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Efficiency of *Allium cepa* and *Commiphora molmol* as a Larvicidal Agent Against Fourth Stage Larvae of *Culex pipiens* (Diptera: Culicidae)

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Abstract: The present study aimed to evaluate the larvicidal activity of two essential oils from *Allium cepa* and *Commiphora molmol* against the fourth stage larvae of *Culex pipiens*. The chemical composition of the two essential oils obtained from *A. cepa* and *Commiphora molmol*, was elucidated by gas chromatography and mass spectrometry (GC-MS) analysis. Three toxic compounds (1,8-Cineole, L-LINALOOL and Camphor) were found in essential oil of *A. cepa* and one toxic compound (dl-Limonene) was found in *C. molmol*. The essential oils extracted from two plants have insecticidal activity against the fourth stage larvae of *C. pipiens*. The larvicidal bioassay results indicated that the *Allium cepa* had the highest activity against the fourth stage larvae *C pipiens*, displaying LC_{50}, LC_{75} and LC_{90} values of 0.383, 0.594 and 0.881, respectively. *Commiphora molmol* exhibited lower activity than *Allium cepa* against *C pipiens* larvae with LC_{50}, LC_{75} and LC_{90} values of 0.992, 2.177 and 4.419, respectively. Scanning electron microscopy was done for the fourth stage larvae of mosquito which was treated with the essential oil of *A. cepa* at dilution 0.38 µL/ml. Treatment with essential oil of *Allium cepa* showed marked changes in abdominal segments of the fourth stage larvae, there was a shrinkage of segments. A concurrent shrinkage was also observed in the cuticle. A separation of anus (anal canal and anal papilla) from segment VIII was obvious with an intrusion of the cavity which had the comb scales. Scattering of protrusions of cuticle existing on the head capsule was clear. It could be concluded that the essential oils of *A. cepa* and *C. molmol* have toxic effect on the fourth stage larvae of *C. pipiens*. *A. cepa* had stronger toxic effect than *C. molmol*.

Key words: Essential oils · *Allium cepa* · *C. molmol* · Mosquito · Larvae · *Culex pipiens* · GC-Msss · SEM

INTRODUCTION

Mosquitoes are the most important vectors of serious human and animal protozoal and arbovirus diseases, such as malaria, encephalitis, yellow fever, dengue and filariasis [1,2]. Current control is based on the use of insecticides (chlorpyrifos, dichlorvos, Cypermethrin), which have a potential toxic effect on public health and the environment. There is considerable international interest in developing benign natural products as an alternative to harmful synthetic pesticides to control invertebrate pests of medical and economic importance. Plants or parts of plants possess a complex of chemicals with unique biological activity [3]. Over 2000 plant species of plants have been shown to have some degree of activity against mosquitoes [4,5]. Essential oils and other components extracted from higher plants such as *Myrtus communis* L, *Origanum syricum* L, *Mentha microcorphylla* Koch, *Pistacia lentiscus* L and *Larvandula stoechas* L have been tested against mosquitoes *C. pipiens* molestus Forskal and *Aedes aegypti* L [2,6]. Many plant oils have proved to be effective for insect control, The efficiency of *citrus* oils (peel oils of Lemon, Grapefruit, navel Orange and *Citrus sinensis*) against egg, larvae and adults of *C. pipeins*, *Musca domestica* and *H dromedarii* were reported [6-8]. Lemon peel and *Citrus sinensis* oils were the most effective against egg, larvae and adults of *C. pipiens* and *H dromedarii*(tick) while grapefruit and navel orange peel oils were more toxic to *M. domestica*. Moreover, gas chromatography and mass spectrometry were used to determine the major components of essential oils [2, 10].

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The current work was carried out to evaluate the efficiency of essential oils of *Commiphora molmol* and *Allium cepa* against the fourth stage larvae of *Culex pipiens*.

