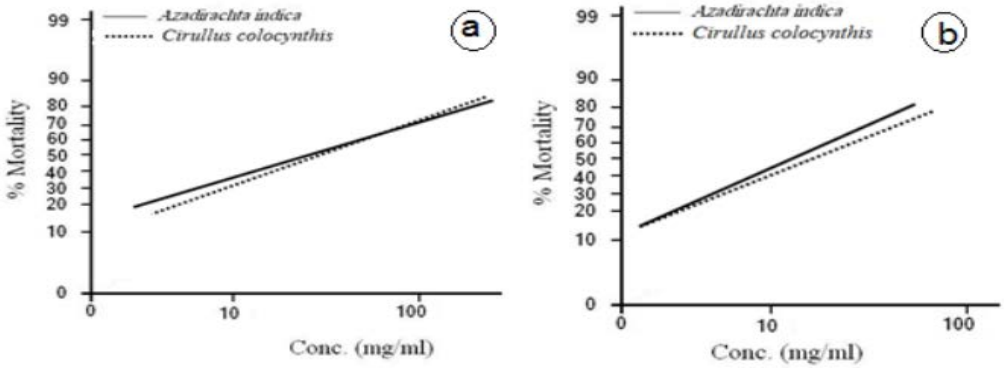

Fig. 3. Mortality percentage of *Sh. gregaria* nymph due to the effect of formulated plant extracts after 7 (a) and 14 (b) days from treatment

Effect on nymph weight:

Nymphs suffered from drastic loss in weights (Table 5) when treated with formulated *A. indica*and *C. colocynthis* extracts. Such decrease was dose dependant. The sever loss was recorded at dose of 100 mg/ml. Weight loss reached 73 and 83% from control groups in 10 days with *A. indica*and *C. colocynthis* extracts, respectively. It seems that *C. colocynthis* extract was effective than *A. indica* ($p<0.05$) in losing weight. It must be noticed that most nymphs died within 15-20 days according to the dose given.

Effects of non-formulated extracts:

The effect of normal hexane extracts of *A. indica, C. colocynthis, A. majus* and *M. Microphylla* on the rate of developmental duration of *Sh. Gregaria* nymph were recorded in Tables (6 and 7). The rate of development duration of treated nymph was increased by increasing concentration of plants extracts. The treated nymph was died and could not form the adult at all the tested concentrations. All the tested plant extracts caused highly significant (P <0.01) elongation of 3rd, 4th and 5th instars duration.

Table 4. The effect of different concentrations of *A. indica* and *C. colocynthis* formulated extract on nymph duration ($\bar{X}$±SE, days) of *Sh. gregaria*

Conc. mg/ml	instar / plant	2nd	3rd	4th	5th	6th
Control		2.33±0.33	4.66±0.33	7.66±0.33	6.33±0.33	7.33±0.33
20	*A. indica*	5.66*±0.33	11.33*±0.33	-	-	-
40		6.33*±0.33	11.66*±0.58	-	-	-
60		6.66*±0.33	12.33*±0.33	-	-	-
80		7.00*±0.58	-	-	-	-
100		7.66*±0.33	-	-	-	-
20	*C. colocynthis*	5.66*±0.33	9.66*±0.33	15.66*±0.33	-	-
40		5.66*±0.33	10.33*±0.33	-	-	-
60		6.66*±0.33	11.33*±0.33	-	-	-
80		7.33*±0.33	-	-	-	-
100		7.33*±0.33	-	-	-	-

n=30, * significant at p<0.05

Effect on nymph duration:

In Table 6, data recorded showed that the normal hexane extract of *A. indica* was more effective on the nymph duration of *Sh. gregaria* more than the other plant extracts. Also the higher tested concentration (100 mg/ml) produced more elongation of the nymph duration particularly at 2nd, 3rd and 4th instars where the recorded nymph duration was 5.66, 13.66 and 16 days, respectively. The lower elongation of nymph duration of *Sh. Gregaria* was obtained by using *C. colocynthis* extract. The recorded duration periods were 3.66, 10.33 and 14.33 days for the 2nd, 3rd and 4th nymph, respetively.

Effect on nymph weight:

As shown in table 7, the botanical extracts of *A. indica, C. colocynthis, A. Majus* and *M. microphylla,* decreased nymph weight. This data revealed that the high decrease in nymph weight could be attributed to the application of *A. indica*. At the maximal tested concentration of *A. Indica* the total average of nymph weight was 1680 mg reduced by 41% as compared to the control level (2860 mg). On the other hand, the same concentration of *C. colocynthis, A. majus*and *M. microphylla* extracts decreased the nymph weight by 31%, 31% and 34%, respectively.

