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Comparative Study of Larvicidal Activity of Clerodendrum Inerme Leaves Extracts and Crude Oil of Roasted Coconut Shell Individually as Well as Mixture of Both

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Abstract: ***Various fractions of leaves and crude oil of coconut shell were obtained in organic solvents like Petroleum ether, Chloroform and Methanol and were subjected to larvicidal activity individually as well as mixture of both against 3rd and 4th instar larvae of Culex pipen fatigan and Aedes aegypti mosquito larvae.The study of larvicidal activity were done by using World Health Organization (WHO) method. They were tested for 24-48 hrs for their mortality. The concentration ranged from 50ppm to 600ppm.Comparative study shows that individual extract of leaves and crude oil with Petroleum ether gave highest activity against Aedes aegypti and Anopheles stephensi whereas mixture of extracts of leaves and crude oil with Petroleum ether gave maximum activity against Culex pipen fatigan mosquito larvae.***

Keywords: Fractionated extraction,Culex pipen fatigan, Aedes aegypti, Anopheles stephensi

1. Introduction

Mosquitoes are responsible for the spread of a wide range of diseases including Malaria, Yellow fever, Dengue, Chikungunya, Japanese encephalitis (JE), West Nile virus and Rift Valley fever.[1,2]Aedes aegypti and Aedes albopictus are implicated as disease vectors for the spread of dengue, chikungunya and Zika virus and breed in a variety of containers. It is a common species in city premises and recorded breeding is predominantly in discarded tyres and solid waste containers in slum areas. [3]Japanese encephalitis, formerly endemic in north-east in India, is currently spreading fast across the state of north region of India with confirmed cases and a high case-fatality rate affecting all ages which is caused by Culex species of mosquito. [4]The incidence of malaria which is caused by Anopheles species is, however, gradually receding, with a consistent decline in cases over the past few years where as the no. of Dengue, Chikungunya and Japanese encephalitis cases has increased gradually due to climate change, rapid urbanization, a growing population and lack of sanitary waste and water disposal are all factors which are responsible for spread of mosquito borne diseases in India.

Clerodendrum inerme is a small tree, shrubs and herbs occurs predominantly in the mangrove forests of coastal India.[5,6]The plant has many medicinal importance such as it is used for treatment of rheumatism, fever, hypertension, cough, skin rashes, muscular pain, dysentery and its Roots are used for venereal diseases.[7,8,9]Also the leaves extract of plant is reported as active against mosquito larvae.[10,11]The Cocos nucifera also known as 'Kalpa vriksha' in India is widely present in the coastal region of India. Its each and every part are used by human in various ways such as food, timber, medicine, fibre, oil and charcoal.[12,13]Various studies shows its highly medicinal value and it's larvicidal activity. [14]

As both the plants are active against mosquitos, it is an attempt to study their effect on mosquito larvae individually as well as mixture of both.

2. Materials and Methods

The leaves were sun dried and 1KG of leaves were fractionated with solvents like Petroleum ether, Chloroform and Methanol respectively by refluction method using soxhlet extraction technique. Crude fractionated solvents extract (Petroleum ether, Chloroform and Methanol) then allowed to evaporate remaining solvent from it and to dry at room temperature. All three concentrated fractionated leaves extract are used to prepare samples of concentration 10ppm to 600 ppm and were tested against mosquito larvae.

1KG of dried coconut shell broken into pieces and subjected to bioactive crude oil extraction by heating in the earthen pot using patented method. [15]The dark viscous crude oil then subjected to fractionated extraction using solvent such as Petroleum ether and Chloroform respectively.Crude fractionated solvents extract (Petroleum ether and Chloroform) then allowed to evaporate remaining solvent from it and to dry at room temperature. The concentrated fractionated solvent extracts of crude oil and whole crude oil were used to prepare samples of concentration 10ppm to 600 ppm and were tested against mosquito larvae.

Mixture was prepared by mixing Petroleum ether leaves extract with Petroleum ether crude oil extract, Chloroform leaves extract with Chloroform crude oil extract and Methanol leaves extract with whole crude oil respectively in the ratio of 1:1.

Bioassay

Bioassay was conducted in 500ml glass beakers containing 250 ml of dechlorinated water for each species separately. The test solution with four replicates was prepared according to the guidelines of WHO (2005) [16].A batch of 3rd and early 4th instar larvae (10 in no.) of Culex pipen fatigan, Aedes aegypti and Anopheles stephensi were collected from the field and introduced in each test solution and in an appropriate control solution. The results were observed at 24hour.

