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Screening of pesticidal activities of some marine sponge extracts against chosen pests

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ABSTRACT

Marine sponges (Porifera) are one of the most productive marine organisms. Eighty per cent of sponges (ethanolic extracts) were found to have insecticidal activity against fifth instar larvae of *Culex quinquefasciatus* (Say.) (Diptera: Culicidae) and *Achaea janata* (Linn.) (Noctuidae: Lepidoptera) and *Pericallia ricini* (Fab.) (Lepidoptera: Arctidae). Of the twelve sponges, five of them like *Clathria longitoxa* (Hentschel) (Poecilosclerida: Microcionidae), *Callyspongia diffusa* (Ridley) (Haplosclerida: Callyspongiidae), *Haliclona pigmentifera* (Dendy), *Sigmadocia carnosa* (Dendy), (Haplosclerida: Chaliniidae), and *Denrilla nigra* (Dendy) (Dendroceratidae: Darwinellidae) showed significant insecticidal activity. This paper clearly elucidated that *C. longitoxa* and *Callyspongia diffusa* (Rid.) were found to be more active than the remaining tested sponges**.** Screened twelve sponges showed the inhibition of larval growth ranged between 9-70% on *A. janata* and 10-96% on *P. ricini* respectively. From the results, we conclude that secondary metabolites of sponges were found to be an effective biopesticides against lepidopteran pests and larvae of *C. quinquefasciatus.*

Key words: Marine sponge, *Culex quinquefasciatus, Achea janata, Pericallia ricini,* Pesticidal, Larvicidal

INTRODUCTION

Increasing use of synthetics leads to serious problems like environmental pollution long term persistence, high toxicity and insect resistance to insecticides. In recent years there has been increasing information on the use of alternative methods (Blunt *et al*., 2005). The marine environment is an exceptional reserviour of bioactive natural products, which produce several novel structures with unique biological properties which may not be found in terrestrial natural products (Thakur and Muller, 2004; Venkateswara *et al*., 2008). The present investigations were aimed at identifying newer drugs and other pharmaceuticals from marine origin, whereas comparatively little attention has been paid with respect to the discovery of pesticide molecules (LiKam *et al*. 2006; Kim *et al*. 2006). Again Venkateswara *et al*. (2003) suggested that the secondary metabolites isolated from the marine sponges may be an alternative source for vector control agents to replace the existing and highly toxic synthetic insecticides and will play an important role in future insecticide development programme. Previously, Bradford *et al*. (1992) described the marine potential natural products to serve as insect control agents *via* mechanisms of toxicity, interference with moulting of metamorphosis and feeding deterrence. Again Donia and Hamanm, 2003; Blunt *et al*. (2005); Haefner (2005); Venkateshwara *et al*. (2008) demonstrated that the sponge consisting of sesquiterpenes and diterpenes - secondary metabolic compounds might be a source of new insecticides; it did not liberate compounds of commercially significant potency against important insect pests.

Muttom coastal area having 3,600 species of flora and fauna is one of the richest coastal areas in the entire mainland in Kanyakumari district. Yet no more work had been done than this kind of sponge activity against these two pests and larvae of mosquito. Hence we had selected the following objectives. The general objectives of this paper are to assess the biodiversity of the marine environment around the Muttom coastal region, and then to isolate and characterize the secondary metabolites from the twelve sponges and screen them for potential larvicidal and pesticidal growth inhibitory effects on polyphagous agricultural pests namely *Achaea janata* (Linn.) (Noctuidae: Lepidoptera) and *Pericallia ricini* (Fab.) (Lepidoptera: Arctidae) properties. The Present study is aimed at evaluating the insecticidal activity of *Clathria longitoxa, Callyspongia diffusa, Haliclona pigmentifera, Sigmadocia carnosa, Petrosia similes, Dendrilla nigra, Ircinia fusa, Sigmadocia fibulata, Clathria reinwardti, Spirastrella inconstans, Cacospongia salaries* and *Ircinia campana* against *C. quinquefasciatus* and *Clathria longitoxa, Callyspongia diffusa, Haliclona pigmentifera, Sigmadocia carnosa, Petrosia similes, Dendrilla nigra, Ircinia fusa, Sigmadocia fibulata, Clathria reinwardii, Spirastrella inconstans, Cacospongia* *salaries* and *Ircinia campana* on larvae of *A. janata* and *P. ricini*.

