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Effect of *Murraya paniculata* Leaf Extract Against *Culex quinquefasciatus* Larva

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ABSTRACT

The mosquito-borne diseases are still the public health's problems. So, in order to cope with these problems the development of ideal and novel natural product is essential for mosquito larvae's control. The efficacy of mosquito larvicide of *Murraya paniculata* leaf aqueous extract was investigated by determining the median lethal concentration, LC_{50}, within 24, 48, 72 and 96 h. Additional, the 25% 24 h LC_{50} of this extract against the *Culex quinquefasciatus* larva was investigated after 24 h exposure by using the histological analysis. The histology of the mid-gut was studied and it was found to be different from the normal larvae such as separation of the epithelial cells from the basement membrane, elongation protruded into its lumen, disruption of the microvilli, appearance several vesicles and cytoplasm masses. The results of this study suggested that the aqueous extract of this leaf had a larvicidal property and it could a used as a novel way to control and eradicate the mosquito's larvae, without the use of harmful pesticides currently available in the market.

Key words: Mosquito, herb, larva, mid-gut, pathology

INTRODUCTION

The mosquito-borne diseases are still the public health's problems which the species belonging to genera *Anopheles*, *Culex* and *Aedes*. They are vectors for the diseases i.e., malaria, dengue, filariasis, encephalitis, yellow fever and chikungunya. The control of these vectors mainly depends on the use of chemical pesticides. However, the adverse risks to the environment and health is known for example disruption of natural biological control systems, outbreaks of other insect species, widespread development of resistance and undesirable effects on non-target organisms (Yang *et al*., 2002). Insecticide resistance has been reported in many country especially Thailand (Southeast Asia) (Sathantriphop *et al*., 2006). These indicate the need to develop environmentally safe, cost effective and preferably locally available agents for the mosquito control. Plant extracts may be the alternative sources of mosquito control due to a lot of bioactive compounds (Govindarajan, 2010). Many studies on plant extracts against mosquito larvae have been conducted around the world. The methanol extract of leave of *Sida acuta* and essential oils from *Cymbopogon citratus*, *Cinnamomum zeylanicum*, *Rosmarinus officinalis* and *Zingiber officinale*

(Govindarajan, 2010, 2011); the ethanolic extract of whole plant of *Melia azedarach* (Al-Mehmadi and Al-Khalaf, 2010) were tested against *A. aegypti* and *A. stephensi* and *C. quinquefasciatus* larvae. Studies have shown the potential of plants for use in *C. quinquefasciatus* control, such as *Piper longum* (Madhu *et al*., 2011), *Azadirachta indica* (Mandal, 2011) and five species of Cucurbitaceous plants, *Momordica charantia*, *Trichosanthes anguina*, *Luffa acutangula*, *Benincasa cerifera* and *Citrullus vulgaris* (Prabakar and Jebanesan, 2004). These plant extracts have several bioactivities i.e., growth regulation, fecundity suppression, male sterility, loss of flying ability, immunodepression and enzyme inhibition (Su and Mulla, 1998).

Murraya paniculata (Linn.) or orange Jessamine belongs to the family Rutaceae and is commonly grown in gardens for its glossy green foliage and large clusters of fragrant flowers (Rout *et al*., 2010). It is a tree found in the tropical and subtropical areas and is often grown in Thailand. Many research groups have found that *M. paniculata* contains several kind of coumarin (Saied *et al*., 2008), indole alkaloid (Wu *et al*., 1989), isoflavonoid (Lapcik *et al*., 2004), carotenoid (Buchecker *et al*., 1970) and essential oil (Raina *et al*., 2006). *M. paniculata* has been used in ethnomedicine (Olawore *et al*., 2005). The dried bark and fruit are used in South East Asia as an astringent, febrifuge and anti-dysenteric (Kong *et al*., 1986). A paste of the leaf mixed with turmeric powder is used in India for the treatment of fractured bones (Nagaraju and Rao, 1990) while the stem bark is used for treating malaria fever (Singh and Ali, 1994).

