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The insecticidal activity of two medicinal plants (*Commiphora molmol*) and (*Balanites aegyptiaca*) against the blowfly *Lucilia sericata* (Diptera: Calliphoridae)

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Abstract

Lucilia sericata larvae (Diptera: Calliphoridae) are facultative ectoparasite infesting suppurative wounds as a primary agent of cutaneous myiasis of sheep. It is important to find safe alternative measures for controlling of this blowfly. Two medicinal plant extracts (*Commiphora molmol* and *Balanites aegyptiaca*) were applied for the three larva stages of *Lucilia sericata*. The calculated LC50 values of *Commiphora molmol* were 6.03, 7.96 and 6.55 mg/ml for 1st, 2nd and 3rd larva instars respectively. The LC50 values of *Balanites aegyptiaca* were 142.27, 216.05 and 371.15 mg/ml for 1st, 2nd, and 3rd larva instars respectively. The most effective concentration for *Commiphora molmol* was 32 mg/ml and for *Balanites aegyptiaca* was128 mg/ml. Morphological abnormalities were noticed for the two applied extracts. Malformations of larvae included small sized, contractile, and damaged larvae with weak cuticle. Abnormalities of pupa included small sized, distorted and larviform pupae and incomplete emergency of adult. Abnormalities of adults included small sized, malformed, poorly developed and deformed wing and legs. Histopathological changes and scanning electron microscope (SEM) for the control and treated 3rd larva instar were studied.

Keywords: *Lucilia sericata*, *Commiphora*, *Balanites*, SEM.

Introduction

Myiasis is the infestation of vertebrate animals or live humans by fly larvae from order diptera which feed on the host's living or dead tissue, usually at the surface of the skin or inside body orifices **(Snoep et al., 2002)**. About 50 species in the family Calliphoridae, act as facultative ectoparasites, belong predominantly to the genera *Lucilia* and *Chrysomya* **(Zumpt, 1965, Smith and Wall, 1997)**.*Lucilia sericata* (Meigen, 1826) commonly known as the sheep blowfly, is the primary agent of cutaneous myiasisin sheep (blowfly strike) in many countries of Europe as Netherlands, England and Germany, also in parts of the southern hemisphere **(Snoep et al., 2002, Bisdorff and Wall, 2008 and Mehlhorn et al., 2010)**.

In Egypt, sheep blowfly infestation represents one of the major problems of sheep industry as it reduces the wool clip. Moreover, infested sheep suffer from anemia, decrease in weight gain, wool production as well as alteration in protein and mineral metabolism **(El-Khateeb, 1999 and Shams-El-Din, 1994)**.

Several years after synthetic insecticides were used in modern agricultural production; they promote

widespread contamination of the environment, toxicity to non-target organisms, resistance development and destructive effects on human and animal health **(Pretty, 2009).** Thus, controlling of insects required natural alternative methods without harming to the environment. Materials derived from plant are considered the alternative insecticides to chemicals and considerable studies gave good results when used some plant extracts in control of medical and veterinary insects such as *Lucilia sericata* **(Mazyad et al., 1999 and El Khateeb et al., 2003).**

The present work was undertaken for studying the effect of two medicinal plants (*Commiphora molmol* and *Balanites aegyptiaca*) against *Lucilia sericata* larvae as an alternative means of control to avoid the harmful effect of insecticides.

Materials and Methods

1. *Lucilia sericata* (Meigen, 1826) colony

Lucilia sericata colony was reared in the laboratory of Parasitology Department, National Research Center, Dokki, Cairo, Egypt. Stock colony of adult *Lucilia sericata* (common green bottle fly) was reared in 20×20×25 cm cage with wooden floor. The other sides and roof were made of wire and the front was provided with a wooden part holding a circular hole fitted with a cloth sleeve to facilitate insertion of pupae, removal of deposited eggs and daily supply of food. Emerging adults were supplied with granulated sugar, water, and meat for stimulating egg maturation and oviposition. Meat was daily examined for egg deposition and possible renewal of food.

The deposited eggs were transferred to the larvae breeding plastic dishes (12 cm in diameter) supplied with fresh meat and covered with muslin, kept tightly in place by rubber bands. As the larvae stopped feeding and reached to the prepupal stage, enough amount of dry sawdust was added to the larva media for pupation. The formed pupae were collected by sieving off the sawdust and then transferred to adult breeding cages for emergence **(El-Khateeb, 1999)**.
The colony was reared under laboratory conditions of room temperature ranged between 14.6 and 32.1°C and mean relative humidity ranged between 32.5 and 53.5%. Laboratory temperature and humidity were recorded daily by means of minimum and maximum thermometer and a hair sensitive hygrometer. Laboratory illumination of the mass colony was controlled during summer and winter seasons by using Philips fluorescent tubes **(Saunders et al., 1986).**

1.1. Obtaining of 1st, 2nd, 3rd instars and adults for identification

In the laboratory, 1st stage larvae obtained after hatching of eggs by 8- 12 hours according to temperature. 2nd stage larvae obtained after 31 hour and 3rd stage larvae obtained after 72 hour according to temperature and humidity. Fifty mature 3rd instar larvae of *Lucilia sericata* were incubated at 32 °C and 30% relative humidity. Each 10 larvae were put in a clean beaker (500cc) containing a suitable amount of sterile sawdust and covered with a gauze fixed with a plastic band to allow the larvae to pupate. The pupae were observed daily to collect the emerging adults, the newly emerged adults were killed with chloroform then the adults were examined using stereoscopic microscope for morphological examination.

1.2. Mounting

1.2.1. Mounting of *Lucilia sericata* larvae

For detailed morphological studies of normal larvae those larvae were washed several times with saline then placed in sufficient amount of 5% caustic soda (NAOH) and left at room temperature for 1-2 hours for the 1st instar and overnight for the second, third instar. The larvae were evacuated from its contents, washed with water then dehydrated through ascending serial concentration of ethanol 70, 80, 90 and 100 % for one hour each. Finally, they were cleared in clove oil then put in xylene for few minutes. The larvae mounted in Canada balsam and left in an oven at 40 °C to dry for 24 hours **(Pritchard and Kruse, 1982)**.

1.2.2. Mounting of *Lucilia sericata* adults

The adult wings, mouth part, antenna and legs were placed in sufficient amount of 5% caustic soda (NAOH) and left at room temperature for suitable time (up to 24 hours) till the cuticles softens and prepared as larvae.

1.3. Identification of *Lucilia sericata* (green bottle blowfly) larvae and adult

The Identification was carried out following the key and morphological characters mentioned by **(Zumpt, 1965)**and**(Beverley, 1991).** The morphological parameters for 20 specimens from each larva stage and adults were measured using stereoscopic light microscope.

2. Plant extract for controlling *Lucilia sericata*

2.1. Preparation of the crude plant extracts

2.1. A. preparation of *Commiphora molmol* (Myrrh) volatile oil

Extraction of volatile oil including the steps of grinding up the myrrh, covering the ground up plant with a layer of water to create a mixture of plant and water, passing steam through the mixture, condensing volatile oil from the steam in a condensing chamber and separating the layer of oil from the aqueous layer was established by **(Hanus et al., 2005)**.The oil was prepared for application by addition of Tween-80 (as emulsifier) and distilled water.

2.1. B. preparation of the alcoholic extracts from *Balanites aegyptiaca* (the desert date or Heglig tree)

The alcoholic extract from (*Balanites aegyptiaca* fruits mesocarp) was prepared at the laboratory of medicinal and aromatic plants research department, National Research Center according to method of **(Tariq et al., 2009).** The plant materials pounded and then extracted with 70% ethanol under reflux. Plant material allowed macerating at room temperature in dark place and percolate collected by filtering through cotton wool. The process of maceration/percolation repeated three times for three weeks. The combined filtrate was completely evaporated in a vacuum rotatory evaporator under pressure at 50°c to obtain a semisoild crude ethanolic extract. The extract was scraped off, transferred to container and kept air tight. It was stored at 4 °c until further use. The extract was prepared for application by addition of distilled water.