Table 5. Effect of different concentrations of *A. indica*and *C. colocynthis* formulated extract on nymph weight ($\bar{X}$±SE, mg) of *Sh. gregaria*

conc. mg/ml	days / plant	initial weight	5 (days)	10 (days)	15 (days)	20 (days)	average weight	% reduction
Control		210±0.012	360±0.012	480±0.012	710±0.012	950±0.012	2710	
20	*A. indica*	210±0.009	290*±0.015	360*±0.01	500*±0.01	550*±0.01	1810	33
40		200±0.015	280*±0.012	330*±0.02	460*±0.02	400*±0.01	1670	38
60		200±0.012	270*±0.012	310*±0.01	420*±0.01	-	1200	56
80		210±0.006	310*±0.01	360*±0.01	-	-	760	72
100		210±0.012	300*±0.01	330*±0.01	-	-	730	73
20	*C. colocynthis*	200±0.012	310*±0.01	360*±0.01	510*±0.01	470*±0.01	1850	32
40		200±0.012	290*±0.01	330*±0.01	470*±0.01	420*±0.01	1720	37
60		210±0.015	270*±0.01	300*±0.02	430*±0.02	380*±0.02	1580	42
80		210±0.012	260*±0.01	300*±0.01	410*±0.01	-	1180	56
100		200±0.012	250*±0.01	-	-	-	450	83

*significant at p<0.05, n= 30

Effect of methylene chloride extracts:

Effect on nymph duration:

Data given in Table 8 revealed that the rate of duration of the treated nymphs was significantly increased ($p<0.5$) by increasing the concentration of methylene chloride extracts of the tested plants. The treated nymphs were died before reaching adult stage.

A. indica extract was more effective than other plants extract on the 2nd instar duration. The duration was 6.66 days at 80 and 100 mg/ml and it was 5.66, 5.33 and 5.33 days at 100 mg/ml for *C. colocynthis, A. majus* and *M. microphylla* extracts, respectively compared to 2.66 days for control.

Also *A. indica* extract was the most effective on 3rd instar duration. The recorded duration of treated nymphs was 13.66, 12, 9.33 and 8.66 days at 100 mg/ml concentration for the extracts of *A. indica, C. colocynthis, M. microphylla* and *A. majus,* respectively, as compared to 4.33 days of the control.

Methylene chloride extracts of the tested plants affected the durational period of the 4th instar. The values recorded were 12.33 days at 20, 40 and 80 mg/ml for *A. indica, C. colocynthis* and *A. majus* extracts and 11.33 days at 80 mg/ml for *M. microphylla* extract compared to 6.66 days of the control. On the other hand, the treated nymphs died before reaching the 5th instar, except at concentration of 20 mg/ml. At such dose level the 5thnymph duration was 14.33 and 13.33 days for *A. majus* and *M. microphylla* extracts comparing to 7.66 days of the control nymph (table 8). On the previous fact, there was no 6thinstar resulted due to the effect of the different plants extract using methylene chloride as solvent.

Effect on nymph weight:

From the data recorded in Table 9, it was clear that the methylene chloride extracts of *A. indica, C. colocynthis, A. majus* and *M. microphylla* reduced significantly ($p < 0.1$) nymph weight of *Sh. gregaria.* Also the weights of the treated nymphs were decreased by increasing the applied concentrations. The maximum percentage reduction in the average body weight occurred at concentration of 100 mg/ml, the values recorded were decreased by 59, 57, 36 and 32% for *A. indica, C. colocynthis, A. majus* and *M. microphylla,* respectively. On the other hand, *A. indica* extract caused the higher decreasing effect of the nymph weight. Also the *M. microphylla* extract had the lower reduced effect on the nymph weight.

Table 6. Effect of different concentrations of normal hexane extract of *A. indica, C. colocynthis, A. majus*and *M. microphylla*on the nymph duration ($\bar{X}$±SE, days) of *Sh. gregaria*

