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Effect of Few Marine Sponges and its Biological Activity against *Aedes aegypti* Linn. *Musca domestica* (Linnaeus, 1758) (Diptera: Culicidae)

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ABSTRACT

Aim of the present study was planned to assess the biodiversity of the marine environment around the Muttom coastal region and then to isolate and characterized the secondary metabolites from the eleven sponges and screen them for their mosquito larvicidal effect. Due to increasing resistance of the vectors mosquitoes causing diseases of importance in public health, to chemical insecticides, is necessary the searching for alternative control methods, as the use of marine sponges extracts with insecticide activity, owing to its capacity of biodegradation and generation of minor environmental damage. In this work the insecticide activity is evaluated for the mixture of chloroform with methanol extracts of the eleven marine sponges on stadium V instar larvae of *Aedes aegypti* (L.) and *Culex quinquefasciatus* (L.) in conditions of laboratory (25°C and 75% Relative Humidity). The sponge extracts of *Clathria gorgonoids* and *Callyspongia diffusa* was found to be the most effective against *A. aegypti* larvae showed LC_{50} values at <50 ppm. A result demonstrates that *Ircinia campana* extract is most active against two insects, as long as the extract of *Sigmadocia carnosa* has a higher toxic effect on *Aedes aegypti* than on *Culex quinquefasciatus*. Between the sponges *Clathria gorgonoids* and *Callyspongia diffusa* were found to be more active towards both larvicidal and insecticidal properties. In view of both these activities, the subsequent sponges *Haliclona pigmentifera, Sigmadocia carnosa, Petrosia similes* and *Ircinia fusca* could be used to obtain novel pesticidal molecules.

Key words: Marine sponge, mosquito, insecticidal, chemical control, larval mortality

INTRODUCTION

Researches have recently concentrated their efforts on the search of active natural products derived from marine sponges as alternatives to conventional insecticides. The results of these developments are new approaches to pest control that differ fundamentally from conventional chemical control. New pest control strategies were needed to eliminate the effects of injudicious pesticide use and the concept of the Integrated Pest Management (IPM) found strong advocates (Vasanthraj, 2008). As sponge produces various novel chemical molecules, they have been a goldmine to chemists and also found their way in to biotechnological applications. Due to increasing resistance of the vectors mosquitoes causing diseases of importance in public health, to chemical insecticides, is necessary the searching for alternative control methods, as the use of marine sponges extracts with insecticide activity, owing to its capacity of biodegradation and generation of minor environmental damage (Martinez *et al.*, 2007). Marine sponges are shown to exhibit antibacterial, insecticidal, antiviral and antiplasmodial activities (Volk *et al.*, 2004; Rao *et al.*, 2003a). Van Wagenen *et al.* (1993) described seventeen years ago investigating the potential of marine natural products to serve as insect control agents via mechanisms of toxicity, interference with moulting or metamorphosis and feeding deterrence. Previous reports of sponge sesquiterpenes and diterpenes from gorgonians and sea pens. These marine invertebrates have evolved chemical defense mechanisms against other invading organisms, which involve the production of secondary metabolites (Blunt *et al.*, 2005; Wah *et al.*, 2006). Among the marine invertebrates, porifera (sponges) remain the most prolific phylum concerning novel pharmacologically active compounds. It has been known for centuries, that sponges contain bioactive compounds that are of potential medical importance (Bokesch *et al.*, 2002). The current thrust of the investigations were involves identifying newer drugs and other pharmaceuticals from marine origin, where as comparatively little attention has been made with respect to the discovery of pesticide molecules (Morlan, 1966). The Secondary metabolites isolated from marine sponges may be an alternative source for vector control agents to replace existing and highly toxic synthetic insecticides and will play an important role in future insecticide development programe.

This term adopts rationalization of pest control techniques to minimize harmful environmental effects. New and more specific target compounds appeared from marine bioactive compounds to have potential role in IPM (Thakur and Muller, 2004). In terrestrial environment Recently, studies have also suggested that some bioactive compounds isolated from marine organisms have been shown to exhibit anti-cancer, anti-microbial, anti-fungal or anti-inflammatory and other pharmacological activities (Venkateswarlu *et al.*, 1993; Proksch *et al.*, 2002; Donia and Hamann, 2003; Haefner, 2003; Kumar and Zi-rong, 2004; Mayer and Hamann, 2005). The Indian Ocean with a unique species of flora and fauna is one of the biologically richest coastal regions in all of mainland of India. The biology of bacterium-sponge relationship has elicited considerable interest among researches, as marine sponges have been considered as a rich reservoiur of bioactive compounds (Bokesch *et al.*, 2002). The general objectives of this study were to evaluate the biodiversity of the marine environment around the seas of Cape Comorin (Indain, Ocean), to isolate and characterize secondary metabolites from sponges and screen them for potential larvicidal and insecticidal properties. This paper reports the taxonomic identification of some potential sponges as a source for further exploration to obtain the pesticidal molecules. These bioactive principles either may be produced from associated microbes or sponge itself. Hence, it is much more important for greater cooperation and well co-ordinated efforts has been seen between the bacteriologists, mycologists, natural product chemists and entomologists for exploring the possibilities of developing newer pesticidal molecules from marine sponges. There is a lot of scope to obtain of pesticidal molecules especially new toxophoric groups, which can be appropriately incorporated in molecules to obtain potent synthetic products with targeted features.