2.2. Ingestion assay (Smith et al., 2000)

Early first, second and third larva instars of *Lucilia sericata* were exposed to oil extract of (*Commiphora molmol)* at five different concentrations: 2mg/ml,4mg/ml, 8mg/ml, 16mg/ml, 32mg/ml and were exposed to alcoholic extract of *(Balanites aegyptiaca)* at four different concentrations: 16mg/ml, 32mg/ml, 64mg/ml and 128mg/ml. The procedures were replicated four times for each concentration of oil, alcoholic extract and for an untreated control group. 25 larvae were used for each replicate (100 larvae were used for each concentration).

Larvae were then transferred to a rearing plastic cups (100 cm^3) each contained a piece of buffalo meat and the test materials were then added. The plastic cups then covered with clean gauze and secured by plastic band. In the control groups, larvae were treated with distilled water and Tween-80. Larvae were maintained under laboratory conditions at 27 ± 2°c, 80 ± 5% relative humidity (RH), and a 16: 8 hour light: dark cycle. Larva behavior (movement and feeding activity) was monitored at 8hours, 12h, 24h, 36h, 48h and 72h (until 3rd instar). Larva mortality counts were determined until pupation. Larvae were considered alive if they exhibited normal behavior when breathed upon or physically stimulated with wooden dowels; larvae which were incapable of movement, maintaining any signs of life, were considered moribund or dead. The developed pupae, at each concentration, were counted and placed in separate cages until adults emerged.

2.3. Evaluation of the extract effect

2.3.1. Statistical analysis

Data was statistically analyzed by ANOVA testing the differences between the five concentrations in *Commiphora molmol*, four concentrations in *Balanites aegyptiaca* and control means. Duncan's test was used to separate means at level ($P< 0.05$) using SPSS computer program version 14. The percentage of mortalities resulted from larvae which treated with *Commiphora molmol* and *Balanites aegyptiaca* were corrected for the natural mortality according to Abbot's formula **(Abbot, 1925).** LC50 (Lethal concentration for 50% of individual) and LC90 (Lethal concentration for 90% of individuals) values were estimated by log concentrations probit model using LdplineR software. This program devoted to calculate probit analyses according to **(Finney, 1971).**

2.3.2. Histopathology of 3rd instar larvae

Samples were taken from control larvae as well as those exposed to 32 mg/ml of *Commiphora molmol* and 128 mg/ml of *Balanites aegyptiaca*, fixed in 10% formalin and processed according to the method of **(Bancroft et al., 1996).** Sections were deparaffinized and stained with hematoxylin and eosin stain for histological examination by light microscopy. The body wall and the gut of larvae were studied and photographed using an Olympus CX41 microscope.

2.3.3. Scanning electron microscopy (SEM) of 3rd instar larvae

Samples were taken from control larvae as well as those exposed to 32 mg/ml of *Commiphora molmol*

and 128 mg/ml of *Balanites aegyptiaca* were first washed several times with saline then immersed in 2.5% glutraldehyde according to **(Mendonca et al., 2014)**. Specimens were then dehydrated through ascending ethanol series, dried in CO2 critical point drier (Autosamdri-815, Germany) the specimens were glued over stubs and coated with 20nm. gold in a sputter coater (Spi-Module sputter Coater, UK), finally the specimens were examined and photographed with scanning electron microscope at a magnification ranging from 35X to 500X (JSM 5200, Electron probeMicro analyzer , Jeol, Japan; at Faculty of Agriculture, Cairo University) also by Quanta FEG 250,at National research center.

Results

The present study described the *in vitro* efficacy of two medicinal plants volatile oil of *Commiphora molmol* and alcoholic extract of *Balanites aegyptiaca* against *Lucilia sericata* larvae

1. Effect of *Commiphora molmol* (Myrrh) on the development of *Lucilia sericata* larvae

1.1. First instar larvae (table, 1)

The results indicated that,*Commiphora molmol* was significantly effective on the development of first instar larvae of *Lucilia sericata*. The insecticidal efficacy of *Commiphora molmol* increased as the concentration increased. One hundred percentage of larva mortality was reached after treatment of larvae with 32 mg/ml concentration. Larva mortality was reached 59% and 75% at concentrations of 8mg/ml and 16 mg/ml respectively, However the larvae which molted to second did not complete their life cycle.

Table (1): The effect of *Commiphora molmol* on the development of *Lucilia sericata* 1st instar larvae

Conc. (mg /ml)	Larval instars						Pupa No.	Emerged fly	
	1st		Molted to 2nd		Molted to 3rd				
	No.	M.%	No.	M.%	No.	M.%		Emergency %	Deformity %
2	100	23 ± 11.94^{b}	77	14.58 ± 13.96^{bc}	67	15.10 ± 23.39^{a}	60	26.19 ± 9.44^{c}	2.40 ± 3.40^{a}
4	100	32 ± 7.30^{b}	68	16.96 ± 13.66^{c}	57	17.90 ± 22.39^{a}	49	11.71 ±5.81^{b}	10.28 ± 6.63^{b}
8	100	59 ± 11.48^{c}	41	100 ± 0.00^{d}	0	0.00^{a}	0	0.00^{a}	0.00^{a}
16	100	75 ± 10.45^{d}	25	100 ± 0.00^{d}	0	0.00^{a}	0	0.00^{a}	0.00^{a}
32	100	100 ± 0.00^{e}	0	0.00^{a}	0	0.00^{a}	0	0.00^{a}	0.00^{a}
C.with D.W. and Tween 80	100	2 ± 4.00^{a}	98	3.26 ± 6.52^{ab}	95	0.00^{a}	95	97.50 ±5.00^{d}	0.00^{a}
F.		69.144		128.06		1.686		235.96	7.36
Sig.		0.00		0.00		0.18		0.00	0.001

a,b…… ect, explain the significant difference between the percent of mortalities. Conc: Concentrations, C: control, D.W: Distilled Water, No: Number, M: Mortality percent, F: F values, Sig: Significance.

The molted pupa (from low concentration) showed some deformities as small, larviform pupa, and incomplete emergence of adult fly. Only 26.1% and 11.7% fly emerged in concentration of 2mg/ml and 4mg/ml.

1.2. Second instar larvae (table, 2)

The results demonstrated that the insecticidal activity of *Commiphora molmol* was also significantly effective on the development of *Lucilia sericata* second instar larvae. The mortality rates were 100% at 32mg/ml, while 87% at 16 mg/ml. The lower mortality rate occurred at 2mg/ml and 4mg/ml, 13% and 17% respectively.

Table (2): The effect of *Commiphora molmol* on the development of *Lucilia sericata* 2nd instar larvae

Conc. (mg /ml)	Larval instars				Pupa No.	Emerged fly	
	2nd		Molted to 3rd				
	No.	M.%	No.	M.%		Emergency %	Deformity %
2	100	13.00 ± 6.00^{b}	87	8.95 ± 8.51^{a}	79	20.22 ± 3.66^{c}	1.30 ± 2.60^{a}
4	100	17 ± 8.86^{b}	83	11.22 ± 7.55^{a}	74	14.71 ± 3.49^{b}	3.10 ± 3.66^{a}
8	100	34 ± 9.52^{c}	66	15.73 ± 10.75^{a}	56	18.09 ± 3.22^{bc}	1.38 ± 2.77^{a}
16	100	87 ± 10.54^{d}	13	75± 50^{d}	3	0.00^{a}	0.00^{a}
32	100	100 ± 0.00^{e}	0	0.00^{a}	0	0.00^{a}	0.00^{a}
C.with D.W. and Tween 80	100	2.00 ± 4.00^{a}	98	2.17 ± 4.34^{a}	96	98.8 ± 2.38^{d}	0.00^{a}
F.		131.62		6.863		789.86	1.31
Sig.		0		0.001		0	0.301

1.3. Third instar larvae (table, 3)

The highest concentration of 32 mg/ml induced 97% mortality and only 3 larvae molted to pupa but failed to emerge to adult fly.