Conc. mg/ml	instar / plant	2nd	3rd	4th	5th	6th
Control		2.66±0.33	4.33±0.33	6.66±0.33	7.66±0.33	8.33±0.33
20	*A. indica*	3.33±0.33	11.33*±0.33	14.66*±0.33	16.33*±0.33	-
40		3.66±0.33	12.00*±0.58	15.33*±0.33	-	-
60		4.66*±0.33	12.33*±0.33	15.66*±0.33	-	-
80		5.00*±0.58	13.33*±0.33	15.66*±0.33	-	-
100		5.66*±0.33	13.66*±0.33	16.00*±0.58	-	-
20	*C. colocynthis*	2.66±0.33	8.33*±0.33	13.00*±0.58	14.33*±0.33	-
40		3.00±0.58	8.66*±0.33	13.33*±0.33	14.66*±0.33	-
60		3.33±0.33	9.33*±0.33	13.66*±0.33	15.33*±0.33	-
80		3.66±0.33	9.66*±0.33	144.33*±0.33	15.66*±0.33	-
100		3.66±0.33	10.33*±0.33	14.33*±0.33	-	-
20	*A. majus*	2.66±0.33	9.66*±0.33	13.66*±0.33	14.66*±0.33	-
40		3.33±0.33	10.33*±0.33	14.33*±0.33	15.66*±0.33	-
60		3.66±0.33	10.33*±0.33	14.66*±0.33	-	-
80		4.33*±0.33	10.66*±0.33	14.66*±0.33	-	-
100		4.66*±0.33	11.33*±0.33	15.33*±0.33	-	-
20	*M. microphylla*	2.66±0.33	8.66*±0.33	13.66*±0.33	15.00*±0.33	-
40		3.33±0.33	9.33*±0.33	13.33*±0.33	15.33*±0.33	-
60		3.66±0.33	9.66*±0.33	14.33*±0.33	15.66*±0.33	-
80		4.00±0.33	10.66*±0.33	14.33*±0.33	-	-
100		4.33±0.33	10.66*±0.33	14.66*±0.33	-	-

*significant at $p<0.05$, number of treated nymphs were 30 for each experiment, nymphs were exposed at the beginning.

Histological effects of extracts:

Effects on cuticle:

Cuticle of control nymphs (as all insects) is composed of cuboid epidermal cells that secret the cuticle layers

(fig. 4). *A. indica* treated (LC_{10}) nymphs suffered from detached epidermis or displaced from the basement membrane (Fig. 5a). Also, there was dissolution of cytoplasmic material beneath the epithelia. Detachment became severer after 28 days' post treatment (fig. 5b). Increased dose (LC_{25}) led to complete dissolution of the epithelial cell walls within 2 days and dissolution and granulation of their cytoplasm (fig. 6a). These effects became more drastic within 28 days as many nuclei were destructed and muscle bundles became fragmented (fig. 6b).

There were no apparent changes in nymph cuticle treated with *C. colocynthis* extract (Fig.7, a and b).

Effects on midgut:

Normal midgut is composed of circular muscle layers, connective tissue, columnar epithelial cells (with brush border) resting on a basement membrane and peritrophic membrane (fig. 8). Treatment with *A. indica* extract (LC_{10}) caused enlargement of the epithelium and rupture of some cells within 2 days (Fig. 9a). These effects were manifested after 28 days' post treatment; vacuoles appeared in the striated border and dissolution of many epithelial cells (fig 9b). A higher dose (LC_{25}) led to sever destruction of the brush border after 2 days of application (fig. 10a), the prolonged effect (28 days) led to dissolution of the cytoplasm and appearance of vacuoles. Chromatin material were clumped (fig. 10 b).

Treatment with *C. Colocynthis* extract (LC_{10}) caused detachment of the nidi and appearance of vacuoles in between within 2 days' post application (Fig. 11a). After 28 days, nuclei lost their appearance and membranes with the chromatin material were fractioned (Fig. 11b). A higher dose (LC_{25}) led to compete dissolution of the nuclei and the chromatin material were liberated (within 2 days) leaving vacuoles instead (Fig. 12a), by time (28 days) the brush border was destructed (Fig. 12b).

Table 7. Effect of different concentrations of normal hexane extract of *A. indica, C. colocynthis M. microphylla,* and *A. majus* on the nymph weight ($\bar{X}$±SE, mg) of *Sh. gregaria*

