EFFECTS OF SOME ESSENTIAL OILS AGAINST *CULEX PIPIENS* LARVAE

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Abstract: Essential oils from linseed, *Linum usitatissimum*, watercress, *Nasturtium officinale* and black seed *Nigella sativa* were evaluated for larvicidal and histological effects against the third instar larvae of the mosquito, *Culex pipiens*. The results showed that all the three tested oils induced larval mortality, watercress oil was more effective followed by linseed and black seed oil and the effects were dose dependent and time of exposure. Under histological study, the watercress oil induced some lesions in the tested organs; midgut lesion i.e. separation of the epithelial cells from the basement membrane, disruption of some epithelial cells, brush border and the peritrophic membrane. The cuticle and hypodermis became thinner and separated from each other. Most of fat cells were degenerated and vacuolated. Muscle fibers were separated from each other.

Key words: *Culex pipiens*, *Linum usitatissimum*, *Nasturtium officinale*, *Nigella sativa*

INTRODUCTION

Mosquitoes are important vector for several diseases on earth. In Egypt, *Culex pipiens* is the most common species and an important vector of several human pathogenic agents, such as filarial worm *Wuchereria bancrofti* (more than 100 million people are infected worldwide with *W. bancrofti* [1] and Rift Valley fever virus [2,3] and West Nile Virus [4]. MOSQUITO CONTROL is becoming increasingly difficult in Egypt because of the emergence of resistance of *C. pipiens* to many insecticides. Traditionally used synthetic insecticides are quick effective and most popular methods of pest control. Because of their unfavorable effects on environment and non-target organisms, there is a growing need to find new, safe effective alternatives. Plant extracts represent the most promising alternative because they are non-pollutant, easy degradable and less toxic to the environment. Plant oils consist of mixture of volatile and terpenoids which affect voltage gated and/or ligand gated ion channels in the CNS such as tyramine, octopamine, GABA, TRP type ion channels and acetylcholinesterase [5].

The mosquito larvicidal activity of many different plant extracts and fractions was reported by several investigators [6-8], who listed and discussed 344 plant species that exhibited mosquitocidal activity. Promsiri et al. [9] evaluated the larvicidal activity of the phytochemicals extracted from 112 medicinal plant species against *Aedes aegypti* mosquito.

Plant-based phytochemicals do not have any hazardous effect on the ecosystem. Recent research has proved that effectiveness of plant derived metabolites, such as isofalvonoids [10], essential oils [11], saponin [12,13], steroids [14] and alkaloids and tannins [15] as potential mosquito larvicides. Several essential oils have been reported to possess insecticidal action against mosquitoes [6, 15-36,37]. Several plant extracts and oils have also been studied.
on histological alterations of many mosquito species [34,38-48,49].

Nevertheless a negligible reference is available regarding the use of essential oil of *Linum usitatissimum*, *Nasturtium officinale* and *Nigella sativa* against *C. pipiens* larvae. Therefore, the present study aims to estimate the effect of these oils against larvae of *C. pipiens* using histological parameters.

**MATERIALS AND METHODS**

**Insect culture:** The mosquito, *C. pipiens* (Diptera: Culicidae) used in the present study was obtained from susceptible reared strain of Research Institute of Medical Entomology, Dokki, Egypt. The colony was maintained in laboratory of Entomology, Zoology Department, Faculty of Science, Menoufiya University at 27± 2°C and 75±5 % R.H. Larvae were reared in dechlorinated water and fed daily on 5% yeast suspension, adult male fed on 10% sugar solution while females received blood meals periodically from pigeons for egg production.

**Tested oils:** Commercially plant oils obtained from the Egyptian natural oils, Cairo, Egypt were used. The tested oils were linseed (*Linum usitatissimum*), watercress (*Nasturtium officinale*) and black seed (*Nigella sativa*).

**Bioassay:** The tested essential oils were carried out by the following standard World Health Organization [50-52]. The oil was first dissolved in acetone at a ratio of 1:1 of acetone and oil. A series of concentrations (0.1, 0.3, 0.6, 0.9 and 1.2% of the dissolved oil was prepared in water. Twenty five early 3rd instar larvae of *C. pipiens* were put into a plastic cup (100 ml) containing the test solution of each concentration. Four replicates were run for each concentration. Control tests were carried out in parallel with the required amount of acetone in water. Mortality counts were made after 24, 48 and 72 h of exposure. The larvae considered dead, when they were immobile and unable to reach the water surface [53]. The corrected mortality was calculated using Abbott formula [54].

**Histological study:** For the histological tests, early third instar larvae of *C. pipiens* were isolated from the standard laboratory colony reared on food which were incorporated with 0.1, 0.3 and 0.6 % of the tested oil of *Nasturtium officinale* for 72 h. Then some treated and untreated larvae were decapitated, the head and respiratory siphon were removed. The thorax and abdomen were fixed in Shina fixative for 24 h. After dehydration, clearing, infiltration, the material was embedded and cut at 5 thick with Rotary microtome (Leica, Japan). The slides were stained with Ehrlichs haematoxylin-eosin, analyzed and photographed with research microscope (Olympus Bx41, Japan).

