International Journal of Zoological Research

ISSN 1811-9778

Academic Journals Inc.

www.academicjournals.com

International Journal of Zoological Research 7 (3): 272-278, 2011
ISSN 1811-9778 / DOI: 10.3923/ijzr.2011.272.278

In vitro Mosquito Larvicidal Activity of Marine Algae Against the Human Vectors, *Culex quinquefasciatus* (Say) and *Aedes aegypti* (Linnaeus) (Diptera: Culicidae)

[1]Aseer Manilal, [1]Nooruddin Thajuddin, [1]Joseph Selvin, [2]Akbar Idhayadhulla, [2]Radhakrishnan Surendra Kumar and [1]Sugathan Sujith

[1]School of Life Sciences, Bharathidasan University, Tiruchirappalli 620 024, South India
[2]P.G and Research Department of Chemistry, Jamal Mohamed College, Tiruchirappalli-620020, South India

Corresponding Author: Aseer Manilal, School of Life Sciences, Bharathidasan University, Tiruchirappalli 620 024, South India

ABSTRACT

A total of twenty marine algae were collected from the rocky intertidal and subtidal regions of the southwest coast of India and extracted in methanol. The extracts were evaluated for larvicidal activity against the second and third instar larvae of the human vector mosquito *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). Analysis on the activity profile of the above marine algae indicated that the early stage larvae were very sensitive to seven seaweed extracts that had been tested. Among the seven marine algae, *Lobophora variegata* was highly potential, showing LD_{50} value of 70.38 and 79.43 μg mL^{-1} on the 2nd instar larvae of *A. aegypti* and *C. quinquefasciatus* respectively. The rank of larvicidal potency in highly active algae in the descending order is as follows: *Lobophora variegata* (Dictyotaceae)>*Spatoglossum asperum* (Dictyotaceae)>*Stoechospermum marginatum* (Dictyotaceae)>*Sargassum wightii* (Sargassaceae) >*Acrosiphonia orientalis* (Acrosiphoniaceae)>*Centroceras clavulatum* (Ceramiacea)>*Padina tetrastromatica* (Dictyotaceae). This is the first report that envisaged the mosquito larvicidal efficacy of *L. variegata* from the Indian coast. Therefore, this marine alga could be recognized as a potential resource of natural insecticide and can be developed to replace synthetic insecticides in future.

Key words: *Lobophora variegata*, seaweed extract, larvicidal activity, mosquito, bioactive compounds, marine brown algae

INTRODUCTION

Mosquitoes coming under the order Diptera, are ravaging humans and other animals for generations. There are nearly 2,500 mosquito species in the world but a mere fraction of them transmit an array of pathogens including viruses (e.g., arboviruses), protozoans (e.g., malaria) and nematode worms (e.g., lymphatic filariasis). According to Taubes (1997), annually more than 700 million people suffer from mosquito-borne diseases. The immense usage of many synthetic aerial, terrestrial and aquatic insecticides offer logistic problems on the environment (Chowdhury *et al*., 2008) and causes resurgence of different mosquito-borne diseases (Milam *et al*., 2000). Costs and complexity of mosquito control have increased strikingly since the passage of the Environmental Protection Act in 1969. Environmental managers practiced the bio-control agents such as fishes, birds, dragonflies and frogs but feedback on the effectiveness of these agents is only

anecdotal and some mosquito species have developed high levels of resistance to microbial control agents (Rajkumar and Jebanesan, 2005). It is therefore high time to develop an alternative strategy in mosquito control management in order to circumvent existing resistance thus reducing the risk of new resistance.

