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Full Length Research Paper

Larvicidal efficacy of *Toddalia asiatica* (Linn.) Lam against two mosquito vectors *Aedes aegypti* and *Culex quinquefasciatus*

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The hexane, acetone and methanol extracts of mature fruits and leaves from *Toddalia asiatica* was investigated to establish its bio-control potentiality under laboratory condition against fourth instars larvae of Dengue vector, *Aedes aegypti* and Filarial vector, *Culex quinquefasciatus.* Hexane extract of fruits of *T. asiatica* showed highest larvicidal activity against both mosquito vector. LC_{50} value of hexane, acetone and methanol extracts of fruits against *A. aegypti* were 37.23, 50.69 and 125.55 ppm and against *C. quinquefasciatus* were 33.23, 82.20 and 215.19 ppm, respectively. Hexane, acetone and methanol extracts of leaves also showed potency against *A. aegypti* with LC_{50} values of 133.80, 177.20 and 79.48 and against *C. quinquefasciatus* with LC_{50} values of 164.53, 175.28 and 87.87 ppm, respectively .These results suggested that *T. asiatica* is promising as larvicide against both targeted mosquitoes.

Key words: *Toddalia asiatica,* larvicidal activity*, Culex quinquefasciatus, Aedes aegypti.*

INTRODUCTION

Insect-transmitted disease remains a major cause of illness and death worldwide (Pavela, 2009). Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc. causing millions of deaths every year (Rahuman et al., 2008). *Aedes aegypti*, a vector of Dengue and Dengue hemorrhagic fever, which is a widely distributed tropical and subtropical disease, is now endemic in more than 100 countries and threatens the health of approximately 2.5 billion people. Worldwide, around 80 million people are infected annually at an attack rate of 4% (Monath, 1994). In recent years, *A. aegypti* (Diptera: Culicidae) spread the virus of chikungunya which affected the southwest Indian Ocean islands in 2005, spread out to India, and resulted in an ongoing outbreak that has involved >1.5 million patients (Taubitz et al., 2007). *Culex quinquefasciatus*, the potential vector of bancroftian filariasis is the most widely distributed mosquito in India (Rahuman et al., 2009). It is responsible for major public health problem in India with around 31 million microfilaraemics, 23 million cases of symptomatic filariasis, and about 473 million individuals potentially at risk of infection (Agrawal et al., 2006).

The most effective way to combat with this mosquito infestation is the prevention of mosquito breeding through the use of larvicides. Synthetic insecticides such as, organophosphates have been used as larvicide in several countries for the last 30 years (Chavasse and Yap, 1997). However, one major drawback with the use of these chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment (Omena et al., 2007). The toxicity problem, together with the growing incidence of insect resistance, underscores the need for development of effective insecticides, which are environmentally safe, target specific and biodegradable.

Economically feasible plant secondary metabolites are considered to be a potential alternative approach against various stages and species of mosquitoes due to their excellent properties like cheap availability, environmental

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Table 1. Larvicidal efficacy of seeds extracts of *T. asiatica* (fruits) against *A. aegypti* and *C. quinquefasciatus.*

Solvent	Mosquito species	LC50 (ppm)	95% confidence interval		LC90 (ppm)	95% confidence interval		Chi-square
			Lower Bound	Upper Bound		Lower Bound	Upper Bound	
Hexane	*Aedes aegypti*	37.23	28.668	44.295	78.75	68.526	96.157	.454
	C. quinquefasciatus	33.23	24.453	39.977	71.64	62.020	88.831	.215
Acetone	*Aedes aegypti*	50.69	21.680	73.013	213.63	176.869	278.347	4.628
	C. quinquefasciatus	82.20	.544	168.247	201.57	134.641	655.708	8.919
Methanol	*Aedes aegypti*	125.55	83.883	164.478	446.86	374.010	566.745	5.189
	C. quinquefasciatus	215.19	136.617	315.137	527.86	399.508	844.975	8.133

LC50 = Lethal concentration that kills 50%of the exposed larvae after 24 h; LC90 = lethal concentration that kills 90%of the exposed larvae after 24 h.

safety nature and the presence of rich source of bioactive compounds, such as larvicidal, repellent, insect growth regulators, antifeedants, ovicidal, oviposition deterrence and reduction of fecundity and fertility (Rajkumar and Jebanesan, 2005; Elango et al., 2009; Kostic et al., 2008; Pavela et al., 2005).

In view of this increasing interest, an attempt was made in the present study to assess the larvicidal efficacy of *Toddalia asiatica* against two mosquito vectors; *A. aegypti* and *C. quinquefasciatus. T. asiatica* (L) Lam (Syn. *T. aculeata Pers*) is locally known as Jaluk bon or Tezmui in Northeast India. It is very rare and found some isolated pockets in Assam in the area of Manas National Park, Sibsagar, Saikhuwa, etc. Traditionally, the twig is used against toothache and gum infection, while the fruits are used against irregular menstrual cycle, fever and weakness (Sarma, 2003).

MATERIALS AND METHODS

Sample collection

The fruits and leaves of *T. asiatica* were collected from plants growing in Sibsagar district of Assam, India. After proper identification, the fruits were air dried under shade for 7 - 10 days at room temperature of 25 ± 2°C.

Preparation of plant extract

Dried fruits and leaves were powdered mechanically with the help of a Remi laboratory blender. Plant materials were then subjected to extraction with hexane, acetone and methanol using a Soxhlet extractor for 6 h. After collecting the extract, it was allowed to condense in a rotary vacuum evaporator. The condensed extract was then preserved in airtight bottles and stored in refrigerator until required for investigation for larvicidal activity.