MATERIALS AND METHODS

Collection of sponges: Samples of sponges (n = 15) were collected in the Cape Comorin coasts of Indian Ocean at depths varying from 10-15 feet by snorkeling and SCUBA-diving during the year of April-2009. Sponges were gently removed from the substratum and cut into small pieces then soaked in methanol for preparing crude extracts. The intact sample specimens were sent to the Central Marine Fisheries Research Institute (CMFRI), Trivandrum and Kerala, India for collect the necessary details only identification purpose.

Preparation of crude extracts: The initial aqueous methanol extract was concentrated in the laboratory under reduced pressure and lyophilized. The lyophilized powder was extracted with 1:1 mixture of methanol and dichloromethane. At the same time the methanol soaked cut pieces (100 g) were further diced and extracted with the same mixture of solvents. The extracts were pooled and the organic portions were evaporated for obtaining solvent free crude extract. Then the test solutions with desired concentrations were prepared by mixing the known amount of crude extract in a carrier solvent, acetone (w/v) and after these preparations were subjected to larvicidal and insecticidal screening.

Biological screening: The cyclic colonies of *Culex quinquefasciatus* were reared in our insectary at 27±1°C and 80±5% RH with a photo period of 11:13 h light and dark cycles followed by the methods of Newman and Cragg (2004) and Keiding *et al.* (1991), respectively with little modifications.

Data analysis: The *Musca domestica* larval mortality in each dose/concentration and control was recorded after 24 h of exposure. Percentage mortalities were corrected for the natural mortality observed in the negative controls using Abbott (1925) formula; P = PI – C / 1 – C, where PI denotes the observed mortality rate and C means the natural mortality. The median lethal concentration or dose (LC_{50} or LD_{50}) was calculated using 'Probit' analysis (Finny, 1971) that has been recommended by OECD guideline as appropriate statistical method for toxicity data analysis. After linearization of response curve by logarithmic transformation of concentrations, 95% confidence limits and slope function were calculated to provide a consistent presentation of the toxicity data.

RESULTS

The use of marine natural products is an alternative pest control method, which helps to minimize the usage of toxic pesticides and their deleterious effects on non target insect species, livestock, wildlife and on the environment. The sponge extracts of *Clathria gorgonoids* and *Callyspongia diffusa* was found to be the most effective against *A. aegypti* larvae showed LC_{50} values at <50 ppm. However, other extracts of *Dendrilla nigra*, *Petrosia testudinaria*, *Petrosia similes*, *Haliclona pigmentifera*, *Ircinia fusca*, *Sigmadocia fibulata* showed LC_{50} values at <100 ppm (Table 1). The above eleven crude extracts were also screened for insecticidal properties using housefly lethality test against *M. domestica* and their LD_{50} values are presented in Table 2. Among the extracts, *Clathria gorgonoids* and *Callyspongia diffusa* sponges were proved to be the most promising extracts with insecticidal properties against female adult *M. domestica* at LD_{50} values at <50 µg/insect. Meanwhile extracts of the *Ircinia fusca, Clathria reinwardti, Spirastrella inconstans* and *Sigmadocia pumila* did not show either larvicidal or insecticidal activities even at higher concentrations or doses (400 ppm for larvicidal and 300 µg/insect for insecticidal activity).

The present preliminary investigations are helped us to short list the bio-active sponge crude extracts, which possess larvicidal and insecticidal activities. These active extracts could be used for obtaining new leads to isolate bioactive pesticidal molecules from marine origin. The percentage of active crude extracts (about 40%) identified in the present experiments is high in comparison to other terrestrial natural products. Use of these invented novel products in mosquito control instead of synthetic insecticides could reduce the environmental pollution. The present results may be useful as blue prints to isolate the active principles from these active crude extracts.