No works have yet been done in this country and near area in regard of these leave extract use for bio-insecticide. The present study was conducted to evaluate the mosquito larvicidal properties of *M. paniculata* leaf aqueous extract against *C. quinquefasciatus* as target species. The susceptibility of *M. paniculata* was evaluated via the histological analysis.

MATERIALS AND METHODS

Plant collection and extraction: Fresh, mature, green leaves of *M. paniculata* were randomly harvested in and around Mahidol University Phayathai campus, Bangkok, Thailand (13°45' 51.77" N, 100°31' 32.47"E). The leaves were initially rinsed with distilled water and air dried. One hundred gram (wet weight) of leaves were crushed with a mixer-grinder machine and the plant juice was filtered by Whatman No. 1 filter paper and the clear filtrate used as a stock solution (100% concentration of crude extract) for bioassay experiments. Required concentrations (10.625, 125, 250, 500 and 1000 ppm) were prepared through the mixing up of stock extract with variable amounts of sterilized distilled water.

Mosquito larvae collection: The fourth instar larvae were collected surrounding the university campus and transferred into a glass beaker containing distilled water and the larvae after sorting were identified as *C. quinquefasciatus* larvae.

Larval bioassay procedure: The larval bioassay was performed using a standard protocol described by WHO (1981). The bioassay was repeated three times using fourth instar larvae of *C. quinquefasciatus*. Twenty larvae were transferred to beakers containing 100 mL of distilled water and five concentrations of leaf extract in a range that causes 0-100% mortality. The bioassay was maintained at 27±1°C throughout the test. Larval mortality was recorded for a maximum of 96 h of exposure. Larvae were considered dead or moribund if they stopped moving for a prolonged

period even after gentle probing with a small spatula. The LC_{50} was analyzed by the probit method of Finny (1971). It estimated the lethal concentrations and the slope of the regression lines with their confidence intervals ($p<0.05$).

Specimen preparation for light microscopic study: For the histological tests, 20 larvae were exposed with 25% 24 h LC_{50} for 24 h. Only live larvae were examined. The procedures for light microscopy were modified (Humason, 1972). Briefly, the larvae were fixed in the 10% buffered formaldehyde for 24 h, dehydrate through a graded series of ethanol and clear with xylene solutions. They were embedded in a block using melted paraffin at the embedding station. The paraffin blocks were sectioned at 5 µm thickness using a rotary microtome and stained with hematoxylin and eosin. The glass slides were examined for abnormalities by a Nikon E600 light microscope and photographed by a Nikon DXM 1200 digital camera (Tokyo, Japan).

RESULTS

Data of the larvicidal activity of the aqueous leaf extract of *M. paniculata* against *C. quinquefasciatus* larvae was presented in Table 1. Result of probit analysis at 95% confidence level revealed that LC_{50} and LC_{90} values gradually decreased with the exposure periods. The dose dependent mortality was observed, as the rate of mortality (Y) was positively correlated with the concentration (X) of the leaf extract as evident from established regression equations. Strong correlation between concentration and mortality (R) was observed from 0.9303-0.9937.

Culex larvae hang the head down with only the tip of the air tube penetrating the water surface. The three body regions, head, thorax and abdomen were distinct (Fig. 1). The head was short and stout becoming darker toward the base. It had the antennae, eyes and mouthparts. The

Table 1: Efficiency of *M. paniculata* leaves extract on the larval mortality against *C. quinquefasciatus*

	M. paniculata (ppm)									
Time (h)	1000	500	250	125	10.625	Control	LC_{50}	LC_{90}	Regression equation	R-value
24	20.00	15.67	8.67	5.33	0.67	0.00	311.32	561.17	Y = 4.2383 X-6.4440	0.9303
48	20.00	16.67	11.33	5.67	1.67	0.00	268.23	509.62	Y = 4.3046 X-5.8427	0.9614
72	20.00	16.67	12.33	7.33	2.33	0.00	246.46	500.76	Y = 4.2291 X-5.0253	0.9404
96	20.00	18.33	13.33	8.00	4.33	0.00	200.59	432.27	Y = 4.2094 X-4.0680	0.9937

