MARINE PLANTS FOR MOSQUITO CONTROL

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Abstract—Insecticides of plant origin have been receiving attention in recent years to overcome the environmental hazards in using synthetic insecticides. Large numbers of plant samples have been screened for their insecticidal and/or repellent activities and a few of them have been found promising and their products are commercially available. We have investigated for the first time seaweeds, seagrasses and mangrove plants for their larvicidal, skin and smoke repellent activities against mosquito species. Some of them were effective in killing the larvae or repelling adult female mosquitoes. Leaves of *Excoecaria agallocha* and *Acanthus ilicifolius* were found to show smoke repellent activity. Isolation and identification of active compounds from the effective samples would be useful in synthesising mosquito larvicides or repellents on a large scale.

INTRODUCTION

Throughout the world there is a long history of plant materials being used for their insecticidal or repellent properties. During the last few decades synthetic chemicals have been used in large quantities to control insect pests as they are cheap and effective. But in due course, the demerits of the synthetic insecticides were understood. The synthetic insecticides are generally non target specific and can cause environmental damage due to their persistant nature. Hence natural insecticides were realised to be ecofriendly and are given preference. In this context a large number of terrestrial plants have been screened for mosquito larvicidal and/or repellent activities (Thangam and Kathiresan, 1990). As there had been no report of the mosquito larvicidal or repellent activities of marine plants, we were prompted to study the same for their larvicidal, skin and/or smoke repellent activities against the mosquito *Aedes aegypti*, the vector of dengue and yellow fever and *Culex quinquefasciatus*, the vector of bancoftian filariasis.

MATERIALS AND METHODS

Extraction of plant samples

Seaweeds, seagrasses and mangrove plant samples were collected from the south-east coast of India. They were washed in tap water, shade-dried, powdered and extracted in petroleum-ether and acetone separately using a Soxhlet apparatus. The solvents from the extracts were removed using a vacuum evaporator. Standard stock solutions were prepared at 2% by dissolving the residues in acetone.

Larval susceptibility tests

Mosquito larval susceptibility tests were conducted according to the standard method (WHO, 1975). Test solutions were prepared at 20 different concentrations from 10 to 200 $mg.l^{-1}$ by dissolving the stock solutions in unchlorinated tap water. Tween-80 was used as an emulsifier at a concentration of 0.02% in the final test solutions. Twenty five larvae of early fourth instar were released in 500ml beakers containing 250 ml of the test or control solutions. Four replicates were run for each concentration including control. The control solution was prepared in tap water using acetone and tween-80 as used in the test solution of the highest concentration. Mortality counts were made at 24 hrs. Experiments were repeated if more than 10% of the larvae pupated during the course of the experiment or more than 10% larval mortality was observed in controls. The LC_{50} values were calculated following the method of Finney (1971).

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Skin repellency tests

Skin repellency tests were carried out as described by Kalyanasundaram *et al.* (1986). On a cleaned fore-arm of a human subject, 0.45ml of the stock solution (2% w/v) was applied on a 3×3 cm area (1 mg of plant material per cm^2). The untreated area of the fore-arm was covered with hand-gloves. The treated arm was inserted into a mosquito cage (30×30×15 cm) containing 100 female *Ae. aegypti* mosquitoes, three to four days old. The mosquitoes were already fed with water-soaked raisins and sucrose solution 5% (w/v), six hrs before the experiment, but not with blood. The treated arm was kept still and exposed continuously to the mosquitoes. The average time taken for the first three bites was calculated and was considered as the protection time of the extract (Thangam and Kathiresan, 1993b). Fore-arms treated with acetone alone were used as controls. Each experiment was repeated four times with different mosquito populations and the average values calculated. All the experiments were conducted at a temperature of 29±2°C and a relative humidity of 80±2% during day time.

Smoke repellency tests

Mosquito-coils were prepared following the method of Saini *et al.* (1986) with minor modifications by using four grams of powdered plant material as the active ingredient, two grams of saw-dust as binding material and two grams of coconut-shell charcoal powder as burning material. All three were thoroughly mixed with distilled water to form a semi-solid paste, from which mosquito coils of 0.6 cm thickness were prepared and shade-dried. Blank coils were prepared without using plant powder.