Table (3): The effect of *Commiphora molmol* on the development of *Lucilia sericata* 3rd instar larvae

Conc. (mg /ml)	3rd larval instars		Pupa No.	Emerged fly	
	No.	M.%		Emergency %	Deformity %
2	100	19 ± 8.86^{b}	81	23.46 ± 6.96^{c}	3.66 ± 2.49^{b}
4	100	28 ± 10.32^{b}	72	15.94 ± 8.57^{b}	5.29 ± 4.31^{b}
8	100	50 ± 14.04^{c}	50	11.73 ± 1.47^{b}	0.00^{a}
16	100	80 ± 7.30^{d}	20	0.00^{a}	0.00^{a}
32	100	97 ± 3.82^{e}	3	0.00^{a}	0.00^{a}
C. with D.W. and Tween 80	100	0.00^{a}	100	100^{d}	0.00^{a}
F.		73.93		307.686	5.41
Sig.		0		0	0.003

2. Effect of *Balanites aegyptiaca* on the development of *Lucilia sericata* larvae

2.1. First instars larvae (table, 4)

Balanites aegyptiaca has low effect on the development of first larva instars. The mortality rate reached 52%, 28%, 18% and 12% at concentration of 128, 64, 32, 16 mg/ml respectively. So these larvae which not affected with the extract were able to complete their development to second and third instar larvae which then pupated and emerged to adult fly.

Table (4): The effect of *Balanites aegyptiaca* on the development of *Lucilia sericata* 1st instar larvae

Conc. (mg /ml)	Larval instars						Pupa No.	Emerged fly	
	1st		Molted to 2nd		Molted to 3rd				
	No.	M.%	No.	M.%	No.	M.%		Emergency %	Deformity %
16	100	12 ±4.61^{ab}	88	6.93 ± 2.99^{a}	82	3.58 ± 4.42^{a}	79	69.09 ± 21.68^{b}	4.97 ± 3.74^{ab}
32	100	18 ±2.30^{bc}	82	11.01 ± 2.66^{ab}	73	4.01 ± 2.68^{a}	70	61.35 ± 8.82^{b}	4.32 ± 2.88^{ab}
64	100	28 ± 7.30^{c}	72	19.91 ± 7.63^{b}	58	10.32 ± 3.42^{ab}	52	36.64 ± 20.73^{a}	9.58 ± 2.84^{b}
128	100	52±16.32^{d}	48	36.34 ± 13.46^{c}	31	23.18 ±19.43^{b}	20	51.04 ±11.46^{ab}	8.33 ± 3.61^{b}
C.with D.W.	100	3 ± 6^{a}	97	0.00^{a}	97	1.13 ± 2.27^{a}	96	97.61 ± 4.76^{c}	0.00^{a}
F.		18.43		15.36		3.75		9.07	2.29
Sig.		0.00		0.00		0.026		0.001	0.10

2.2. Second instar larvae (table, 5)

Balanites aegyptiaca caused mortality rate for second instar larvae only reached 41% for concentration of 128 mg/ml but it has high effect on emergency of adult fly which reached only 19%.For concentration of 16, 32, 64mg/ml the mortality rate was 8%, 13%, and 20% respectively.

Table (5): The effect of *Balanites aegyptiaca* on the development of *Lucilia sericata* 2nd instar larvae

Conc. (mg /ml)	Larval instars				Pupa No.	Emerged fly	
	2nd		Molted to 3rd				
	No.	M.%	No.	M.%		Emergency %	Deformity %
16	100	8 ± 3.26^{ab}	92	3.26 ± 2.17^{a}	89	56.79 ± 21.18^{b}	4.54 ± 3.71^{b}
32	100	13 ±5.03^{bc}	87	11.6 ± 3.18^{b}	77	50.16 ± 18.89^{b}	3.94 ± 2.66^{b}
64	100	20 ± 3.26^{c}	80	17.58 ± 6.71^{b}	66	38.32 ± 15.46^{ab}	4.71 ± 3.18^{b}
128	100	41 ± 9.45^{d}	59	29.24 ± 7.63^{c}	44	19.84 ± 9.28^{a}	0.00^{a}
C. with D.W.	100	2 ± 4^{a}	98	0.00^{a}	98	96.73 ± 6.52^{c}	0.00^{a}
F.		29.82		23.18		13.83	3.79
Sig.		0.00		0.00		0.00	0.02

2.3. Third instar larvae (table, 6)

The highest concentration of *Balanites aegyptiaca* (128mg/ml) produced mortality rate not exceeded 30% and other non-affected larvae pupated and only 20.6% were able to emerge to adult fly.

The calculated LC50 values of *Commiphora molmol* were 6.03, 7.96 and 6.55 for 1st, 2nd, and 3rd larva instars respectively. The LC50 values of *Balanites aegyptiaca* were 142.27, 216.05 and 371.15 for 1st, 2nd, and 3rd larva instars respectively (table, 7).

Table (6): The effect of *Balanites aegyptiaca* on the development of *Lucilia sericata* 3rd instar larvae

Conc. (mg /ml)	3rd larval instars		Pupa No.	Emerged fly	
	No.	M.%		Emergency %	Deformity %
16	100	5 ± 3.82^{ab}	95	60.37 ± 16.57^{c}	0.0 ± 0.0^{a}
32	100	9 ± 2^{ab}	91	47.32 ± 7.24^{bc}	2.17 ± 0.0^{b}
64	100	14 ± 5.16^{b}	86	33.94 ± 10.57^{ab}	0.0 ± 0.0^{a}
128	100	30 ± 13.26^{c}	70	20.60 ± 11.31^{a}	0.0 ± 0.0^{a}
C.with D.W.	100	0.00^{a}	100	100 ± 00^{d}	0.0 ± 0.0^{a}
F.		11.95		32.67	2.76
Sig.		0.00		0.00	0.07

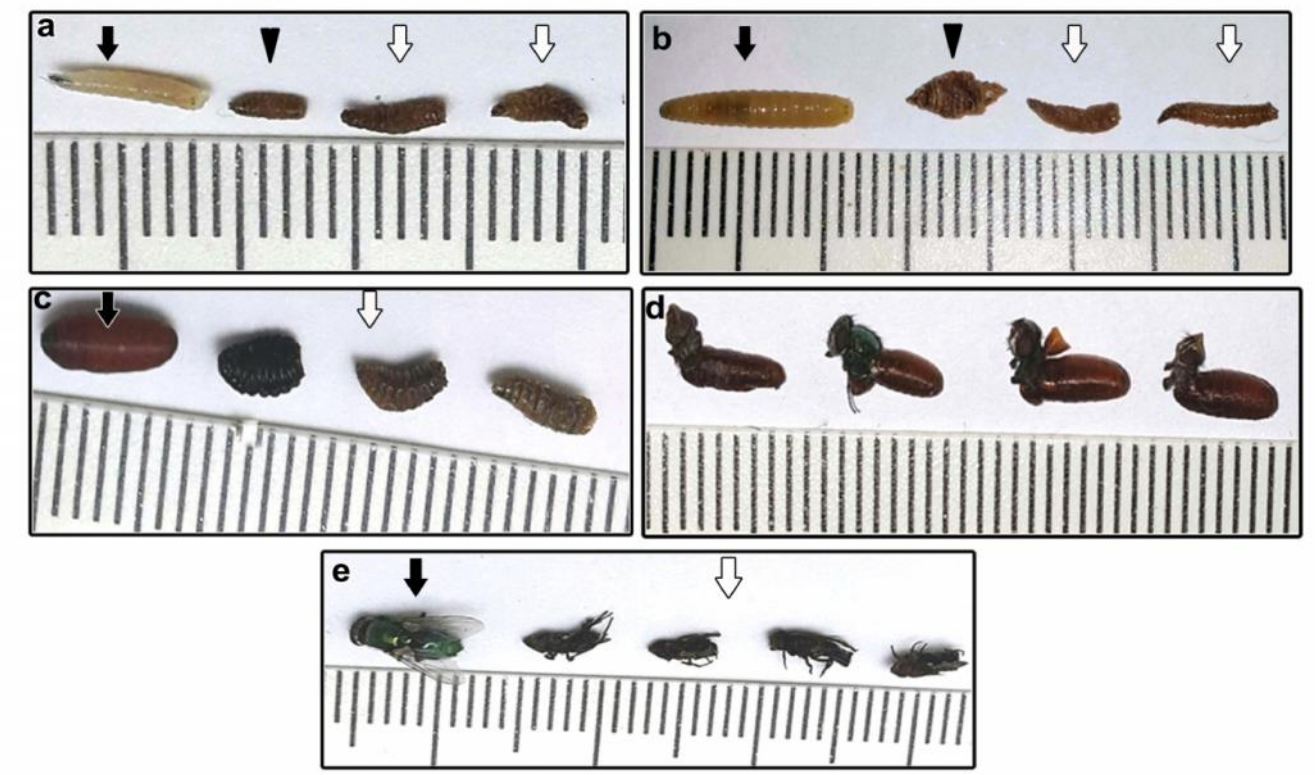
Table (7): Calculated LC50 and LC90 values

Applied extract	*Commiphora molmol*		*Balanites aegyptiaca*	
LC50/LC90	LC50	LC90	LC50	LC90
First larval instar	6.03	24.55	142.278	1005.99
Second larval instar	7.96	21.77	216.054	1649.41
Third larval instar	6.55	25.47	371.15	3865.91

LC50: Lethal concentration for 50% of individuals, LC90: Lethal concentration for 90% of individuals.