n = 20

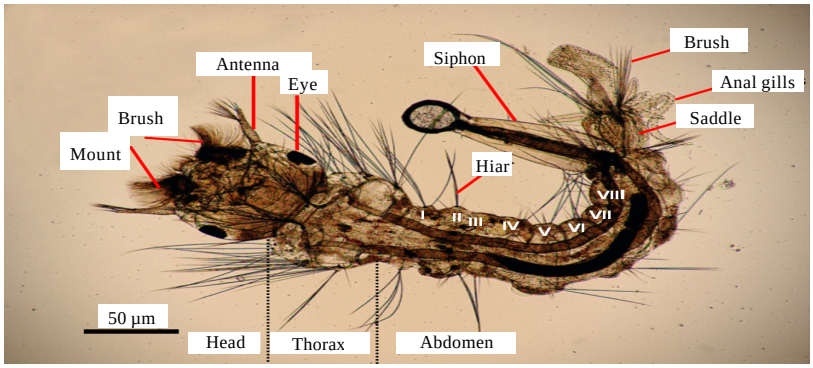

Fig. 1: Light micrograph of the fresh whole body of *C. quinquefasciatus* larvae showing the composition of three body regions, head, thorax and abdomen, Bar = 50 µm

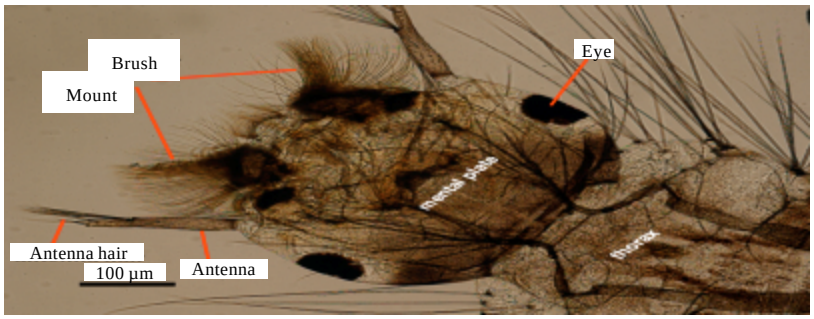

Fig. 2: Light micrograph of the head part of *C. quinquefasciatus* larvae, Bar = 100 µm

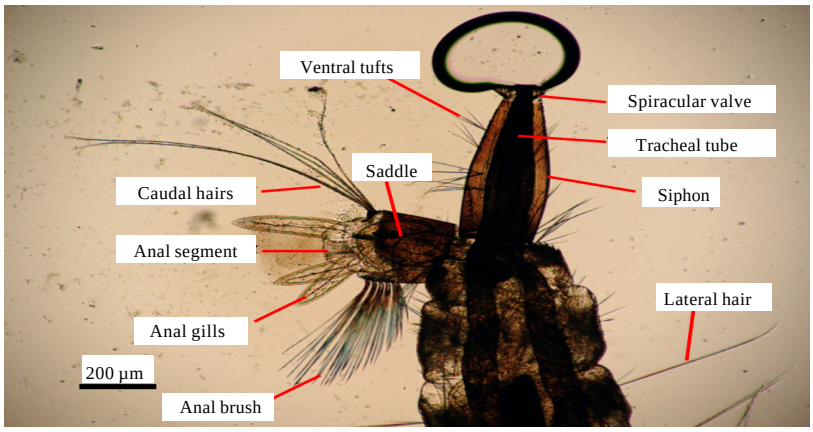

Fig. 3: Light micrograph of the anal part of *C. quinquefasciatus* larvae, Bar = 200 µm

antennae were located on each side of the head toward the front. Behind the antennae near the hind margin of the head were the dark eyes. The mouthparts were at the underside of the head. They consisted of a series of brushes which have long filaments that are used for filtering materials (Fig. 2). The thorax was broader than head or abdomen and somewhat flattened. It had several groups of hairs. The abdomen was long and cylindrical, consisting of eight segments, the siphon and the saddle. Each segment had a unique setae pattern. The siphon was on the dorsal side of the abdomen and in *C. quinquefasciatus* the siphon was four times longer than it was wide with multiple setae tufts. The saddle was barrel shaped and located on the ventral side of the abdomen with four long anal papillae or anal gills protruding from the posterior end (Fig. 3).

