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ANTIFUNGAL AND INSECTICIDAL ACTIVITY OF A LECTIN ISOLATED FROM MARINE SPONGE FASCIOSPONGIA CAVERNOSA

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Abstract

The present study was intended to explore antifungal and insecticidal activity of a lectin isolated from marine sponge *Fasciospongia cavernosa* (FCL). The lectin was subjected to evaluation for inhibition of microbial growth of five phytopathogenic fungi based on disc diffusion method. The results showed that the FCL (1mg/ml) had a potent antifungal activity against *Penicillium chrysogenum* and *Candida albicans*. A comparison of the antifungal activity of FCL antibiotics such as Amphotericin B was also carried out. Also the toxic effects of FCL with different concentrations (0.1-1 mg/mL) were tested against cowpea aphid, *Aphis craccivora* by artificial diet. FCL showed insecticidal activity in a time and dose dependent manner. These findings indicate that the lectin may be of importance in therapeutic and pesticidal applications.

Keywords: *Fasciospongia cavernosa*, Lectin, Disc diffusion method, antifungal activity, *Aphis craccivora*, artificial diet

Introduction

Various researchers were concentrated on the marine natural products and their different medicinal functions to unfold new potent drugs. Sponges (phylum Porifera) are the oldest multicellular animals (Metazoa) and among the leading sources of appealing chemicals produced by marine organisms (Bakus *etal*., 1974 & Sepcic *etal*., 2010). Marine sponges are a depository of structurally diverse bioactive lectins, (Takahashi *etal*., 2008; Moura *etal.*, 2006, Molchanova et al., 2005). Lectins comprised of all sugar-specific agglutinins of non immune descent; regardless of source and blood type selectivity (Sharon and Lis, 1972).These lectins from marine sponges have great potential for biotechnological applications, shown by activities ranging from mitogenic, chemotactic, antibacterial, anticancer and modulation in the mammalian nervous system. (Gomes Filho *etal*., 2014).

The indiscriminate use of chemicals as fungicides and insecticides, has developed high levels of immunity to the conventional pesticides and majority of them have diverse side effects (Varma and Dubey *etal*., 1999; Kranthi *etal*., 2002).On these basis, probing for naturally arising potential antifungal and insecticidal agents to be used for crop defence , food safety and low mammalian toxicity is foremost and takes much consideration, due to the importance of natural and biological food products (Arora *etal*.,2005; Blunt *etal*., 2005:

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Boeke *etal*.,2004; Pretorius and Van der Watt *etal*., 2011).There are many reports of insecticidal and fungicidal activities of lectins towards pests and pathogens of economically importance (Vasconcelos *et al*., 2004; Mohsen *etal*.,2018).This feature of lectins can be used as a potent naturally occurring insecticide/ fungicide and thus improve crop production and has a severe impact on food safety and quality of the environment.

The aim of this work was to check the antifungal and insecticidal properties of lectin from the marine sponge *Fasciospongia cavernosa*.

Materials and methods

Chemicals

Agar, dextrose, peptone, beef extract were purchased from SRL Ltd., and Amphotericin B from Himedia., India. All other biochemicals and chemicals used in the investigation were of analytical grade.

Preparation of *Fasciospongia cavernosa* lectin (FCL)

Lectin from the marine sponge *Fasciospongia cavernosa* (FCL) was purified essentially as previously described (Sadanandan *etal*., 2018). In short, the lectin were extracted with Phosphate buffered saline (PBS) buffer (pH - 7.4), fractionated by ammonium sulphate precipitation and purified by Guar gum affinity chromatography. The purified lectin (FCL) was then lyophilized and kept under 4˚C until use.

Fungal strains

The fungal strains used in this study were obtained from the, Department of Biotechnology, University of Kerala, Trivandrum, Kerala, India. The fungal pathogens were *Penicillium chrysogenum, Candida albicans, Aspergillus flavus, Collectrichum corchori* and *Rhizopus oryzae.*

Maintenance of test insects

Aphis craccivora were reared on cowpea (*Vigna unguiculata*) plants raised in experimental fields. All experiments are conducted in following conditions: a temperature of 25°C, 85% humidity.

Antifungal Activity of FCL

The *in vitro* antifungal activity of FCL was determined by disc diffusion method (Ye et al., 2000). SDA medium was used for the culture of fungi. After preparation of the media, SDS was melted in hot water bath and allowed to cool. On cooling, it was poured to sterile Petri dishes and allowed to solidify in horizontal position. The culture colonies from the stock cultures were selected and 100 µl was transferred to the culture medium. A sterile spreader was dipped into the properly diluted inoculums and spread evenly. Different concentrations of FCL (0.1-1mg/ml) were prepared with PBS (pH -7.4) for the antifungal studies. Sterile paper disc of diameter 5mm (made from Whatmann No-1 filter paper) impregnated with FCL (0.1-1mg/ml) were placed on the surface of the SDA plates seeded with test organisms. They were touched down with a sterile forceps to ensure complete contact with surface. The plates were incubated at 37°C for about 24 hrs. Control disc impregnated with PBS was also used along with the test disc in each experiment as negative control. After incubation, the test cultures were plated on air dried nutrient SDA plates using a sterile glass spreader. Using a clean forceps, the sterile discs loaded with the lectin was placed on

the surface of SDA plates seeded with the test fungal strains. Commercially available Hi combs of Amphotericin B (Himedia, Mumbai) were used as the positive control and PBS was used as the negative control. The plates were then incubated at 35±2°C for 72 hours. The zone of fungal growth inhibition was observed and its diameter was measured in millimeters.