3. Morphological changes of treated larvae

Morphological abnormalities were noticed for two applied extracts (Fig. 1). Malformations of larvae included small sized, contractile, and damaged larvae with weak cuticle (Fig. 1a, b). Abnormalities of pupa included small sized, distorted and larviform pupae (Fig. 1c). Incomplete emergency of adult (Fig. 1d).Abnormalities of adults included small sized, malformed, poorly developed and deformed wing and legs (Fig. 1e).



(Fig. 1): Morphological abnormalities occurred after treatment of larvae with plant extract. a- Normal second instar larva (black arrow), Small sized larva (head arrow), contractile or shrunken larva (white arrow), b- Normal third instar larva (black arrow), damaged or broken larva (head arrow), small sized larvae (white arrow), c- Normal pupa (black arrow), larvifom, distorted and small sized pupa (white arrow). d- Incomplete emergency of adult. e- Normal adult (black arrow), small, malformed and poorly developed adult with deformed wing and legs (white arrow).

4. Histopathological examination of 3rd instar larvae

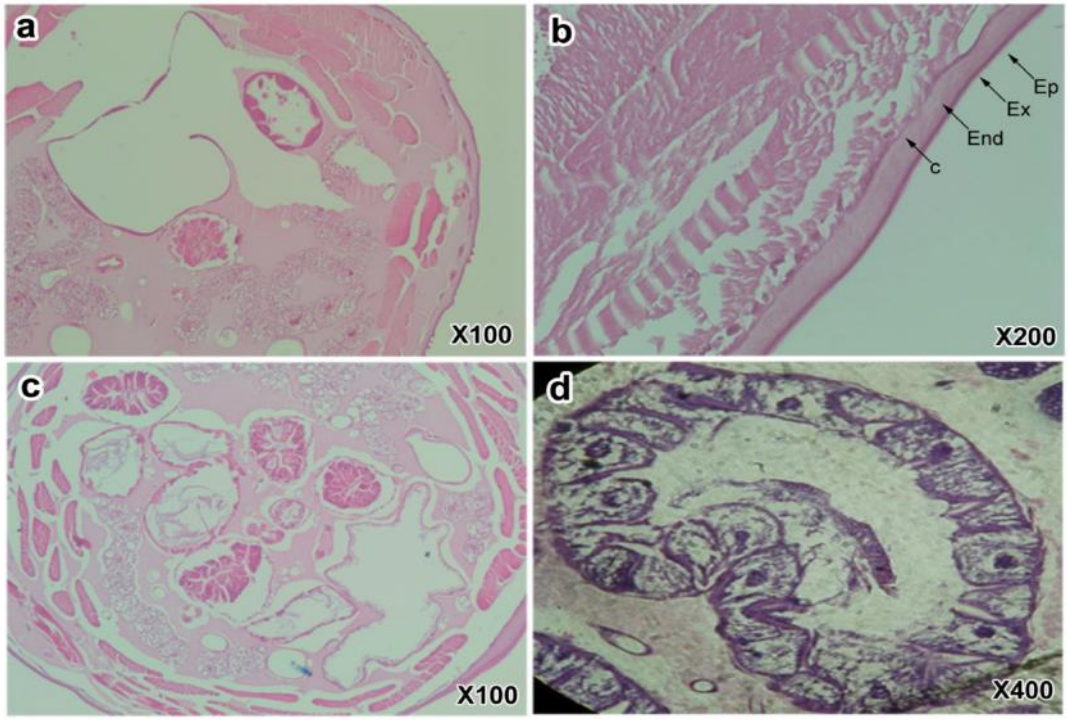
4.1. Normal control larvae

Histological study of *Lucilia sericata* larvae for the control group showed the structure of cuticle which formed from outer-dense layer called epicuticle that forms a continuous layer covering cuticular surface. The region of the cuticle; present between epicuticle and the epidermal cell layer; is called procuticle which represents the main part of the total cuticle consisting of the outermost layer (exocuticle) and the innermost layer (endocuticle) followed by the epidermal cell layer (Fig. 2a, b). Epithelial cells of the gut are simple cuboidal epithelium which appeared normal without any damage and well adhesive to gut wall beside each other (Fig. 2c, d).

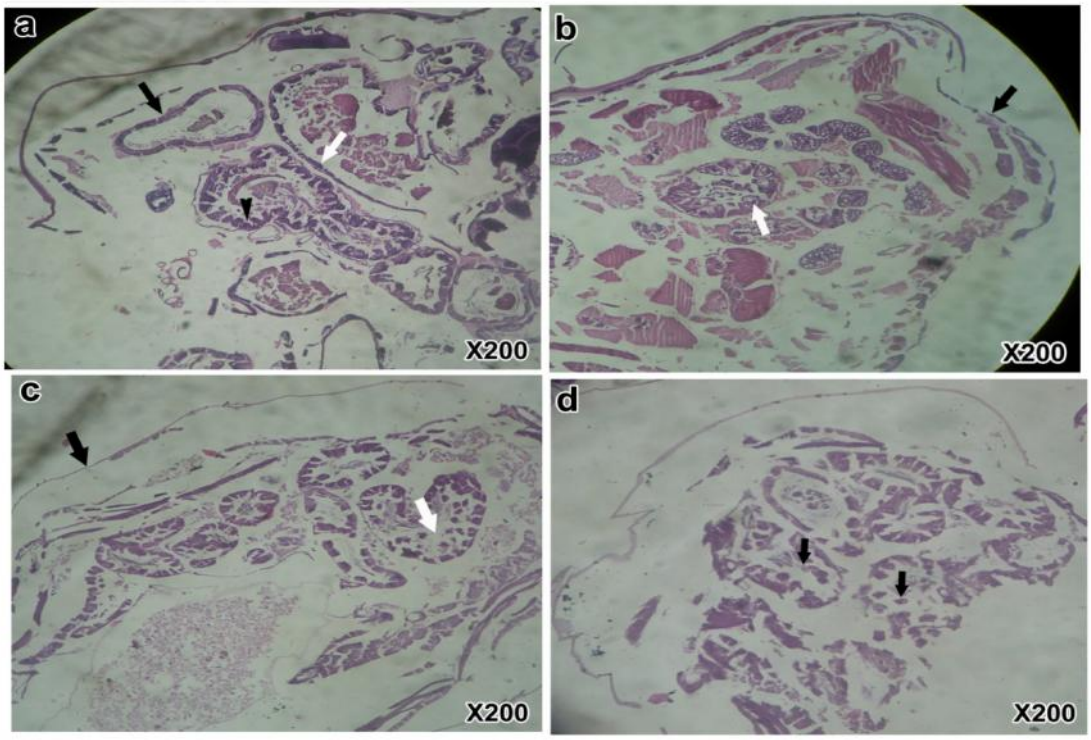
4.2.Histopathological examination of treated larvae

Larvae of *Lucilia sericata* treated with volatile oil of *Commiphora molmol* suffered from various pathological changes especially cuticle and gut. Epithelial cells of gut severely necrosed and degenerated with destructed gut wall (Fig. 3a, white arrow). Also some epithelial cells of gut slightly degenerated and diffused together with separation of inner cellular layer of epidermal cells in some regions of procuticle (Fig. 3a, black arrow). Gut epithelial cells were reduced in size and separated from each others but still adhesive to wall of the gut with few cells were destroyed (Fig. 3a, head arrow). Destruction of cuticular surface (Fig. 3b, black arrow) Vacuolization of epithelial cells with cellular disorganization and the nucleus being absent (Fig. 3b, white arrow).

