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Research Article

Isolation and Detection of Marine Microorganisms and Evaluation of *In-Vitro* Insecticidal Activity of Ethanolic Crude Extracts of Marine *Streptomyces sp*. Against Larvae of *Sitophilus oryzae*

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Abstract: Marine microorganisms, whose immense genetic and biochemical diversity is only beginning to be appreciated, look likely to become a rich source of novel chemical entities for the discovery of more effective drugs. The present study was performed to evaluate the insecticidal potential of Actinomycetes isolated from the soils of the Dublarchar and Kochikhali, Bangladesh. The actinomycete isolates were identified by various parameters such as colony morphology, spore arrangement, staining, and biochemical reactions. Insecticidal activity of different concentrations of ethanolic extract of the isolates was determined against the second instar larvae of *Sitophilus oryzae.* The larvicidal effect, in terms of mortality of larvae, of the extracts was determined by counting the number of dead larvae after 24 hours. Ten actinomycete isolates were recovered from the soil sample and one was subjected to identify (Due to potent antibacterial activity) as the species of Streptomyces on the basis of microscopic, biochemical, and staining characteristics. The insecticidal potential of ethanolic extracts, in terms of larval mortality, was found to be dose dependent. The isolate showed a marked insecticidal activity. At a concentration of 24mg/ml, the isolate caused 100% mortality of the larvae. The lethal conc. 50 was found 3.16mg/ml. Isolation and characterization of active constituents from the ethanol extract possessing insecticidal potential are to be investigated.
Keywords: Streptomyces, Antibiotics and Insecticides, *Sitophilus oryzae*,

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INTRODUCTION

Over 3500 species of Streptomyces sp. are known to possess some insecticidal activity, by containing either antifeedant, repellent or insecticidal compounds that enable the crude extracts or an isolated active compound to protect stored products. As a rich source of bioactive chemicals, Streptomyces sp. may provide potential alternatives to used insect-control agents. Bioactive chemicals of Streptomyces sp. can also be less toxic, readily biodegradable and of great economic interest both from the agronomic and preventive medicine points of view. Also, about 60% of the new insecticides and herbicides reported in the past 5 years originate from *Streptomyces.* The problems of drug resistance, patient's sensitivity and inability to control certain infectious diseases have given an impetus for continuous search of new antibiotic all over the world. To combat the multidrug resistantorganisms, production of new antimicrobial compound or antibiotics from new source is essential. Cotton leafworm (*Spodoptera littoralis*), is considered one of the most injurious and destructive polyphagous lepidopterous insect pests attacking crops, vegetables and fruit trees all over the world [1].It has the ability todevelop relatively quick resistance to most conventional insecticides. As the environmental contamination by toxic chemicals increases, alternative approaches for controlling pest populations have become research priorities. These have includedbiological or ecological control methods for limiting the destructive impacts of pest populations, especially in agriculture [2, 3, 4]. Egypt lost 50% of the national yield of cottons due to country wide resistance to Toxaphene in 1961 [5]. Many reports indicated that actinomycetes play an important role in the biological control against insect including the cotton leaf worm *spodopetra liltoralis*, house fly *Musca domestica*[6], *Culex quinquefasciatus*, *Drosophila melanogaster* [7],

Helicoverpa armigera [8], *Anopheles mosquito* larvae [9] and *Culex pippins* [10]. Investigate the biological activity of secondary metabolites of some actinomycetes isolates on last instar larvae of the cotton leaf worm *Spodoptera littoralis* through the food plant (Castor leaves) .They showed that Streptomyces and Streptoverticillum were the most potent actinomycetes, which cause larval and pupal mortality. Osman *et al*., reported that the pellets of some strepyomyces isolates were more active against cotton leaf worm than culture filtrate reported that the direct introduction of parasites, pathogenand predators to target insect is very effective method of biological control. Also, using of dead spores of varieties of the natural soil bacterium and actinomycetes show interfere in the digestion systems of larvae. These spores are no longer effective after the larvae turn into pupae because they stop eating. *Streptomyces* strains isolated from sea water and sea sediments from Beidiahe and Dagang of the east coast of China, screened for their insecticidal activities using bioassay against *Helicoverpa armigera*. Many of bio product which produced from actinomycetes showed different mode of action against different insect. Avermectin produced by the soil microorganism, *Streptomyces avermitilis* act on GABA- and glutamate-gated chloride channels of insect. Spinosad is a neurotoxin mixture produced during fermentation of a soil actinomycete that has high activity towards Lepidoptera. It blocking the chloride channel associated with GABA receptor of the insect [11].Spinosad and indoxacarb were introduced at the same time against insect pests of cotton in Pakistan which cause paralysis in the larvae on eating them. A rational approach is being developed to use Streptomyces sp. derived bioactive compounds as an insecticide. The insecticidal activity is due to the presence of active molecules.Thus, the object of this work was to assess the insecticidal activity of Ethanolic extracts of Bangladeshi *Streptomyces sp*. against the storage pest *Sitophilus oryzae*.

