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RESEARCH ARTICLE

A PRELIMINARY STUDY ON LARVICIDAL EFFICACY OF THREE TRADITIONAL MEDICINAL PLANTS AGAINST DENGUE VECTOR, AEDES AEGYPTI

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ABSTRACT

Synthetic insecticides used to control mosquito vectors have led to the development of mosquito resistance, environmental pollution and undesirable adverse effects on non-target organisms. Thus use of herbal insecticides as a means of vector control is getting wide acceptance. The present study was conducted to determine the effectiveness of leaf extracts of *Glyricidia maculate*, *Manihot esculanta* and *Glycosmis pentaphylla* against dengue and chikunguniya vector *Aedes aegypti*. The larvicidal efficacy was tested against the early 4th instar larvae of *Aedes aegypti* at concentrations of 500 and1000 ppm. Larval mortality was observed against petroleum ether, methanol and ethyl acetate extracts after 24h and 48 h. *Manihot esculanta* extract in ethyl acetate and *Glycosmis pentaphylla* extract in petroleum ether provided highest mortality rates of 69.9% and 69.6% respectively with 500ppm in 24 hrs against *Aedes aegypti*. Further investigation is needed to explore the bioactive principles involved in action.

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INTRODUCTION

Mosquito-borne diseases have an economic impact, including loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates. Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, filariasis, schistosomiasis, japanese encephalitis (JE), etc., causing millions of deaths every year (James, 1992). Mosquito transmitted disease continues to be a major source of illness and death. Most parasitic diseases are tropical, and the intensifying globalization and climatic change are increasing the risk of contracting arthropod-borne illnesses (Brower and Chalk, 2003; Guenier *et al.,* 2004). *Aedes aegypti*, the main carrier for viruses that cause dengue and dengue hemorrhagic and yellow fevers, is found majorly in the tropics and subtropics. There is no effective vaccine against dengue, and thus the only way of significantly lowering the incidence of this disease is through mosquito control (Malavige *et al.*, 2004). The main vector *Aedes aegypti* is a cosmotropical species that proliferates in water containers in and around houses. They breed in many types of household containers, such as water storage jars, drums, tanks, and plant or flower containers (Honorio*et al.,* 2003; Harrington *et al.,* 2005). Vector control is of serious concern in developing countries like India due to the lack of general awareness, development of resistance and socioeconomic reasons. Every year, a large part of the population is affected by one or more vector borne diseases. Vector control, which includes both anti-larval and anti adult measures, constitutes an important aspect of any mosquito control programs. Control either by biological or chemical means is the basic requirement for planning an effective vector control strategy. Chemical control is an effective strategy used extensively in daily life. Synthetic insecticides are today at the forefront of mosquito-controlling agents. However, the environmental threat that these chemicals pose, effects on non-target organisms, and the resistance of mosquitoes to insecticides have all increased during the last five decades (Wattanachai and Tintanon, 1999; Amer and Mehlhorn, 2006a, b).

There is a critical need for cheap and effective control campaigns, as were implemented during the DDT era. A creative and organized search for new strategies, perhaps based on new technologies, is urgently required irrespective of future climate change (Reiter 2008). Several studies have emphasized the importance of research and development of herbal

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substances for controlling mosquitoes (Shaala *et al.,* 2005). Their results may vary, but natural plant products may be a possible alternative to synthetic substances, as they are effective and compatible with human and animal life and the environment (Chaithong *et al.,* 2006).The plant materials are non-toxic to non-target animals, have no phytotoxic properties, and leave no residue in the environment. Scientists therefore have embarked on a mission to survey the flora extensively to discover more and more potential plants having insecticidal properties (Dwivedi and Kavitha, 2001). The present study investigated the larvicidal efficacy of *Gliricidia maculate, Manihot esculenta* and *Glycosmis pentaphylla* against *Aedes aegypti*.

MATERIALS AND METHODS

Phytoextraction

The dried leaves of *Gliricidia maculate, Manihot esculenta* and *Glycosmis pentaphylla* were mechanically grinded to get fine powder. 20g of dried powder were taken within a thimble made of filter paper. The solvent (250 ml of ethanol or petroleum ether or ethyl acetate) was added to a round bottom flask, which is attached to a Soxhlet extractor and condenser on an isomantle. The crushed plant material was loaded into the thimble, which is placed inside the Soxhlet extractor. The side arm is lagged with glass wool. The solvent was heated using the isomantle and began to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. The process should run for a total of 24 hours. Once the process has finished, the ethanol should be evaporated, leaving a small yield of extracted plant material (about 2 to 3 ml) in the glass bottom flask. The mass of this remaining extract is measured and stored in an air tight bottle for further experiment.

Mosquito Larvae

Larvae of *Aedes aegypti* were reared in plastic trays (36x24x7) containing dechlorinated water. Larvae were fed a diet of yeast and powdered dog biscuits in the ratio of 2:1, kept at 26 ± 2º C and 75% - 80% relative humidity, with a photoperiod of 12:12 Light and Dark h for the larval growth. Early fourth instar larvae were used for bioassay.