MATERIALS AND METHODS

Collection of sponges and preparation of crude extracts

Sponges were collected from Muttom coastal region in Cape Comorin coasts of Indian Ocean, at depths varying from 10 - 15 feet by snorkeling and SCUBA-diving process. Sponges were gently removed from the substratum and cut into small pieces and then soaked in methanol for preparing crude extracts. The intact sample specimens were sent to the Central Marine Fisheries Research Institute (CMFRI), Trivandram, Kerala, India for identification. The initial aqueous ethanol extract was concentrated in the laboratory under reduced pressure and lyophilized. The lyophilized powder was extracted with 1:1 mixture of ethanol solvent. At the same time the ethanol soaked cut pieces (100g) were further diced and extracted with the same mixture of solvents. The extracts were pooled and the organic portions 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, ethanol (w/v).

Collection and maintenance of pests

Castor semiloopers were collected from castor fields as well as the horticultural plains in Cheruvarakonam, whereas *Pericallia ricini* were collected from Kaliakkavilai, Kanyakumari district. Then the various life stages of both pests were maintained at 28±1.5°C with photoperiod of 11L: 13 D. A laboratory reared cyclic colonies of *C. quinquefasciatus* were used for this study followed by the little modifications made on the methods of Keiding *et al*. (1991) and Newman and Cragg (2004).

Screening bioassay on insects

Ethanolic extracts of sponge were sprayed on to the castor leaf and pomegranate leaf at the concentrations of 250ppm and 500ppm for *A. janata* and 0.050 ppm for *P. ricini* and evaluated their impacts described by the method of Venkateswaru *et al.* (2005) were evaluated whereas, the control category was treated with carrier alone. After three days, mortality rate was observed both in control and experimental categories. The mortality data were subjected to correction. Moreover, for *C. quiquefasciatus*, the median lethal concentration (LC_{50}) was calculated using 'Probit' analysis (Finney, 1971). 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. Corrected mortality data were subjected to Analysis of Variance (ANOVA) and the differences among treatment means were analysed using the Least Significant Difference (LSD) test and dose response data were analysed using linear regression in Microsoft Excel 2003.

RESULTS AND DISCUSSION

The sponge extracts of *C. longitoxa* and *C. diffusa* were found to be most effective against *C. quiquefasciatus* larvae which showed LC_{50} values at < 50 ppm. However, other extracts of *Dendrilla nigra* (Den.), *Petrosia similes*, *Haliclona pigmentifera*, *Ircinia fusa*, *Sigmadocia fibulata* showed LC_{50} values at <100 ppm (Table 1). The medium level of larvicidal effects revealed that five sponge extracts [*Haliclona pigmentifera, Sigmadocia carnosa, Petrosia similes, Dendrilla nigra* (Den.) *and Ircinia fusca* (Cartor)] showed medium response against these

Table 1. LC_{50} values (ppm) for 24h with their 95% fiducial (lower and upper) limits, regression equation, (χ^2) and P-levels of certain marine sponges against 5th instar larvae of *Culex quinquefasciatus* (Say)