conc. mg/ml	days / plant	initial weight	5 (days)	10 (days)	15 (days)	20 (days)	average weight	% reduction
Control	*A. indica*	230±0.007	470±0.01	510±0.01	760±0.01	940±0.01	2860	
20		200±0.013	350*±0.01	410*±0.01	600*±0.01	550*±0.01	2110	26
40		190±0.003	330*±0.01	380*±0.01	560*±0.01	500*±0.01	1960	31
60		200±0.006	320*±0.01	370*±0.01	540*±0.06	480*±0.01	1910	33
80		210±0.006	310*±0.01	360*±0.01	510*±0.01	440*±0.01	1830	36
100		210±0.012	300*±0.01	330*±0.01	330*±0.01	380*±0.02	1550	45
20	*C. colocynthis*	220±0.006	380*±0.06	460*±0.01	690*±0.01	650*±0.01	2400	16
40		210±0.012	360*±0.01	450*±0.01	660*±0.01	610*±0.01	2290	20
60		210±0.015	350*±0.02	430*±0.02	630*±0.02	570*±0.02	2190	23
80		200±0.009	330*±0.01	400*±0.01	600*±0.01	530*±0.01	2060	28
100		190±0.006	320*±0.01	380*±0.01	570*±0.01	500*±0.01	1960	31
20	*M. microphylla*	210±0.02	370*±0.02	460*±0.02	690*±0.02	640*±0.01	2370	17
40		230±0.01	380*±0.01	440*±0.01	660*±0.01	610*±0.01	2320	19
60		190±0.006	350*±0.01	420*±0.01	640*±0.01	560*±0.01	2200	23
80		210±0.006	310*±0.01	380*±0.01	590*±0.01	510*±0.01	1980	31
100		220±0.006	310*±0.01	380*±0.01	570*±0.01	480*±0.01	1950	31
20	*A. majus*	200±0.065	380*±0.01	440*±0.01	660*±0.01	610*±0.01	2310	19
40		200±0.015	350*±0.02	410*±0.02	620*±0.02	560*±0.02	2014	25
60		220±0.09	350*±0.01	400*±0.01	600*±0.01	540*±0.01	2110	26
80		200±0.015	330*±0.02	380*±0.02	570*±0.02	500*±0.02	1980	31
100		200±0.006	320*±0.01	360*±0.01	540*±0.01	470*±0.01	1890	34

*significant at $p<0.05$, number of treated nymphs were 30 for each experiment, nymphs were exposed at the beginning.

Table 8. Effect of different concentrations of methylene chloride extract of *A. indica, C. colocynthis, A. majus*and *M. microphylla* on the nymph duration ($\bar{X}$±SE, days) of *Sh. gregaria*

Conc. mg/ml	instar / plant	2nd	3rd	4th	5th	6th
Control	*A. indica*	2.66±0.33	4.33±0.33	6.66±0.33	7.66±0.33	8.33±0.33
20		4.66*±0.33	10.33*±0.33	12.66*±0.33	-	-
40		5.66*±0.33	10.00*±0.33	12.33*±0.33	-	-
60		6.33*±0.33	11.33*±0.33	-	-	-
80		6.66*±0.33	12.33*±0.33	-	-	-
100		6.66*±0.33	13.66*±0.33	-	-	-

20	*C. colocynthis*	4.00±0.58	10.00*±0.58	11.33*±0.33	-	-
40		4.66*±0.33	10.66*±0.66	12.33*±0.33	-	-
60		4.66*±0.66	11.00*±0.58	-	-	-
80		5.33*±0.33	11.33*±0.33	-	-	-
100		5.66*±0.33	12.00*±0.58	-	-	-
20	*A. majus*	3.66±0.33	7.33*±0.33	10.66*±0.33	14.33*±0.33	-
40		4.33*±0.33	7.66*±0.33	11.33*±0.33	-	-
60		4.66*±0.66	8.33*±0.33	11.66*±0.33	-	-
80		5.33*±0.33	8.33*±0.33	12.33*±0.33	-	-
100		5.33*±0.33	8.66*±0.33	-	-	-
20	*M. microphylla*	4.33*±0.33	7.33*±0.33	10.00*±0.58	13.33*±0.33	-
40		4.33*±0.33	7.66*±0.33	10.33*±0.33	-	-
60		4.66*±0.33	8.33*±0.33	10.33*±0.33	-	-
80		5.00*±0.58	8.66*±0.33	11.33*±0.33	-	-
100		5.33*±0.33	9.33*±0.33	-	-	-

*significant at p<0.05, number of treated nymphs were 30 for each experiment, nymphs were exposed at the beginning.