**RESULTS AND DISCUSSION**

**Larvicidal effect:** Linseed oil, *Linum usitatissimum* induced 8, 67, 80, 87 and 93% larval mortality after 24 h of exposure at 0.1, 0.3, 0.6, 0.9 and 1.2%, respectively. After 48 h of exposure, this oil induced 6.19, 76.29, 86.60, 92.78 and 100 % larval mortality at 0.1, 0.3, 0.6, 0.9 and 1.2%, respectively. While after 72 h post exposure, this oil induced 4.25, 80.85, 93.60, 100 and 100% larval mortality at 0.1, 0.3, 0.6, 0.9 and 1.2%, respectively (Tables 1,2).

Watercress oil, *Nasturtium officinale* induced 46, 60, 83 and 90 % larval mortality after 24 h from treatment at 0.1, 0.3, 0.6, 0.9 and 1.2%, respectively. After 48 h post exposure, this oil elicited 64.95, 72.16, 82.47, 92.78 and 96.91 % larval mortality at 0.1, 0.3, 0.6, 0.9 and 1.2%, respectively. While at 72 h from treatment, this oil induced 74.47, 82.98, 93.62, 100 and 100 % larval mortality at 0.1, 0.3, 0.6, 0.9 and 1.2%, respectively (Tables 1,2).

Black seed oil, *Nigella sativum* induced 3, 10, 23, 33 and 50% larval mortality at 0.1, 0.3, 0.6, 0.9 and 1.2%, respectively. After 48 h of exposure, this oil elicited 7.22, 14.34, 30.93, 45.36 and 76.92 % larval mortality 0.1, 0.3, 0.6, 0.9 and 1.2%, respectively. While after 72 h of exposure, it induced 7.45, 22.34, 36.17, 64.89 and 100 larval mortality at 0.1, 0.3, 0.6, 0.9 and 1.2%, respectively (Tables 1,2).

All the three tested essential oils induced larval mortality. This effect was dose dependent and the time of exposure. The susceptibility of the mosquito larvae was positively correlated with the concentration tested and the period of exposure. Watercress oil was more effective on the larval mortality followed by linseed oil and black seed oil, which induced high mortality at low concentrations.
Plate 1:
Fig. 1: Midgut sections. 1a is a midgut section of control larvae. 1b, 1c, 1d are midgut sections of larvae treated with 0.1, 0.3 and 0.6 % of watercress oil, respectively.
Fig 2: Sections of the body wall. 2a is a section of the body wall of control larvae. 2b, 2c, 2d are sections of the body wall of larvae treated with 0.1, 0.3 and 0.6 % of watercress oil, respectively.
The essential oils; linseed, watercress and black seed have been found to exhibit larvicidal activity against the third instar larvae of *C. pipiens*. The biological activity of the oils might be as a result of the various compounds that exist in plants, jointly or independently. These compounds may contribute to the production of larvicidal activity against *C. pipiens*. *Nigella sativa* or balck cumin contains monoterpenes, including P- Cymene, α-thujene, γ-terpinene, carvacol, α-pinene and β-pinene, to be the main components of the essential oil from black cumin [55].

The literature does not reveal other studies on the larvicidal properties of the tested oils for *C. pipiens*. However, studies considering other plant oils as bases for lethal concentrations served as reference for this study of the potential of linseed, watercress and black seed oil for usage in *C. pipiens* control.

Percent mortality of *C. pipiens* larvae after 24, 48 and 72 h. of exposure under laboratory conditions showed that watercress oil had the highest percent mortality at all concentrations, followed by linseed oil and black seed oil as shown in (Tables 1,2). Therefore, watercress oil has been selected for investigating its histological effect on *C. pipiens* larvae.

The obtained results were similar with the studies of different investigators on the same insect *C. pipiens* by other essential oils. Mohamed et al. [43], El-Husseiny et al. [34] and El- Husseiny and El-Kholy [37] observed the larvicidal efficacy of jujuoba oil (*Zizyphus jujube*). Khater and Shalaby [28] reported the larvicidal efficacy of 6 plant oils, fenugreek (*Trigonella foenum*), earthalmond (*Cyperus esculentus*), mustard (*Brassica compestris*), olibanum (*Boswellia serrata*), rocket (*Eruca sativa*), and parsely (*Carum ptoselinum*). Abo Elnga [35] observed the larvicidal efficacy of sage oil and nutmeg oil, while Mahmood et al. [56] used *Allium sativum* and *Nigella sativa* against *C. pipiens* larvae control.

The present data are in harmony with the results obtained by other investigators using different essential oils against *C. quinquefasciatus* and other mosquito species such as *Aedes* and *Anopheles* species [21,22,24,25,29,31,33,45]. Nawaz et al. [57] studied the adulticidal activity of linseed oil (linum usitatissimum) against *A. aegypti* and *Anopheles stephensi*.