3. Results and Discussion

Among all samples Petroleum ether extract (Individual as well as combination sample) shown maximum activity against all three species of mosquito. With LC_{50} – 31.85 Petroleum ether extract of crude oil(individually) had shown maximum activity for Culex pipen fatigun species of mosquito larvae followed by Chloroform extract of combination sample with LC_{50} – 34.55 and Petroleum ether extract of combination sample. For Aedes aegypti and Anopheles stephensi, Petroleum ether extract of crude oil (individually) found more active with LC_{50} – 295.54 and LC_{50} – 298.54 respectively. followed by Chloroform extract of crude oil(individually) and whole crude oil.

4. Conclusion

Present comparative study suggests that Petroleum ether extract individually as well as in combination is quite effective against all three types of mosquito species larvae and possess effective larvicidal properties. Hence can be a better replaceable option to chemical substituent which can be used as herbal insecticide for treating the mosquito larvae.

Table 1: Lethal concentration of organic solvent extracts of C. inerme plant leaves and crude oil of roasted coconut shell against third and fourth instar larvae of Culex mosquito species

Sr. No.	Sample/Extracts	Mosquito species	Sample concentration	% Mortality	LC50	LC90 (Estimated)	Regression equation
1	Petroleum ether (Leaves)		300ppm	100	91.38	196.384	Y=1.7465x-2.9662
2	Chloroform(Leaves)		300ppm	100	81.43	175.593	Y=1.5687x-1.8169
3	Methanol(Leaves)		600ppm	0	-	-	-
1	Petroleum ether (Crude oil)		100ppm	100	31.85	61.283	Y=2.617x-4.06
2	Chloroform (Crude oil)	Culex pipen fatigan	400ppm	100	176.30	346.264	Y=1.961x-5.14
3	Whole Crude oil		300ppm	100	149.35	260.014	Y=2.213x-6.08
1	Petroleum ether (Leaves+ Crude oil)		100ppm	100	36.525	86.525	Y=2.3271x-3.1674
2	Chloroform (Leaves+ Crude oil)		100ppm	100	34.55	85.162	Y=2.3748x-3.4123
3	Methanol+ Whole crude oil (Leaves+ Crude oil)		-	-	-	-	-

Table 2: Lethal concentration of organic solvent extracts of C. inerme plant leaves and crude oil of roasted coconut shell against third and fourth instar larvae of Aedes mosquito species

Sr. No.	Sample/Extracts	Mosquito species	Sample concentration	% Mortality	LC50	LC90 (Estimated)	Regression equation
1	Petroleum ether (Leaves)		600ppm	50	434.96	884.609	Y=1.064x-2.8647
2	Chloroform(Leaves)		600ppm	0	-	-	-
3	Methanol(Leaves)		600ppm	0	-	-	-
1	Petroleum ether (Crude oil)		600ppm	90	295.54	543.023	Y=2.958x-11.18
2	Chloroform (Crude oil)	Aedes aegypti	600ppm	80	295.07	656.865	Y=2.385x-8.56
3	Whole Crude oil		600ppm	80	565.56	691.059	Y=1.367x-4.0
1	Petroleum ether (Leaves+ Crude oil)		600ppm	0	-	-	-
2	Chloroform (Leaves+ Crude oil)		600ppm	0	-	-	-
3	Methanol+ Whole crude oil (Leaves+ Crude oil)		600ppm	0	-	-	-

Table 3: Lethal concentration of organic solvent extracts of C. inerme plant leaves and crude oil of roasted coconut shell against third and fourth instar larvae of Anopheles mosquito species

Sr. No.	Sample/Extracts	Mosquito species	Sample concentration	% Mortality	LC50	LC90 (Estimated)	Regression equation
1	Petroleum ether (Leaves)		600ppm	0	-	-	-
2	Chloroform(Leaves)		600ppm	0	-	-	-
3	Methanol(Leaves)		600ppm	0	-	-	-
1	Petroleum ether (Crude oil)		600ppm	90	298.54	784.609	Y=2.958x-11.18
2	Chloroform (Crude oil)	Anopheles stephensi	600ppm	50	746	1242.526	Y=2.385x-8.56
3	Whole Crude oil		600ppm	60	725.37	964.335	Y=1.367x-4.0
1	Petroleum ether (Leaves+ Crude oil)		600ppm	0	-	-	-
2	Chloroform (Leaves+ Crude oil)		600ppm	0	-	-	-
3	Methanol+ Whole crude oil (Leaves+ Crude oil)		600ppm	0	-	-	-

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