Histology of the whole body of *C. quinquefasciatus* larvae in the control group showed the normal appearance of the three body regions, head, thorax and abdomen (Fig. 4). The mid-gut epithelium consisted of a single layer of digestive cells exhibiting a well developed brush border or microvilli and cytoplasm with acidophilic regions (Fig. 5).

After 24 h exposure, partial lyses of the epithelial cells began through local detachment or dilated basal membrane, the degeneration of the microvilli and the cells were elongated. It also was containing vesicles of different sizes and broken membranes, at the apical side of the epithelial cells (Fig. 6). Cytoplasm masses looked like the bubbles were also observed in several areas.

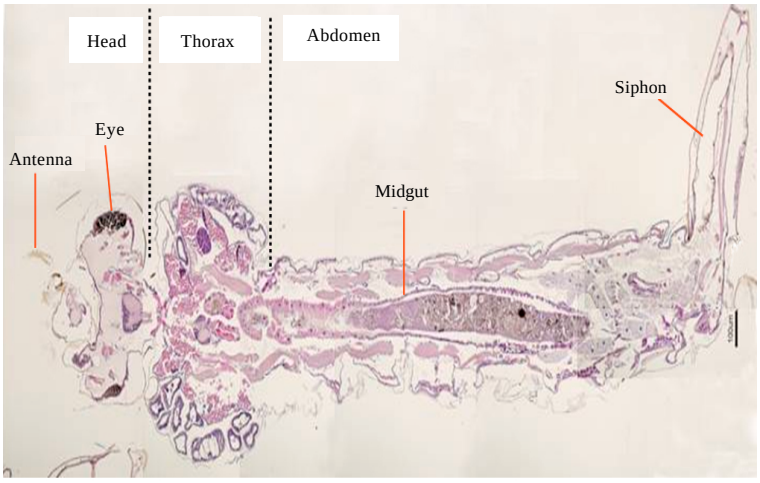

Fig. 4: Histology of the whole body of *C. quinquefasciatus* larvae showing the three body regions, head, thorax and abdomen, Bar = 100 µm

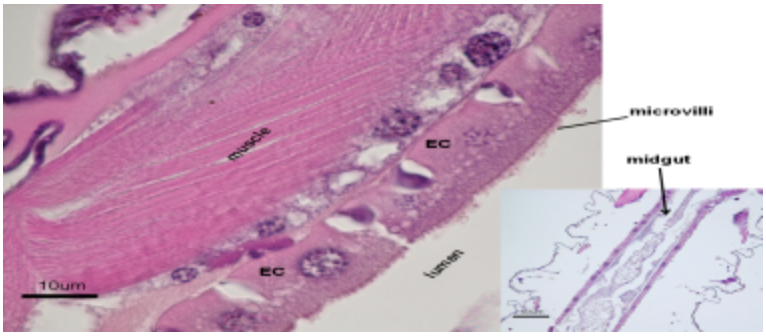

Fig. 5: Histology of the mid-gut of control group showing cylindrical epithelial cells (EC) with microvilli (arrow), Bar =10 µm (inset = 50 µm)