Smoke repellent tests were conducted in a glass chambermeasuring 140×120×60 cm (Thangam and Kathiresan, 1992b). A window measuring 60×30 cm was situated at the mid-bottom of one side of the chamber. 100 adult female mosquitoes (three to four days old) were released into the chamber. The mosquitoes were already fed with glucose solution six hours before the experiment but not with blood. A belly shaven pigeon was kept tied inside the cage in an immobilized condition. The coil was kept on a stand inside the cage and was allowed to burn out. The window was tightly closed. The experiments were conducted at night for eight hrs from 10p.m. to 6 p.m. Each experiment was repeated three times using mosquitoes of the same age group at a temperature of 29±2%, and relative humidity of 80±2% and the average values were subsequently used for calculations. After the experiment was over, the fed and unfed (alive and dead) mosquitoes were counted. Protection given by the smoke from the plant samples against the biting of mosquitoes was calculated in terms of percentage of unfed mosquitoes after treatment (Thangam and Kathiresan, 1992b).

$$\text{Unfed mosquitoes in treated (\%)} = \frac{\text{No. of unfed mosquitoes in treated} - \text{No. of unfed mosquitoes in control}}{\text{No. of mosquitoes treated}} \times 100$$

RESULTS

The list of plant samples tested for larvicidal activity is given in Table 1 (1988a,b; 1989; 1991a,b; 1992a; 1993a; 1994). Extracts of *Caulerpa scalpelliformis*, *Dictyota dichotoma* and the stilt root of *Rhizophora apiculata* were found promising in causing LC_{50}s below 50 $mg.l^{-1}$. Among these, *R. apiculata* showed the highest mosquito larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus* with LC_{50}s of 23 and 25 $mg.l^{-1}$ respectively. Four seaweeds and 11 mangrove plant samples were tested against the females of *Ae. aegypti* (Table 2). The Stilt root of *R. apiculata* gave the maximum protection for 70min (Thangam and Kathiresan, 1993b). The smoke repellent activity of 10 mangrove plant samples was studied against *Ae. aegypti* (Table 3), and two seaweeds and five mangrove plant samples against *Cx. quinquefasciatus* (Table 4). The leaf of *Excoecaria agallocha* was found most effective against *Cx. quinquefasciatus* by giving 56% of protection

Table 1. Plant samples studied for larvicidal activity against Aedes aegypti and Culex quinquefasciatus

Plant species	Plant part	Activity
Seaweed		
Caulerpa peltata Lamour	whole	++
C. racemosa (Forssk.) Weber V. Bosse	"	++
C. scalpelliformis (R. Br.) Weber V. Bosse	"	++
Chaetomorpha linum (O.F. Muell.) Kuetz.	"	+
Codium decorticatum (Woodward) Harvey	"	–
Dictyota dichotoma (Huds.) Lamour.	"	++
Enteromorpha clathrata (Roth) J. Agardh	"	++
E. intestinalis (L.) Link	"	++
Halimeda opuntia f. typica Barton	"	–
Hypnea valentia (Turn.) Mont.	"	+
Sargassam tenerrimum J. Ag.	"	–
S. wightii Greville	"	–
Turbinaria conoides Kuetz	"	–
T. oranata J. Ag.	"	–
Ulva lactuca L.	"	–
Seagrass		
Halophila beccarii Aschers	whole	+
H. ovalis (R. Br.) Hook. F.	"	+
Halodule pinifolia (Miki) den Hortog	"	+
Mangrove plant		
Acanthus ilicifolius L.	leaf	–
	root-bark	–
Aegiceras corniculatum (L.) Blanco	leaf	+
	fruit	–
Avicennia marina (Forssk.) Vierh.	leaf	++
	stem-bark	–
A. officinalis L.	leaf	+
	stem-bark	–
Ceriops decandra (Griff.) Ding Hou.	leaf	++
	stem-bark	–
	hypocotyl	–
Excoecaria agallocha L.	eaf	+
	fruit	+
	stem-bark	+
	root-bark	–
Lumnitzera racemosa Willd.	leaf	–
Rhizophora apiculata Blume	leaf	+
	hypocotyl	–
	stilt-root	++
R. lamarckii Montr.	leaf	+
	hypocotyl	–
	stilt-root	++
R. mucronata Lamk.	leaf	+
	hypocotyl	–
	stilt-root	++
Salicornia brachiata Roxb.	stem	–
Sesuvium portulacastrum L.	leaf	–
	stem	–
Sonneratia apetala Buch. Ham.	leaf	–
	stem-bark	–
	root-bark	–
Suaeda maritima (L.) Dumort	leaf	+
	stem	–
S. monoica Forssk.	leaf	+
	stem	–
Xylocarpus granatum Koenig	leaf	–
	trunk-bark	–
	root-bark	–

