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ANTAGONISTIC AND INSECTICIDAL ACTIVITIES OF SOME *STREPTOMYCES* ISOLATES

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ABSTRACT

Fifteen local isolates of *Streptomyces* named, S01, S02, S03, S04, S05, S06, S07, S08, S09, S10, S11, S12, S13, S14, and S15, isolated from different soils and geographical areas in Egypt were used in this investigation. These isolates were propagated for screening studies and to evaluate their efficiency as antagonistic agents against some phytopathogenic fungi (such as, *Rhizopus nigricans*, *Aspergillus niger*, *Fusarium oxysporum* and *Helminthisporum gramenium)* and insect pest, (cotton leaf worm, *Spodopetra littoralis*). The *Streptomyces* isolates were grown on starch nitrate broth medium under shaking condition at 28°C for 6 days. Culture filtrates were then tested against the five phytopathogenic fungi. Results revealed that most of the isolates were varied in their antagonistic activities. Isolate S08 was active against *R. nigricans*, *A. niger* and *F. oxysporum* while isolates S01, S05, S11 and S14 were active against *A. niger* and *F. oxysporum* and isolates S04, S09 and S13 were active against *R. nigricans* and *A. niger*. On the other hand, no antifungal activity was found against *Helminthisporum sp*. The insecticidal activity of both culture filtrates and cell pellets were tested against cotton leaf worm. The experimental results showed that the pellets of some *Streptomyces* isolates were more active against cotton leaf worm than culture filtrates. Generally, isolates S05, S08, S10 and S15 showed 80, 100, 70 and 80% mortality against cotton leaf worm, respectively. The protein(s) of isolate S08 cells was purified through ammonium sulfate saturation 40, 60 and 80%. Results of SDS-PAGE analysis showed that a 40 KDa protein was purified and showed high activity against four instars of the cotton leaf worm. This result demonstrated the ability to use such *Streptomyces* isolates as effective biopesticide agents.

INTRODUCTION

Integrated pest management (IPM) programs create the need for novel fungicides or insecticides having more selective modes of action. Attempts have been made to develop *Streptomyces* species as fungal root disease control agents, since *Streptomyces* spp. are capable of producing a remarkably wide spectrum of antibiotics as secondary metabolites (Lechevalier and Waksman, 1962; Lechevalier, 1988 and Franklin *et al*., 1989). Microorganisms produce many useful anthelmintic and insecticidal antibiotics (Pachlatko, 1998; Ghazal *et al*., 2001 and Yuhui *et al*., 2002).

In 2001, Bream *et al*. investigated the biological activity of the secondary metabolites of 41 Egyptian actinomycete strains on the cotton leaf worm *S. littoralis*. They found that 58% of the tested strains caused larval mortality ranging from 10-60%; *Streptomyces* and *Streptoverticillum* were the most potent actinomycetes affecting the biological and physiological criteria of the present insect species. *Streptomyces* strains isolated from seawater and sea sediments from Beidiahe and Dagang of the east coast of China, screened for their insecticidal activities using

bioassay against Helicoverpa *armigera*. Results revealed that 40 out of the 331 (12.08%) isolates showed potential insecticidal activities. Of the 40 isolates, one isolate, designated *Streptomyces* sp. 173, was found to have strong insecticidal activity against *H. armigera*. (Xiong *et al*., 2004).

Recently, new antimycin antibiotic A9 has been isolated from a cultured broth of *Streptomyces* sp. K01-0031 together with antimycins A3a, A3b, A4, A7, and flazin methyl ester. Antimycin A9 is the first antimycin having an aromatic 8-acyl residue. It showed potent nematocidal and insecticidal activities against *Caeno-rhabditis elegans* and *Artemia salina*, respectively but inhibited bovine heart NADH oxidase at nanomolar level like other known antimycins. (Shiomi *et al*., 2005)

(Mourad *et al*., (2004) showed that the Egyptian cotton leaf worm (*S. littoralis*) is an important polyphagous insect pest attacking cotton, several cultivated crops and ornamental plants. The aim of this study was to determine the antifungal and insecticidal activities of fifteen soil-*Streptomyces* isolates against some phytopathogenic fungi and cotton leaf worm insect.