Table 1: LC_{50} values (ppm) for 24 h with their 95% fiducial (lower and upper) limits, regression equation, Chi-square (χ^2) and p-levels of certain marine sponges against 5th instar larvae of *Aedes aegypti*

Species	LC_{50} with fiducial upper and -- Upper lower	Lower limits	Regression equation LogY = (Y-bx) +bX	Median LC_{50} (mg mL^{-1})	p-level
Clathria gorgonoids	25.90	29.78	Y = 1.40+2.55X	3.12	0.63
Callyspongia diffusa	33.46	44.49	Y = 0.57+2.96X	5.65	0.58
Haliclona pigmentifera	67.99	73.08	Y = -0.09+2.82X	3.47	0.67
Sigmadocia carnosa	75.84	84.62	Y = 0.11+2.62X	3.56	0.72
Petrosia similes	76.83	87.23	Y = -0.37+2.85X	4.65	0.82
Dendrilla nigra	87.92	103.78	Y = 0.76+2.18X	1.97	0.84
Ircinia fusca	80.368	102.20	Y = -0.73+2.93X	1.58	0.67
Sigmadocia fibulata	91.08	108.35	Y = -1.15+3.09X	0.97	0.97
Clathria reinwardti	109.87	116.05	Y = -0.16+2.53X	4.45	0.57
Spirastrella inconstans	112.03	129.03	Y = 0.40+2.24X	2.35	0.67
Ircinia campana	137.05	131.24	Y = -6.84+5.68X	0.65	0.87

Table 2: LD_{50} values (μg/fifth instar larvae) for 24 h with their 95% fiducial (lower and upper) limits, regression equation, of certain marine sponges against 3-4 day old of female *Musca domestica* (L.)

Species	LD_{50} with fiducial upper and -- Upper lower	Lower limits	Regression equation LogY=(Y-bx) +bX	Median LC_{50} (mg)	p-level
Clathria gorgonoids	21.14	33.56	Y = 3.15+1.08X	3.31	0.64
Callyspongia diffusa	39.57	43.47	Y = 3.73+0.04X	3.02	0.77
Dendrilla nigra	55.08	59.31	Y = 0.55+2.36X	1.47	0.69
Sigmadocia carnosa	77.27	80.03	Y = 0.79+2.41X	1.12	0.77
Petrosia similes	97.51	138.00	Y = 0.51+2.36X	5.18	0.63
Haliclona pigmentifera	105.18	134.64	Y = 0.50+2.64X	0.74	0.67
Ircinia fusca	135.52	135.52 141.36	Y = 0.48+2.13X	1.87	0.85
Sigmadocia fibulata	139.36	1654.91	Y = -0.51+1.65X	1.69	0.71
Clathria reinwardti	159.61	217.23	Y = -0.87+2.74X	6.98	0.34
Spirastrella inconstans	165.38	235.21	Y =2.06+2.64X	6.35	0.38
Ircinia campana	196.36	211.67	Y = 3.01+3.98X	3.56	0.65

The larvicidal activities of sponge extracts were evaluated against the fourth-instar larvae of *Culex quinquefasciatus*. The bioassays were performed at a room temperature of 30±1°C by exposing 25 larvae in each concentration of the extract. Triplicates for each concentration and the control (Distilled water), were tested for fifth instar larval bio-control potential. Table 1 show the relative activity among the eleven experimental sponges denoted the highest as well as lowest relative activity showed 4.67 and 1.0 responsible sponge species such as Ircinia campana and *Clathria gorgonoids* respectively. Though minimum and also with average relative activity observed with *Petrosia similes* 2.97 as well as moderate activity reflected as 3.40 particularly with *Haliclona pigmentifera* sponge.

Healthy female houseflies of three to four day old were treated topically on the dorsal surface of the thorax with five to six different doses (ranging from 100 to 500 μg/insect) of test solutions (1 μL/insect) with the help of a microapplicator.

A minimum of three replicates (35 flies each) were used for every test concentration. Simultaneously, control groups were also run with zero concentration of the test substance. After treatment the flies were transferred into observation jars and the mortality was recorded after 24 h. Marine sponge terpenoids have dominated the subject of chemical ecology since they have been studied for their activities against a variety of insect models. The relative activity showed that the Ircinia campana sponge having maximum activity 7.19 and moderate relative activity shows in *Petrosia similes* 3.67 also *Clathria gorgonoids* having the almost lowest relative activity.

Among the extracts evaluated, five of the sponge extracts were *C. diffusa, C. longitoxa* (Henschel), *C. diffusa* (Ridley) *S. carnosa* (Dendy), *H. pigmentifera* (Den.) and *D. nigra* (Dend.) showed significant activity in both larvicidal and insecticidal assays. Based on the results the most promising extracts are from *C. longitoxa* (Hen.) and *C. diffusa* that showed both larvicidal activities with LC_{50} at <50 ppm and <50 μg per insect, respectively. From this study, over all results were clearly indicated the better performance on both larvicidal and pesticidal activities shown marine sponge mainly belongs to the family of Microcionidae as well as Chaliniidae and the order of Poecilosceridae and Haplosclerida. Based on the present findings envisaged that the above said sponges (crude extracts) consisted secondary metabolites could be useful for searching new novel pesticidal molecules from marine origin.

