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RESEARCH ARTICLE

STUDIES ON CONTROL OF MOSQUITO, *AEDES AEGYPTI* (CULICIDAE : DIPTERA) USING THE CHLOROFORM LEAF EXTRACT OF *CENTELLA ASIATICA* AS A BIOCIDE

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Abstract:
In recent years much interest has been evinced in the use of plant products as insecticides to control vector as pests, in view of the environmental and health hazards by synthetic organic insecticides. In the present study, *Centella asiatica* plant extract was prepared using the solvent chloroform. The plant extract was tested against the various developmental stages of mosquito, *Aedes aegypti*.The level of the toxicity of *Centella asiatica* was expressed in terms of LC_{50}/24 hours values. The LC_{50} values of chloroform leaf extract for I, II, III & IV instar larvae of *Aedes aegypti* were 1.04%, 1.369%, 1.937% and 2.642% respectively. In the present study the preliminary phytochemical analysis showed the presence of flavanoids, alkaloids, terpenoids, glycosides and steroids in the chloroform leaf extract of *Centella asiatica*.The morphogenetic abnormalities are commonly caused by botanical extracts and the disturbance from the growth regulating hormones. It is therefore suggested that *Centella asiatica* derivatives are considered for vector control operations besides their use in other fields after exploring field trails.

Keywords: *Centella asiatica*, *Aedes aegypti*, Insecticide, Morphogenetic abnormalities, Biocide.

Introduction

Among the various groups of invertebrate animals, insects have a very close relationship with life and existance of mankind (Venkitaraman PR, 1983). In the insect group, many insects of the order Diptera act as vectors and play a role in spreading disease among man.Vector-borne diseases constitute the major cause for morbidity in most of the tropical and sub tropical countries and have always been a challenge to the medical professionals struggling for the welfare of humanity (Ramanathan, 2012).

WHO (2006) has declared the mosquitoes as “public enemy number one”. Mosquito borne diseases are prevalent in more than 100 countries across the world infecting over 700,000,000 people every year globally and 40,000,000 of the Indian population. *Aedes aegypti* is the main carrier for viruses that cause dengue haemorrhagic fever (DHF) and chikunguniya is found majorly in the tropics and subtropics. There is no effective vaccine against dengue and thus the only way of reducing the incidence of this disease is through mosquito control (Malavige *et al*., 2004).

Among the various control measures for mosquitoes, use of synthetic insecticides is still the most convenient and indispensable method all over the world. Chemical measures were first tried but they failed since their overuse caused irreparable hazards to the environment and humans also developing resistance among mosquito species and undesirable effect on non - target organisms, (Thangam and Kathiresan, 1990). The vast number of mosquitoes present naturally causes the civilians to use mosquito coils and liquidators which release CFC (chloro fluoro carbon) in a considerable amount that depletes the ozone which is harmful for the earth and human's future. These mosquitoes are vastly present in the developing countries where not much importance is given to the sanitation. Rain water and sewage can easily get stagnant in the roads and in the open spaces, these water stagnant and open sewage passages acts as a very good habitat for the mosquitoes to breed which seriously concerns the civilian's day to day life.

Several studies have emphasized the importance of research and development of herbal substances for controlling mosquitoes (Shaalan *et al*., 2005). Botanical insecticides may serve as suitable alternatives to synthetic ones in future, as they are relatively, environmentally safe effective and comparatively with human and animal life and environment and also inexpensive (Chaithong *et al*., 2006). Ethano botanical search reveals use of many traditional herbs in the treatment of various diseases which are usually free from side effects are economical and also accessible to humans and provide significant potential for the development of novel biomolecules. (Jiang Shiou Hwang , 2012).

A large number of plant extracts have been reported to have mosquitocidal as repellent activity against mosquito vector. (Ansari *et al*., 2000). Many studies on plant extracts against mosquito larvae have been conducted around the world. Extracts or essential oil from plants may be alternative sources of mosquito larval control agents as they constitute a rich source of bioactive compounds that are biodegradable into non toxic products and potentially suitable for use in control of mosquito larvae. Much effort has been focused on photo chemicals and their essential oils (Dharmagadda *et al.,* 2005) as potential sources of mosquito control agents as they are environment friendly and easily biodegradable. The study biologically active materials derived from plant sources can act as larvicides, insect growth regulators, repellents and oviposition attractants and have deterrent activities are observed many researches. (Venkatachalam & Jebanesan 2001).