Larvae of *Lucilia sericata* treated with *Balanites aegyptiaca* showed thinning of cuticular surface with separation of inner cellular layer of epidermal cells (Fig. 3c, black arrow). Epithelial cells of gut destructed, reduced in size, separated from each other's and some of them detached in the lumen (Fig. 3c, white arrow). Severe destruction of epithelial cells and detached from the wall of gut into the lumen with destruction of the wall of the gut (Fig. 3d, black arrow).



(Fig. 2): Light micrograph of the cuticle and midgut of normal third instar larvae of *Lucilia sericata*. a, b normal control of cuticle. c, d normal control of midgut. Ep: Epicuticle, Ex: Exocuticle, End: Endocuticle, C: Ceullar layer of epidermal cell.

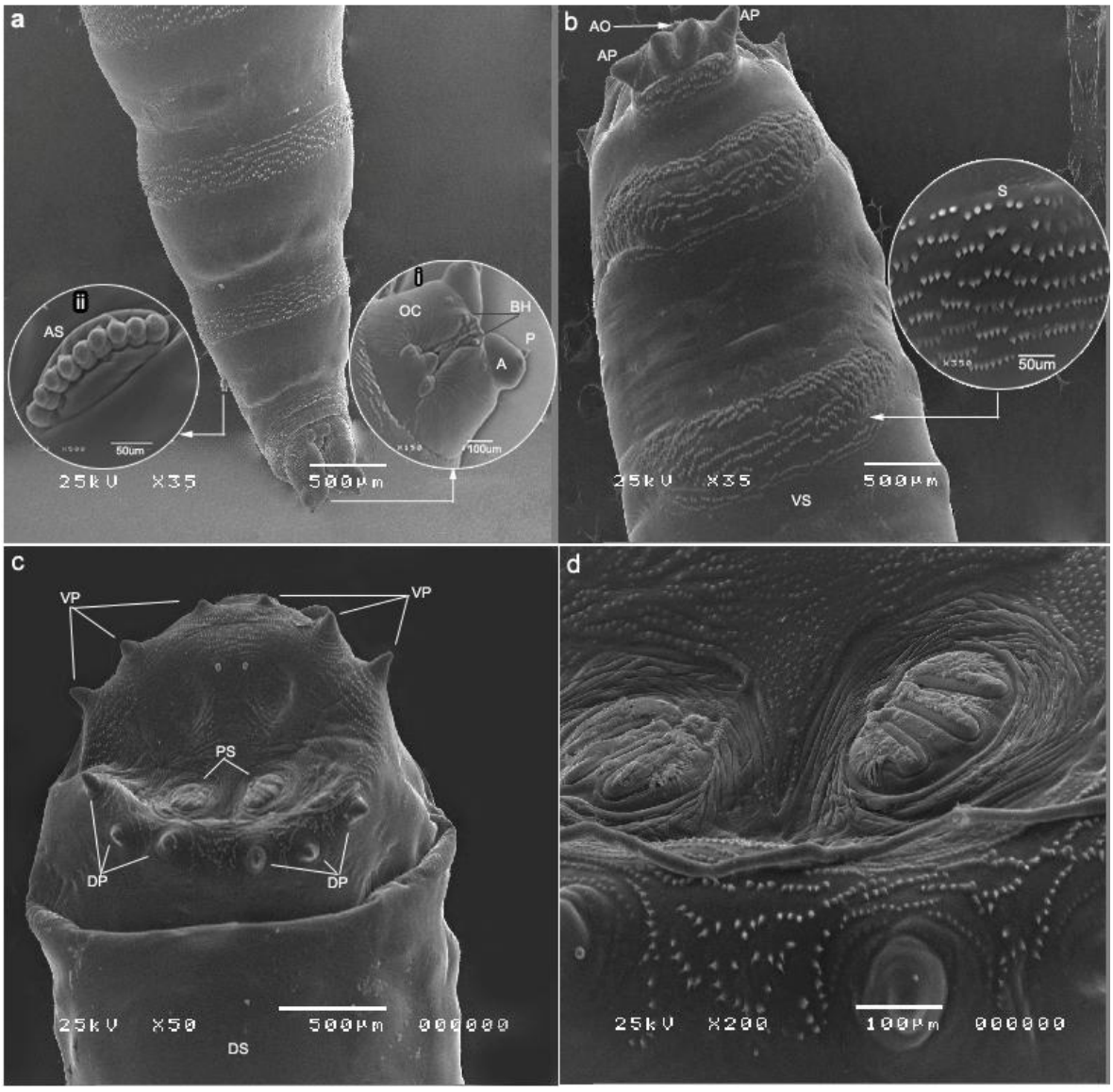


(Fig. 3): Light micrograph of third larva instars of *Lucilia sericata* treated with *Commiphora molmol* (a, b) and *Balanites aegyptiaca* (c, d). a, necrosis of epithelial cells with destruction of the gut wall (white arrow). Epithelial cells degenerated and diffused together (black arrow). Epithelial cells reduced in size and separated from each other (head arrow). b, destruction of the cuticular surface (black arrow).Vacuolization of epithelial cells (white arrow). c, thinning of cuticular surface (black arrow). Destruction of epithelial cells with some detached in the lumen (white arrow).d, severe destruction of epithelium with destruction of gut wall (black arrow).

5.Scanning electron microscope of 3rd instar larvae

5.1. Normal control larvae

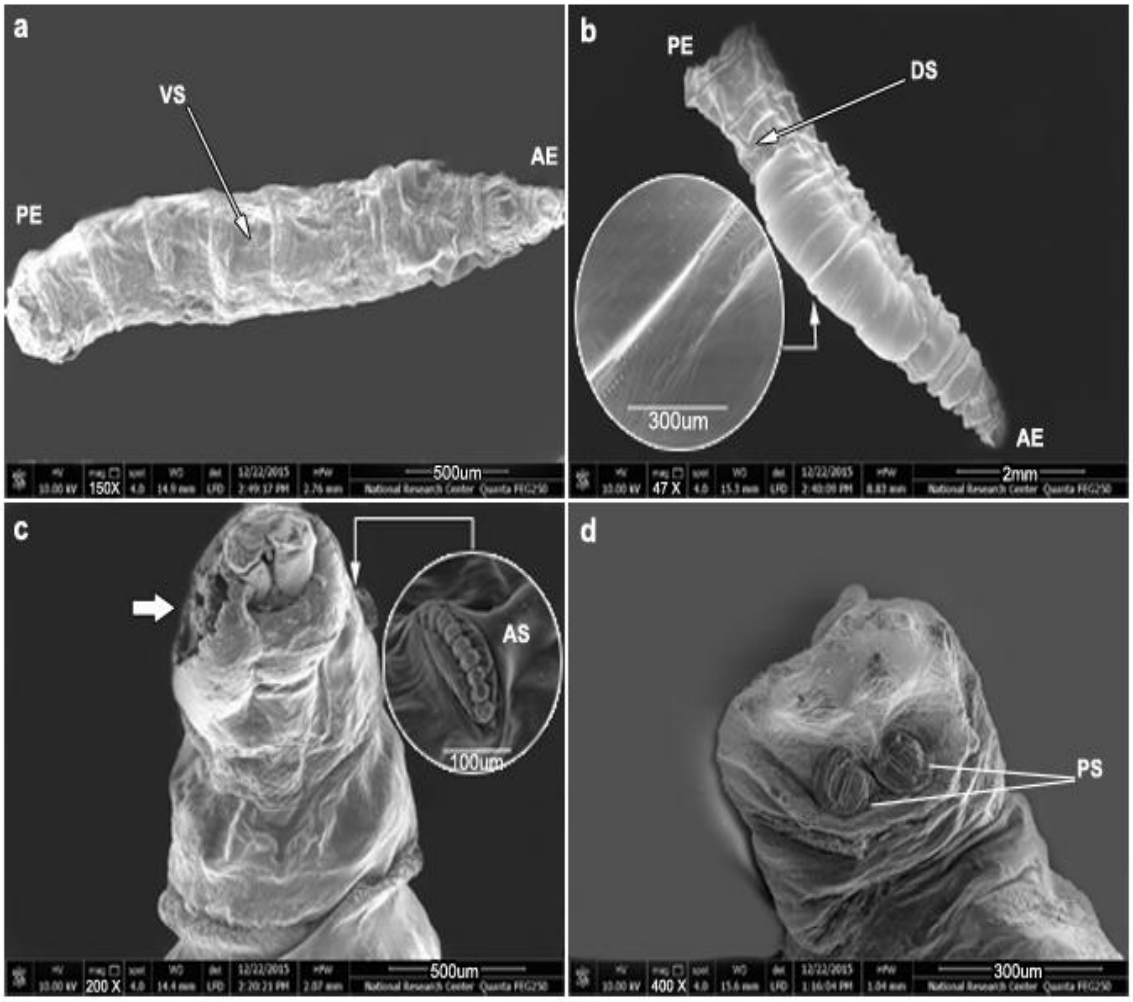
Normal *Lucilia sericata* larva consisted of 12 segments; one cephalic, three thoracic and eight abdominal segments. The anterior end was pointed and the posterior end was blunt. The cephalic region was bi-lobed and had the antennal sensory papillae. It also has two buccal hooks and oral cristae (Fig. 4a, i).The anterior spiracles, present in the first thoracic segment, each with seven to nine branches (Fig. 4a,ii).The body surface was covered with many tiny short spines. The body spines were sharp with a single point and arranged in distinct bands around the body showing clearly defined spine bands. The anal opening had one pair of anal papillae surrounded by numerous tiny spines and was located under the posterior spiracular plate in the last segment(Fig. 4b). Posterior end also had dorsal and ventral papillae which were important for protection of posterior spiracles(Fig. 4c).The last segment had a pair of posterior spiracles. Posterior spiracular plates were with three straight, approximately parallel slits. Peritreme which completely surrounded the spiracular plate was thin and less sclerotized (Fig. 4d).