For many centuries, phyto-products have been used traditionally by human communities to fend off vector and pest species of insects (Jang *et al*., 2002; Pavela, 2007). The application of phyto-products is considered to be one of the counter measures to control pests and vectors (Manilal *et al*., 2009b). The first observation pertaining to larvicidal activities of botanicals was reported by Campbell *et al*. (1933). In recent years, marine natural products from sponges, microbes and algae have been screened for larvicidal activity (Rao *et al*., 2008; Selvin and Lipton, 2004). Marine flora and fauna produce diverse novel bioactive metabolites unique to the environment (Manilal *et al*., 2009a, 2010a, 2011a,b). Over the last decade, numerous secondary metabolites with complex chemical structures and different bioactivity spectra have been characterized from marine algae and recognized as a virtually untapped reservoir of novel drug leads (Manilal *et al*., 2010c). Many of these metabolites have toxicological, pharmacological and ecological importance (Manilal *et al*., 2010b). Therefore, the marine derived algal products may serve as a suitable alternatives to synthetic insecticides in future as they are relatively safe, biodegradable and are easily available around the world. The objective of the current study was to evaluate the larvicidal potency of 20 marine algae collected from the southwest coast of India against second and third instar larvae of two urban mosquito species.

MATERIALS AND METHODS

The present study was carried out in Marine Bio-Prospecting Laboratory, Bharathidasan University, Trichy, India between April to December 2010. Algae belonging to Chlorophyta, Phaeophyta and Rhodophyta were collected from the intertidal and subtidal habitat of Kollam (08° 89' N and 76°54'E) area (Table 1) during April to December 2010 when algal diversity remains greater. The study area, southwest coast of India exhibits small intertidal pools, large and shallow sand bottom flats, numerous irregularly distributed laterite rocks, cobbles and scattered granite boulders inhabited with a wide variety of seaweed floristic biomass (Sulekha and Panikkar, 2006; Mantri, 2006). Live and healthy plants were handpicked and washed thoroughly in running water to remove adhering detritus matters. Cleaned algal samples were wrung between blotting paper and shade dried until it becomes brittle. After completing the shade drying process, they were chopped into small pieces and dried in an oven at 40°C. The completely dried material was weighed and ground coarsely in a mechanical grinder. Algae were extracted in methanol as per Manilal *et al*. (2010b). The resultant extractive were collected in air–tight plastic vials and stored in the refrigerator at 4°C for further activity studies.

Bioassays: The assessment of larvicidal activity of various extracts was tested against the urban mosquitoes *C. quinquefasciatus* and *A. aegypti* using standard bioassay protocol. Eggs of respective mosquitoes were obtained from drainage systems of Tiruchirappalli city (Tamilnadu, South India). Eggs were reared under standard insectary conditions at ambient temperature (29±3°C), relative humidity 80±5%, 12:12 light: dark photoperiod and fed with ground shrimp feed daily. Larval development was monitored for seven days. The second and third stage larvae were collected at the tip of a pasture pipette and placed in cotton bud to remove excess water and transferred gently to the test vial (10 / vial) by tapping. The larval mortality was observed using various concentrations

Table 1: Extract yields of algae collected from the southwest coast of India

Division	Species	Dry wt.(g)	Yield (g dry^{-1} wt.)
Chlorophyta	*Valoniopsis pachynema*	550	0.074
	Chaetomorpha antennina	700	0.094
	Enteromorpha intestinalis	500	0.034
	Acrosiphonia orientalis	850	0.056
	Ulva fasciata	600	0.083
	Caulerpa racemosa	700	0.096
	Bryopsis plumosa	1080	0.146
Phaeophyta	*Dictyota dichotoma*	600	0.079
	Padina tetrastromatica	800	0.063
	Chnoospora bicanaliculata	700	0.179
	Sargassum wightii	750	0.088
	Stoechospermum marginatum	780	0.075
	Lobophora variegata	600	0.084
	Spatoglossum asperum	600	0.055
Rhodophyta	*Gracilaria corticata*	1000	0.185
	Hypnea pannosa	750	0.061
	Centroceras clavulatum	1500	0.096
	Cheilosporum spectabile	600	0.083
	Portieria hornemannii	600	0.095
	Gelidium sp.	800	0.093

of seaweed extracts (100, 200, 300 and 400 μg mL^{-1}) including positive (with 2% methanol) and negative controls (without vehicle). The number of larvae surviving after 24 h of exposure was recorded. Wriggling movement after probing it with a needle identified deceased larvae. Toxicological effects were recorded in all trials in order to maximize consistency. All the experiments were repeated six times to validate the findings statistically (Wardlaw, 1985).