MATERIALS AND METHODS

Insects: A local strain of *Culex pipiens* larvae was collected from a lake in Giza Governorate, Egypt during September, 2007 and placed into plastic dishes (35×25×12 cm) with 2000 ml of water at 25°C. Larvae were fed on a powdered mixture of equal parts of biscuits, dried yeast and dried milk powder using the methods of Traboulsi *et al.* [2]. The fourth-instar larvae were identified according to Kettel [11] and were used in these assays.

Plant Extracts: Two essential oils were used in this study, *Commiphora molmol* and *Allium cepa*, this products were obtained from El-Captain Company (CAP PHARM) for extracting natural oils, Herbs and Cosmetics, Cairo, Egypt.

Chemical Analysis of the Essential Oils: The components of the essential oils were determined. Twenty µl of the respective essential oils were individually diluted with 1000 µl diethyl ether and then 2 µl of diluted oils were injected individually in Perkin Elmer gas. Chromatograph model XL with a split ratio 1:10. The oil constituents were separated on 60 m D 13.5 capillary column having 0.32mm internal diameter [8,12].

Toxicity Test of Essential Oils: Each essential oil was diluted in 0.5% of tween 20 (GERHAM, USA) as solvent to give a range of four concentration, 0.5% of tween 20 was used alone as a control treatment. Concentrations of *Allium cepa* (1 µL, 0.5 µL, 0.25 µL and 0.12 µL/ ml or *Commiphora molmol* (3 µL, 1.5 µL, 0.75 µL, 0.4 µL/ ml) were used in this experiment. Each concentration or control treatment was replicates three times and the replicates included twenty fourth stage larvae. The treatments were applied according to Traboulsi *et al.* [2]. Mortality was recorded after 2 and 12 hrs. Calculated mortality percentage of fourth instars larvae were based on larvae not moving in water (paralysis). LC50, LC75 and LC90 values for each concentration from *Commiphora molmol* or *Allium cepa* were calculated according to Finney [13].

Scanning Electron Microscopy of Fourth Instars Larvae: Fourth stage larvae were dipped for 30 second in the essential oil of *Allium cepa* at 0.38 µL/ ml (LC_{50} value of fourth instars larvae in toxicity test). Control treatments were immersed at the same time in water. Then larvae samples of treatment and control were immersed in 2.5% glutraldehyde for 48 hrs, washed in buffer and post fixed in 1% osmium tetra oxide in 0.1 M cacodylate buffer before being dehydrated in an ethanol series [14]. Alternatively, larvae were dehydrated in ethanol to 100%, dried at Co2 critical point drier (Blazzer Union F1-9496 Blazzer/ Furstenun Liechtenstein, Germany), glued over specimens stubs and coated with 20 nm gold in a sputter coater (SI50A Sputter Coter Edward, UK).Finally larvae samples were examined and photographed with scanning electron microscope (JXA 840, Electron Probe Microanalyzer, Jeol, Japan).Morphological description was done according to Kettel, [11] and Schaper and Hernandez-Chavarria [15].

RESULTS

GC/MS Analysis of the *Allium cepa*: The GC/MS analysis of *Allium cepa* oil (Table1) revealed the presence of 30 compounds. It was found that, L-LINALOOL 34.36%, Benzene,1-methoxy-4-(2-propenyl) 21.16% and a-Cadinol 11.37% are the major identified compounds. These compounds are oxygenated hydrocarbon compounds. It was found that the volatile oil of *Allium cepa* contains non oxygenated monoterpenoid hydrocarbon compounds (33.3%) as well as oxygenated hydrocarbon compounds (66.6%). The main constituent of hydrocarbon and oxygenated compounds was c-Cadinene (4.08%) and L-LINALOOL (34.36%).

GC/MS Analysis of the *Commiphora molmol*: Over 30 components were detected from essential oil of *Commiphora molmol*. The main constituent of hydrocarbon compounds was dI-Limonene 12.25% and the oxygenated compounds were 2-Cyclohexen-1-one,2-methyl-5-(1-methylethenyl) 21.10% respectively, (Table 2).