(Fig. 4): Scanning electron microscopy (SEM) of *Lucilia sericata* normal third instar larvae. Key: (a) AS: Anterior spiracle, A: Antenna, P: Papilla, OC: Oral cristae, BH: Buccal hook, (b) AO: Anal opening, AP: Anal papilla, S: Spines, VS: Ventral surface, (c) DP: Dorsal papilla, VP: Ventral papilla, PS: Posterior spiracle. (d) Three slits opening with closed peritreme.

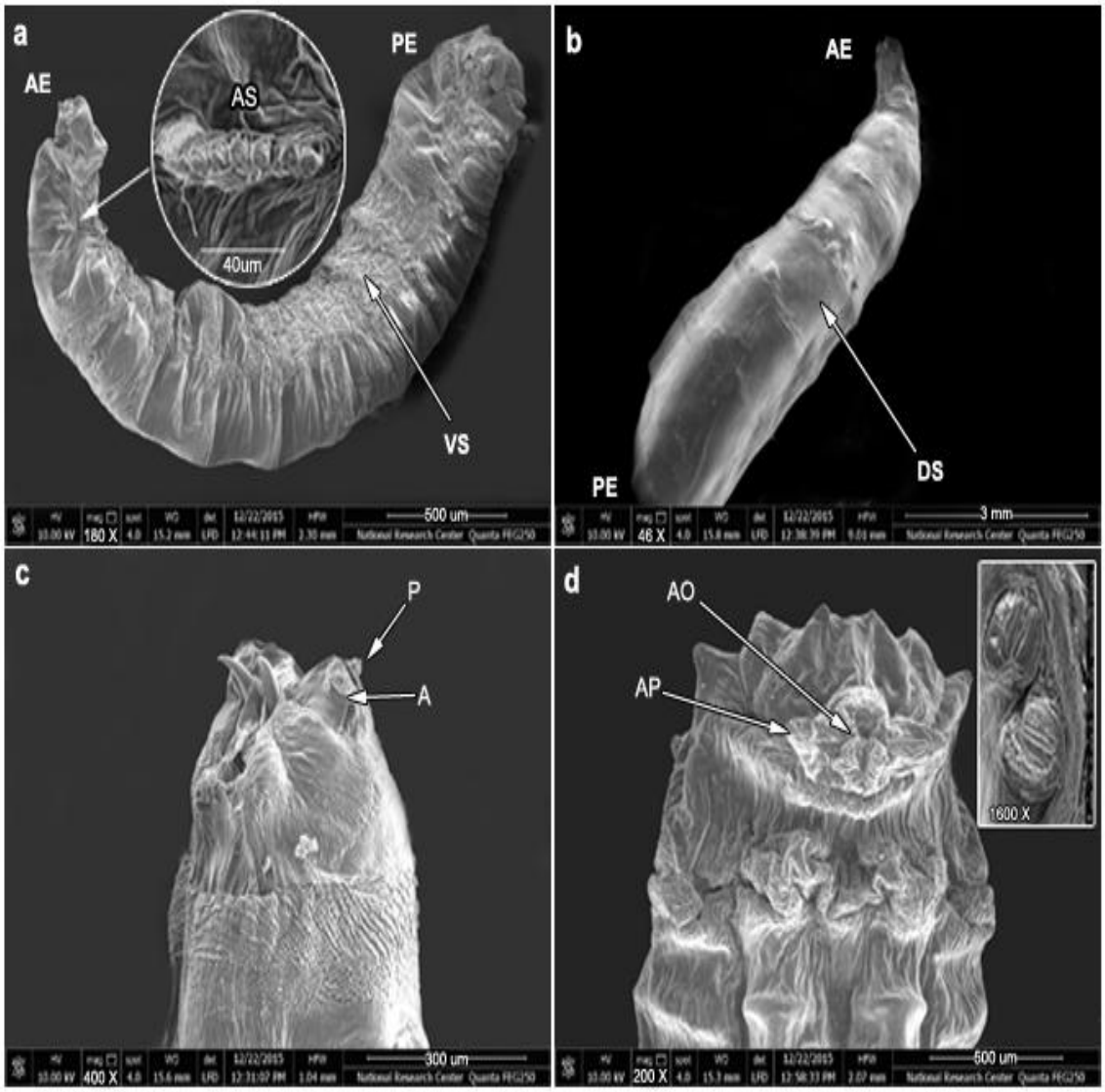
5.2. Treated larvae

Scanning electron microscopy of third instar larvae after treatment with *Commiphora molmol* showed some alterations on dorsal and ventral surface. There were small areas of swelling appeared at the ventral surface with severely corrugated cuticle which leads to disappearance of spines (Fig. 5a). At the dorsal surface there was extensive area of swelling so spines of the dorsal surface appeared submerged or sunken in the swelling (Fig. 5b). Anteriorly in addition to swelling large area appeared to be eroded and sloughed with slight degeneration of anterior spiracles (Fig. 5c). At the posterior end degeneration of dorsal, ventral and anal papillae was occurred with sever wrinkled and folded cuticle (Fig. 5d).



(Fig. 5): Scanning electron microscopy (SEM) of *Lucilia sericata* third instar larvae treated with *Commiphora molmol*. (a) SEM of ventral surface of larva showed swelling with losing of spines. (b) SEM of dorsal surface shows extensive or huge swelling appeared on the dorsal surface with spines submerged in the swelling. (c) SEM of anterior end shows erosion and sloughing of anterior end with degenerated anterior spiracles. (d) SEM of posterior end shows degeneration of anal, dorsal and ventral papillae. AE: Anterior end, PE: Posterior end, VS: Ventral surface, DS: Dorsal surface, AS: Anterior spiracle, PS: Posterior spiracle.

While *Balanites aegyptiaca* cause severe cuticular shrinkage on the ventral surface so spines could not be observed and sever degeneration of anterior spiracles (Fig. 6a). Dorsally there were several areas of swelling (Fig. 6b). The anterior end showed degeneration and distortion of antennal sensory papillae with wrinkled cuticle (Fig. 6c). Distortion of the anal papillae, sever wrinkled cuticle and longitudinal folds appeared on the posterior end (Fig.6d).



(Fig. 6): Scanning electron microscopy (SEM) of *Lucilia sericata* third instar larvae treated with *Balanites aegyptiaca* (a) SEM of ventral surface shows severe shrinkage with severe degeneration of anterior spiracles. (b) SEM of the dorsal surface shows cuticular swelling in different areas. (c) SEM of anterior end shows degeneration of antennal sensory papillae. (d) SEM of posterior end shows distortion of anal papillae with severe wrinkled cuticle. AE: Anterior end, PE: Posterior end, VS: Ventral surface, DS: Dorsal surface, AS: Anterior spiracles, A: Antenna, P: Papilla. AO: Anal opening, AP: Anal papillae.

