African Journal of Biomedical Research, Vol. 8 (2005); 31 - 33
ISSN 1119 – 5096 © Ibadan Biomedical Communications Group
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Full Length Research Article

Larvicidal and Adult Emergence Inhibition Effect of *Centella asiatica* Brahmi (Umbelliferae) against Mosquito *Culex quinquefasciatus* Say (Diptera : Culicidae)

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Received: August, 2004
Accepted: January, 2005

Abstract
Ethanolic extract of *Centella asiatica* leaves were evaluated for the larvicidal and adult emergence inhibition activity against mosquito *Culex quinquefasciatus* under five constant temperatures 19, 22, 25, 28 and 31°C in the laboratory. Toxicity of this extract increased with temperature. The 50% medium lethal concentrations ranged between 6.84 ppm at 19°C and 1.12 ppm at 31°C. A similar trend was observed for the 90% lethal concentrations which varied from 9.12 to 3.63 ppm at the two temperatures, respectively. The adult emergence inhibition activity of this extract at LC_{50}s of different temperatures was generally more pronounced in increased temperatures. These results suggest that the leaf extract of *C. asiatica* is promising as larvicide and adult emergence inhibitor against *Culex quinquefasciatus* and might be used directly in small volume aquatic habitats or breeding sites of limited size around human dwellings.

Keywords
Centella asiatica, *Culex quinquefasciatus*, Larvicidal activity, Adult emergence inhibition activity.

INTRODUCTION

Mosquitoes not only cause nuisance by their bites but also transmit deadly diseases like malaria, filariasis, yellow fever, dengue and Japanese encephalitis, contribute significantly to poverty and social debility in tropical countries (Jang *et al*., 2002). The mosquito *Culex quinquefasciatus* acts as a vector for *Wuchereria bancrofti* responsible for filariasis in India. However, control of such diseases are becoming increasingly difficult because the over production of detoxifying mechanisms of chemical insecticides has been reported for *Culex* species (Severini *et al*., 1993). On the other hand, some mosquito species have developed high levels of resistance to microbial control agents (Rao *et al*., 1995). One alternative approach is the use of natural products from plant origin (Consoli and Oliveira, 1994). The botanical insecticides are generally pest specific and are relatively harmless to non-target organisms including man. They are also biodegradable and harmless to the environment. One plant species may possess substances with a wide range of activities, for example extracts from the neem tree *Azadirachta indica* showed antifeedant, antioviposition, repellent and growth–regulating activity (Schmutterrer, 1995).

The species *Centella asiatica* Brahmi (umbelliferae) grows as a climber in most part of the Asia. The leaves of this plant used for skin diseases like chronic ulceration, psoriasis and leprosy (Vishnurao, 1996). The objectives of this study were to determine the efficacy of *Centella asiatica* against the larvae of *Culex quinquefasciatus* under constant temperatures ranging between 19 and 31°C.

MATERIALS AND METHODS

Preparation of phyto-chemical extract
Fully developed leaves of *C. asiatica* were collected from the medicinal plant garden of our university campus. The dried and powdered leaves (1.0 kg) of *C. asiatica* were extracted with ethanol (3 l) at soxhlet apparatus for 8 hrs. The

extract was concentrated in a rotary vacuum evaporator to yield a dark greenish, gummy extract (143.31 g). The residue was then made into 1.0% stock solution with acetone.

Test organisms

The test organism namely *Culex quinquefasciatus* was reared in the laboratory. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and 1-week – old chick for blood feeding. Mosquitoes were held at 28 ± 2°C, 70 ± 5% RH, and a photoregime of 16:8 (L:D) h.

Larvicidal activity

The larvicidal activity of the extract against *Culex quinquefasciatus* at constant temperatures of 19, 22, 25, 28 and 31°C was evaluated as per the standard procedure (WHO, 1996). The stock solution of the extract was volumetrically diluted to 250 ml filtered tap water to obtain the test solutions of 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 ppm. The acetone was served as a control. Early third instar larvae (25) were introduced to each of the test solutions as well as control. For each dose five replicates were run at a time. The larval mortality was recorded after 24 h post treatment. Probit analysis (Finney 1971) was used to determine the median lethal concentration (LC_{50}) and lethal concentration (LC_{90}) at all the tested temperatures.

Adult emergence inhibition activity

The effect of leaf extract on the inhibition of adult emergence was determined by the use of the LC_{50} at each of the tested temperatures. Twenty five larvae were placed in capped one litre glass jar containing 250 ml of water in which the LC_{50} of the extract for each temperature was mixed. Control groups were set up for each temperature in a similar fashion. The number of emerged adults was recorded until adult emergence was completed in the control jars.

RESULTS

C. asiatica leaf extract caused mortality against larvae of *Culex quinquefasciatus* at all the tested temperatures. However, the sensitivity of the insects was positively correlated to temperatures. Thus, the 24 h LC_{50} was 1.12 ppm at 31°C and increased the LC_{50} value with the decrease in temperature to reach 6.84 ppm at 19°C (Table 1).