It was found that the volatile oil of *Commiphora molmol* contains non oxygenated monoterpenoid hydrocarbon compounds (60%) as well as oxygenated hydrocarbon compounds which represent (40%).

Table1: GC/MS. Analysis of *Allium cepa* L. (onion) oil extract

Peak No.	Identified compounds	Molecular formula	M.wt	RT	Area %
1	1,8-Cineole	C10H18O	154	13.26	2.11
2	L-LINALOOL	C10H18O	154	16.04	34.36
3	Camphor	C10H16O	152	17.05	0.17
4	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	C10H18O	154	18.05	1.34
5	Benzene, 1-methoxy-4-(2-propenyl)-	C10H12O	148	18.66	21.16
6	trans-Geraniol	C10H18O	154	19.93	0.26
7	ENDOBORNYL ACETATE	C12H20O2	196	20.85	0.61
8	(1'-butenyl)thiophene	C8H10S	138	21.88	0.21
9	Phenol, 2-methoxy-4-(2-propenyl)	C10H12O2	164	22.76	5.07
10	LAVANDUYL ACETATE	C12H20O2	196	23.34	0.55
11	(1R)-cis-10-Isopropyl-7-methyl-cis-1-cisoid-1,2-cis-2-tricyclo [5.3.0.0(2,6))dec-4-en-3-one	C14H20O	204	23.55	0.07
12	ä ELEMENE	C15H24	204	23.73	3.46
13	2,2-bis[.eta(5).-Cyclopentadienyl]-3,4-dimethyl-1,5-dipheny l-2-titanabicyclo[3.1.0]hex-3-ene	C29H28Ti	424	23.90	0.16
14	1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro- 3,6,8,8-tetramethyl-, [3R-(3à,3aá,7á,8aà)]-(CAS)	C15H24	204	24.83	3.01
15	1-methoxy-1-(4-methylphenyl)cyclohexane	C14H20O	204	25.13	0.23
16	à-Humulene	C15H24	204	25.41	0.82
17	2,2a,4,8b-tetrahydro-8b-acetoxy-1-methyl-1-methoxycyclobuta [c]quinolin-3(1H)-one	C15H17NO4	275	25.56	0.98
18	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methy l-4-methylene-1-(1-methylethyl)-, (1à,4aà,8aà)-(CAS)	C15H24	204	26.07	3.81
19	bicyclogermacrene	C15H24	204	26.39	0.81
20	1,5-Methano-1H-indene, octahydro-6-isocyano-1,3a-dimethy l-6-(1-methylethyl)-, (1à,3aá,5à,6á,7aá)-(+)-(CAS)	C16H25N	231	26.54	1.10
21	ç-Cadinene	C15H24	204	26.86	4.08
22	MIXTURE OF 1-ISOPROPYL-4,6-DIMETHYL-1,2, 3,4-TETRAHYDRO-NAPHTHALENE AND4-ISOPROPYL-1, 6-DIMETHYL-1,2,3,4-TETRAHYDRO-NAPHTHALENE	C15H22	202	27.00	0.18
23	(4bà,4cà,9bà,9cà)-4b,4c,9b,9c-Tetrahydro-2,4,7,9-tetramethoxy-4c, 9c-dimethylcyclobuta[1,2-a:3,4-a']diindene-5,10-dione	C24H24O6	408	28.20	0.23
24	1,4-Dimethyl-1,4-benzodiazepin-2,5-dione	C11H12N2O2	204	28.36	0.13
25	Isocubenol	C15H26O	222	29.27	1.34
26	à-Cadinol	C15H26O	222	29.97	11.37
27	t-Muurolol	C15H26O	222	30.20	0.33
28	Hexadecanoic acid (CAS)	C16H32O2	256	36.62	0.26
29	7-Dodecenol	C12H24O	184	39.95	1.13
30	9-Octadecenoic acid (Z)-(CAS)	C18H34O2	282	40.07	0.65

Table 2: GC/MS. Analysis of *Commiphora molmol* oil extract.