MATERIAL AND METHODS

Sample collection

The soil samples were collected from the Dublarchor and Kochikhali,Sundarbans, Bnagldesh for screening of insecticidal activity against *Sitophilus oryzae*. Samples were collected as aseptically as possible. Samples were collected from various depth of the earth surface, ranging from layers just beneath the upper surface to 1.5 feet depth. A trowel was used to dig the soil. The samples were collected in the sterile polythene bags with a clipped border and properly labeled indicating the date of collection and the depth.

Isolation and Identication of Streptomyces

Ten marine microorganisms were isolateddesigned as AIAH-1 to AIAH-10 and one of them(AIAH-10) showed most potent antibacterial and insecticidal activity.The Streptomyce used in this study was isolated from the Dublarchor and Kochikhali,Sundarbans, Bangladesh. The actinomycetes specie, and stored microbiology lab of Rajshahi University, Bangladesh. The actinomycetes isolates were culturally characterized based on colour, dryness, rough, with irregular or regular margin, and generally convex colony morphology, tough leathery colonies, branched vegetative mycelia, and when present, aerial mycelia and spore formation as described by Ghanem and grouped into generic morphotypes including the Streptomyces.

Preparation of *Streptomyces* suspension

The test *Streptomyces* suspensions were prepared by suspendinga loopful of pure *Streptomyces* colony in 2 mL sterile normal saline,vortexed to homogenize and stored at 4°C until ready for use. This suspension was used as *Streptomyces* inoculants in all cultivations.

Fermentation and preparation of crude ethyl acetate extracts

Fermentation for production of antibiotic and subsequent extraction of the antibiotics was done as described by Ilic *et al*. (2007) with modification. Yeast extract Glucose broth (YGB) was prepared and 20 mL dispensed into 100 mL Erlenmeyer flask capacity, sterilized, allowed to cool and inoculated with 0.5 mL *Streptomyces* isolate suspension and incubated at 28°C for 48 h at 230 rpm. About 500 mL of YGB was prepared in 1L Erlenmeyer flask and inoculated with the 48 h old pre-culture of *Streptomyces* isolate and incubated for 10 days at 28°C at 230 rpm. At the end of the incubation period, the culture was harvested by centrifugation at maximum speed for 15 min. The culture supernatant was extracted twice with equal volumes of ethyl acetate (1:1 v/v) and vaporized to dryness in a rotary evaporator at 50°C. The extract was re-constituted in 50% filter sterilized methanol to obtain the desired concentration at every stage of screening

Insecticidal activity

Test materials used for the study:

The crude ethanolic extract of isolated Streptomyces was used for the investigation of insecticidal activity. The extract was dissolved in ethanol.

Test Larva used for the study:

For determining the insecticidal activity of the isolated extracts, the larva of *Sitophilus oryzae* was collected from the department of Zoology. University of Rajshahi, Bangladesh.

Apparatus and reagents

Sample Beaker,Ethanol, Measuring cylinder, Micropipette and Tap water.

Procedure:

For each compound, six beaker were taken and they were marking as 1 to 6.Then 0, 1, 2, 4, 8, 16 and 32μg/ml of previously prepared solution of the compounds were added in the 1 to 7 no beaker respectively, where beaker no 1 is used as control. Then twenty larva of *Sitophilus oryzae* were added in each of the beaker. The insecticidal activity of the compounds was determined by counting the number of dead larva after 24 hours. Death larva was identified when they failed to move after probing with a needle in siphon or cervical region. The percentage of larval mortality was determined.