Table 9. Effect of different concentrations of methylene chloride extract of *A. indica, C. colocynth is, M. microphylla*and *A. majus* on the nymph weight ($\bar{X}$±SE, mg) of *Sh. gregaria*

conc. mg/ml	Days / plant	initial weight	5 (days)	10 (days)	15 (days)	20 (days)	average weight	% reduction
Control	*A. indica*	210±0.01	360±0.01	480±0.01	710±0.01	890±0.03	2650	
20		190±0.03	320*±0.03	360*±0.03	520*±0.03	460*±0.03	1850	30
40		190±0.02	310*±0.02	350*±0.02	510*±0.02	440*±0.02	1800	32
60		170±0.01	300*±0.01	340*±0.01	480*±0.01	390*±0.01	1700	36
80		190±0.01	280*±0.01	310*±0.01	420*±0.01	330*±0.01	1530	42
100		170±0.01	250*±0.01	280*±0.01	380*±0.01	-	1080	59
20	*C. colocynthis*	190±0.02	330*±0.02	400*±0.02	560*±0.02	520*±0.02	2000	24
40		200±0.01	320*±0.01	370*±0.01	520*±0.01	470*±0.01	1880	29
60		200±0.02	300*±0.02	350*±0.02	490*±0.02	420*±0.02	1760	34
80		170±0.02	280*±0.03	300*±0.02	440*±0.02	360*±0.02	1550	41
100		170±0.03	260*±0.03	280*±0.03	410*±0.03	-	1130	57
20	*M. microphylla*	190±0.01	360*±0.01	450*±0.01	620*±0.01	570*±0.01	2190	17
40		200±0.01	360*±0.01	430*±0.01	590*±0.01	530*±0.01	2110	20
60		180±0.02	330*±0.02	400*±0.02	560*±0.02	490*±0.02	1960	26
80		190±0.02	330*±0.02	390*±0.02	530*±0.02	450*±0.02	1890	29
100		190±0.01	320*±0.01	370*±0.01	500*±0.01	410*±0.01	1790	32
20	*A. majus*	180±0.01	330*±0.01	420*±0.01	590*±0.01	550*±0.01	2070	22
40		200±0.02	340*±0.01	410*±0.02	570*±0.02	530*±0.02	2050	23
60		180±0.01	320*±0.01	390*±0.01	540*±0.01	490*±0.01	1920	28
80		200±0.01	330*±0.01	380*±0.01	520*±0.01	460*±0.01	1890	29
100		200±0.01	320*±0.01	340*±0.01	470*±0.01	400*±0.01	1700	36

*significant at p<0.05, number of treated nymphs were 30 for each experiment, nymphs were exposed at the beginning

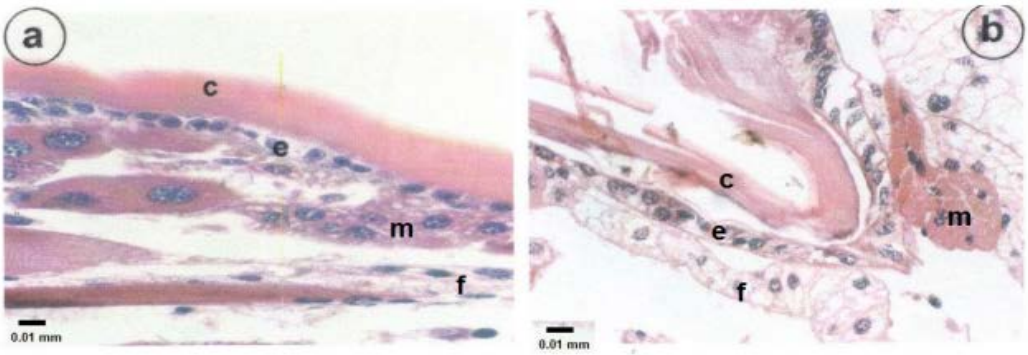

Fig. 4. a: T.S. in body wall of control *Sh. gregaria* nymph 2 days from the beginning of the experiment showing normal cuticle and epidermis; b: the same after 28 days after the beginning of the experiment. c, cuticle; e, epithelium; f, fat body; m, muscle.

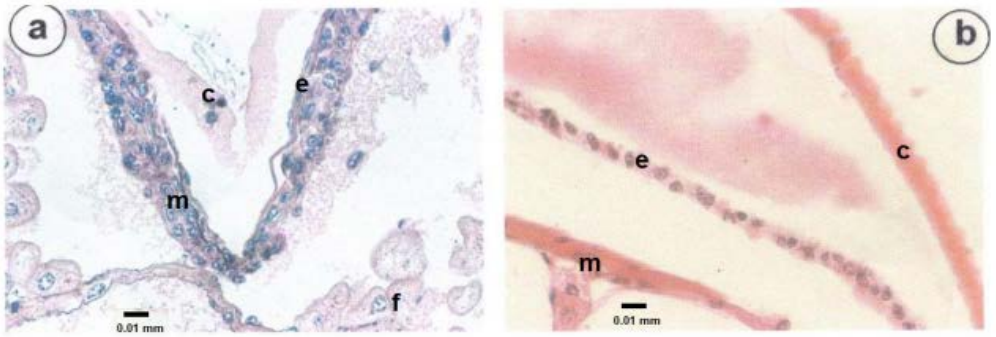

Fig. 5. a: T.S. in body wall of *Sh. gregaria* nymph treated with LC_{10} formulated extract of *A. indica* after 2 (a) and 28 (b) days from the beginning of the experiment. c, cuticle; e, epithelium; f, fat body; m, muscle.