DISCUSSION

The use of marine natural products is an alternative pest control method, which helps to minimize the usage of toxic pesticides and their deleterious effects on non target insect species, livestock, wildlife and on the environment (Fatope *et al.*, 1993). Earlier, we have isolated a molecule, ethylene bisisobutyl xanthate, from marine green alga, *Dictyosphaeria favulosa* (Kim and Mendis, 2006) based on the larvicidal activity (LC_{50} value at <50 ppm). The investigation further revealed that this molecule also exhibited Insect Growth Regulator (IGR) properties against *A. aegypti* (Rao *et al.*, 1995). Based on the bio-active properties, several analogues of alkylxanthates were synthesized and evaluated against lepidopteron pest, *Spodoptera litura* and *Helicoverpa armigera.* Three of the analogues i.e., methylene bis (tetrahydrofurfuryl xanthate), m-Fluorobenzyl n-butylxanthate and m-Fluorobenzyl isobutylxanthate have shown antifeedent and IGR activities against mosquitoes and agricultural pests (Rao *et al.*, 2003b; Taylor *et al.*, 2005). During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides (Thomas *et al.*, 2004; Dharmagadda *et al.*, 2005; Singh *et al.*, 2005). Previous literature also indicated that the marine organisms possess maximum percentage of bioactive substances with novel biological properties than the molecules originated from terrestrial origin (DOD, 1991).

Another findings relate this kind of sponge based pesticidal activity clearly described by Balbin-Oliveros *et al.* (1998) and marine biomolecules effect in rat brain enzyme synthesis level (Rao *et al.*, 1998) that is Philippine marine sponge of the genus Strongylophora yielded a new meroditerpenoid-strongylophorine dimer (1) and the known meroditerpenoids, strongylophorine-2 (2), strongylophorine - 3 (3) and strongylophorine-4 (4). The structures of the compounds were established on the basis of NMR spectroscopic also compound three was active against the phytopathogenic fungus *Cladosporium cucumerinum* and also against the neonate larvae of the polyphagous pest insect *Spodoptera littura* (Fab) demonstrated earlier by Okada *et al.* (2006).

Further studies on identification of active compounds, toxicity and field trials are needed to recommend the active fraction of these sponge extracts for development of eco-friendly chemicals for control of insect vectors. Edrada *et al.* (1996) demonstrated that the seven compounds were exhibited insecticidal activity toward neonate larvae of the polyphagous pest insect *Spodoptera* *littura* (with an ED_{50} of 35 ppm) when incorporated in artificial diet and offered to larvae in a chronic feeding bioassay. Again *Okada et al.* (2006) reported two novel insecticidal metabolites, calyculin E (1) and F (2), which had insecticial activity against the German cockroach and mosquito larvae, were isolated from a Japanese marine sponge, *Discodermia* sp. Van Wagenen *et al.* (1993) demonstrated that the validity of the premise that the marine biosphere might be source of new insecticides, did not disclose compounds of commercially significant potency against important insects pests. In case of that report clearly described a simple, but novel sponge metabolite with striking activity against not only our primary assay insect the tobacco hornworm, but also one of the more intransigent insect pests the cockroach. Again Funda (2007) described the insecticidal compounds derived from tropical marine sponge *A. carti* demonstrated that the guanidine alkaloids, hymenialdisine and debromo-hymenialdisine exhibited insecticidal activity towards neonate larvae of the polyphagous pest insects *Spodoptera littoralis* (LD_{50} 88 and 125 ppm, respectively).

CONCLUSION

Along with the eleven extracts evaluated seven of the sponge extracts, i.e., *S. carnosa, H. pigmentifera, I. fusa, I. campana* and *C. gorgoniods, S. inconstans* and *Petrosia similis* showed significantly higher activity on both larvicidal and pesticidal evaluation. Based on the results the most promising extracts are from *C. gorgonoids* and *C. diffusa* that showed both larvicidal and insecticidal activities with LC_{50} and LD_{50} at < 50 ppm and < 50 μg per insect, respectively. The results obtained from this study suggested that the above said sponges could be useful for searching new novel pesticidal as well as insecticidal molecules from marine origin. From the results, able to identify the secondary metabolites in sponges that were found to be effective pesticidal activity against lepidopteran pests and also effective insecticidal activity against the larvae of *Aedes aegypti* (Say).

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