Discussion

The present work described in details the effect of two plant extracts *Commiphora molmol* and *Balanites aegyptiaca* on the development of *Lucilia sericata* larvae. No previous data studied the effect of these extracts on these larvae until now, However *Commiphora molmol* has insecticidal effect against different insects as *Culex pipiens* larva **(Shonouda and Mehanney, 2000, Massoudand Labib, 2000 and Habeeb et al., 2009)**; *Musca domestica* **(Shonouda and Mehanney, 2000**) and *Aedes caspius* **(Massoud and Labib, 2000).** Also *Balanites aegyptiaca* has effect on larvae of *Aedes aegypti* **(Wiesman and Chapagain, 2006, Chapagain et al., 2008);** *Culex quinquefasciatus* **(Abdalla, 2011)** and *Culex pipiens* **(Chapagain and Wiesman, 2005).**

The results showed that, both extracts have toxicity against the three instar larvae of *Lucilia sericata* and different biological changes were recorded after treatment of larvae with these plant extracts as larva molting, pupation rate and emergency of adult**.**

The resin extract of *Commiphora molmol* has Fasciolicide, schistosomicide, heterophycide, dicrocoelicide, and molluscicide effect as reviewed in detail by **(Abdul- ghani et al., 2009).**

Some of the chief components in myrrh were sesquiterpense which were a large family of C15-isoprenoid molecules found in plants, microbes and some marine organisms. Isoprenoids also called terpenoids, were unsaturated hydrocarbons found in essential oils and oleoresins of plants. They passed through the cell wall and cytoplasmic membrane, disrupted the structure of their different layers of polysaccharides, fatty acids and phospholipids and permeabilized them. Cytotoxicity appeared to include such membrane damage **(Massoud et al., 2012).**

Interaction of saponins compounds of *Balanites aegyptiaca* with the cuticle membrane of the larvae, ultimately disarranging this membrane by association of the saponins molecules with these membrane could be the most probable reason for death of larvae **(Kamel et al., 1991).**

The present study indicated that, *Commiphora molmol* was more effective against the three larva instars of *Lucilia sericata* than *Balanites aegyptiaca*. However *Commiphora molmol* showed approximately complete mortality of the three larvae instars at the highest concentration (32mg/ml) with LC50 values 6.03, 7.96 and 6.55 mg/ml for 1st, 2nd, and 3rd larvae instars respectively. While *Balanites aegyptiaca* at highest concentration (128mg/ml) caused lower mortality than *Commiphora molmol* and LC50 values were 142.27, 216.05 and 371.15 mg/ml for 1st, 2nd, and 3rd larvae instars respectively. Comparable results have been indicated after using *Commiphora molmol* at different concentrations that ranged from 25.000 to 100.000 ppm on *Musca domestica* 3rd instar larvae, the mortality rate ranged from ranged from 40%-100% respectively with LD50 30856ppmAlso the concentration of myrrh oil ranged from 40ppm to320 ppm on *Culex pipiens* resulted in mortality rates ranged from 24 to 91% respectively with LD50 138.13 ppm **(Shonouda and Mehanney, 2000)**. Concerning *Balanites aegyptiac*a fruit pulp when applied against larvae of *Culex pipiens* mosquitoes at the highest concentration of 2% killed approximately 90% of larvae after 3 days**(Chapagain and Wiesman, 2005**). These variations between the present results and that of **(Chapagain and Wiesman, 2005**) using *Balanites aegyptiac*a attributed to the habit of mosquito larva and the permanent contact between these larvae and the extract as well as using of saponins or crude extract.

The two extracts induced morphological malformation of larvae included small sized, contractile, and damaged larvae with weak cuticle. Abnormalities of pupa includedsmall sized, distorted and larviform pupae. Incomplete emergency of adult. Abnormalities of adults included small sized, malformed, poorly developed and deformed wing and legs.

Nearly the same results have been reported after treatment the larvae of *Lucilia sericata* with other extracts including lettuce, chamomile, anise, and rosemary oils **(Khater et al., 2011)** as well as Neem extracts **(El-Khateeb et al., 2003).** These abnormalities also have been indicated in other myiasis causing flies as *Chrysomya albiceps* after exposure to pomegranate (*Punica granatum*) (**Morsy et al., 1998)** as well as sesame (*Sesamum indicum*), nigella (*Nigella sativa*) and onion (*Allium cepa*) oils badly affect pupation rates and emergence of adult *Musca domestica* and *Culex pipiens* (**Khater, 2003)**.

In the workers opinion and according to **(Hussien, 1995)** the occurrence of larviform pupae may be contributed to failure of larvae to contract to the pupa stage due to paralysis of the muscle but the cuticle of the pupaemelanizes because continuation of the tanning process. Also the failure of adult to emerge may be combination of two or more reasons; unsaturated fatty acids accelerate the melanization process and hardening of larvae (thus adults are unable to emerge from the pupalexcuviae) and there is insufficient pressure in the ptilinumas well as hardening of the opercular suture occurs.

It is generally accepted that the developmental anomalies induced by plant compounds are due to an interference with the neuroendocrine control of molting and ecdysis **(Schmutterer, 1990).** In larva insects three endocrine glands are known to be responsible for releasing neuro-hormones essential for growth, development and differentiation; the prothoracic gland (PTG), the corpus allatum (CA), and the corpus cardiacum (CC). It has been shown that plant compounds cause progressive degeneration of all these endocrine glands in larvae **(Meurant et al., 1994).** This morphological degeneration implies a generalized dysfunction of the neuroendocrine system.

(Shalaby et al., 2015) recorded good larvicidal activity with *Lavender* and *Camphor oil* against *Lucilia sericata* third instar larvae. Several plant extract have been known to have larvicidal effect

against *Lucilia sericata* larvae such plant include Lettuce (*Lactuca sativa*), chamomile (*Matricaria chamomilla*), anise (*Pimpinella anisum*), and rosemary (*Rosmarinus officinalis*), **(Khater et al.,2011)** with LC50 0.57%, 0.85%, 2.74%, and 6.77%.Also, the larvae of *Lucilia sericata* were sensitive to Neem Azal T/S, Nivaar, and Bio Dux, LC50 values were 1.3%, 0.4%, and 4%, respectively **(El-Khateeb et al., 2003).** In addition to after treatment with 16% fenugreek (*Trigonellafoenum- graecum*) and celery (*Apium graveolens*) the pupation rate was strongly decreased in *Lucilia sericata*. Moreover, adult emergence was suppressed after treatment of larvae with 8% mustard (*Brassica compestris*) 12% radish (*Raphanus sativus*) and 16% fenugreek and celery oils **(Khater and Khater, 2009).**

Histopathology and Scanning electron microscopy were used to define the aim of the tested products. Histopathological changes showed damage of cuticular structure and gut of the larvae as thinning and destruction of cuticular surface with separation of inner cell layer of epidermal cells in some regions of procuticle, destruction of epithelial cells lining the gut, vacuolization of the epithelial cells of the gut and destruction of the gut wall. SEM showed morphological changes in the cuticular surface. These changes included, cuticular swelling, degeneration of anterior spiracles, wrinkled and shrinkage cuticle, degeneration of papillae. On the other hand, histopathology and Scanning electron microscopy was done only one time for the larvae of *Lucilia sericata* but using camphor and lavender oil **(Shalaby et al., 2015)** Who mentioned that, there were corrugation and thinning of cuticular surface and severely folded cuticle with camphor oil, while with lavender oil it showed disruption of inner cell layer of epidermal cells. SEM of lavender showed cuticular distortion at both dorsal and ventral surface and cuticular surface blebbing. While SEM of camphor showed damage of the cuticle with loss of spines at the ventral surface, slight degeneration of anterior spiracles, deformed and wrinkled cuticular surface.

References

Abbot, W. S. (1925): A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265 – 267.

Abdalla, M. M. O. (2011): Acute toxicity of some parts of *Balanites aegyptiaca*. (Doctoral dissertation, University of Khartoum).

Abdul-Ghani, R. A., Loutfy, N. and Hassan, A. (2009): Myrrh and trematodoses in Egypt: an overview of safety, efficacy and effectiveness profiles. Int. J. Parasitol. 58(3): 210-214.

