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Larvicidal Activity of Crude Plant Extract against Fourth Instar Larvae of *Anopheles subpictus* and *Culex quinquefasiatus* Mosquitoes

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Abstract: ***The present study was carried out to evaluate the Larvicidal activity of crude Chloroform, Ethyl alcohol, Petroleum ether and leaf extract of Murraya koenigii were tested against fourth instar larvae. It is reared in the laboratory and used for larvicidal bioassay against malarial vector, Anopheles subpictus (LC_{50}=169.55) (LC_{90}=588.27) and filarial vector , Culex quinquefasiatus (LC_{50}=186.47) (LD_{90}=669.76).And higher mortality of larvae is measured at 1000 ppm concentration., the larval mortality were observed after 24 hr of exposure. These result suggested that higest mortality of Anopheles subpictus and Culex quinquefasiatus was found in Murrya chloroform extract. This study investigates the larvicidal potential of indigenous plant extracts from commonly used medicinal plants as an environmentally safe measure to control mosquito larvae. An attempt is made to emphasize and create awareness of the great potential of the plant in India for its application as herbal pesticides to kill mosquito larvae.***

Keywords: Larvicidal, *Murraya koenigii*, *Anopheles subpictus and Culex quinquefasiatus.*

1. Introduction

Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, filariasis and Japanese encephalitis (Das, 2003). Mosquito alone transmit diseases to more than 700million people annually and the disease are endemic in more than 100 countries (Jang *et al.*, 2002). Mosquito in the larval stage are attractive targets for pesticides because they breed in water and thus, are easy to deal with them in this habitat(Nandita,2008). Continued and repeated use of conventional mosquitocides such as organophosphorus (op) and carbmate insecticides, insect growth regulators and bacterial larvicides has often resulted in the widespread development of resistance and has undesirable effects on non-target organisms. (Rozendal, 1997; WHO, 2006).

Plants are rich source of alternative agents for the control of mosquitoes, because they possess bioactive chemical which act against a number of species including specific target insect and are more environmentally friendly when used in pest control (Lok and Singh, 2003).

Mosquitoes control has become increasingly difficult because of the indiscriminate uses of synthetic chemical insecticides which have an adverse impact on the environment and disturb the ecological balance. The majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects (Ghosh, 1991).

Murraya koenigii L. (curry leaf) belonging to family Rutaceae is used as a spice for its characteristic flavour and aroma. It is reported to have antioxidant, anti-diabetic, anticarcinogenic, antidysenteric, stimulant, hypoglycaemic and antimicrobial activities (Khanum, Anilakumar, Sudarshana Krishna, Viswanathan and Santhanam 2000). Biologically active carbazole alkaloids are reported to have antimicrobial properties (Ramsewak, Nair, Strasburg, De Witt and Nitiss 1999). Curry leaves have been reported to contain tocopherol, b-carotene, lutein and alkaloids (Khanum *et al.* 2000).

The misuse and excessive use of synthetic insecticides may cause some undesirable effects not only to the agricultural ecosystem but also to human health due to insecticide residue in food (Dadang *et al.* 2009).

This study of medicinal plants is used for the effectiveness to control mosquito larva.

2. Materials and Methods

2.1. Collection of plant material

The Leaves of *Murraya koenigii* L.(Rutaceae) were collected from Jawadhu Hills, Tiruvannamalai region (altitude 705 m), Tamil Nadu, South India. In the present study, the experimental plants were selected based on the ethnobotanical information collected through different literature sources. The taxonomic identification of plants was made by Dr.C.Hema, Department of Botany, Arignar Anna Government Arts College for Women , Walajapet, India. The voucher specimen was numbered and kept in our research laboratory for further reference.

2.2. Preparation of plant extracts

The leaves were dried for 7-10 days in the shade at the environmental temperatures (27-37^{o} C day time). The leaves (500 g) were powdered mechanically using commercial electrical stainless steel blender and extracted with chloroform (1,000 ml, Fine Chemicals, Qualigens), ethyl acetate (1,500 ml, Qualigens, Fine Chemicals, Mumbai,

India), and petroleum ether (1,800 ml, Qualigens), in a soxhlet apparatus (boiling point range 60–80°C) for 6hr. The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper. The extract was concentrated under reduced pressure 22 - 26 mm Hg at 45°C and the residue obtained was stored at 4°C.The residues were then made in to a 1 per cent stock solution with acetone (stock solution). From the stock solution, 1000-31ppm, dilutions were prepared with dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05per cent in the final test solution(Mehra and Hiradhar 2000).

2.3 Mosquito culture

Anopheles subpictus and Culex quinquefasciatus were collected from rice field and stagnant water areas of Melvisharam and identified in Zonal Entomological Research Centre, Vellore, and TamilNadu. To start the colony and larvae were kept in plastic and enamel trays containing tap water. They were maintained and all the experiments were carried out at27 ± 2°C and 75–85 percent relative humidity under14:10 h light and dark cycles. Larvae were fed a diet of Brewer's yeast, dog biscuits and algae collected from ponds in a ratio of 3:1:1, respectively as per the method of (Kamaraj *et al*. 2009).