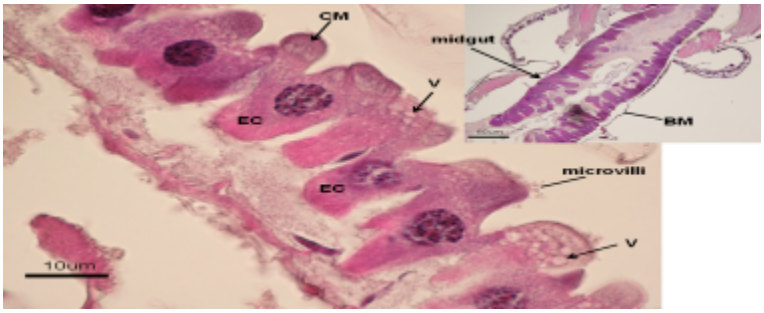

Fig. 6: Longitudinal section in the mid-gut of *C. quinquefasciatus* larvae treated with 25%-24 h LC_{50} of *M. paniculata* after 24 h, EC: Epithelial cells, BM: Basement membrane, V: Vesicle and CM: Cytoplasm mass, Bar = 10 µm (inset = 50 µm)

DISCUSSION

The mosquito control at the larval stage of development with phytochemicals that occur in the several parts i.e., fruit, flower, leaf and root of plants is one of the techniques which affords a cheap, easy to use and environment friendly method. The larvicidal properties of different plants have been reported in terms of lethal concentrations for 50% mortality. This study has shown the potential of this plant for use in *C. quinquefasciatus* larvae control. The present LC_{50} values at 24, 48, 72 and 96 h of *M. paniculata* against *C. quinquefasciatus* larvae were 311.32, 268.23, 246.46 and 200.59 ppm, respectively. The larval mortality was high on the first 24th h and it continued up to the 96th h. The larvicidal activity of this leaf extract result was also comparable with earlier reports. The activities of *Murraya koenigii* acetone extract against *Aedes aegypti* were reported in the range 25-900 ppm (Harve and Kamath, 2004). The efficiency of *Carica papaya*, *M. paniculata* and *Cleistanthus collinus* were evaluated against *C. quinquefasciatus* and the qualitative phytochemical analysis of all the plants were revealed the presence of many bioactive principles such as steroid, alkaloid, terpene, saponin (Rawani *et al*., 2009). Kumari (2010) reported the 24 h LC_{50} and LC_{90} of petroleum ether extract of *M. koenigii* against *C. quinquefasciatus* were 89.50 and 350.40 ppm, respectively. The LC_{50} values of ethyl acetate extract of *Swertia chirata* against first, second, third and fourth instar larvae of *C. quinquefasciatus* were 164.91, 220.10, 284.05 and 326.46 ppm, respectively (Balaraju *et al*., 2009).

The mid-gut possesses well-developed microvilli in the cell apex because it is the main absorption area in the mosquito gut (Alves *et al*., 2010). In this study, histopathological alteration were seen in the mid-gut, included separation of the epithelial cells from the basement membrane, sometime distinct elongations protruded into its lumen, disruption of the microvilli. These observations were in agreement with earlier reports. The mid-gut of *Culex pipiens* that treated with *Artemisia judaica* was affected, the epithelial layer was vacuolated, swollen cells, masses of cellular material appeared in the lumen and finally the epithelium lost their normal appearance (Hamouda *et al*., 1996). The histological effects of *Melia azedarach* extract on *C. quinquefasciatus* were analysis (Al-Mehmadi and Al-Khalaf, 2010). These lesions were seen in the anterior and posterior regions of the midgut, included separation of the epithelial cells from the basement membrane with damage of the peritrophic membrane. The mixing of the gut contents with the hemolymph caused the larval mortality. The border of mid-gut showed a striated appearance due to the presence of the microvilli which line the inner edge of the epithelial cells. These microvilli enhanced the rate of absorption. The gut apical portion of columnar cells was swollen and sometimes, distinct protruded into its lumen as a ballooned section showed vacuolated cytoplasm, cells were dislodged, sloughed and detached from each other. Several reports suggested that the larvicide substances lead to morphological damage in epithelial cells of the mid-gut which are likely where these substances are absorbed. Regardless of the type of substances used, the similarity of detrimental changes in the organism indicates that these alterations are a common response to cellular intoxication.

In conclusion, the aqueous extract of *M. paniculata* can be recommended in field areas and can be effectively used as the natural larvicidal product in the mosquito control program. However, further studies are needed to know what the active substances are and how they do or the mechanism of them in the target species.

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