The current study involved extraction and evaluation of leaf extract of *Centella asiatica* for larvicidal activities of *Aedes aegypti*. The primary objective of this work is to find a biological way to solution using the plant extract of *Centella asiatica* for the problem caused by the mosquito to the public.

MATERIALS AND METHODS:

For the present study the chloroform extract of *Centella asiatica* was used to test the efficiency and insecticidal effects against different developmental stages of the mosquitoes of *Aedes aegypti*.

Rearing and maintanance of the mosquitoes

Collection of eggs

The eggs of *Aedes aegypti* were collected from National Institute of Communicable Diseases (NICD) at Mettupapalyam and were placed separately in sterilized glass throughs containing two liters of unchlorinated tap water, (Size 18cm diameter and 9cm height) under laboratory conditions.

Rearing of larvae

A small container with water was kept inside the cage to facilitate the female to lay the eggs. The eggs in the container were removed carefully and allowed to hatch. Eggs were laid singly and placed on the dry surface water. Usually the eggs are laid early in the morning. They are oblong shaped. The incubation period of the normal eggs were 48 hours. The plastic trays were examined every six hours (6, 12, 18 and 24 hours) and the number of larvae was recorded. The mosquito colony was maintained at 7.0 - 8.5 pH, 28 ± 2^0c temperature and 14:10 light and dark photoperiod cycle. After 24 hours freshly hatched larvae were collected and maintained in separate containers with tap water (capacity 2liters), glucose biscuit and yeast (2:1) were given as the source of food.

Preparation of phytochemical extract

The leaves collected from the field were brought to the laboratory, washed with tap water followed by distilled water and were dried under shade. The dried leaves were ground to fine powder. The powdered leaves (100 gm) were extracted with chloroform (300 ml) by using soxhlet apparatus for 8 hours (Vogel, 1978). The extract was concentrated in a vacuum evaporator to yield dark greenish gummy extract. The residue was than made into 1% stock solution with acetone and taken for further bioassay test.

Preliminary phytochemical studies

The extract was subjected to determine the presence of groups of secondary metabolites present in the plant

materials such as alkaloids (Ciulci, 1994), flavanoids (Sofowara, 1993), glycosides (Gokhale *et al*., 2008), steroids (Ciulci, 1994), tannins (Mace and Gorbach, 1963:Ciului, 1994), saponins (Brain and Tum, 1975) and terpenoids (Harbone, 1973).

Larvicidal Bioassay

To obtain different concentrations of test medium the crude extract, 1 to 10ml of the stock solution were dissolved in water and mixed thoroughly with the dry ingredients of the diet as suggested by Miller *et al.,* (1993). Newly emerged batch of 20 early instars 1^{st}-4^{th} instar larvae of *Aedes aegypti* were transferred in 25 ml of water to a 500 ml bowl containing 240 ml of distilled water and 1 ml of the varying concentration of each plant extract. Three replicate tests were carried out simultaneously, the toxicity of each plant extract was evaluated with four to five concentrations yielding range of 0-100% mortality. The untreated larvae were maintained in water only. These bioassays were performed at 25-30^{o}C. Mortality in control was negligible. The mortality at the different concentrations, LC_{50} of the plant extract that can kill 50% of the treated stages of each samples were calculated and presented in the table 2.

RESULTS AND DISCUSSION

Vector control is facing a serious threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides or development of newer insecticides. S However due to the continuous increase in resistance of mosquitoes to familiar synthetic insecticides, better alternative means are sought. A considerable number of plant derivatives have been screened effective against mosquitoes (Sukumar *et al.,* 1991). There for it is necessary to look for and find a better larvicide which could provide a safer and long lasting control against *Aedes aegypti* mosquitoes.