Table 1:
Larvicidal activity of *Centella asiatica* leaf extract against *Culex quinquefasciatus* at five constant temperatures.

Temperature (°C)	LC_{50} (ppm)	95% Confidence limit (ppm)	LC_{90} (ppm)	95% Confidence limit (ppm)
19	6.84 ± 1.32^{a}	4.85 – 8.79	9.12 ± 2.12^{a}	5.92 – 12.57
22	5.64 ± 1.57^{b}	3.78 – 7.56	8.32 ± 1.82^{b}	4.98 – 11.39
25	3.92 ± 1.23^{c}	2.22 – 4.82	6.78 ± 1.47^{c}	4.06 – 8.71
28	2.79 ± 1.43^{d}	1.37 – 3.57	5.28 ± 1.43^{d}	3.32 – 7.19
31	1.12 ± 1.23^{e}	0.22 – 2.08	3.63 ± 1.57^{e}	2.68 – 4.52

Values in a column with a different superscript are significantly different at P<0.05 level (DMRT test). Each value ($\overline{X} \pm$ S.D) represents mean of five values

Table 2:
Emergence inhibition activity of median lethal concentrations of *Centella asiatica* leaf extract against *Culex quinquefasciatus* at five constant temperatures.

Temperature (°C)	Adult emergence (%)	
	Control	Treated
19	95.2 ± 0.8^{a}	7.0 ± 1.2^{a}
22	98.8 ± 1.4^{b}	9.2 ± 0.8^{b}
25	94.4 ± 1.8^{c}	6.4 ± 1.4^{c}
28	93.0 ± 0.8^{d}	4.0 ± 0.8^{d}
31	91.2 ± 1.6^{e}	2.2 ± 1.6^{e}

Values In A Column With A Different Superscript Are Significantly Different At P<0.05 Level (DMRT Test). Each Value ($\overline{X} \pm$ S.D) Represents Mean of Five Values

The larvae susceptible in 31°C was 6.10, 5.03, 3.5 and 2.49 times more effect than 19, 22, 25 and 28°C, respectively. The results showed that this extract can be used to control larvae of *Culex quinquefasciatus* over wide range of temperature.

The adult emergence inhibition of *Culex quinquefasciatus* by *C. asiatica* leaf extract presented in Table II. The emergence inhibition of adult from the treatment of survivors with LC_{50}s of different temperature generally increased with temperature. Therefore, the data suggest that the leaf extract of *C. asiatica* also act as a adult emergence inhibition against mosquito *Culex quinquefasciatus*.

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). Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive, and are readily available in many areas of the world. According to Bowers *et al*. (1995) 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.

The crude extracts of the leaves of *C. asiatica* has been found to possess larvicidal and adult emergence inhibition activity against the mosquito *Culex quinquefasciatus.* The biological activity of the plant extract might be due to the various compound, including phenolics, terpenoids, and alkaloids, exist in plants, these compounds may jointly or independently contribute to produce larvicidal and adult emergence inhibition activity against *Culex quinquefasciatus*.

The larvicidal efficacy of *C. asiatica* is comparable to well established insecticidal plant species. Pizzarro *et al* (1999) studied the activity of the saponine fraction of *Agave sisalana* and estimated the LC_{50} and LC_{90} against 3^{d} instar larvae of *Culex quinquefasciatus,* that were 183 and 408 ppm, respectively. These concentrations were much higher than those reported in this study, but these authors suggested its use for control of this mosquito. The leaf extract of *C. asiatica* is superior to various neem extracts, which are reported to be effective with LC_{50} values ranging from 55-65 ppm against mosquito larvae (Ascher and Meisner 1989). The median lethal concentrations (LC_{50}) of various parts of *Melia azederach* ranging from 30-40 ppm against larva of *Culex pipiens* (Al-Sharook *et al*., 1991). The effect of the various neem extracts and various parts of *Melia azederach* was slightly lower than that reported for the *C. asiatica* leaf extract. The adult emergence inhibition activity of *C. asiatica* is also comparable to different species of plant extract in different families (Muthukrishnan *et al*., 1999; Pushpalatha and Muthukrishnan, 1999).

The findings of the present investigation revealed that the leaf extract of *C. asiatica* possess remarkable larvicidal and adult emergence inhibition activity against mosquito *Culex quinquefasciatus.* Further investigations are needed to elucidate this activity against a wide range of mosquito species and also the active ingredient(s) of the extract responsible for larvicidal and adult emergence inhibition activity in *Culex quinquefasciatus* should be identified and utilized, if possible, in preparing a commercial product / formulation to be used as a mosquitocidal.

Acknowledgement

The authors are grateful to the Professor and Head, Department of Zoology, Annamalai University for the facilities provided.

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