MATERIALS AND METHODS

***Streptomyces* isolates:** Fifteen unidentified *Streptomyces* isolates (S01, S02, S03, S04, S05, S06, S07, S08, S09, S10, S11, S12, S13, S14, and S15) isolated from different soils in Egypt were kindly obtained from, Department of Agricultural Microbiology, Soil, Water and Environment Research Institute (SWERI), ARC, Giza, Egypt.

Isolates propagation and preparation: Standard inoculums for each applied *Streptomyces* species was prepared by scraping the heavy spores from the surface of the growth of starch nitrate slant in the presence of 5 ml sterilized d.H_2O. An aliquot of 2 ml standard inoculum was transferred aseptically to 50 ml of a broth medium (data not shown) modified from starch nitrate broth medium as reported by Waksman and Lechvalier (1961) in a 250 ml conical flask. Inoculated flasks were incubated at 28±2^oC for 6 days on a rotary shaker (160 rpm/min). Thereafter, growth was centrifuged at 10000 rpm at 4^oC for 5 minutes. The supernatants and pellets were then subjected for the evaluation of their antagonistic or insecticidal activities.

Pellets preparation: The pellet of each *Streptomyces* isolates was re-suspended in equal volume of Tris buffer pH 7.5 then sonicated and spun at 14000 rpm.

Antagonistic activities of *Streptomyces* isolates: The antagonistic activities of the supernatants of the 15 *Streptomyces* isolates under investigation were tested against four phytopathogenic fungi, i.e., *Rhizopus nigricans*, *Aspergillus niger*, *Fusarium oxysporum* and *Helminthisporum gramenium*, (provided by Cairo MIRCEN, Faculty of Agriculture, Ain Shams University), as described by Mohamed *et al.,* (2001).

Source of insect: Sets of the cotton leaf worm (*S. littoralis*) insect belonging to family *Noctuidae* were provided by the insectary at AGERI, ARC, Giza, Egypt.

Insecticidal activities of *Streptomyces* isolates: The supernatants and pellets of the 15 *Streptomyces* isolates of this study were used to determine their insecticidal activities against the 1st instar larvae of cotton leaf worm. For each treatment, three bioassay cups 5-cm in diameter containing 2.5 ml of cooled-dried semi-artificial diet (Levinson and Navon, 1969) supplemented with 500 µl of the supernatant or pellet-suspension and 10 larvae of cotton leaf worm were added. Cups were then kept at

28°C, and the mortality (%) was recorded after 3-5 days according to Finny (1962). As a control, 3 replicates of the bioassay cups with the same medium were also used.

Protein purification: The protein(s) of the *Streptomyces* isolate No. 8 were extracted and purified as follows: the pellet-suspension was separately treated with 40, 60 and 80% ammonium sulfate, incubated for three hours at 4°C and centrifuged at 14000 rpm for 15 min at 4°C. The supernatant was discarded and the pellet was then resuspended in a suitable volume of 20 mM Tris-HCl pH 7.5 and dialyzed against phosphate buffer saline (PBS). The crude proteins prepared by 40, 60 and 80% ammonium sulfate treatments were subjected fort the evaluation of their insecticidal activities against 1st instar of cotton leaf worm larvae. Moreover, the protein of 40% was tested against 2nd, 3rd and 4th instars as mentioned above.

RESULTS AND DISCUSSION

Streptomycetes produce a wide variety of commercially important secondary metabolites, including antibiotics, which exhibit antibacterial, antifungal, anthe-lmintic, antitumour and immunosuppre-ssive activities (Hopwood, 1997, Katz, 1997 and Leadlay, 1997).

In this study, sets of fifteen soil-streptomycete isolates from Egypt were used to investigate the biological activities of their secondary metabolites against some phytopathogenic fungi as well as cotton leaf worm (*S. littoralis*). Results in Table-1. showed that culture filtrates of the applied streptomycete isolates were varied in their antifungal activities. *Streptomyces* isolate S08 was active against *R. nigricans*, *A. niger* and *F. oxysporum* while isolates S01, S05, S11 and S14 were active against *A. niger* and *F. oxysporum* and S04, S09 and S13 isolates were active against *R. nigricans* and *A. niger*. On the other hand, no antifungal activity was found against *Helminthisporum sp*. This finding confirmed the possibility of presence of secondary metabolites, i.e., fungicides in the culture filtrates of propagated isolates.

Several investigators (Mohamed, 1998, Mohamed *et al*., 2000, Mahfouz and Mohamed, 2002, Abdel-Fattah 2005 and Mohamed *et al*., 2005 reported the ability of Streptomycetes to inhibit the growth of different phytopathogenic fungi as well as soil-borne fungi.