Larvicidal bioassay

The larvicidal activity and the level of effectiveness of tested extracts against the targeted mosquitoes were analyzed using the standard World Health Organization procedure (WHO, 2005) with slight modification. Late third instars or early fourth instars larvae (25) were then distributed in each of the bowls (approximately 7.5 - 10 cm in diameter); each containing 25 ml of pre boiled distilled water. The necessary test concentrations were maintained accordingly. The experiments were carried out at 25 ± 2°C. The test mosquitoes were a laboratory strain of *C. quinquefasciatus* and *A. aegypti.* The authentic eggs of both strain were collected from previous culture that was maintained in Environmental Biotechnology Laboratory, Gauhati University, Assam. The mosquito colonies were maintained at 25 - 28°C and relative humidity 80 - 90% under a photoperiod of 14:10 h (light/night) without exposure to any insecticides and pathogen. During this course, larva were feed on finely ground dog biscuits and yeast powered (1:1) and the adult colony was provide with 10% glucose soaked cotton pad and blood meal in every three days with laboratory mice.

Data management and statistical analysis

Mortality counts were taken after 24 h exposure. The larvae that have pupated during the test were discarded. If more than 10% of the control larvae pupate in the course of the experiment, the test was discarded. The test with the control mortality of over 20% was unsatisfactory and in such case, the test was repeated. The average larval mortality data were subjected to probit analysis. For calculating LC_{50}, LC_{90} and other statistics at 95% fiducial limits of upper confidence limit, lower confidence limits and the chi-square values, statistical package for the social sciences (SPSS) software was used.

RESULTS AND DISCUSSION

In the preliminary screening, results reveal that the hexane extracts of fruits of *T. asciatic* was effective, compared with the other solvent extracts of fruits and leaves (Table 1 and 2). It showed highest larvicidal potency with LC_{50} value of 37.23 and 33.23 ppm against *A. aegypti* and *C. quinquefasciatus*, respectively. Acetone and methanol extracts of fruits also have moderate larvicidal activity against both mosquito vectors. LC_{50} value of the acetone and methanol extract of fruits against *A. aegypti* was found to be 50.69 and 125.55 ppm and that for *C. quinquefasciatus* was found to be 82.20 and 215.19 ppm respectively. Among the leaves extracts, methanol extract

Table 2. Larvicidal efficacy leaves extracts of *T. asiatica* against *A. aegypti* and *C. quinquefasciatus.*

Solvent	Mosquito species	LC50 (ppm)	95% confidence interval		LC90 (ppm)	95% confidence interval		Chi-square
			Lower bound	Upper bound		Lower bound	Upper bound	
Hexane	*A. aegypti*	133.80	109.286	161.074	320.59	273.913	394.119	2.307
	C. quinquefasciatus	164.53	47.775	287.333	478.42	335.375	994.149	2.639
Acetone	*A. aegypti*	177.20	145.097	212.275	434.67	374.651	525.196	4.637
	C. quinquefasciatus	175.28	61.397	300.813	499.34	352.246	1011.943	2.187
Methanol	*A. aegypti*	79.48	2.262	133.040	456.09	376.800	596.013	2.439
	C. quinquefasciatus	87.87	62.326	112.006	268.25	225.219	340.196	2.036

LC50 = Lethal concentration that kills 50%of the exposed larvae after 24 h; LC90 = lethal concentration that kills 90%of the exposed larvae after 24 h.

showed comparatively high potency with LC_{50} value of 79.48 and 87.87 against *A. aegypti* and *C. quinquefasciatus*, respectively. Hexane and acetone extract of leaves however showed less potency against *A. aegypti* (LC_{50} 133.80, 177.20ppm) and *C. quinquefasciatus* (LC_{50} 164.53 and 175.28 ppm), respectively. No mortality was observed in controls. Allethrin, which was taken as a reference compound for the evaluation of the toxicity to larvae recorded LC_{50} of 102.2 ppm against *A. aegypti* and 132.7 ppm against *C. quinquefasciatus*.

The secondary compounds of plants make up a vast repository of compounds with a wide range of biological activities (Chowdhury et al., 2008). This secondary metabolite is known to be effective against a wide range of insect pests as well as mosquito vectors (Sriwattanarungsee et al., 2008). These compounds may jointly or independently prove its efficacy against the mosquito targets by its ovicidal, larvicidal, pupicidal, adulticidal and by inhibition of growth activity. Several compounds of plant origin have already been reported. Still further researches are required to produce an alternative to synthetic pesticides. Earlier, Arunachalam and Kadarkarai, (2009) reported that *T. asiatica* leaf extracts has larvicidal activity against all larvae and pupae stages of *A. aegypti* with LC_{50} and LC_{90} ranging from 47.90 to 61.28 and 93.98 to 116.22 ppm, respectively. In the present study, LC_{50} and LC_{90} values of leaves extracts against larvae of *A. aegypti* varies from the earlier reports. This discrepancy is due to the fact that all individuals in the same plant specie do not always have an identical chemical composition and quantities, as the synthesis of bioactive compounds is regulated by different stress factors, such as soil temperature, soil type, pressure and overall climate of the area (Pandey et al., 2007).

However the present study revealed that *T. asiatica* contain active anti-larvicidal compound in its fruits. This makes it a more suitable candidate for the development of new potential eco-friendly larvicides. Further investigation is needed to identify the active compounds of these extracts responsible for its activity.

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