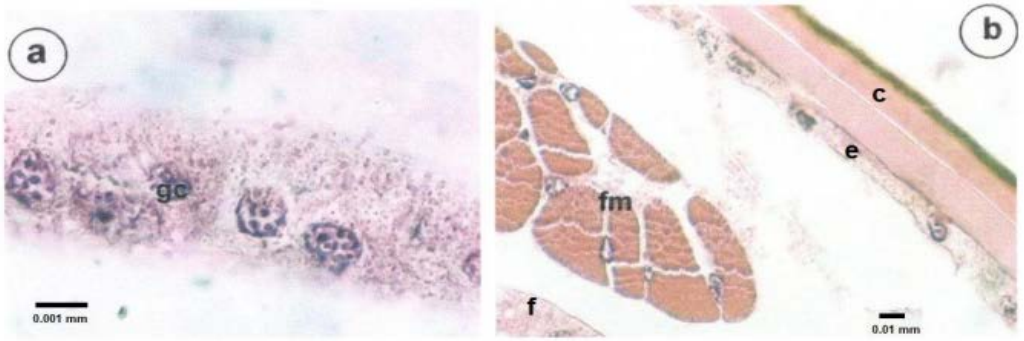

Fig. 6. T.S. in body wall of *Sh. gregaria* nymph treated with LC_{25} formulated extract of *A. indica* after 2 and 28 days (a and b, respectively) from the beginning of the experiment. c, cuticle; e, epithelium; f, fat body; fm, fragmented muscle; gc, granulated cytoplasm.

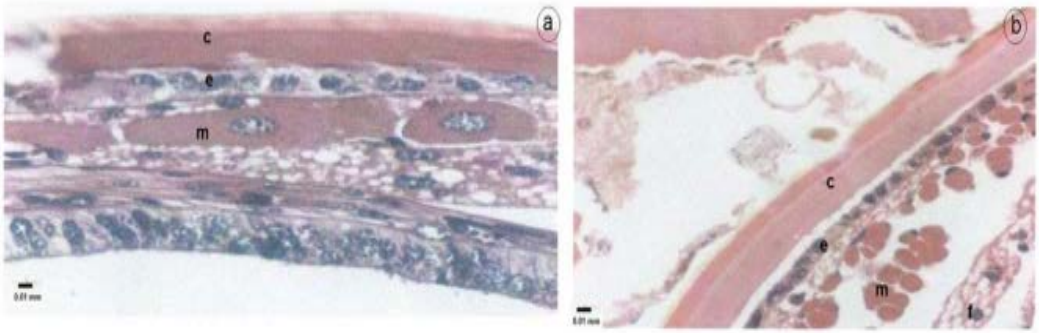

Fig. 7. T.S. in body wall of *Sh. gregaria* nymph treated with formulated extract of *C. colocynthis*.a: (LC_{10}, 2 days) and b: (LC_{25}, 28 days) c, cuticle; e, epithelium; m, muscle

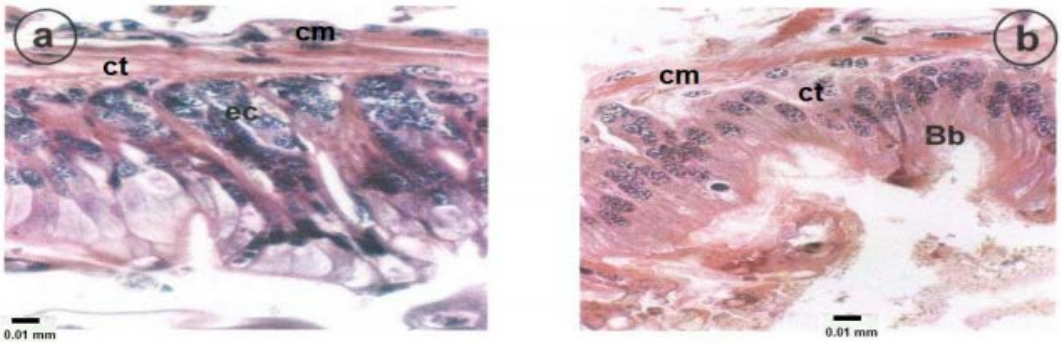

Fig. 8. T.S. in the midgut of control *Sh. gregaria*nymph after 2 and 28 days (a and b, respectively) from the beginning of the experiment. Bb, brush border; cm, circular muscle fibres; ct, connective tissue; ec, epithelial cells.

