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Larvicidal activity of methanolic leaf extracts of plant, *Chromolaena odorata* L. (Asteraceae) against vector mosquitoes

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Abstract
Mosquitoes transmit malaria, filariasis, dengue, chikungunya, etc. Repeated use of insecticides for mosquito control has caused development of resistance, adverse effects on non-target organisms and serious environmental concerns. Hence alternative control measures are being explored *inter alia* plant based insecticides. We carried out larvicidal bioassays with methanolic extract of leaves of *Chromolaena odorata* (family Asteraceae) against late instar larvae of disease vectors *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. The highest mortality was observed in *Cx. quinquefasciatus* [LC_{50} = 43 ppm, (95% CI: 34 - 48 ppm); LC_{90} = 110 ppm (CI: 94 - 135 ppm)] followed by *Ae. aegypti* [LC_{50} = 138 ppm, (CI: 121 - 157 ppm); LC_{90} = 463 ppm (CI: 386 - 584 ppm)] and *An. stephensi* [LC_{50} = 1613 ppm (CI: 1364 - 1890 ppm); LC_{90} = 8306 ppm (CI: 6598 - 11076 ppm)]. Being larvicidal, leaf extracts of *Chromolaena odorata* could be explored further.

Keywords: *Chromolaena odorata*, leaf extract, *Anopheles stephensi*, *Culex quinquefasciatus*, *Aedes aegypti*, Larvicidal activity.

1. Introduction
Mosquitoes are the major vectors for the transmission of Malaria, Dengue, Chikungunya, Filariasis, and Japanese encephalitis, etc. posing a major public health problem and resulting in extensive morbidity and mortality each year globally. Malaria is one of the most important causes of direct or indirect mortality in infants, children and adults with approximately two to three million new cases arising every year. Approximately 2,400 million (about 40%) of the world's population live under the risk of malaria alone. And India contributes 77 per cent of the total malaria burden in Southeast Asia [1]. Approximately 1.2 billion of the global population is at risk of lymphatic filariasis of which currently more than 120 million people are affected including 25 million men who suffer from the genital swellings and 15 million people from severe lymphoedema or elephantiasis of the legs [2]. In India, seventeen States and six Union Territories are endemic to filariasis with about 553 million people exposed to the risk of infection, 31 million mf carriers and 23 million suffer from disease manifestations [3] and the annual economic loss is $1 billion USD [4]. An estimated suspected chikungunya fever cases reported in India between 2007 and 2012 ranged between 15,783 and 59,535 annually [5]. Similarly from 2007 to 2012, 24 States/UTs reported suspected Dengue cases ranging from 5,534 to 47,029 annually and deaths from 69 to 242 with a rising trend [5].
It is well established that repeated use of synthetic chemical insecticides for mosquito control has led to interference in the natural biological control eco-systems, which in turn in part, might have led to resurgences in the target mosquito populations. It has also resulted in the development of resistance [6] undesirable effects on non-target organisms [7] and serious environmental and human health concerns. These phenomena have resulted in search for alternative control measures *inter alia* herbal based insecticides. Plants are rich source of bioactive chemical compounds with insecticidal properties. The activity of crude plant extracts is often attributed to the complex mixture of active compounds. Crude extracts of leaves or bark of these plants have been tested earlier by several investigators [8-19]. In view of an increasing interest in developing insecticides of plant origin as alternative to chemical insecticide, we tested methanol extract of dried leaves of *C. odorata*, of family Asteraceae for larvicidal activity against III & IV instar larvae of urban

malaria vector, *Anopheles stephensi* Liston 1901, filaria vector, *Culex quinquefasciatus* Say 1823; Dengue and Chikungunya vector, *Aedes aegypti* Linn 1762.

2. Materials and Methods

2.1 Collection of plant material

Healthy leaves of *C. odorata* were collected from the road sides of Mayem village, Bicholim 'taluka', Goa, India. All leaves were washed and dried in shade for a week for further processing.

