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## **Larvicidal, Histopathological and Ultra-structure Studies of *Matricharia chamomella* Extracts Against the Rift Valley Fever Mosquito *Culex quinquefasciatus* (Culicidae: Diptera)**

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### **ABSTRACT**

The present study was carried out to establish the larvicidal properties and histopathological effect of *Matricharia chamomella* plant extract on third larval instar of the *Culex quinquefasciatus*, the mosquito vector of Rift valley fever in Saudi Arabia using stock solution from King Saud University. Results of log-probit analysis( at 95% confidence level ) revealed that  $LC_{50}$  and  $LC_{90}$  values gradually decreased with the exposure periods ( 24 and 48 h ). This study shows that the larvicidal action of the *M. chamomella* plant extract is positively correlated to the concentration tested. The histopathological changes caused by  $LC_{50}$  have been investigated using light and an electron microscope. Observations were revealed over time (24 and 48 h). The most noticeable effect is the increasing damage to the larvae mid-gut epithelium, including cell vacuolization and rupture of the epithelial walls, microvillus damage and the epithelium cell contents passing into the mid-gut lumen. This article is the first report of the toxicity and histopathological effects of the *M. chamomella* as a bioinsecticide in the mid-gut of *Cx. quinquefasciatus* larvae. These results indicate that the tested extract may affect delayed larval development.

**Key words:** *Cx. quinquefasciatus*, *Matricharia chamomella*, larvicidal , histopathology, mid-gut, ultra-structure

### **INTRODUCTION**

Vector control is threatened by the emergence of resistance to conventional synthetic insecticides among vector mosquitoes, a trend that warrants either stronger countermeasures or the development of newer insecticides (Chandre *et al.*, 1998). Besides of their adverse effects of synthetic acaricides on ecosystem, some recent studies have shown that chemical substances widely used for pest control have a considerable genotoxic and cytotoxic effect on human target cells. Natural biological control of *Aedes*, *Anopheles* and *Culex* has become one of the most important alternative to prevent development of these mosquitoes vectors of many human diseases. Botanical insecticides may serve as a suitable alternatives to synthetic insecticides because they are relatively safe, degradable and readily available in many areas of the world. Though several plants from various families have exhibited larvicidal activity . Many plant extracts and/or their isolated active compounds have been tested by researchers worldwide in order to study the insecticidal effects against mosquitoes (Dua *et al.*, 1995; Pathak *et al.*, 2000; Sun *et al.*, 2001; Singh *et al.*, 2002; Sivagnaname and Kalyanasundaram, 2004; Obomanu *et al.*, 2006; Singh *et al.*, 2006). *Cx. quinquefasciatus* is responsible for transmitting the filarial nematode, *Wuchereria bancrofti* (Tropical Africa and Southeast Asia), Chikungunya virus (CHIKV)

(Africa, India and Asia) and Rift Valley fever virus (RVF) (Africa) (Foster and Walker, 2002). *Wuchereria bancrofti* is a filarial nematode that can cause lymphatic filariasis. Currently, worldwide there are approximately 120 million cases of lymphatic filariasis (WHO 2000). Saudi Arabia recorded cases of Rift Valley fever appears to be a declining trend in the rate of occurrence of cases and deaths as reported by the World Health Organization (WHO) in its Global Alert and Response (GAR) report Disease Outbreak of October 2000 entitled Rift Valley fever in Saudi Arabia. The factors accounting for Disease Outbreak are: uncontrolled demographic increase, poor urban planning, reduced epidemiological surveillance and progressive resistance of the vector mosquito to several insecticides produced by the chemical industry (Hemingway and Ranson, 2000; Nazni *et al.*, 1998; Selvi *et al.*, 2005).

The histopathological effects of plant extracts or their active compounds on insects have also been studied often; however, only Al-Mehmadi and Al-Khalaf (2009) have examined *Cx. quinquefasciatus* in Saudi Arabia.