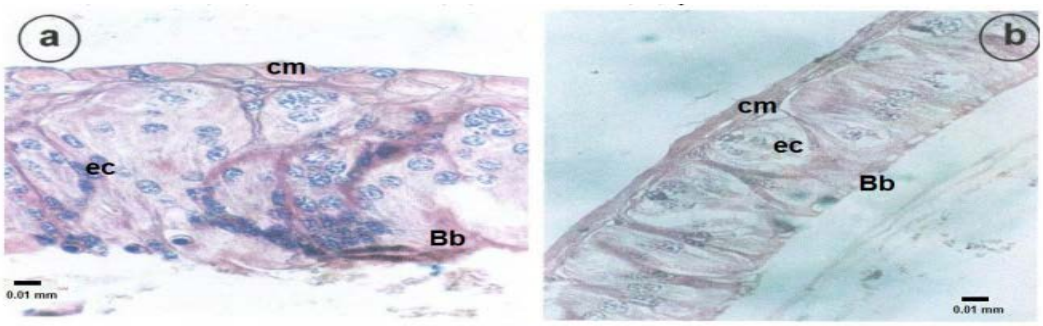

Fig. 9. T.S. in the midgut of *Sh. gregaria*nymph treated with LC_{10} formulated extract of *A. indica* after 2 and 28 days (a and b, respectively) from the beginning of the experiment. Bb, brush border; cm, circular muscle fibres; ec, epithelial cells.

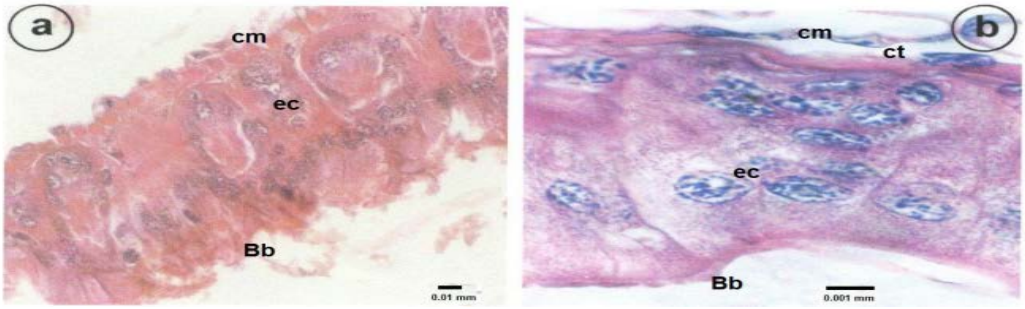

Fig. 10. T.S. in the midgut of *Sh. gregaria* nymph treated with LC_{25} formulated extract of *A. indica* after 2 and 28 days (a and b, respectively) from the beginning of the experiment. Bb, brush border; cm, circular muscle fibres; ct, connective tissue; ec, epithelial cells.

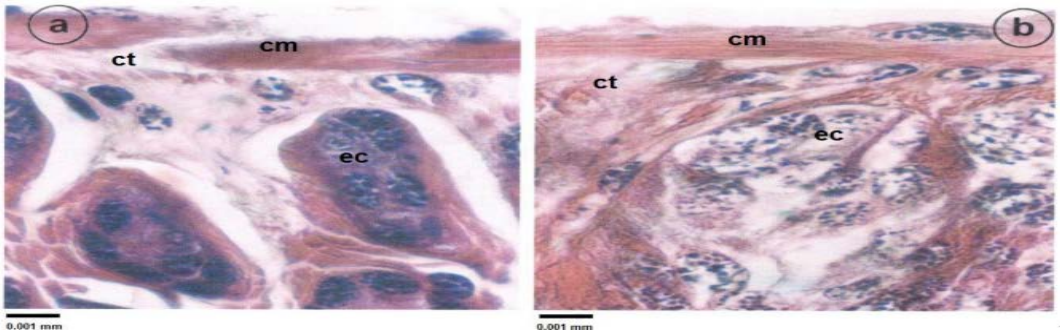

Fig. 11. T.S. in the midgut of *Sh. gregaria* nymph treated with LC_{10} formulated extract of *C. colocynthis* after 2 and 28 days (a and b, respectively) from the beginning of the experiment. cm, circular muscle fibres; ct, connective tissue; ec, epithelial cells.