2.2 Preparation of plant leaf extract

The leaves were powdered mechanically using commercial electrical stainless steel blender and was further macerated with (1:2.5w/v) methanol at room temperature for three days and then filtered by suction through Buckner funnel (Whatman filter paper 0.25 mm pore size). The solvent was removed by rotary evaporation under reduced pressure 22-26 mm Hg at room temperature and the residues obtained were stored at 4 ºC. The residues were then used to prepare one per cent stock solution with methanol. From the stock solution, different dilutions were prepared with distilled water.

2.3 Mosquito culture

Bioassays were carried out against the larvae of the mosquito species, *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*. These mosquito species were reared [20] in the insectary of the National Institute of Malaria Research, Field unit, Campal, Panaji-Goa. Larvae of these three species were fed on a diet of commercially available baby food of trusted brand mixed with ground fish food in a ratio of 2:1. Late 3rd and early 4th instar larvae were used to screen the larvicidal activity of the methanolic extract of the leaves.

2.4 Larvicidal bioassay

To perform larvicidal activity, 25 healthy larvae of 3rd/4th in star were introduced into each 450 ml capacity plastic bowls containing 200 ml of leaf extract of desired concentration adjusted in water. All the experiments were carried out at room temperature of 27±2 °C and relative humidity of 75–85 per cent. Bioassays were performed as per WHO [21] procedure with some modification as per the method of Rahuman, *et al.* [19]. From the stock solution, different concentrations ranging from 100 to 10,000 ppm were prepared. For all the bioassays, four replicates of 25 larvae were taken in 200 ml of water with desired concentration of the plant extract along with controls with methanol at the same concentration as used for dissolution and preparation of leaf extract and water. Mortality was recorded after 24 hours. The numbers of dead larvae were counted and the percentage of mortality was recorded from the average of four replicates. All the experiments were carried out in 5 replicates and repeated 4 times.

2.5 Statistical analysis

The percent mortalities were corrected using Abbott's formula [22] and the average larval mortality data were subjected to probit analysis for calculating LC_{50} and LC_{90}, 95% confidence limits and Z test values by using the SPSS (SPSS-PASW-1.8.0) software. $P<0.05$ was considered to be statistically significant.

3. Results & Discussion

Results of the study to determine the effect of treatment of methanolic leaf extracts of *C. odorata* on the larval stages of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* indicated deleterious effect resulting in larval mortality. There was no mortality in control. The results of screening are shown in Table 1 & Fig. 1, 2 and 3

Table 1: Laboratory evaluation of leaf extract of *C. odorata* against larval stages of different mosquito vector species

Mosquito species	LC_{50} (ppm) (95% CI: Lower bound-upper bound)	LC_{90} (ppm) (95% CI: Lower bound-upper bound)	X^{2}* (df)	SE	Z	p	S/ NS
Culex quinquefasciatus	43 (38-48)	110 (94-135)	23.09* (4)	0.108	16.636	<0.001	**S**
Aedes aegypti	138 (121-157)	463 (386-584)	5.043* (6)	0.194	12.612	<0.001	**S**
Anopheles stephensi	1613 (1364 -1890)	8306 (6598-11076)	91.56* (8)	0.108	16.636	<0.001	**S**

*25 number of 3rd and 4th instar larvae exposed per replica & 20 total replicates (5 replicates each time) were carried out

SE= Standard Error: Z= Z-test p= Probability S/NS=Significant/Not Significant

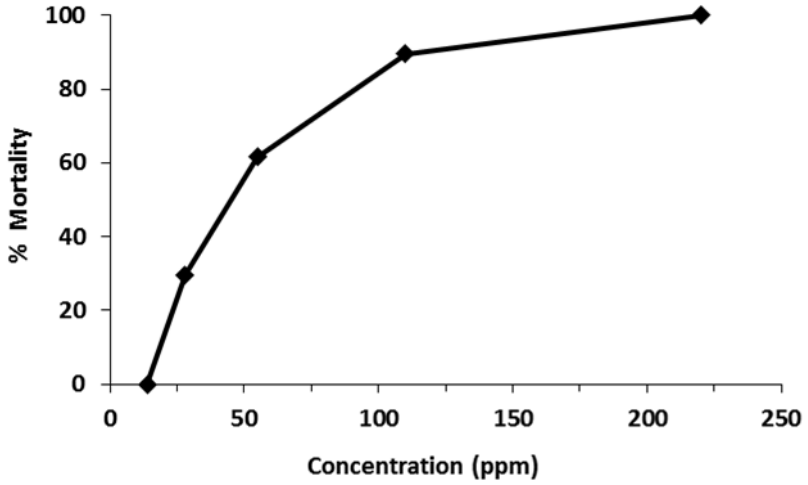

Fig 1: Bioassay of leaf extract of *C. odorata* against 3rd/4th instar larvae of *Culex quinquefasciatus*