	Identified compounds	Molecular formula	M.wt	RT	Area %
1	dl-Limonene	C10H16	136	13.33	12.25
2	Benzenemethanol (CAS)	C7H8O	108	13.71	5.63
3	2-Cyclohexen-1-one,2-methyl-5-(1-methylethenyl)-, (R)-(CAS)	C10H14O	150	20.14	21.10
4	Heptadecane (CAS)	C17H36	240	31.22	0.99
5	Octadecane (CAS)	C18H38	254	33.44	2.66

Table 2: Continued

6	Hexadecane, 2,6,10,14-tetramethyl-(CAS)	C20H42		33.56	1.19
7	Nonadecane (CAS)	C19H40	282	35.58	3.37
8	1,2-Benzenedicarboxylic acid, dibutyl ester (CAS)	C16H22O4	278	36.68	8.26
9	6-Imino-8-phenyl-2,3,4,6-tetrahydro-pyrido[2,1-b][1,3]thiazine-7-carbonitrile	C15H13N3S	267	36.97	1.25
10	Eicosane (CAS)	C20H42	282	37.61	4.20
11	Nonadecane (CAS)	C19H40	268	38.30	1.70
12	1-[(2-trimethylsiloxy)vinyl]-4-trimethylsiloxy-2,6-dideuteriobenzene	C14H22D2O2Si2	258	38.79	0.89
13	Eicosane, 3-methyl-(CAS)	C21H44	296	38.98	3.24
14	Pentadecane (CAS)	C15H32	212	39.51	3.02
15	Eicosane (CAS)	C20H42	282	40.20	3.03
16	Methyl 11a-methyl-9-oxo-5,6,9,11a-tetrahydro-4H-pyrido [3,2,1-jk]carbazole-11-carboxylate	C18H17NO3	295	40.84	1.81
17	Cyclohexane, undecyl-(CAS)	C17H34	238	40.96	1.73
18	Docosane (CAS)	C22H46	310	41.34	1.43
19	(S)-N-Phenyl-2-methoxy-2-phenyl-3,3,3-trifluoropropionamide	C16H14F3NO2	309	41.99	3.18
20	Methyl 8,11a-dimethyl-9-oxo-5,6,9,11a-tetrahydro-4H-pyrido [3,2,1-jk]carbazole-11-carboxylate	C19H19NO3	309	42.46	0.71
21	Methyl 8,11a-dimethyl-9-oxo-5,6,9,11a-tetrahydro-4H-pyrido [3,2,1-jk]carbazole-11-carboxylate	C19H19NO3	309	42.63	1.42
22	Cyclohexane, eicosyl-(CAS)	C26H52	364	42.81	1.96
23	3-{2-[(tert-Butyldimethylsilyl)oxy]phenyl}-1-iodopropane	C15H25IOSi	376	43.09	1.06
24	5,6-Dihydro-1,2,3-trimethoxy-8H-isoquino[2,1-b][2,7]naphthyridin-8-one	C19H18N2O4	338	43.31	2.03
25	Heptadecane, 8-methyl-(CAS)	C18H38	254	43.71	3.08
26	Cyclohexane, eicosyl-(CAS)	C26H52	364	44.55	1.57
27	Heneicosane (CAS)	C21H44	296	45.33	2.25
28	(3S,3'R)-2,2',3,3'-tetrahydro-3,3'-diisopropyl-5,5'-bi{furo[3,2-b]pyridine}	C20H24N2O2	324	45.47	1.16
29	Cyclohexane, eicosyl-(CAS)	C26H52	364	46.33	2.27
30	Benzyl (dideuterated)methyl ether	C8H8D2O	114	46.94	1.55