RESULTS

Overall objective of the present study was to compare the ability of organic extract of marine algae collected from the same habitat to inhibit the survival of mosquito larvae. Larvicidal assay was performed with the extract of twenty marine macro algae against the second and third instar larvae of *C. quinquefasciatus* and *A. aegypti*. The data for the amount of algal extract yielded are presented in Table 1. Of the 20 marine algae, yield of extract was highest (0.185 g dry^{-1} weight) for the *Gracilaria corticata* and lowest (0.034 g dry^{-1} weight) for the *Enteromorpha intestinalis*. The data also revealed the considerable variability in the concentration of the extract and larvicidal activity. Comparing the two species representing the different mosquito genera, *C. quinquefasciatus* was found to be more sensitive to seaweed extracts than *A. aegypti*. It was also found that the red and brown seaweed groups had high potency than green algae. Data obtained from the larvicidal activity of seaweed extracts at the end of 24 h suggested that the second instar larvae of both species were highly sensitive than the third instar. Of the 20 seaweed extracts tested, the dose level 200 μg mL^{-1} of seven species showed 100% mortality in both species of second instar larvae. These algal extracts showed high larvicidal activity, their LD_{50} after 24 h ranged between 70.38 and 97.41 μg mL^{-1} against second instar larvae of *A. aegypti* (Table 3) and 79.43 and 97.94 μg mL^{-1} against *C. quinquefasciatus* (Table 2). Among the algae tested, *L. variegata* was the highly potential and showed LD_{50} value of 70.38 and 95.52 μg mL^{-1}, respectively against the second

Table 2: LD_{50} values of *C. quinquefasciatus* larvae against effective seaweed extracts

Algae	2nd instar LD_{50} (µg mL^{-1})	3rd instar LD_{50} (µg mL^{-1})
L. variegata	79.43±1.5	96.52±2.1
S. asperum	83.17±2.4	97.71±3.2
S. marginatum	85.11±3.5	98.59±1.4
S. wightii	87.09±3.2	107.15±1.6
A. orientalis	94.42±1.7	131.82±3.5
C. clavulatum	97.72±2.4	178.76±2.5
P. tetrastromatica	97.94±1.5	226.31±3.2

Values are Mean±SD (n = 6)

Table 3: LD_{50} value of *A. aegypti* larvae against effective seaweed extracts

Algae	2nd instar LD_{50} (µg mL^{-1})	3rd instar LD_{50} (µg mL^{-1})
L. variegata	70.38±1.1	95.52±2.8
S. asperum	81.28±3.5	96.13±3.7
S. marginatum	82.95±1.7	97.83±2.4
S. wightii	84.82±1.2	97.28±1.5
A. orientalis	86.13±3.7	125.43±3.1
C. clavulatum	91.54±2.8	158.26±1.8
P. tetrastromatica	97.41±2.1	199.34±2.3

Values are Mean±SD (n = 6)

and the third instar larvae of *A. aegypti*. The same algae produced a LD_{50} value 79.43 and 96.52 µg mL^{-1}, respectively against *C. quinquefasciatus*. The second most effective seaweed was *S. asperum* which showed LD_{50} at 81.28 and 96.13 µg mL^{-1} against *A. aegypti* larvae and in *C. quinquefasciatus,* LD_{50} was 83.17 and 97.71 µg mL^{-1}, respectively. However, a lower level of activity was observed in other species of algae and their LD_{50} value was above 150 µg mL^{-1} (data not shown). Although the trend of larval mortality within 24 h was consistent between the larval stages and the mosquito exposed to 100 µg mL^{-1} of *L. variegata* extracts showed exceptionally 80% mortality at the end of 24 h. In the highest dose level of *L. variegata* (200 µg mL^{-1}), the 80% larvae (second and third instar) of both vectors showed behavioral changes. After 1 h of exposure, the larvae exhibited primary phase of behavioral changes including restive wriggling with rapid surface surge and loss of co-ordination while the secondary phase began after 6 h and larvae exhibit loss of wriggling activity. During the tertiary phase (12-24 h) the larvae showed loss of response to tapping and probing with needle and finally inclined to the bottom of the test vial followed by mortality.