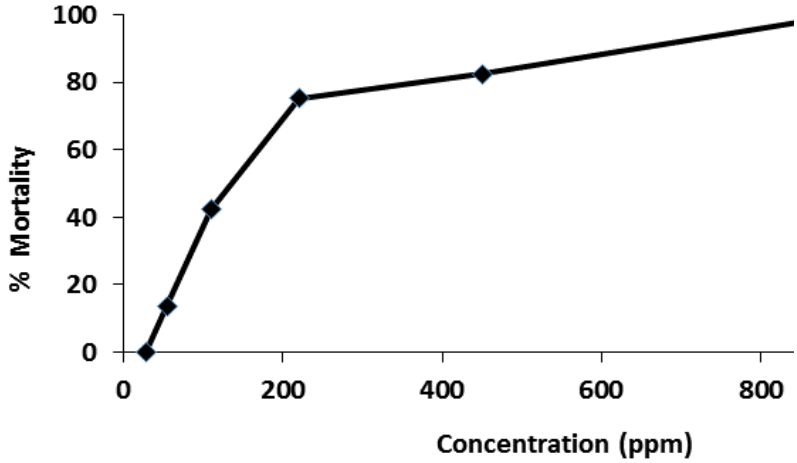

Fig 2: Bioassay of leaf extract of *C. odorata* against 3rd/4th larvae of *Ae. aegypti*

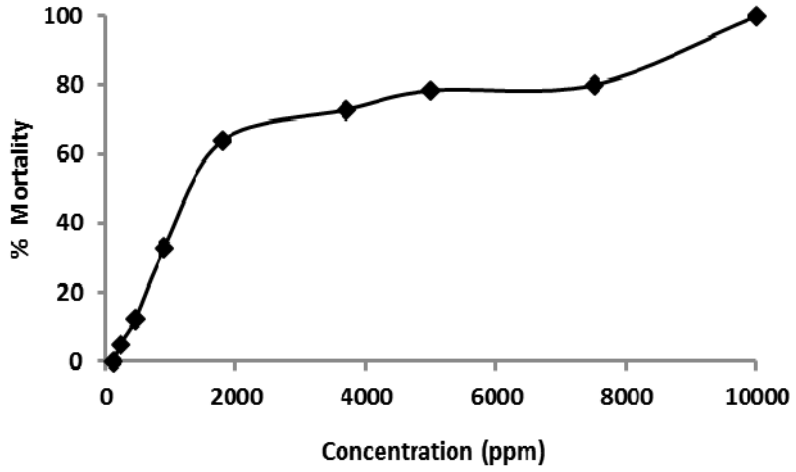

Fig 3: Bioassay of leaf extract of *C. odorata* against 3rd/4th instar larvae of *An. stephensi*

Under laboratory conditions 125 3rd/4th instar larvae (5 replicates with 25 larvae each) of *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* were exposed to each of the concentrations of methanolic leaf extract separately. The methanolic extract tested against *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* resulted in 100 per cent larval mortality after 24 h exposure at the concentrations of 220, 900 and 10,000 ppm respectively. The LC_{50} values for *Cx.*