Bancroft, J. D., Stevens, A. and Turner, D. R. (1996): Theory and practice of histological techniques, 4th ed. Churchill Livingstone, New York.

Beverley, A. H. (1991): Morphological characters to identify adult *Lucilia sericata* (Meigen, 1826) and *Lucilia cuprina* (Wiedemann, 1830) (Diptera: Calliphoridae), New Zealand J. Zool. 18 (4): 413-420.

Bisdorff, B. and Wall, R. (2008): Sheep blowfly strike risk and management in Great Britain: a survey of current practice. Med. Vet. Entomol.22: 303–308.

Chapagain, B. and Wiesman, Z. (2005): Larvicidal effects of aqueous extracts of *Balanites aegyptiaca* (desert date) against the larvae of *Culex pipiens* mosquitoes. Afr. J. Biotechnol. 4(11): 1351-1354

Chapagain, B. P., Saharan, V. and Wiesman, Z. (2008): Larvicidal activity of saponins from *Balanites aegyptiaca* callus against *Aedes aegypti* mosquito. Bioresour. Technol. 99(5): 1165-1168.

El-Khateeb, R. M. (1999): Seasonal incidence and some biological and physiological studies on blowflies, *Lucilia sericata* (Meigen) and *Chrysomya albiceps* (Calliphoridae: Diptera) with special reference to the effect of cutaneous myiasis on sheep host. (Ph. D. Thesis. Faculty of Science, Cairo University).

El-Khateeb, R.M., Abdel-Shafy, S. and Zayed, A.A. (2003): Insecticidal effects of neem seed and vegetable oils on larval and pupal stages of sheep blowfly, *Lucilia sericata* (Diptera: Calliphoridae). J. Egypt. Vet. Med. Assoc.63: 255- 268.

Finney, D.J. (1971): Probit analysis, Campridge University Press, 3rd Ed, pp. 333.

Habeeb, S. M., El-Namaky, A. H. and Salama, M. A. (2009): Efficiency of *Allium cepa* and *Commiphora molmol* as a larvicidal agent against fourth stage larvae of *Culex pipiens* (Diptera: Culicidae). Am. Euras. J. Agric. Environ. Sci. 5(2): 196-203.

Hanus, L.O., Rezanka, T.S., Dembitsky, V.M. and Moussaieff, A. (2005): *Myrrh Commiphora* chemistry. Biomed.Pap. 149(1): 3-28.

Hussien, K. T. (1995): Effect of some plant extracts in the control of a non-biting muscoid fly. PhD thesis, Cairo University: Cairo.

Kamel, M.S., Ohtani, K., Kurokawa, T., Assaf, M.H., El-Shanawany, M.A., Ali, A.A., Kasai, R., Ishibashi, S. and Tanaka, O. (1991): Studies on the *Balanites aegyptiaca* fruits, an antidiabetic Egyptian folk medicine. Chem. Pharm. Bull. (Tokyo), 39(5): 1229-33.

Khater, H. F. (2003): Biocontrol of some insects. PhD thesis, Zagazig University: Benha Branch, Benha.

Khater, H. F. and Khater, D. F. (2009): The insecticidal activity of four medicinal plants against the blowfly *Lucilia sericata* (Diptera: Calliphoridae). Int. J. Dermatol. 48(5): 492-497.

Khater, H. F., Hanafy, A., Abdel-Mageed, A. D., Ramadan, M. Y. and El-Madawy, R. S. (2011): Control of the myiasis-producing fly, *Lucilia sericata*, with Egyptian essential oils. Int. J. Dermatol. 50(2): 187-194.

Massoud, A. M. and Labib, I. M. (2000): Larvicidal activity of *Commiphora molmol* against *Culex pipiens* and *Aedes caspius* larvae. J. Egypt. Soc. Parasitol. 30(1): 101-115.

Massoud, A. M., Shalaby, H. A., El Khateeb, R. M., Mahmoud, M. S. and Kutkat, M. A. (2012): Effects of Mirazid and myrrh volatile oil on adult *Fasciola gigantica* under laboratory conditions**.** Asian pacific J. Trop. Biomed., 2(11): 875-884.

Mazyad, S.A.M., El-Serougi, A.O.M. and Morsy, T.A. (1999): The efficacy of the volatile oils of three plants for controlling *Lucilia sericata*. J. Egypt. Soc. Parasitol. 29: 91–100.

Mehlhorn, H., Al-Rasheid, K. A., Abdel-Ghaffar, F., Klimpel, S. and Pohle, H. (2010): Life cycle and attacks of ectoparasites on ruminants during the year in Central Europe: recommendations for treatment with insecticides (eg, Butox®). Parasitol. Res. 107 (2): 425-431.

Mendonca, P.M., Barbosa, R.R., Carrico, C., Cortinhas, L.B., Santos-Malleta, J.R. and CarvalhoQueiroz, M.M. (2014): Ultrastructure of immature stages of *Lucilia cuprina* (Diptera: Calliphoridae) using scanning electron microscopy. ActaTropica, 136: 123–128.

Meurant, K.; Sernia, C. and Rembold, H. (1994): The effects of azadirachtin A on the morphology of the ring complex of *Lucilia cuprina* (Wied) larvae (Diptera: Insecta). Cell Tissue Res. 275: 247–254.

Morsy, T. A., Mazyad. S. A. M. and El-Sharkawy, I. M. A.(1998): The larvicidal activity of solvent extracts of three medicinal plants against third instar larvae of *Chrysomya albiceps*. J. Egypt. Soc. Parasitol. 29: 91–100.

Pretty, J. (2009): The pesticide detox, towards a more sustainable agriculture. Earth scan, London.

Pritchard, M.H. and Kruse, G.O.W. (1982): The collection and preservation of animal parasites, University of Nebraska Press, Lincoln and London.

Saunders, D.S., Macpherson, J.N. and Cairncross, R.D. (1986): Maternal and larval effects of photoperiod on the induction of larval diapause in two fly *Calliphora vicina* and *Luciliasericata*.Exp. Biol. Med. 46: 51-58.

Schmutterer, H. (1990): Properties and potential of natural pesticides from the Neem tree, *Azadirachta indica.* Annu. Rev. Entomol. 35: 271–297.

Shalaby, H. A., El Khateeb, R. M., El Namaky, A. H., Ashry, H. M.,Kandil, O. M. and El Dobal, S. K. A. (2015): Larvicidal activity of camphor and lavender oils against sheep blowfly, *Lucilia sericata* (Diptera: Calliphoridae). J. Parasit. Dis. 1-8.

Shams-El-Din, H. M. H. (1994): Studies on diptera affected farm animals in Egypt with special reference to those producing myiasis (M.V.Sc. Thesis Faculty of Veterinary Medicine, Cairo University).

Shonouda, M. L. and Mehanney, S. M. (2000): New botanical derivatives, used in medicinal preparations, showing bioactive action on insect pests II: Effect on the vectors *Culex pipiens* and *Musca domestica* larvae. Pak. J. Biol. Sci. 3: 1039-1041.

Smith, K.E. and Wall, R. (1997): The use of carrion as breeding sites by the blowfly *Lucilia sericata* and other Calliphoridae. Med. Vet. Entomol.11: 38–44.

Smith, K.E., Wall, R. and Howard, J.J. (2000): In vitro insecticidal effect of fipronil and beta-cyfluthin on larvae of the blowfly *Lucilia sericata*. Vet. Parasitol. 88: 261–268.

Snoep, J. J., Sol, J., Sampimon, O. C., Roeters, N., Elbers, A. R. W., Scholten, H. W. and Borgsteede, F. H. M. (2002): Myiasis in sheep in The Netherlands. Vet. Parasitol. 106 (4): 357-363.

Tariq, K. A., Chisti, M. Z., Ahmed, F. and Shwl, A. S. (2009): Anthelmentic activity of extracts of *Artemesia apthensium* against ovine nematodes. Vet. Parasitol. 160 (1-2): 83-88.

Wiesman, Z. and Chapagain, B. P. (2006): Larvicidal activity of saponin containing extracts and fractions of fruit mesocarp of *Balanites aegyptiaca.*Fitoterapia, 77(6): 420-424.

Zumpt, F. (1965): Myiasis in man and animals in the Old World. Butterworths, London. 257pp.

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