DISCUSSION

Many marine plants synthesize a variety of chemically diverse secondary metabolites in response to selection pressures from herbivores and microorganisms and some of these compounds are recognized as insecticides. The management of adult mosquito stocks using adulticides is not a successful strategy, as the adult stage occurs beside human inhabitation and they can simply overcome remedial measures (Service, 1983, 1992). Mosquito control management including periodic larviciding (WHO, 1975; Becker *et al*., 2003) is very helpful in favorable conditions. Larval stage of mosquitoes are exclusively aquatic, systematic treatment of plant based larvicides in their breeding habitat is a successful and safer way to interrupt larval stages of vectors rather than the infective adult stage (Thangam and Kathiresan, 1991).

Mosquitoes are the vector for large number of human pathogens than any other group of arthropods (El-Hag *et al*., 1999; Cepleanu, 1993). The uncontrollable breeding of mosquitoes is posing a serious threat to the humanity. The mosquito borne diseases not only cause high levels of morbidity and mortality but also cause great economic loss and social chaos in the developing countries including costs of health care and negative impacts on tourism and real estate values. India alone carries around 40% of global filariasis burden and the estimated annual economic loss is about 720 crores (Hotez *et al*., 2004). Recent figures from the World Health Organization (WHO) evidenced that malaria accounts for at least 500 million infections and 3 million deaths annually. The prevalence of dengue fever has increased over the last 50 years and over 2 billion people are under risk in more than 100 countries. In 2007, incurable crippling disease Chikun Gunya, spread primarily by *A. aegypti* species has resurfaced in Kerala, a southern state of India and claimed more than hundred lives.

Many plant derived natural compounds was tested for mosquito control (Oladimeji *et al*., 2007, 2008; Vinayagam *et al*., 2008; Chakkaravarthy *et al*., 2011; Almehmadi, 2011). Ishibashi *et al*. (1993) reported the extracts of *Aglaia elliptifolia* showed insecticidal activity. Polyhalogenated monoterpenes, aplysiaterpenoid A and telfairine isolated from *Plocamium* sp. exhibited insecticidal activity against the mosquito larvae (Watanabe *et al*., 1989). Early studies envisaged that the Indian marine plant extracts possessed potential larvicidal activity (Thangam *et al*., 1993; Rao *et al*., 1995). Furthermore, studies with secondary metabolites isolated from algae showed significant larvicidal activity (Selvin and Lipton, 2004; Alarif *et al*., 2010).

Of the algae screened, *L. variegata* was effective against, both mosquito species in relatively lower concentration. There is no previous report on the mosquito larvicidal activity of *L. variegata* from the Indian coast.

The organic extract of algae, *D. dichotoma, H. musciformis* and *U. fasciata* found to be larvicidal in the previous reports were less active in the present study. This may be due to the fact that the bioactivity of same taxa can vary with geographical zone and could depend on ecological parameters such as irradiance and nutrients (Marti *et al*., 2004).

In conclusion, overall data of this study illustrate that the marine alga, *L. variegata* is a potential candidate for the eco-congenial larvicide development programme as an alternate to chemical insecticides that are being currently used in mosquito vector control programs. Our investigations leads to the following suggestions: (1) *L. variegata* can be cultivated in coastal areas (2) the plant bioactives can be extracted by cost effective methods with high yield (3) larvicidal activity was potent and safe to non target cohabitants (4) synergistic activity will defend the development of resistance in vectors. Till date, no seaweed bioactives have been operationally used for mosquito control. Therefore, systemic bio-efficacy evaluation in field condition is further warranted.

ACKNOWLEDGMENT

Manilal, A is grateful to the Council of Scientific and Industrial Research (CSIR), New Delhi, India, for the award of Senior Research Fellowship.

REFERENCES

Alarif, W.M., Z.S.A. Elnaga, S.E.N. Ayyad and S.S. Al-Lihaibi, 2010. Insecticidal metabolites from the green alga *Caulerpa racemosa*. Clean Soil Air Water, 38: 548-557.

Almehmadi, R.M., 2011. Larvicidal, histopathological and ultra-structure studies of *Matricharia chamomella* extracts against the rift valley fever mosquito *Culex quinquefasciatus* (Culicidae: Diptera). J. Entomol., 8: 63-72.