quinquefasciatus, *Ae. aegypti* and *An. stephensi* were 43, 138 and 1,613 ppm respectively. Similarly the LC_{90} values for these three species were 110, 463 and 8,306 ppm respectively. All 500 larvae of *Cx. quinquefasciatus* exposed to 220 ppm concentration, all died in 24 h. Whereas, with same concentration of leaf extract, the mortality of the *Ae. aegypti* larvae was 75% and in *An. stephensi* 4.3% respectively in 24 h under laboratory conditions. In case of *An. stephensi* increase in test concentrations between 110 ppm and 1,800 ppm resulted in a steady increase in the percent mortality, whereas at higher concentrations beyond 1,800 ppm per cent further increase in larval mortality was not so significant (Fig. 1). Similar trends in larval mortality were observed in case of *Ae. aegypti*, *Cx. quinquefasciatus* (Fig 2 & 3). These observations revealed that the methanolic extract was relatively more toxic to *Cx. quinquefasciatus* than against *Ae. aegypti* and the least in case of *An. stephensi.*

In the present study with leaf extract of *C. odorata* only 1.2% mortality was observed in malaria vector *An. stephensi* at 220 ppm concentration and the mortality increased as the concentration was increased in a dose dependant manner and absolute mortality was observed at a concentration of 10,000 ppm. Against filaria vector, *Cx. quinquefasciatus* only 3.7% mortality was recorded at a concentration of 28 ppm. There was no marked difference in mortality at the concentrations of 55 and 110 ppm. However, cent per cent mortality was observed at a concentration of 220 ppm. Investigations against larvae of dengue, chikungunya and West Nile virus vector *Ae. aegypti* showed that 42.5% mortality occurred at a concentration of 55 ppm and total mortality was observed at a concentration of 900 ppm.

Many plant chemicals have larvicidal effects. The differential responses induced by the active ingredients on various species of mosquitoes are influenced by many factors such as the species of plant, the parts of the plant, the solvents used for extractions, the geographical location where the plants grow and the methods employed for extraction [23-24]. Preliminary screening is a good approach to evaluate the potential larvicidal activity of plants and their parts [25-27]. Approximately 2,000 plant derivatives have been screened for insecticidal properties [28] and good deal of work is being done in India to search for alternative eco-friendly and effective larvicides of plant origin [29-31]. Our observations are similar to that made by Sujatha *et al* [32] with petroleum ether extract of six plants *Acorus calamus*, *Ageratum conyzoides*, *Annona squamosa*, *Bambusa arundanasia*, *Madhuca longifolia* and *Citrus medica* which were found effective against 3 vector species of mosquitoes, *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus.* Similarly Raghavendra *et al.* [33] have reported toxicity of hexane extract of the dried fruit of *Solanum nigrum* against five mosquito species of three genera: *An. culicifacies* sibling species A, *Ae. aegypti*, *An. culicifacies* sibling species C, *Cx. quinquefasciatus* and *An. stephensi*. Our results show that the methanol leaf extract of *C. odorata* was relatively more toxic to *Cx. quinquefasciatus* followed by *Ae. aegypti* and *An. stephensi.* Similar findings were reported by Das *et al.* [9], who have reported that the ethanol leaf extract of *A. squamosa* and found promising larvicidal activity against *Cx. quinquefasciatus* larvae and larvae of *An. stephensi*.

Though larvicides play a vital role in controlling mosquitoes in their breeding sites, these could also be detrimental to the beneficial and non-target organisms due to their indiscriminate action. Hence new alternative insecticides need to be evaluated against non-target organisms which share habitats with mosquito larvae so is the case of *C. odorata* and if found safe, it can be developed as an eco-friendly larvicide. The feasibility of its large scale use in field, however, will encompass toxicity testing against non-target organisms, formulation as insecticide, conduction of phase II and III field trials and mandatory regulatory approvals. In conclusion, our findings suggest that the methanol leaf extract of *C. odorata* was effective against larvae of 3 important vector species.

4. Conclusion

The present study on preliminary screening has shown that methanolic leaf extract of the plant *Chromolaena odorata,* L has anti-larval activity against the vectors, *An. stephensi. Cx quinquefasciatus* and *Ae. aegypti.* The extract was relatively more toxic to *Cx. quinquefasciatus* than against *Ae. aegypti* and the least in case of *An. stephensi.* The leaf extract of this plant therefore could be a potential source of herbal larvicide for vector control and could be used in integrated vector management which is being encouraged by WHO [34].

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