In the present study the dried leaves of *Centella asiatica* was extracted with chloroform and the extract was evaluated for mosquitocidal effect against *Aedes aegypti.* Table 1 reveals the results of phytochemical screening of *Centella asiatica*. Chloroform extract showed the presence of alkaloids, flavanoids, glycosides, steroids and terpenoids. These compounds may jointly or independently contribute to produce maximum larvicidal activity against *Aedes aegypti* mosquitoes. Although these compounds arose as defences against phytophagous insects, they also effective against mosquitoes and other biting dipterans (Moore and Lenglet, 2004).

In the present investigation larvicidal efficacy of leaf extract of *Centella asiatica* was tested against the developmental stages of *Aedes aegypti.* The LC_{50} values and other associated statistics of a 24 hour bioassay study was calculated in the I,II,III,IV instar larvae of *Aedes aegypti.* The I, II, III and IV instar larvae of *Aedes aegypti* were exposed to 0.5, 1.0, 1.5, 2.0, 2.5% concentration. LC_{50} values of chloroform leaf extract for I, II, III, IV instar larvae of *Aedes aegypti* were 1.04%, 1.369%, 1.937% and 2.642% respectively.

Among the various results obtained with chloroform leaf extract of *Centella asiatica* acting on the I, II, III, IV instar larvae of *Aedes aegypti* at 24 hours exposure the III and IV instar larvae showed maximum activity, with LC_{50} values are 1.937% and 2.642%. From the overall results, it was interesting to note that I and II instar larvae were more susceptible than III and IV instar larvae. All these values are statistically significance at 5% level. Moreover behaviour changes were observed in the movement of the larvae. These effects may be due to the presence of neuro toxin compounds in the plant extracts. No behavioural changes were obtained in control. Similar results were obtained for D*atura stramonium, Lautana camera, Tridax procumbeus.* The petroleum ether extracts of the leaves of *Vitex negunde* were calculated for larvicidal activity with LC_{50} values of 2.4883mg/l against the 4^{th} instar larvae of *Culex tritacniorbynehus* (Karunamoorthi *et al*., 2008). Rehuman *et al*., 2007 reported that the larvicidal activity of petroleum ether leaf extracts of *Jatropa curcus, Pedilonthus amarus, Euphorbia hirta and Euphorbia tirucalli* with LC_{50} values of 8.79, 55.36 and 90.92ppm respectively against *Aedes aegypti.*

It was clear that, all concentrations eventually produced a high mortality at a characteristic point in larval stage, whereas the lower dose treatments give more dispersed actions. It caused harm to larvae during moulting especially at the time of metamorphosis. This strongly suggested that the action was a hormone mimic. However from the experiments it is difficult to speculate on the actual mode of action of the plant extract. All the high dose, death occurred most in the larval stage.

The findings of the present investigation revealed that the leaf extract of *Centella asiatica* possess remarkable larvicidal properties against *Aedes aegypti.* Further research undoubtedly will lead to improve formulations with enhanced activity which may eventually become environmentally acceptable and replace objectionable conventional insecticides for mosquito control.

Table 1. Results of preliminary phytochemical screening of *Centella asiatica* using chloroform extract

SECONDARY METABOLITES						
Alkaloids	Flavonoids	Glycosides	Steroids	Tannins	Saponins	Terpenoids
+	+	+	+	-	-	+

Table 2. LC_{50} values of chloroform leaf extract of *Centella asiatica* on I,II,III and IV instar larvae of *Aedes aegypti*

Larval stages	% of mortality						LC50 (%)	Regression equation	Chi-square
	Control	0.5 %	1.0 %	1.5 %	2.0 %	2.5 %			
I- instar	0	26	52	76	100	100	1.04(1.453-0.734)	Y=39.2x+12	0.413
II- instar	0	20	35	57	76	96	1.369(1.685-1.042)	Y=38.6x-1.1	0.209
III- instar	0	18	32	50	69	93	1.937(2.3715-1.437)	Y=37.4x-22.4	0.548
IV- instar	0	15	30	46	62	86	2.642(2.965-2.174)	Y=34.8x-39.2	0.716

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