Becker, N., D. Petric, M. Zgomba, C. Boase, C. Dahl, J. Lane and A. Kaiser, 2003. Mosquitoes and their Control. Kluwer Academic/Plenum Publishers, New York, USA., pp: 5-23.

Campbell, F.L., W.N. Sullivan and C.R. Smith, 1933. The relative toxicity of nicotine, anabasine, methyl anabasine and lupinine for culicine mosquito larvae. J. Econ. Entomol., 26: 500-509.

Cepleanu, F., 1993. Validation and application of three bench-top bioassays for screening of crude extracts and subsequent activity-guided isolation. Ph.D Thesis, Facultedes Sciences, Universite de Lausanne, Lausanne, pp: 307-310.

Chakkaravarthy, V.M., T. Ambrose, S. Vincent, R. Arunachalam, M.G. Paulraj, S. Ignacimuthu and G. Annadurai, 2011. Bioefficacy of *Azadirachta indica* (A. juss) and *Datura metel* (Linn.) leaves extracts in controlling *Culex quinquefasciatus* (Diptera: Culicidae). Trends Applied Sci. Res., 8: 191-197.

Chowdhury, N., A. Ghosh and G. Chandra, 2008. Mosquito larvicidal activities of Solanum villosum berry extract against the dengue vector *Stegomyia aegypti*. BMC Complementary Altern. Med., 3: 8-10.

El-Hag, E.A., A.H. Nadi and A.A. Zaiton, 1999. Toxic and growth retarding effects of three plant extracts on *Culex pipiens* larvae (Diptera: Culicidae). Phytother. Res., 13: 388-392.

Hotez, P.J., J.H.F. Remme, P. Buss, G. Alleyne, C. Morel and J.G. Breman, 2004. Combating tropical infectious diseases: Report of the disease control priorities in developing countries project. Clin. Infect. Dis., 38: 871-878.

Ishibashi, F., C. Satasook, M.B. Isman and G.H.N. Towers, 1993. Insecticidal 1H-Cyclopentatetrahydro[b] Benzofurans from *Aglaia odorata*. Phytochemistry, 32: 307-310.

Jang, Y.S., M.K. Kim, Y.J. Ahn and H.S. Lee, 2002. Larvicidal activity of Brazilian plants against *A. aegypti* and *C. pipiens pallens* (Diptera: Culicidae). Agric. Chem. Biotechnol., 45: 131-134.

Manilal, A., S. Sujith, G.S. Kiran, J. Selvin, C. Shakir, R. Gandhimathi and A.P. Lipton, 2009a. Antimicrobial potential and seasonality of red algae collected from the Southwest coast of India tested against shrimp, human and phytopathogens. Ann. Microbiol., 59: 207-219.

Manilal, A., S. Sujith, G.S. Kiran, J. Selvin, C. Shakir, R. Gandhimathi and M.V.N. Panikkar, 2009b. Biopotentials of seaweeds collected from Southwest Coast of India. J. Mar. Sci. Technol., 17: 67-73.

Manilal, A., S. Sujith, G.S. Kiran, J. Selvin, C. Shakir and A.P. Lipton, 2010a. Antifouling potentials of seaweeds collected from the Southwest coast of India. World J. Agric. Sci., 6: 243-248.

Manilal, A., S. Sujith, B. Sabarathnam, J. Selvin, G.S. Kiran, C. Shakir and A.P. Lipton, 2010b. Bioactivity of the red algae *Asparagopsis taxiformis* collected from the Southwestern coast of India. Braz. J. Oceanogr., 58: 93-100.

Manilal, A., S. Sujith, G.S. Kiran, J. Selvin, C. Shakir and A.P. Lipton, 2010c. Antimicrobial potential of marine organisms collected from the Southwest coast of India against multiresistant human and shrimp pathogens. Sci. Mar., 74: 287-296.

Manilal, A., S. Sujith, B. Sabarathnam, J. Selvin, G.S. Kiran, C. Shakir and A.P. Lipton, 2011a. Biological activity of the red alga *Laurencia brandenii*. Acta Bot. Croat., 70: 81-90.