Many species of chamomile grow throughout the world. The type commonly available in the Saudi Arabia is known as German chamomile, or *Matricharia chamomella*. The objective of this study is to evaluate the larvicidal activity of *M. chamomella* extract on third larval instar of *Cx. quinquefasciatus* and to observe the dynamic changes in mid-gut epithelial cells following treatment with  $LC_{50}$  of plant extract. This study will hopefully find a viable alternative to synthetic insecticides for *Cx. quinquefasciatus* control.

## MATERIALS AND METHODS

**Rearing technique:** A laboratory-sensitive strain of *Cx. quinquefasciatus* mosquitoes was obtained from a susceptible in 2006-2007, reared strain at the College of Food and Agricultural Science, King Saud University. The larvae used in the tests were reared in plastic cups with water from the public water supply, under standard conditions of  $27\pm 2^{\circ}C$ ,  $70\pm 5\%$  R.H. and 12 h photoperiod. The larvae were reared in dechlorinated water and fed daily on tetra mine (tropical fish food). Adults were maintained on a 10% sugar solution and females were also fed on chicken. The third instar larvae were used in the bioassay tests.

**Preparation of stock solution:** Ethanolic extract from a whole plant of *M. chamomella* was obtained from Pharmacy College, King Saud University. This extract was dark green and crude and was used to prepare stock solution. The known amount ( $10\text{ mg L}^{-1}$ ) of filtered crude extract obtained from the above source was serially diluted to obtain the desired concentration. The stock solution was gradually diluted to prepare test solutions of 0.12, 0.25, 0.50, 0.75, 1.00 and  $1.25\text{ mg L}^{-1}$ . One drop of emulsifier (Tween 20, Sigma Chemical Company) was mixed with the extract to ensure complete solubility of the material in water.

**Bioassays and larval mortality:** In four replicates, ten larvae were pipetted into each 20 mL volume and observed for a maximum of 48 h, when mortality was recorded. Larvae were considered dead or moribund if they stopped moving for a prolonged period even after gentle probing with a small spatula, as described in the World Health Organization's technical report series. Each experiment examined a minimum of 40 larvae per concentration. Larvae maintained in distilled water served as a control. The  $LC_{50}$  and  $LC_{90}$  were calculated using probit analysis (Finney, 1971). When necessary, percentage mortality in the treatments was corrected for mortality in the controls using Abbott (1925).

**Histological studies:** For histological purposes, the treated and untreated newly ecdysed third instar larvae of *Cx. quinquefasciatus* were isolated from the standard laboratory colony, which were incorporated with the LC<sub>50</sub> of *M. chamomella* after 24 h at 1.035 mg L<sup>-1</sup>. Only live larvae were examined and then they were fixed in bouins solution 24 and 48 h after exposures. After dehydration in a graded ethanol series, the material was embedded and cut with glass knives in a rotary microtome. The sections were stained with haematoxylin-eosin, analyzed and photographed with a photomicroscope. After 24 and 48 h of electron microscopic studies, the mid-gut was fixed in a solution containing 2.5% glutaraldehyde and 4% paraformaldehyde in a 0.1 M phosphate buffer (pH 7.3). The mid-gut was then post-fixed in a 1% osmium tetroxide solution in the same buffer, dehydrated in a graded acetone solution and embedded. Ultra-thin sections were stained with uranyl acetate and lead citrate before examination.

**RESULTS**

The results of susceptibility tests for the extract of *M. chamomella* are shown in Table 1, the *M. chamomella* extract caused mortality among larvae of *Cx. quinquefasciatus* at all tested concentrations. However, the susceptibility of the mosquito larvae was positively correlated with the concentration tested and the period of exposure. The inclination of regression lines indicates that concentration increases mortalities. Thus, the LC<sub>50</sub> value was 0.505 mg L<sup>-1</sup> after 24 h and decreased with the exposure time, reaching 0.301 mg L<sup>-1</sup> after 48 h Table 2.

