Ultrastructural Effects of *Matricharia chamomella* Extracts on Cuticle of 3\(^{rd}\) Larval Instar of *Culex quinquefasciatus*

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**Abstract** The present study aimed to estimate the effect of botanical extracts on the cuticle of the 3\(^{rd}\) larval instar of *Cx. quinquefasciatus*, by using the LC\(_{50}\) of the methanol extract of *Matricharia chamomella* for periods of 6 hours, 12 hours, 24 hours and 48 hours. The ultrastructural studies on the cuticle indicated that the larva were affected according the time elapse after exposure to the LC\(_{50}\) of the extracts, these effects appeared in separating epicuticle from cuticle, then the spaces between the epicuticle and exocuticle were increased after 12 hours.

**Keywords** Mosquito; *Matricharia chamomella*; *Culex quinquefasciatus*; Ultrastructural

**Introduction**

*Culex quinquefasciatus* is one of the most important pests medically because it transmits diseases to mankind and animals (Goddard et al., 2003; Zinser et al., 2004).

Insects have become the most diverse and numerous animal groups partly because of the evolution of a multifunctional integument, which provides for growth, mobility, protection and communication (Kramer et al., 1988).

Insect cuticle is a highly adaptive material that fulfils a wide spectrum of different functions. Cuticle does not only build the exoskeleton with diverse moveable parts but is also an important component of a stunning variety of mechanosensory receptors. Therefore, the mechanical properties of these specialized cuticular systems are of crucial importance (Müller et al., 2008).

The insect cuticle is a bio composite material with a remarkable range of physical properties. Insect cuticles can be flexible or rigid; ductile or hard, porous or impermeable. An insect cuticle is composed of chitin nanofibers, proteins, water, and polyphenols along with trace amounts of metals, lipids and waxes (Gorb, 2001).

In view of the dangerous diseases transmitted by mosquitoes, efforts exerted to control them. Medically and veterinary, mosquitoes control became an important issue all over world. Botanical insecticides are from the promising material (Gorb, 2001).

The effectiveness of insecticide mosquito larvae from plant extracts led to the exploitation of different types of plants to control mosquitoes in communities in different parts of the world by a number of researchers (Pathak et al., 2000; Sun et al., 2001; Singh et al., 2002 and 2006; Sivagnanaame and Kalyanasundaram, 2004; Obomanu et al., 2006). The fine structure and the distribution of an esterase have been studied in the cuticle of *Culex quinquefasciatus* larvae. The present study aimed to describe the cuticle surface of the 3\(^{rd}\) larval instar of *Cx. quinquefasciatus*, to
consider it from a mechanistic point of view, and to
discuss potential consequences of the integument surface
in the predator-prey relationships by using the LC_{50} of
the methanol extract of *Matricharia chamomella* has
been identified in the present study alone for periods of
6 hours, 12 hours, 24 hours and 48 hours.

**1 Results and Discussion**

Insect exoskeletons, called cuticles, can stop chemical
and physical attack while also providing structure for
the insect's muscles and wings. The remarkable
material is very thin and has a low density. It can also
vary its properties, from rigid along the insect's body
segments and wings to elastic along its limb joints.
Insect cuticle is a composite made up of layers of
chitin, which is a polysaccharide polymer, and protein
organized in a laminar, plywood-like structure.
Mechanical and chemical interactions between these
materials provide the exoskeleton with unique
mechanical and chemical properties. The cuticle
consists of a relatively soft and colourless endocuticle,
hardened and darkened in its outer part in some places
to form a rigid exocuticle, and a complex epicuticle
made up of several layers.

Development of resistance among target insects and
concern for environmental pollution limit the use of
chemical insecticides. Biological control is an
important component of integrated vector control
strategy and is being practiced in many countries to
control mosquitoes (Fraenkel and Rudall, 1940;
Vincent and Ablett, 1988). However, biocontrol
agents effective against adult mosquitoes are limited,
and hence chemicals are being used for indoor
residual spray. Mosquito control is very necessary by
discovering new way to prevent its distribution by
using botanical extract. The first organ face the
botanical is the body wall or exoskeleton which it is very
rigid. The botanical extracts work in broken this power
by sequences changes gradually according to the time.

Figure 1 shows the that the effect of extract was
slightly appear after 6 hours by separating Ep from
cuticle; maybe because it is the first barrier faces the
extract from outside insect body, the Endocuticle and
Epidermis are not affected.