Species	LC_{50} withfiducial limits		Regression equation	Median	Plevel
	upper	lower	LogY=(Y-bx) +bX	LC_{50} (mg/ml)	
Clathria longitoxa	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 fusa	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
Cacospongia salaries	128.3	127.05	Y=3.87+4.71X	0.68	0.74
Ircinia campana	137.05	131.24	Y =-6.84+5.68X	0.65	0.87

respective sponges. Meanwhile, the remaining three experimental sponges articulated minimum larvicidal activities. They were *C. reinwardii, Spirastrella inconstans, Ircinia campana* (Rao.). The larvicidal activities of sponge extracts were evaluated against the fourthinstar larvae of *C. quinquefasciatus* (Say). The relative activity of these twelve experimental sponges was observed. The highest as well as the lowest relative activity showed 4.67 and 1.0 in responsible sponge species such as *I. campana* and *Clathria longitoxa* (Hentschel) respectively. Furthermore minimum with average relative activity was observed in *Petrosia similes* (Ridly and Dendy) moderate activity has also been observed particularly in the sponge of *H. pigmentifera* extracts on *C. quinquefasciatus.*

Among the tested sponges, high and low mortality was caused by *Ircinia campana* and *C. longitoxa* in *A. janata* and *P. ricini* (Figure 1). Our results are comparable to those of Venkateshwara *et al.* (2008) which showed marine sponge methanolic crude extracts screened for insecticidal properties using housefly lethality test against *Musca domestica.* Structure activity relationship (SAR) studies have shown that adjacent acetogenins having two bis tetrahydrofuran (THF) rings are more potent than those having only one and adjacent bis-THF dibenzoate (Bradford *et al*., 1992; Balbin *et al.*, 1998; Bokesch *et al*., 2002; Akihiko *et al*., 2006). In addition recent studies of Jiangnan *et al*. (2003) reported 18 structurally diverse derived compounds examined for insecticidal, herbicidal, and fungicidal activities. Several new classes of compounds have been shown to be insecticidal, herbicidal, and fungicidal, which suggests that marine natural marine products represent an intriguing source for the discovery of new agrochemical agents.

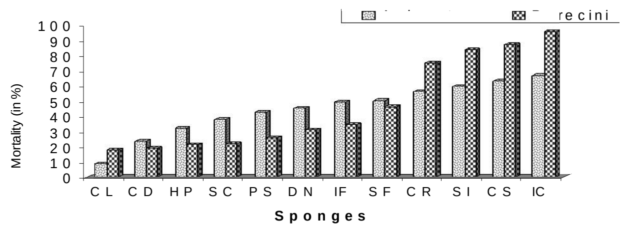

Figure 1. Impact of crude ethanolic extract of twelve marine sponges on *Achaea janata* and *Pericallia ricini*

The use of natural marine 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; Funda, 2007). The investigation further revealed that this molecule also exhibited Insect Growth Regulator (IGR) properties against *A. aegypti* (Venkateswara Rao *et al*., 1995; Martinez *et al*., 2007). Previously several authors explained the two agricultural pests and based on the bioactive properties, several analogues of alkylxanthates were synthesized and evaluated against the lepidopteron pests, such as *Spodoptera litura* and *Helicoverpa armigera*. Three of the analogues such as, methylene bis (Tetrahydrofurfuryl Xanthate), m-Fluorobenzyl n-Butylxanthate and m - Fluorobenzyl isobutylxanthate have shown antifeedent and IGR activities against mosquitoes and agricultural pests (Venkateswara *et al*., 2003; Taylor *et al.*, 2005). The present preliminary investigations have helped us to shortlist the bioactive 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 resources. 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).

Among the extracts evaluated, *Clathria longitoxa* (Henschel), *Callyspongia diffusa* (Ridley) *Sigmadocia carnosa* (Dendy), *Haliclona pigmentifera* (Den.), and *Dendrilla nigra* (Dend.) showed significant insecticidal activity. Based on the results the most promising extracts are from *Clathria longitoxa* (Hen.) and *Callyspongia diffusa* that showed both larvicidal activities with LC_{50} at < 50 ppm and < 50 ìg per insect, respectively. These promising results in relation with *in vitro* insecticidal activity open the way for complementary investigation in order to purify and identify active molecules.

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