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: Chalinidae), and Dendrilla nigra (Dendy) (Dendroceratidae: Darwiniellidae) 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, Achaea 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 reservoir 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 molting of metamorphosis and feeding deterrence. Again Donia and Hamann, 2003; Blunt et al. (2005); Haefner (2005); Venkateshwarar (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 similis, Dendrilla nigra, Ircinia fusa, Sigmadocia fibulata, Clathria reinwardii, Spirastrella inconstans, Cacospongia salinaria and Ircinia campana against C. quinquefasciatus and Clathria longitoxa, Callyspongia diffusa, Haliclona pigmentifera, Sigmadocia carnosa, Petrosia similis, Dendrilla nigra, Ircinia fusa, Sigmadocia fibulata, Clathria reinwardii, Spirastrella inconstans Cacospongia...
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 and water. The extracts were pooled and the organic portions evaporated for obtaining solvent free mixture of solvents. The extracts were further diluted and extracted with the same solvent. The 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. quinquefasciatus, 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. quinquefasciatus larvae which showed LC$_{50}$ values at $<50$ ppm. However, other extracts of Dendrilla nigra (Den.), Petrosia similes, Haliclona pigmentifera, Ircinia fusca, 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 pests.

Table 1. LC$_{50}$ values (ppm) for 24h with their 95% fiducial (lower and upper) limits, regression equation, ($y^2$) and P-levels of certain marine sponges against 5th instar larvae of Culex quinquefasciatus (Say)
Among the tested sponges, high and low mortality was particularly in the sponge of Dendy moderate activity has also been observed activity was observed in respective sponges. Furthermore minimum with average relative activity showed 4.67 and 1.0 in responsible sponge species such as I. campana and Clathria longitoxa (Hentschel) respectively. The highest as well as the lowest relative activity of these twelve experimental sponges was observed. The highest 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 Venkateshwar et al. (2008) which showed marine sponge methanolic crude extracts screened for insecticidal properties using houself 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; Akikho 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 Achatia 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 (Venkateshwar 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 littura and Helicoverpa armigera. Three of the analogues such as, methylene bis (Tetrahydrofurfuryl Xanthate), m-Fluorobenzyl n-Butylxanthate and m-Fluorobenzyl isobutylxanthate have shown antifeedant and IGR activities against mosquitoes and agricultural pests (Venkateshwar 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 (Rudley Sigmadicia 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 LC50 at < 50 ppm and < 50 μg 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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