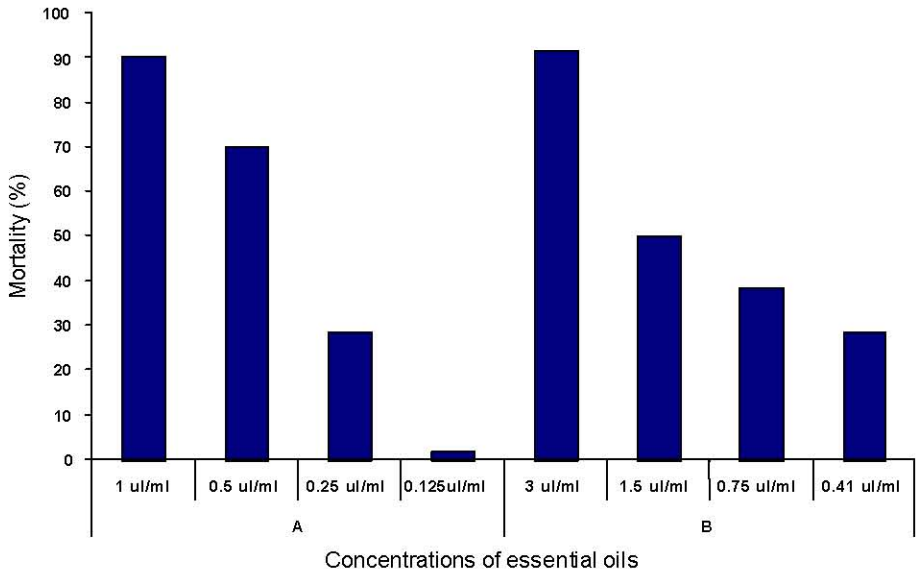

Fig. 1: Activity of essential oils against fourth instar larvae of *Culex pipiens*. (A) *Allium cepa*; (B) *Commophora molmol*

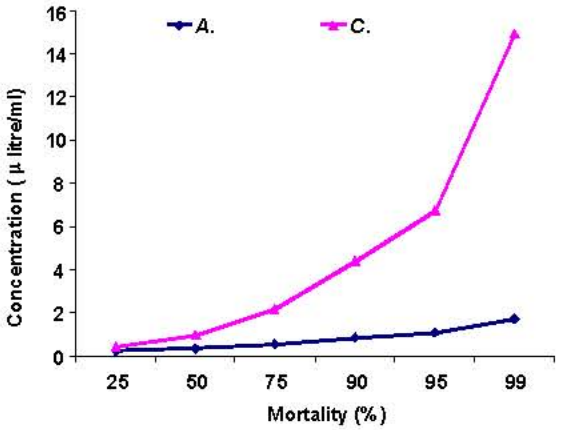

Fig. 2: Toxicity limits for the essential oil of *Allium cepa* and *Commiphora molmo*l against the fourth stage larvae of *Culex pipiens*

There are not common toxic compounds between GC/MS analysis of essential oils of *Allium cepa* and *Commiphora molmol*.

Toxicity Assays: The activity of different concentrations of essential oils against the larvae of *C pipiens* are shown in Figures 1 and 2. The Results showed that essential oil of *Allium cepa* was more effective than *Commiphora molmol*.

There was a significant difference($P<0.001$) between mortalities of all concentrations and the control of the two oils *Commiphora molmol* and *Allium cepa*. The control treatment had no effect on the fourth-instars larvae.

The larvicidal bioassay results indicated that the *Allium cepa* had the highest activity against *C pipiens*, displaying LC_{50}, LC_{75} and LC_{90} values of 0.383, 0.594 and