++ Effective (LC_{50} < 100 mg.l^{-1}); + Less effective (LC_{50} between 100 and 200 mg.l^{-1}); - Ineffective (No LC_{50} < 200 mg l^{-1})

Table 2. Marine plant samples studied for skin repellent activity against *Aedes aegypti*

Plant species	Plant part	Activity
Seaweed		
Caulerpa peltata Lamour.	whole	–
C. racemosa (Forssk.) Weber V.Bosse	"	–
C. scalpelliformis (R.Br.) Weber V.Bosse	"	–
Dictyota dichotoma (Huds.) Lamour.	"	–
Mangrove plant		
Avicennia marina (Forssk.) vierh. leaf + A. officinalis L.	leaf	+
Excoecaria agallocha L.	leaf	++
Lumnitzera racemosa Willd.	leaf	+
Rhizophora apiculata Blume	leaf	++
R. apiculata Blume	stilt-root	++
R. lamarckii montr.	leaf	++
R. mucronata Lamk.	leaf	++
Salicornia brachiata Roxb.	leaf	++
Sonneratia apetala Buch. Ham.	leaf	+
Xylocarpus granatum Koenig	leaf	+

++ Protection > 30 min; + Protection between 15 and 30 min; – protection < 15min

Table 3. Mangrove plant samples studied for smoke repellent activity against *Aedes aegypti*

Plant species	Plant part	Activity
Acanthus ilicifolius L.	leaf	++
Aegiceras corniculatum (L.)	"	++
Avicennia marina (Forssk.) Vierh	"	++
A. officinalis L.	"	++
Bruguiera cylindrica (L.)	"	++
Ceriops decandra (Griff.) Ding Hou.	"	+
Excoecaria agallocha L.	"	++
Lumnitzera racemosa Willd.	"	++
Rhizophora apiculata Blume	stilt-root	++
R. lamarckii Montr.	leaf	++

++ Protection > 50%; + protection between 50 and 25%; Protection < 25%

Table 4. Marine plant samples studied for smoke repellent activity against *Culex quinquefasciatus*

Plant species	Plant part	Activity
Seaweed:		
Caulerpa scalpelliformis (R.Br.) Weber V.Bosse	whole plant	+
Dictyota dichotoma (Huds.) Lamour.	whole plant	–
Mangrove plant:		
Avicennia marina (Forssk.)	leaf	–
Excoecaria agallocha L.	leaf	++
Rhizophora apiculata Blume	leaf	–
R. apiculata Blume	stilt-root	+
Xylocarpus granatum Koenig	leaf	–

++ protection > 50%; + protection between 50 and 25%; protection < 25%

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(Thangam and Kathiresan, 1992b) while *Acanthus ilicifolius* was most effective against *Ae. aegypti* by giving 74% of protection (Thangam *et al.*, 1992).

DISCUSSION

There have been numerous reports on the mosquito larvicidal activity of terrestrial plants. Ours was the first study on mosquito larvicidal and repellent activity of marine plants (Kathiresan and Thangam, 1987). Subsequently the mosquito larvicidal activity of seaweeds, *Plocamium telfairiae* and *Laurencia nipponica* was reported by Watanabe *et al.* (1989a,b; 1990). Mosquito larvicidal compounds were also isolated by them. Effective repellent compounds, like dimethyl pthalate, available in the market are very costly and moreover they can give protection only for a short period of one or two hours (Kalyanasundaram *et al.*, 1986). In view of these facts, the purified active compounds from the most effective samples found in our studies could be effective in killing mosquito larvae or repelling adult female mosquitoes in an economic and safe manner. This finding would be useful in the field of mosquito control without polluting the environment.

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