**Histological and histopathological studies:** This study examined a series of cross-sections through the thoracic and abdominal regions of normal untreated and treated third instar larvae of *Cx. quinquefasciatus*. The normal mid-gut wall in *Cx. quinquefasciatus* consists of columnar

Table 1: Susceptibility of *Cx. quinquefasciatus* larvae after treated with *M. chamomilla* for 24 and 48 h

Plant extract LC <sub>50</sub> (mg L <sup>-1</sup> )	Conc. Mg L <sup>-1</sup>	Means±SD of mortality (%)	
		Exposure period (h)	
		24	48
<i>M. chamomilla</i>	0.12	5.00±5.77	15.00±5.77
	0.25	20.00±11.54	32.50±9.57
	0.50	40.00±8.16	67.50±22.17
	0.75	65.00±19.14	87.50±12.58
	1.00	80.00±8.16	95.00±5.77
	1.25	97.50±5.00	100.00±0.00
Statistical analysis			
b <sup>1</sup>		81.49	76.29
Sig.		****	***

b<sup>1</sup>: Regression coefficient. \*\*\*Significant p≤0.001, \*\*\*\*Significant p≤0.0001

Table 2: Comparing the efficacy of *M. chamomella* extract on third larval instar of *Cx. quinquefasciatus* at various periods of exposure.

No. of insects treated	Exposure time (h)	LC <sub>50</sub> (mg L <sup>-1</sup> )	Lower limit	Upper limit	Index	RR	Slope±SD	Chi square	LC <sub>90</sub>
40	<i>M. chamomella</i> for 48 h	0.301	0.250	0.358	100.00	1.00	2.566±0.271	4.52	0.95
40	<i>M. chamomella</i> for 24 h	0.505	0.432	0.583	59.604	1.678	3.049±0.344	6.01	1.329

Index compared with *M. chamomella* after 48 h. Resistance Ratio (RR) compared with *M. chamomella* after 48 h

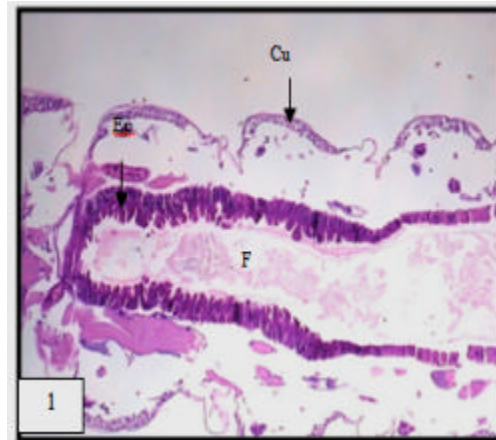


Fig. 1: Longitudinal sections on the middle and posterior regions of *Cx. quinquefasciatus* third instar larva showing the mid-gut epithelia cells x200

epithelium cells; each one is cylindrical, containing a large, coarsely granular nucleus that occupies a middle position within the cells. The columnar epithelium has a striated border (microvilli) covered by the peritrophic membrane (Fig. 1).

**Structure of the untreated epithelium:** The mid-gut of the control third instar of *Cx. quinquefasciatus* consists of a unicellular layer (epithelium) resting upon a basement membrane. This membrane is surrounded externally by circular and longitudinal muscle fibers, respectively. The epithelium consists of columnar cells with clusters of small regenerative cells, each of which contains a relatively large nucleus and strongly basophilic cytoplasm. The epithelium is also protected from food particles by a detached sheath—a peritrophic membrane—surrounding a lumen. In the longitudinal section, from the anterior, middle and posterior regions of the mid-gut, the columnar cells seem to be unsaturated in position, shape and size (Fig. 1-3).