**Histological studies:** The histological structure of midgut of the control third instar larvae of *C. pipiens* consists of a single columnar epithelial cells resting upon a basement membrane. This membrane is surrounded externally by circular and longitudinal muscle fibers, respectively. The midgut epithelium exhibited well developed brush border. Each epithelial cell contains a relatively large nucleus and strongly acidophilic cytoplasm. The epithelium

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Plate 2:

Fig. 1: Sections of fat body. 1a is a section in the fat body of control larvae. 1b, 1c, 1d are sections of the fat body of larvae treated with 0.1, 0.3 and 0.6 % of watercress oil, respectively.

Fig. 2: Sections of muscles. 2a is a section of muscles of control larvae. 2b, 2c, 2d are sections of muscles of larvae treated with 0.1, 0.3 and 0.6 % of watercress oil, respectively.
is protected from food particles by a peritrophic membrane (plate 1 Fig. 1a).

Cross sections in the midgut of 3rd instar larvae treated with the essential oil watercress at 0.1, 0.3 and 0.6 % induced histological damages 24 h post treatment. Watercress oil induced degeneration, vacuolation and separation of some epithelial cells from the basement membrane, destruction in both peritrophic membrane and the brush border. (Plate 1 Figs. 1b, 1c,1d). This effect was dose dependent. The noticed histological effects of watercress oil on midgut of C. p. larvae were in agreement with the results obtained by earlier worker on the same insect [37,38,39,41,42,43].

Hamouda et al. [38] stated that midgut of C. p. larvae treated with Artemisia judaica was affected, the epithelial layer was vacuolated, swollen cells and masses of cellular material appeared in anterior part of midgut and finally the epithelium lost their normal appearance. Moreover, they found that larvae treated with A. arvensis showed rupture of cell wall and destruction of the peritrophic membrane. In addition, Massoud and labib [41] mentioned that the oil and oleo-resin of Myrrh (Commiphora molmol) induced histological deformities in different tissues of C. p. The gut apical portion of columnar cells was swollen and sometimes distinct elongations protruded into its lumen as a bulbous aversion. Sometimes the apical part of columnar cells appeared empty. In completely paralyzed larvae, sections showed vacuolated cytoplasm with elongated nuclei of gut cells, cells were dislodged, sloughed and detached from each other.

Assar and El-Sobky [42] observed that the water extract of Eichhornia crassipes, revealed drastic effect on larval midgut as the brush border and some of the epithelial cells were apically degenerated after 48 h and 72 h, most of the epithelial cells completely degenerated and vacuolated.

The present observations are in accordance with earlier reports on C. quinquefasciatus by different plant extracts viz., extract of fenugreek (Trigonella foenumgraceum) [47], Melia azedarach [46], Matricaria chamomella extract [48] and leaf extract of Andrographis paniculata [36]).

Plate 1 Fig, 2a shows the normal histological structure of the body wall (integument). Abnormalities appeared in the integument of C. p. third instar larvae treated with watercress oil at 0.1, 0.3 and 0.6 % (Figs. 2b,2c,2d). The cuticle and hypodermis became thinner than the normal, the cuticle was separated from the hypodermis and some hypodermal cells were degenerated. The observed histopathological effects on the body wall were in harmony with results obtained by Assar and El-Sobky [42] and El-Husseiny et al. [34] on the same species, C. p.

Cells of normal fat body possessed a nucleus with a variable size and shape and demonstrated obvious areas of condensed and decondensed chromatin. Furthermore, these cells also exhibited large nucleoli and a cytoplasm rich in large clear areas which likely represent lipid inclusions and small protein granules (plate 2 Fig. 1a). Some effects were seen in the fat body 72 h post treatment as damage (degeneration), high vacuolation and absence of nuclei (plate 2 Figs. 1b,1c,1d) when the C. p. larvae treated with 0.1, 0.3 and 0.6 % of watercress oil, respectively. These effects were dose dependent. The fat tissue lost its compact form, cells appeared scattered, sometimes without the oily droplets and complete disintegration of that tissue in most treated sections. These results are in accordance with those obtained by Assar and El-Sobky [42], El- Husseiny et al. [34] and El-Monairy [49] on the same insect species.

The normal histological structure of muscles is shown in (plate 2 Fig. 2a). Treatment of 3rd instar larvae of C. p. with 0.1, 0.3 and 0.6 % of watercress oil after 72 h induced separation and degeneration of muscle fibers (plate 2 Figs. 2b,2c,2d), respectively. The effects were dose dependent. These histological alterations were recorded in C. p. treated with other plant extract [42]. All these symptoms on different tested organs indicate that watercress oil had a poisonous effect on C. p. larvae. The same is also reported by El- Husseiny et al. [34] and El-Monairy [49]. Thus it can be concluded that watercress oil proved to be a promising controlling agent against C. p. larvae.

CONCLUSIONS

Finally, we concluded that the tested essential oils have potential to kill the larvae of C. p. and induced several histological damage in the
midgut, body wall, fat bodies and the muscles, are environmentally safe, low mammalian toxicity, easily obtained and low cost. In addition, these oils eliminated the need and risk associated with insecticides. Thus, these oils can be used or play an important role for control of the mosquito, C. pipiens.

REFERENCES