RESULT AND DISCUSSION:

Isolation:

The observation of the test plates were done after 5 days of incubation at 30°C for the growth of marine microorganisms on the surface of the isolation medium (Figure 1).

The colonies were counted on the 10^{-2}dilution plates and the number of actinomycetes per gram of soil was determined by multiplying with the dilution factor. The total actinomycete count on isolation medium supplemented with nystatin as antifungal agent was being in the range of 0.7×10^4 to 1.2×10^4 c.f.u.g^{-1} of dried weight soil. The count was highest in the middle of the Dublarchor, Sundarbans. Some isolated strains were shown in the Figure 2.

Identification:

From the morphological, cultural and physiological characteristics, pattern of utilization of carbon sources and growth pattern of AIAH-10, it may be concluded that the organism belongs to *Streptomyces* genus and for the identification scheme of an organism, its molecular level characterization is also necessary. To assign the strain to the specie level, the 16S rDNA of the *Streptomyces* had already been isolated. The sequencing of 16S rDNA for both of the organisms is under process.

Isolation of Ethanolic crude extract by Fermentation:

The ethanolic extract obtained from the broth culture medium was subjected to insecticidal activity.

Insecticidal activity crude ethanolic extract

Insecticidal activity of the isolated ethanolic extract against the larva of *Sitophilus oryzae* in different concentrations was shown in the Table 1 and Figure 3. Concentration dependent mortality was observed. The larval mortality was recorded as 100% in case of 24mg/ml and higher concentrations. The lethal concentration 50 (LC_{50}) of the extract was found to be 3.16 mg/ml (Figure 4).

Table 1. Insecticidal activity of the isolated crude extract against *Sitophilus oryzae* larva.

Group	Conc. of sample (mg/ml)	LogC	No. of larva added	No. of death in each beaker			Average No. of death	(%) of Mortality	LC_{50}(mg/ml)
				1	2	3			
Control	0	0.00	20	0	0	0	0.00	0.00	0.00
Extract	1.5	0.17	20	3	7	2	4.00	20.00	3.16
	3	0.47	20	8	12	7	9.00	45.00	
	6	0.77	20	14	17	15	15.3	76.50	
	12	1.07	20	19	18	20	19.00	95.00	
	24	1.38	20	20	20	20	20.00	100.00	

Figure -1: **Bacterial colony appeared on the dilution plates of the marine soil samples collected from marine sediments and soil samples of different location of mangrove forest (Sundarbans), Bangladesh**.

Figure-2: Some isolated strains marine microorganisms.

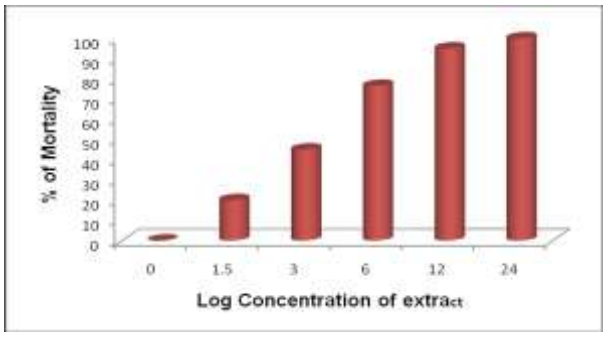

Figure-3: Insecticidal activity of the isolated crude extract against *Sitophilus oryzae* larva.

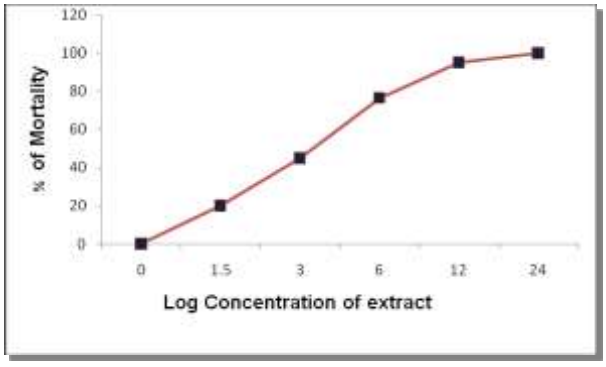

Figure 4: LC_{50} of the isolated crude extract against *Sitophilus oryzae* larva.

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