Larval bioassay

Larval bioassays were performed according to the standard guidelines of WHO (2005). Stock solutions of the solvent extracts were prepared and used to make different concentrations. Twenty five larvae were introduced into a 500 ml beaker containing 249 ml of water with one ml of acetone dissolved with desired concentration of the extract. A minimum of three replicates were kept for each concentration along with the control. Mortality was recorded after 24 and 48h at 26^{o} C.

Data analysis

The control mortality was corrected using Abbott's (1925) formula, if mortality was between 5-20%. Standard deviations were calculated to pool the bioassay data of duplicates.

RESULTS

The relative larvicidal efficacy of three plants extracted in different solvents were recorded (Table 1, 2 and 3). Among them, *Manihot esculanta* extract in ethyl acetate and *Glycosmis pentaphylla* extract in petroleum ether provided highest mortality rates of 69.9% and 69.6% respectively against *Aedes aegypti*. The mortality rates considerably varied between two test concentrations taken for bioassay such as 500 and 1000 ppm. Ethyl acetate extract of *Manihot esculenta* shows highest variation between 500 and 1000ppm at 24 hrs.

Table 1. Percentage of larvicidal efficacy of *Glycosmis pentaphylla* leaf extracts against *Aedes aegypti*

Extracts	Concentration in ppm			
	500		1000	
	%mortality 24 hour	%mortality 48 hour	%mortality 24 hour	%mortality 48 hour
Ethyl Acetate	52.05±0.05	80.09±0.09	61.75±0.75	79.99±0.99
Methanol	42.03 ±0.03	65.23±0.23	81.21±0.21	88.09±0.09
Petroleum Ether	69.6 ±0.20	80.10±0.10	75.20±0.20	90.40 ±0.40

Table 2. Percentage of larvicidal efficacy of *Manihot esculenta* leaf extracts against *Aedes aegypti.*

Extracts	Concentration in ppm			
	500		1000	
	%mortality 24 hour	%mortality 48 hour	%mortality 24 hour	%mortality 48 hour
Ethyl Acetate	69.9 ± 0.00	82.06 ± 0.06	78.4 ± 0.4	89.06 ± 0.06
Methanol	54.6 ± 0.6	79.08 ± 0.08	66.00 ±0.00	71.09 ± 0.09
Petroleum Ether	58.6 ± 0.6	82.10 ± 0.10	68.8 ± 0.80	73.00 ± 0.00

Table 3. Percentage of larvicidal efficacy of *Glyricidia maculate* leaf extracts against *Aedes aegypti*

Extracts	Concentration in ppm			
	500		1000	
	%mortality 24 hour	%mortality 48 hour	%mortality 24 hour	%mortality 48 hour
Ethyl Acetate	30.72 ± 0.72	37.70 ± 0.70	32.31 ± 0.31	49.08 ± 0.08
Methanol	46.09 ±0.09	65.03 ± 0.03	27.70 ± 0.70	59.20 ± 0.20
Petroleum Ether	33.03 ±0.03	47.08 ± 0.08	36.07 ±0.07	62.03 ±0.03

In general, it is observed that the methanolic extracts show highest variation in mortality between 500ppm and 1000ppm at 24 hrs. Mortality rate increases to more than 90% in 1000 ppm while it remains around 30%in 500 ppm. Different extracts of the same plant have also shown similarity in mortality percentages. *Gliricidia maculate* extract in ethyl acetate & petroleum ether and *Manihot esculanta* extract in ethyl acetate provided the least mortality rates of 30.72% and 33.03%.

DISCUSSION

According to Bowers *et al*. (1995) the screening of locally available medicinal plants for mosquito control would reduce dependence on expensive imported products and stimulate local efforts to enhance public health. The biological activity of these plant extracts might be due to the various compounds, including phenolics, terpenoids and alkaloids existing in plants (Park *et al.*, 2000). These compounds may jointly or independently contribute to produce larvicidal activity against mosquito larvae. The results came out of the current investigation is also promising and comparable to earlier reports. Sharma *et al*.in 2005 studied the acetone extract of *Neriumindicum* and *Thenus orientalis* with LC_{50} values of 200.87, 127.53, 209.00, and 155.97 ppm against the larvae of *An. Stephensi* and *Cx. Quinque fasciatus* respectively. Singh and Bansal (2003) have tested the aqueous extracts of different parts of *Solanum xanthocarpum* against the larvae of a few mosquito species. The efficacy of water extract of citrus-seed extract showed LC_{50} values of 135, 319.40 and 127, 411.88 ppm against the larvae of *Ae. Aegypti* and *Cx. Quinque fasciatus* (Sumroiphon *et al.,* 2006).

The benzene extracts of *C. vulgaris* exerted 100% mortality at 250 ppm, a very low hatchability (11.8%) at 200 ppm, and complete ovicidal activity at 300 ppm. The fraction I at 80 ppm exerted a very low hatchability rate of 3.2% followed by fraction II (6.9%), and fractions III and IV afforded 4.9% and 5.3% hatchability recorded against *Anopheles stephens i*and *Aeades aegypti* respectively. The results of this study also open the possibility for further investigations of the efficacy of larvicidal properties of plant extracts, which in turn raise the opportunity for eco-friendly control of mosquito vectors by herbal insecticides.

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