2.4. Larvicidal bioassay

Early fourth-instar larvae were used for bioassay test. A total of 100 larvae were exposed in five replicates of 20 larvae each. Experiments were conducted for 24 h at room temperature (28±2°C). The control was setup with solvent and polysorbate 80. The experimental media, in which 100% mortality of larvae occurs alone, were selected. The different fractions isolated were tested against the early fourth-instar larvae of mosquitoes by the procedure of WHO (1996) with some modification and as per the method of (Rahuman *et al.* 2000). For Bioassay test, larvae were taken in five batches of 20 in 249 ml of water and 1.0 ml of plant extract concentration. The numbers of dead larvae were counted after 24 hr of exposure, and the percentage mortality was reported from the average of five replicates.

3. Results and Discussion

The activity of crude plant extracts is often attributed to the complex mixture of active compounds .Hence larvicidal activity of different solvent that is chloroform,ethyl alcohol and petroleum ether extracts of *Murraya koenigii* were tested against *Anopheles subpictus* and *culex quinquefasciatus*.

3.1 Larvicidal activity of different concentrations leaves chloroform extracts against Anopheles subpictus and culex quinquefasciatus.

The preliminary screening is a better mean of larvicidal activity different solvents crude leaf extracts of *Murraya koenigii* are noted and presented(Table 1).Among the crude extracts tested larvicidal activity showed that the leaf *Murrya Koengii* with chloroform extract shows 100% mortality (Table 1) against *Anopheles subpictus*(Table 2) Lc_{50}=169.55 and LC_{90} =588.27 and *culex quinquefasciatus* (Table 2) Lc_{50} =186.47 and LC_{90}=669.76 at 1000ppm (Highest concentration) 31.25(lowest concentration) *Murrya Koengii* extract against *Anopheles subpictus* (Table 1) 7%Mortality and *culex quinquefasciatus* 8% Mortality.

Table 1: Larvicidal activity of different concentrations leaves Chloroform extracts against *Anopheles subpictus and Culex quinquefasciatus.*

Murrayakoenigii (Linn.)	1000	100±0.00	100±0.00
	500	89±1.15	86±1.20
	250	55±1.72	52±1.71
	125	36±1.87	28±1.78
	62.5	16±2.69	15±2.62
	31.25	07±1.30	08±1.12

Control—nil mortality. * Mean value of five replicates ± SD standard deviation.

Table 2: LC_{50}, LC_{90}, and other statistical analysis of different test samples larvicidal activity of different concentrations leaves of chloroform, ethyl alcohol and petroleum ether extracts against *Anopheles subpictus and Culex quinquefasciatus.*

S.No.	Plant name	Extract	Parasite	Lc_{50}± SE	LC_{90}±SE	$\chi 2$ (df =4)
1.	*Murraya koenigii*	Chloroform	*Anopheles subpictus*	169.55±10.52	588.27±59.87	10.85
2.	*Murraya koenigii*	Chloroform	*Culex quinquefasciatus*	186.47±11.89	669.76±72.11	16.51

LC_{50}- Lethal concentration that kills 50% of the exposed larvae, LC_{90} - Lethal concentration that kills 90% of the exposed larvae, UCL= Upper confidence Limit, LC = Lower confidence Limit, $\chi 2$ -Chi-square, df-Degree of freedom, Significant at P< 0.05 level.

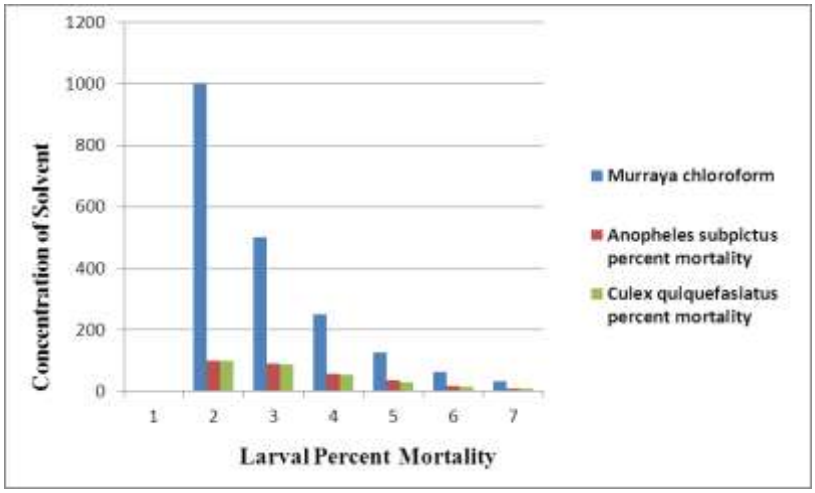

Figure 1: Larvicidal Activity of *Anopheles subpictus* and *Culex quinquefasciatus* in *Murraya* chloroform extract.(1000 ppm – 100% mortality)

4. Conclusion

The present experimental result showed that the fractionated chloroform extract possess effective larvicidal properties against *Culex* larvae and *Anopheles* larvae. Whereas Ethyl alcohol and petroleum ether is non-effective against Culex species of mosquito larvae and *Anopheles* species of mosquito larvae. Hence *Murraya koenigii* can be used as a botanical insecticide for treating the mosquito larvae as it is a commonly available plant and easily affordable.

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