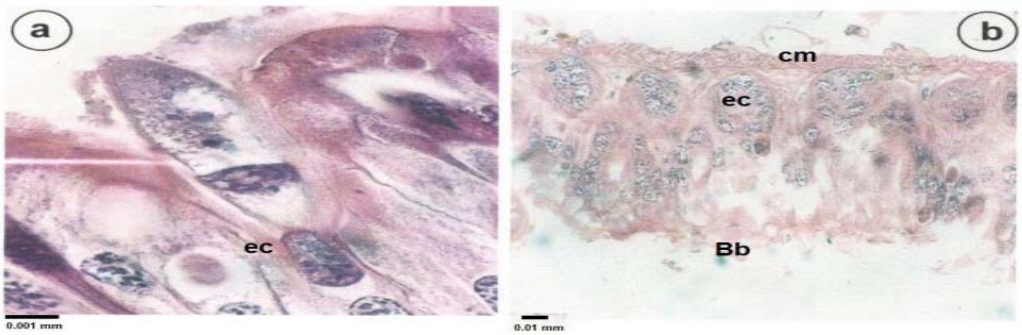

Fig. 12. T.S. in the midgut of *Sh. gregaria* nymph treated with LC_{25} formulated extract of *C. colocynthis* after 2 and 28 days (a and b, respectively) from the beginning of the experiment. Bb, brush border; cm, circular muscle fibres; ec, epithelial cells.

DISCUSSION

Mortality of *Sh. gregaria*, as many other insects, depends basically on the plant extract type and its mode of action [8-10] Extracts mostly have delayed effects on insects [11]. *A. Indica* and *C. colocynthis* extracts were more potent than the other tested plants; this could be attributed to the active ingredients, method of extraction, as well as mode of action [12-15] It is now well established that the activity of many plants including *A. indica* is attributed to the presence of saponin components that perhaps affects cell membranes as well as reduces digestion and absorption [16, 17].

Since the mortality potency of the investigated plant extract differs from the conventional pesticides, the different mortality percentages were recorded 7 & 14 days after treatment. According to Stark and Rangus (1994), neem acts so slowly and perhaps a week is a short application time [18]. On the other hand, Ghazawy *et al.*, (2007) and Asiri (2015) reported LC_{50} within 24 Hrs on 2nd (*Sh. gregaria*), 4th, 5th and 6th (*Heteracris littoralis*) instars [2, 19].

On basis of the LC_{50} values, methylene chloride extracts were superior to that of normal hexane. The potency was in favour of *A. indica* followed by *C. colocynthis, M. microphylla* and *A. majus.* This also was reached by El-Khawas and Abd El-Gawad, (2002) and Bream *et al.*, (2001) [20, 21].

On the basis of LC_{50} value, the detected values showed a considerable decrease by -91, -101, -107 and -105 for *Sh. gregaria* nymph post 7 and 14 days' treatment with *A. indica* and *C. colocynthis*, respectively. This means increased extracts toxicity by formulation. 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 of pH and surface tension and increase of conductivity and viscosity are the main causes [22, 23].

Both formulated and non-formulated forms of methylene chloride and normal hexane extracts prolonged the nymph duration so that nymphs were unable to be adult especially at the higher dose of *A. indica.* Data recorded revealed marked reduction in nymph weights in the all tested plants. These data could be confirmed by Schmidt and Assembe-Tsoungui (2002) and Ghazawy *et al.*, (2007) [2, 24].

Formulated extracts of *A. indica* and *C. colocynthis* caused detachment of the cuticular layers, destruction of epidermal cells and separation of epidermis from cuticle in the body wall. Such observations were recorded in *Heteracrislittoralis* [3]. Also, larvae of *Galleria* suffered from degeneration of epithelia and cuticular abnormalities when treated with azadirachtin [25].

The extent of injury by plant insecticides depend on the susceptibility of tissues to particular poison. Some poisons the lipid layers of membranes resulting in destroying the permeability of plasma membrane. This in turn results in water loss and appearing of vacuoles [26].

Histopathological changes took place in the mid gut were vaculation and necrosis of the epithelia and destruction of the cells and their boundaries. Similar results were obtained by El-Shazly *et al.*, (2008) and Sharaby y*et al.*, (2012) on *Heteracrislittoralis* [3, 26].

CONCUSION:

Perhaps natural insecticides could be effective when used in proper concentrations since they are decomposable in nature and have remarkable selectivity.

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