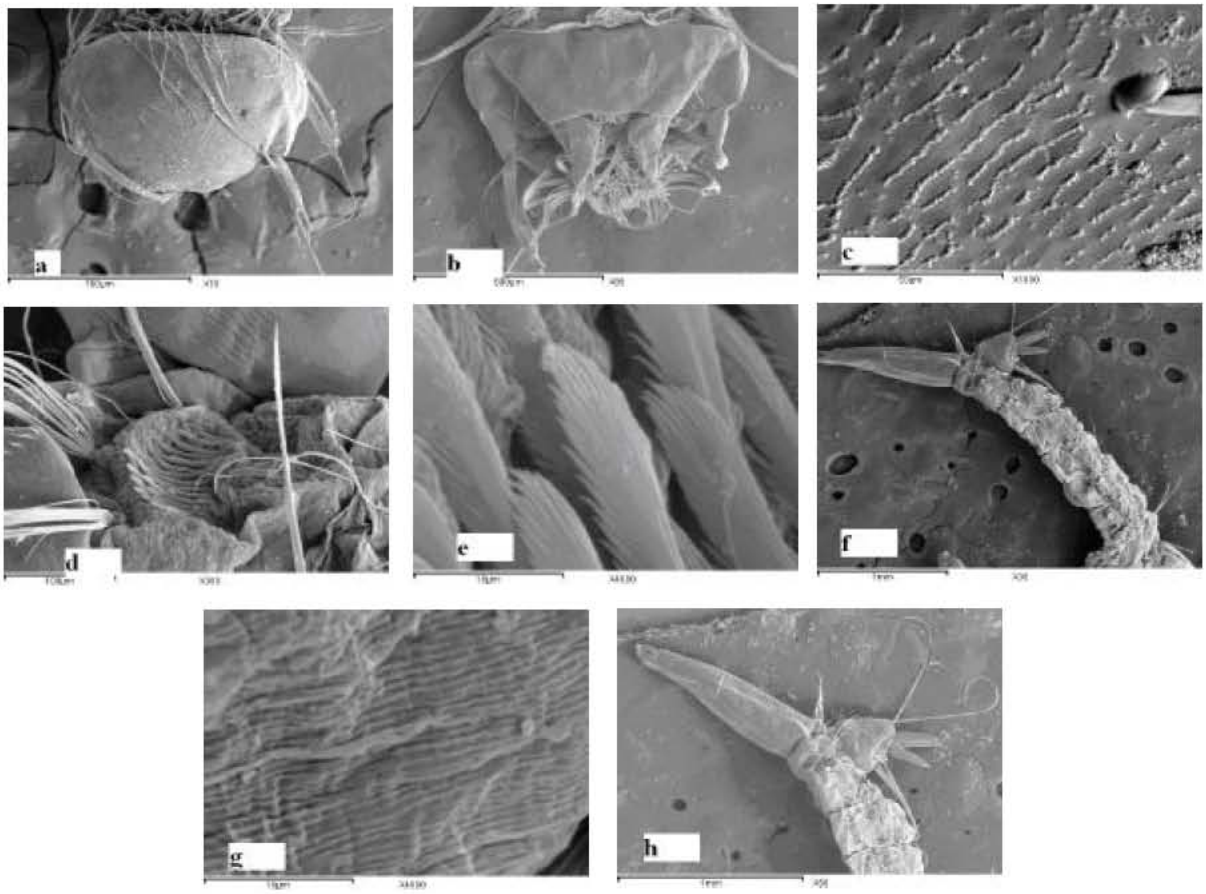

Fig. 3: (a) dorsal view of head capsule had three pairs of small brush, palpis evident on lateral sides in head capsule; (b) ventral view of head capsule revealed palatal brushes appeared as conglomerates of uniformly shaped filaments, palpi, palatum and maxillae are evident. (c) semi regular rows of protrusions were found on dorsal view of head capsule (d, e) segment VIII had a conglomerates of uniformly shaped comb scales with one of row of uniform teeth. (f, g, h) abdominal segment,cuticle of segments had bulging wirinkles and siphon had a row of ten pectin spines