When treated with *M. chamomilla* extract, all larvae developed dramatic lesions, affecting mainly the mid-gut epithelium. The histopathological effects differed qualitatively according to their location along the mid-gut and quantitatively according to the duration of the treatment. The cross-section in the middle gut also showed disarrangement in appearance of the columnar cells, swelling and extruded masses of cellular material in the anterior portion of the mid-gut. The epithelial cells appeared to have expanded into the gut lumen (Fig. 4) and contained large cytoplasmic spaces in the posterior mid-gut (Fig. 5). Disintegration of the mid-gut appeared more extensive after 48 h in all three regions (Fig. 6-8).

**Electronic microscopic observations:** Electron micrographs of the untreated control mid-gut epithelial cells revealed long and regularly placed microvilli borders toward the lumen (Fig. 9, 10). Some cells in the mid-gut of the larvae exposed to *M. chamomilla* presented an irregularly structured brush border within 24 h (Fig. 11, 12). The cells began to swell via a slight vacuolization, with disorganized, shortened and confluent microvilli membranes. The mid-gut was analyzed after 48 h. At this time, the structural disorganization of the mid-gut epithelium was evident; cells did not show the characteristic morphology, had become more destroyed and were sometimes budding into the lumen (Fig. 13).

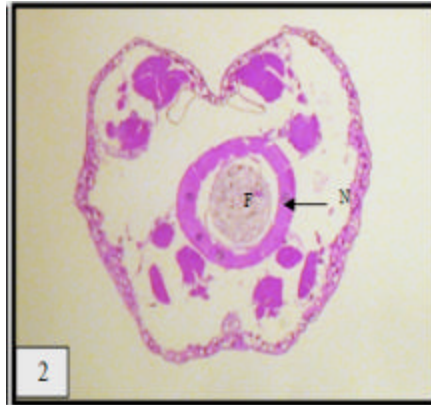


Fig. 2: Cross-section in the normal untreated *Cx. quinquefasciatus* third instar larva showing the anterior mid-gut epithelia cells x200. N: nucleus, Ep: epithelium cell, PM: Peritrophic membrane

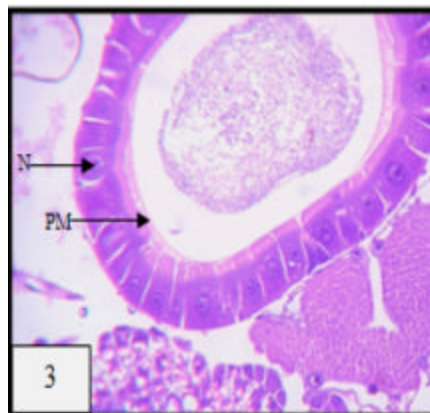


Fig. 3: Cross-section in the normal untreated *Cx. quinquefasciatus* third instar larva showing the middle mid-gut epithelia cells x200



Fig. 4: Cross-section in the normal untreated *Cx. quinquefasciatus* third instar larva showing the posterior mid-gut epithelia cells x200. N: nucleus, Ep: epithelium cell, PM: Peritrophic membrane, Mg: Malpighian tubules, Microvilli: arrow

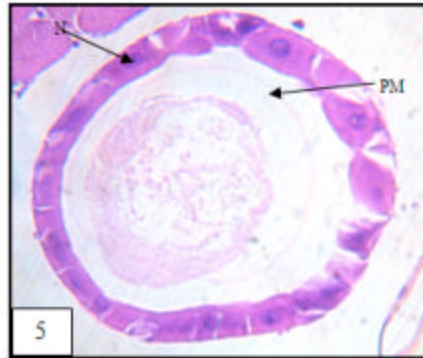


Fig. 5: Cross-section through the anterior mid - gut region of third instar larvae of *Cx. quinquefasciatus* treated with  $LC_{50}$  of *M. chamomella* extract, showing the effects after 24 h of exposure x400



Fig. 6: Cross-section through the middle mid-gut region of third instar larvae of *Cx. quinquefasciatus* treated with  $LC_{50}$  of *M. chamomella* extract, showing the effects after 24 h of exposure x400. N: Nucleus, PM: peritrophic membrane, BM: Basement membrane