Manilal, A., S. Sujith, G.S. Kiran, J. Selvin and M.V.N. Panikkar, 2011b. Evaluation of seaweed bioactives on common aquatic floral and faunal weeds of shrimp ponds. Thalassas. Int. J. Mar. Sci., 27: 47-56.

Mantri, V.A., 2006. Seaweed floristic studies along tsunami affected Indian coasts: A litmus test scenario after 26th December 2004. J. Earth Syst. Sci., 115: 371-378.

Marti, R., M.J. Uriz and X. Turon, 2004. Seasonal and spatial variation of species toxicity in Mediterranean seaweed communities: Correlation to biotic and abiotic factors. Maine. Mar. Ecol. Progr., 282: 73-85.

Milam, C.D., J.L. Farris and J.D. Wilhide, 2000. Evaluating mosquito control pesticides for effect on target and non target organisms. Arch. Environ. Contam. Toxicol., 39: 324-328.

Oladimeji, H.O., P.M. Ubulom, E.I. Akpabio, I.E. Etim and E. Nyong, 2008. Larvicidal and anti-microbial potentials of *Nymphaea odorata*. J. Pharmacol. Toxicol., 3: 357-362.

Oladimeji, H.O., R. Nia, N. Kalu and E.E. Attih, 2007. *In vitro* biological activities of *Carica papaya*. Res. J. Medicinal Plant, 1: 92-99.

Pavela, R., 2007. Possibilities of botanical insecticide exploitation in plant protection. Pest Tech., 1: 47-52.

Rajkumar, S. and A. Jebanesan, 2005. Larvicidal and adult emergence inhibition effect of *Centella asiatica* Brahmi (Umbelliferae) against mosquito *Culex quinquefasciatus* Say (Diptera: Culcidae). Afr. J. Biomed. Res., 8: 31-33.

Rao, D.R., T.R. Mani, R. Rajendran, A.S. Joseph, A. Gjanana and R. Reuben, 1995. Development of high level of resistance to *Bacillus sphaericus* in a field population of *C. quinquefasciatus* from Kochi, India. J. Am. Mosq. Control Assoc., 11: 1-5.

Rao, V.J., P.K. Usman and J. Bharat Kumar, 2008. Larvicidal and insecticidal properties of some marine sponges collected in Palk Bay and Gulf of Mannar waters. Afr. J. Biotechnol., 7: 109-113.

Selvin, J. and A.P. Lipton, 2004. Biopotentials of *Ulva fasciata* and *Hypnea musiformis* collected from the peninsular coast of India. J. Mar. Sci. Technol., 12: 1-6.

Service, M.W., 1983. Biological control of mosquitoes: Has it a future?. Mosquito News, 43: 113-120.

Service, M.W., 1992. Importance of ecology in *A. aegypti* control. Southeast Asian J. Trop. Med. Publ. Health, 23: 681-690.

Sulekha, S. and M.V.N. Panikkar, 2006. Marine green algal flora of Kollam coast, Kerala, South India. Seaweed Res. Utiln., 28: 5-21.

Taubes, G., 1997. A mosquito bites back. New York Times Magazine, August, 24, pp: 40-46.

Thangam, T.S. and K. Kathiresan, 1991. Mosquito larvicidal activity of marine plant extracts with synthetic insecticides. Bot. Mar., 34: 537-539.

Thangam, T.S., K. Kathiresan and T. Mabbett, 1993. Mosquito larvicidal activity of seaweed extract against *A. aegypti* and *C. quinquefasciatus*. Int. Pest. Contr., 35: 94-95.

Vinayagam, A., N. Senthilkumar and A. Umamaheswari, 2008. Larvicidal activity of some medicinal plant extracts against malaria vector *Anopheles stephensi*. Res. J. Parasitol., 3: 50-58.

WHO, 1975. Instructions for determining the susceptibility of resistance mosquito larvae to insecticides. Mimeographed Document WHO/VBC/75 583.

Wardlaw, A.C., 1985. Practical Statistics for Experimental Biologists. John Wiley and Sons, Chichester, England.

Watanabe, K., M. Miyakado, N. Ohno, A. Okada, K. Yanagi and K. Moriguchi, 1989. A polyhalogenated insecticidal monoterpene from the red alga, *Plocamium telfairiae*. Phytochemistry, 28: 77-78.