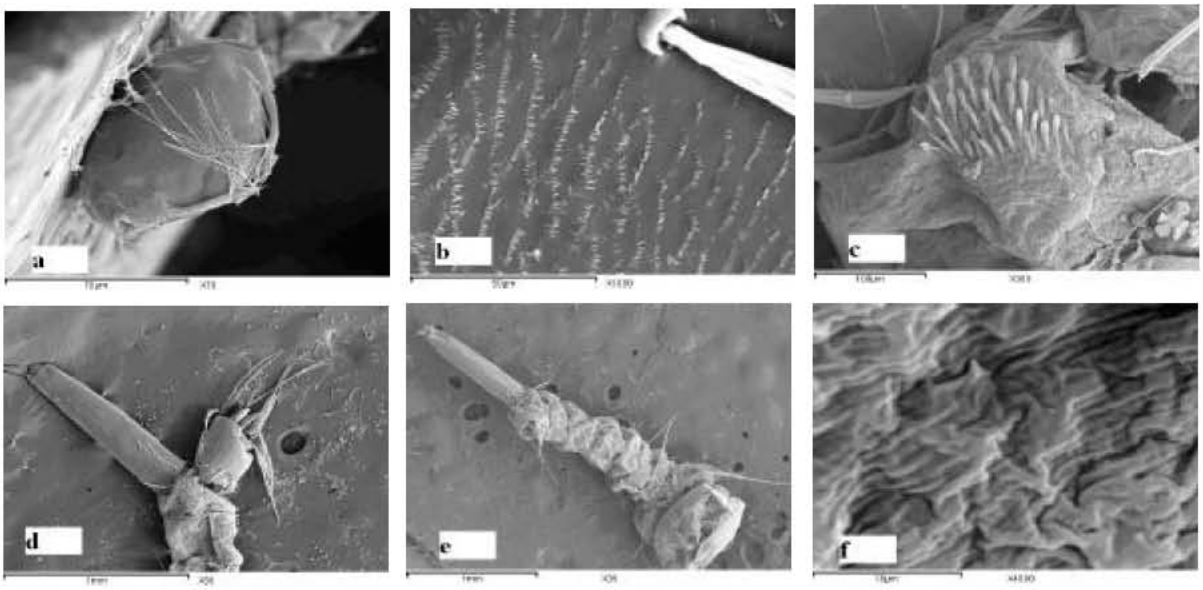

Fig. 4: (a, b) revealed cracking and scatter of protrusions of head capsule. (d, c) revealed of intrusion of segment VIII and a separation of anal canal and anal papilla from segment VIII. (e, f) showed abdominal segments was a shrinkage and concurrent shrinkage was observed in the cuticle of segments

0.881, respectively. *Commiphora molmol* exhibited lower activity than *Allium cepa* against *C. pipiens* larvae displaying, LC_{50}, LC_{75} and LC_{90} values of 0.992, 2.177 and 4.419, respectively.

Scanning Electron Microscope: The structure of head capsule of the fourth stage larvae of *Culex pipiens* is shown in Fig. (3a,b,c).Three pairs of small brush of setae were found in dorsal view of head capsule, each brush had four thin setae. One pair of palpi is evident on lateral sides of the head capsule and protrusions of cuticle were found in semi regular rows on it, (Fig. 3c). Palatal brushes of the fourth stage larvae appeared as conglomerates of uniformly shaped filaments. Thus, the palpi of both the palatum and maxillae are evident (Fig. 3b). The morphological development of ventral brush of segment VIII (eight) are evident. The ventral brushes were composed of three brush of thin setae, each brush had nine thin setae. Lasted segment (VIII) had a conglomerate of uniformly shaped comb scales with one or two rows of uniform teeth in the cavity of the cuticle. Segment VIII (eight) had a development of long spine and three thin setae (Fig. 3d,e). Abdominal segments of larva cuticle had bulging wrinkles and the siphon had a row of ten pectin spines (Fig. 3f,g,h).

Treatment with essential oil of *Allium cepa* induced marked changes in abdominal of fourth stage larvae, in the form of shrinkage of segments. A concurrent shrinkage was also observed in the cuticle (Fig. 4a,b). A separation of anus (anal canal and anal papilla) from segment VIII occurred, with an intrusion of the cavity witch had a comb scales (Fig. 4c,d). Cracking and scatter of protrusions of cuticle existing on the head capsule occurred (Fig. 4e).