After 12 hours, the penetrate of extract material take it
way through the cuticle layers causing interruption of
structure form of it, and the spaces between the Ep and
Ex were increased (Figure 2). Because it is lethal to
mosquito larvae, which its body wall contact the
water, the activity is by cuticular contact. Al-Mehmadi
and Al-Khalaf (2010) also found activity by stomach
poisoning.

After 24 hours, the result shows the effective basement
membrane which was split from the epithelium
(Figure 3; Figure 4).

After 24 hours (Figure 5) the epidermis structure beginning

![Figure 1: Treated larva cuticle after 6 hours](image1)

Note: Ep: Epicuticle, En: Endocuticle, EPD: Epidermis cells, Bm: Basment membrane

![Figure 2: After 12 hours of treating](image2)

![Figure 3: After 24 hours of treating](image3)
Ultrastructural Effects of *Matricaria chamomella* Extracts on Cuticle of 3rd Larval Instar of *Cx. quinquefasciatus*

Figure 4 After 24 hours of treating

Figure 5 After 24 hours of treating

Figure 6 After 48 hours of treating

Figure 7 After 48 hours of treating

in disruption by separating of basement membrane from the cell line, or from epidermis cell layer.

By increasing time of expose period (48 hours, Figure 6 and 7) the effect of treatment reach its higher rate causing damage in cell layer with interruption and the cuticle space decrease with big change in the structure of it shown in its different appearance from control one (the surface of cuticle being edges, sub cuticle disappeared, separate of En from Ep). These dramatically changes causing abnormal state of insect life.

Insect cuticle remains the second most widely distributed material (the first is plant cell wall and wood). Under experimental conditions the cuticle can be shown to be extremely sensitive to water content, suggesting that an important source of stabilization is H-bonding (Vincent and Ablett, 1988).

2 Materials and Methods

Histological study of the cuticle of the third larval instar of *Cx. quinquefasciatus*.

Lotions and dyes used in the preparation of electron microscopy samples.

2.1 0.1 M Na-cacodylate buffer (Glauert, 1991)

Solution (a): Dissolve 21.4 grams of in liters of distilled water.

Solution (b): Add 8.5 mL of concentrated hydrochloric acid in a liter of distilled water.

Add 500 mL of solution (a) to 41.5 mL of solution (b) and adjust the pH at 7.2.

2.2 Prepare glutaraldehyde 3% (Hayat, 1970)

Add 12 mL of glutaraldehyde concentration of 25% to 88 mL Organizer 0.1 Molar sodium cacodylate record according to the previous method.

2.3 Prepare osmium tetroxide 1%

Prepare 1% of the fourth osmium oxide Osmium tetroxide 1%. Melt 1 gram of crystals fourth osmium oxide in 100 mL of solution Organizer 0.1 Molar sodium cacodylate record according to the previous method.

2.4 Toluidine blue (Bancroft and Steven, 1982)

1. Dissolve 1 gram of borax (Sodium tetra borate) in 100 cm³ of distilled water.

2. Add 1 gram of Toluidine blue with continuous stirring.

3. Filter the dye before use.

2.5 Uranyl acetate (Bozzola and Russel, 1992)

1. Dissolve 2 grams of powder uranyl acetate in 100 cm³ of distilled water.
2. The solution is shaken until smooth and kept in a dark bottle.

2.6 Lead citrate (Reynold, 1963)
1. Dissolve 1.33 of lead nitrate (Lead nitrate) and 1.76 grams of distilled water into a glass beaker.
2. Shak the flask well for one minute the turbidity severe white where consists
2.7 Continued shaking (once every five minutes during half-hour)
1. A d dt o f l a s k8c m3 of (1 N Sodium hydroxide) free of carbonates with shake.
2. A size until 50 cm³ and add distilled water.
3. A good solution is filtered before use. It can be saved at room temperature for a period of six months of preparation.

2.8 Resin material
Following materials were used in the preparation of this plastic material:

Landfill Resin: SPI-EPON 812, 10 g; Araldite 502, 10 g; DDSA (Dodecenyl Succinc Anhydrite), 24 g; DMP-30 (Dimethyl almmo methyl phenol), 0.7 g. All of this materials are shaking well before using, all of them from SPI-CHEM, USA Company.

It has been prepared at electron microscope Research Center at King Saud University.

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