Insecticidal activity of FCL

The insecticidal activity of FCL was assessed by ingestion in artificial diet according to Douglas et al., 2006. FCL (0.1-1 mg / mL) with 0.5M Sucrose either supplemented with or without the antibiotic rifampicin at 50 µg/ mL in the diet. The diet was provided to the aphids in a feeding chamber consists of a food sachet containing 100 µL of the artificial diet sandwiched between two layers of Parafilm stretched over a plastic ring (3.0 cm diameter and 3.0 cm high) as described in Sadeghi *etal.*, (2009). Adults of *A.craccivora* (10 numbers) were transferred from the *Vigna unguiculata* plants onto the sachet using a artist's brush and then the sachet was confined within a small ventilated Petri dish. Mortality percentages were determined after 24, 48, and 72 hours after treatment. Each treatment was replicated three times under the same environmental conditions described for the insect rearing.

Statistical analysis

All the experiments were performed in triplicate (n = 3). The data were expressed as mean ± standard deviation.

Results and Discussion

FCL (1mg/ml in SDA medium) showed significant inhibition (10 mm) of mycelial growth against *Penicillium chrysogenum,* and *Candida albicans* (11 mm) *among* tested fungi(Table -1). On the other hand, the growth of all the five fungi was totally inhibited by antifungal antibiotic Amphotericin (10 µg/ml). Similarly antifungal effects of a 30 kDa D-Galactoside-Specific Lectin from the demosponge, *Halichondria okadai* lectins have reported and showed strong antifungal activity (Kawser *etal*., 2011, Hasan *etal*., 2019).A potent antifungal activity of lectin from marine sponge *Axinella donnani* against *Penicllium crysogenum* and *Aspergillus flavus* was reported by Ratheesh and Rauf *etal*., (2018). It was recommended that role of lectin in the resistance system against pathogens was a result of potential sites of invasion by infectious agents, as well as the binding of lectins to several fungi and their ability to inhibit fungal growth and development(Vaz *etal*.,2010; Trindade *etal*.,2006). As per Wong *etal*., (2008) lectins do not directly inhibit the fungal growth by shifting the structure or penetrability of the fungal membrane but preferably by secondary effects produced by the binding of the lectins to carbohydrates on the surface of the fungal cell wall.

The insecticidal activity of FCL on *A.craccivora* was time and dose-dependent manner (Table -2). After 72 h exposure, food added with 0.1 to 1 mg/ml of FCL showed 13.33 % to 100% of *A.craccivora* mortality. Previously Fitches *et al.,* (2008) demonstrated that the heterodimeric and homodimeric garlic lectins, ASAI and ASAII, produced as recombinant proteins, showed insecticidal effects when fed to pea aphids (*A. pisum*). Lectin toxicity against several aphid species from *P. australis* (Zapata *et al.,* 2015), Allium *porrum* (Sadeghi *etal.*, 2009), *Allium sativum* (Dutta *et al.,* 2005), *Canavalia ensiformis* (Sauvion *et al.,*2004) showed acute and chronical -

insecticidal activity. Similarly snowdrop lectin (*Galanthus nivalis* agglutinin, GNA) fused to an insecticidal spider venom neurotoxin (*Segestria florentina* toxin 1, SFI1) were found to be toxic against the rice brown plant hopper *Nilaparvata lugens* and the peach-potato aphid *Myzus persicae* (Sulzer) by incorporation into artificial diets (Down *etal*., 2006) . Insecticidal activity of lectins was by obstruction of feeding (Sprawka and Golawska , 2010) delayed reproductive status (Down *et al.,* 2006) interfere with the normal absorption of nutrients (Rahbé et al., 1995) binding of carbohydrates on the surface of responsive cells, including the epithelial cells of animal digestive tracts (Harper *et al*., 1995; Zhu-Salzmann *et al*., 1998). To our knowledge, this is the first report on the insecticidal activity of lectin from a marine sponge lectin.

Conclusion

A galactose specific lectin isolated from marine sponge *Fasciospongia cavernosa* (FCL) exhibited antifungal and insecticidal activity in a dose dependent manner. These results indicate that the lectin may be utilized in therapeutic and pesticidal applications.

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Table 1. Antifungal activity of the purified lectin (FCL) against phytopathogenic fungi

Name of fungus	Percentage inhibition of fungal mycelia growth (1mg/ml ADL)(mm)	Percentage inhibition of fungal mycelia growth Amphotericin* (10 µg/ml) (mm)
Penicillium chrysogenum	10± 0	10.33 ± 0.58
Candida albicans	10.67±1.15	13 ± 0.41
Aspergillus flavus	Nil	10 ± 1.15
Colletotrichum corchori	Nil	12 ± 0.53
Rhizopus oryzae	Nil	10 ± 1.15

*Standard antifungal antibiotic, growth measured-radial growth in cm.

Table 2. Insecticidal activity of the purified lectin (FCL) against *Aphis craccivora*

Concentration of FCL (mg/ml)	**Mortality of *A.craccivora* after (hours)**		
	24	**48**	**72**
Control	0 ± 0	0 ± 0	6.67 ± 5.78
0.1	0 ± 0	6.67 ± 5.78	13.33 ± 5.78
0.25	6.67 ± 5.78	16.67 ± 5.78^{a}	26.67 ± $5.78^{a,b}$
0.5	16.67 ± $5.78^{a,b}$	43.33 ± $5.78^{a,b,c}$	60 ± $10^{a,b,c}$
0.75	50 ± $10^{a,b,c,d}$	80 ± $10^{a,b,c,d}$	100 ± $0^{a,b,c,d}$
1	83.33 ± $5.78^{a,b,c,d,e}$	100 ± $0^{a,b,c,d,e}$	100 ± $0^{a,b,c,d}$

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