DISCUSSION

Plants contain a complex of chemicals with unique biological activity [3]. This is probably due to toxins and secondary metabolites acting as attractants or deterrents, [16]. The current results showed that the essential oil extracted from two plant species have insecticidal activity against *C. pipiens*.

Several products have been evaluated and have different types of activities against mosquitoes and ticks [8,9,17]. The essential oil of *C. sinensis* and *C. lemon* have insecticidal effects against, larvae and adults of *Culex pipiens* and *Musca domestica* [7], fourth-instar larvae of *Culex pipiens* [2] and egg, larvae and engorged females cattle tick *Boophilus microplus* and camel tick *Hyalomma dromedarii* [8,9,18].

In this study, the most toxic effects were found in the essential oil from *Allium cepa*, with LC_{50}, LC_{75}, LC_{90} values of 0.0003, 0.0005 and 0.0007 mg/liter^{-1} respectively. Although pure components were not tested in this study, over 30 major components have been identified in essential oil from each plant species, (*Allium cepa* and

Commiphora molmol). Three toxic components were found in *Allium cepa* (1,8-Cineole l2.11%, L-LINALOOL 34.36%and Camphor 0.17%) and one toxic component (dl-Limonene 12.25%) was found in *Commiphora molmol*.

The toxicity of the oil was attributed to these four main groups of compounds. This explained why the essential oil of *A. cepa* had more toxic effect against the fourth stage larvae of *C. pipiens* than essential oil of *C. molmol*, because it contained three toxic compounds. In this respect, [19] detected only 78.36 % limonene in fruit peels of balady orange *C. sinensis*. while, [20] reported that Limonene of *C. sinensis* (L.); Osbrck cv. Maltese was 92.6%.The toxicity of the oil was attributed to these two main compounds. However [8,9] revealed that main compounds of essential oil of *C. sinensis* and *C. lemon* were limonene 83.28%, linalool 3.97 for *C. sinensis* and limonene 45.99%, Myrcenol 21.85% and Cis-ocimene 15.49% for *C. lemon*. Chungsamarnayrt and Jansawan [18] found that the peel oils of *Citrus reticulate* and *C. maxima* showed 2 times higher acaricidal activity than that of (+)-limonene against the engorged females of *B. microplus*. They added that *C. sinensis* and *C. maxima* oils exhibited a higher larvicidal activity 1.5 times stronger than that of (+)-limonene. Traboulsi *et al.* [2] found that four out of five plants tested against *C. pipiens* contained linalool, a-thjene, a-B-pinene or 1,8-cineol. 1,8-ceneol is present in a wide range of plants [21,22]. In this study linalool, 1,8-ceneol,camphor and Limonene components were found in the two plant species tested and the 90-91.7% mortality achieved with two essential oils against the mosquito larvae were recorded. Our results agree with Traboulsi *et al.* [2], Habeeb *et al.* [8] and Habeeb *et al.* [9]. On the other hand, [21] reported that when plant oils were used alone, they become less effective against the beetle *Sitophilus granaries* L than when oils were combined with either 1,8-ceneole, eugenol or camphor. Traboulsi *et al.* [2] revealed that when the combination between LC_{50} of essential oil of five plants, *Ferula hermonis* Boiss, *Citrus sinensis* Osbeck, *Pinus pinea* L, *Laurus nobilis* L and *Eucalyptus* spp were used against larvae of *Culex pipiens*, the mortality was higher than the essential oils of plants were used individually (90-100%). Changes in abdominal segments and head capsules of treated larvae were observed by Scanning Electron Microscopy, it could be that the essential oil of *A. cepa* dehydrated the abdominal cuticle. A concurrent shrinkage of segments, was also observed in the cuticle. A separation of the anus (anal canal and anal papilla) from segment VIII occurred, with an intrusion of the cavity which had comb scales. Cracking and scatter of protrusions of cuticle existing on the head capsule occurred.

It could be concluded that the fourth stage larvae of *C. pipiens* is sensitive to the essential oils of *A. cepa* and *C. molmol*, *A. cepa* had more toxic effect on *C. pipiens* larvae.

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