Fig. 7: Cross-section through the posterior mid-gut region of third instar larvae of *Cx. quinquefasciatus* treated with  $LC_{50}$  of *M. chamomella* extract, showing the effects after 24 h of exposure x400. PM: Peritrophic membrane, Mg: Malpighian tubules, Ep: Epithelial cells

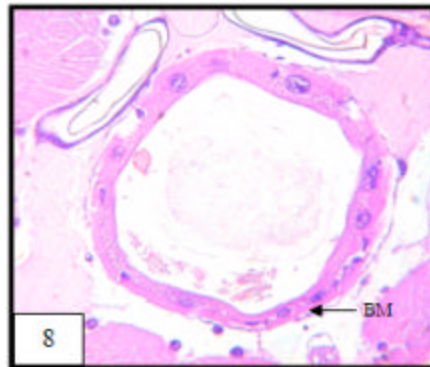


Fig. 8: Cross-section through the anterior mid-gut region of third instar larvae of *Cx. quinquefasciatus* treated with  $LC_{50}$  of *M. chamomella* extract, showing the effects after 48 h of exposure x400. BM: Basement membrane, N: Nucleus

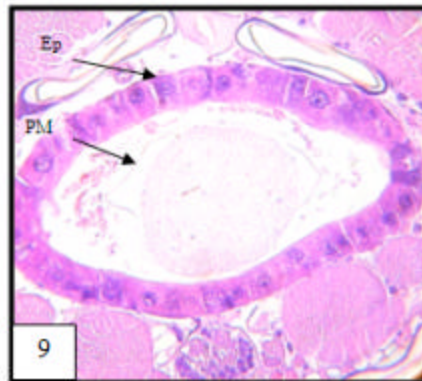


Fig. 9: Cross-section through the middle of the mid-gut region of third instar larvae of *Cx. quinquefasciatus* treated with  $LC_{50}$  of *M. chamomella* extract, showing the effects after 48 h of exposure x400. PM: Peritrophic membrane, BM: Basement membrane

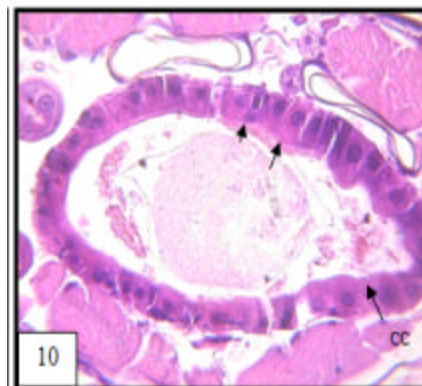


Fig. 10: Cross-section through the posterior of the mid-gut region of third instar larvae of *Cx. quinquefasciatus* treated with  $LC_{50}$  of *M. chamomella* extract, showing the effects after 48 h of exposure x400. CC: Cell content, Microvilli: arrows



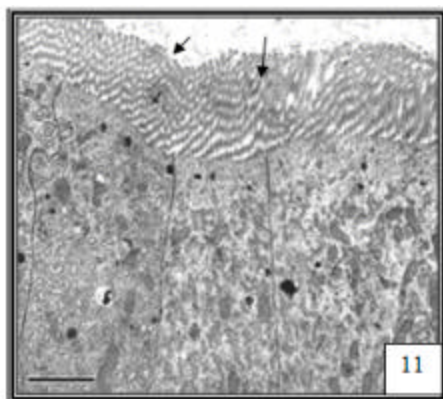


Fig. 11: The continuous microvilli (arrows) characterize the mid-gut epithelium in control larvae of *Cx. quinquefasciatus*

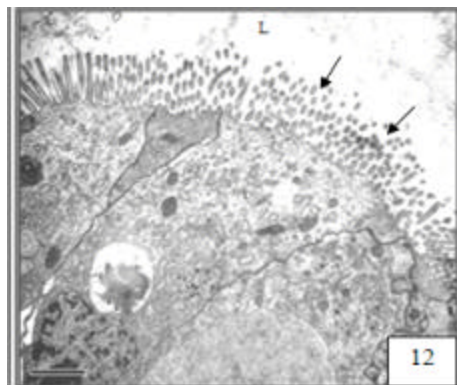


Fig. 12: Longitudinal section of a columnar cell of the mid-gut in a treated larvae of *Cx. quinquefasciatus* after 24 h via transmission electron micrograph. L: Lumen, Microvilli: arrow

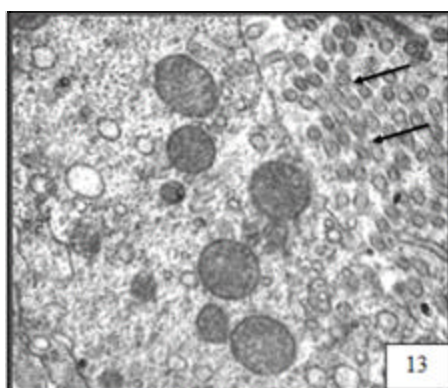


Fig. 13: Longitudinal section of microvilli of a columnar cell of the mid-gut in a treated larvae of *Cx. quinquefasciatus* after 48 h via transmission electron micrograph. Microvilli: arrow

## DISCUSSION

Today, the environmental safety of an insecticide is considered to be of paramount importance. An insecticide does not have to cause high mortality on target organisms in order to be acceptable (Kabaru and Gichia, 2001). In the future, phytochemicals may serve as suitable alternatives to synthetic insecticides because they are relatively safe, inexpensive and readily available in many areas of the world. The screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health (Bowers *et al.*, 1995).

The crude extract of *M. chamomella* has been found to exhibit larvicidal activity against the third larval instar of *Cx quinquefasciatus*. The biological activity of the plant extract might be a result of the various compounds that exist in plants; jointly or independently, these compounds may contribute to the production of larvicidal activity against *Cx. quinquefasciatus*.

The literature does not reveal other studies on the larvicidal properties of *M. chamomella* for culicids. However, studies considering other plants as bases for lethal concentrations served as reference for this study of the potential of *M. chamomella* for usage in *Cx. quinquefasciatus* control. Pizarro *et al.* (1999) studied the activity of the dehydrated gross extract and saponine fraction of *Agave sisalana* and estimated lethal concentrations of LC<sub>50</sub>, LC<sub>90</sub> and LC<sub>95</sub> for third instar *Cx. quinquefasciatus* that were 183, 408 and 512 ppm, respectively. These concentrations are much higher than those reported in this study, but these authors suggest their use for control of this mosquito. The observed histopathological effects of ethanolic extract of *M. chamomella* on the mid-gut of third larval instar of *Cx. quinquefasciatus* are consistent with the results of other studies on *Cx. pipiens* larvae (Hamouda *et al.*, 1996; Hussin and Shoukry, 1997; Massoud and Labib, 2000; Assar and El-Sobky, 2003). These studies found that the treated larvae were affected in the epithelial layer, which was vacuolated. They also observed swollen cells, masses of cellular material in the anterior part of the mid-gut and the loss of appearance of the epithelium. The gut's apical portion of columnar cells was swollen and sometimes distinct elongations protruded into its lumen as bulbous eversions. The findings of the present investigation reveal that the extract of *M. chamomella* displays remarkable larvicidal activity against mosquito *Cx. quinquefasciatus*.

Further investigations are needed to examine this activity against a wide range of mosquito species and to determine the active ingredient(s) in the extract that are responsible for its larvicidal activity against *Cx. quinquefasciatus*. These ingredients should be identified and if possible, utilized in a commercial product or formula to be used as a mosquitocidal agent.

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