Saroj *et al*., (1987) screened metabolites from 942 microbial isolates for insecticidal properties. The isolates included 302 streptomycetes, 502 novel actinomycetes including representatives of 18 genera, 28 unidentified aerobic actinomycetes, 70 fungi and 40 bacteria other than actinomycetes. They showed that the metabolites from 55 isolates at a dilution of 10^{-1} caused nearly one hundred percent mortality in mosquito larvae (*Aedes aegypti*) within 24 h. These isolates included 27 isolates of *Streptomyces*, four of *Actinoplanes*, three isolates each of *Actinomadura* and *Streptoverticillium*, two isolates each of *Micromonospora, Bacillus* and *Paecilomyces* and one isolate each of *Micropolyspora, Nocardiopsis, Streptos-porangium, Oerskovia, Thermomonospora, Chainia, Pseudomonas, Fusarium, Monilia* and *Syncephalestrum*.

In this investigation, the insecticidal activities of both culture filtrates and cell pellets had been tested against cotton leaf worm. Results in Table-2 illustrated in Figure-1 revealed that the pellets of some *Streptomyces* isolates were more active against cotton leaf worm than culture filtrates. Streptomycete isolates S09; S14; S02 & S07, S12 & S13, S01, S11, S03 & S04, S06 showed percentages of mortalities against the 1st instar of cotton leaf warm as

followed 32.5, 27.5, 25, 22.5, 20, 17.5, 12.5 and 7.5, respectively. On the other hand, four Streptomycete isolates namely S05, S08, S10 and S15 were recorded as the most effective as they showed 80, 100, 70 and 80% mortality, respectively.

In a trial to define which protein is responsible for toxicity, the protein(s) of isolate S08 cells was purified through ammonium sulfate saturation (ASS) 40, 60 and 80%. Results of SDS-PAGE analysis showed that a 40KDa protein was obtained from the 40% ASS (Data not shown). As interestingly, data in Table-3 showed 100% mortality for that purified protein against four instars of the cotton leaf (Figures 2 and 3).

In China*, Streptomyces nanchangensis* isolated from the soil in Nanchang, China produces at least two kinds of insecticidal antibiotics (Ouyang *et al.*, 1993, 1984), first resembles dianemycin and second resembles milbemycin (Takahashi *et al.*, 1993). Both (broad spectrum) meiling-mycin and nanchangmycin are very active against harmful nematodes and insects, which are non-toxic for mammals as well as plants (Ouyang *et al.*, 1993).

Table-1: Antagonistic activities of the 15 *Streptomyces* isolates under investigation against four phytopathogenic fungi

Streptomyces isolates	Phytopathogenic fungi used			
	R.nigricans	*A.niger*	*F.oxysporum*	*H.grameniu m*
S01	-	+	+	-
S02	-	+	-	-
S03	+	-	-	-
S04	+	+	-	-
S05	-	+	+	-
S06	+	-	-	-
S07	-	-	+	-
S08	+	+	+	-
S09	+	+	-	-
S10	+	-	-	-
S11	-	+	+	-
S12	+	-	-	-
S13	+	+	-	-
S14	-	+	+	-
S15	+	-	-	-

-:Negative +:Positive

Table -2: Insecticidal activities of the 15 *Streptomyces* isolates under investigation against 1st instar larvae of cotton leaf worm (*S. littoralis*).

Streptomyces isolates	Insecticidal activities			
	Alive (%)		Dead (%)	
	Filtrate	Cells	Filtrate	Cells
S01	86.0	80.0	14.0	20.0
S02	94.0	75.0	6.00	25.0
S03	98.0	87.5	2.00	12.5
S04	92.0	87.5	8.00	12.5
S05	94.0	20.0	6.00	80.0
S06	96.0	92.5	4.00	7.50
S07	96.0	75.0	4.00	25.0
S08	0.00	0.00	100	100
S09	94.0	67.5	6.00	32.5
S10	86.0	30.0	14.0	70.0
S11	68.0	82.5	32.0	17.5
S12	96.0	77.5	4.00	22.5
S13	96.0	77.5	4.00	22.5
S14	88.0	72.5	12.0	27.5
S15	94.0	20.0	6.00	80.0
Control	100	100	0.00	0.00

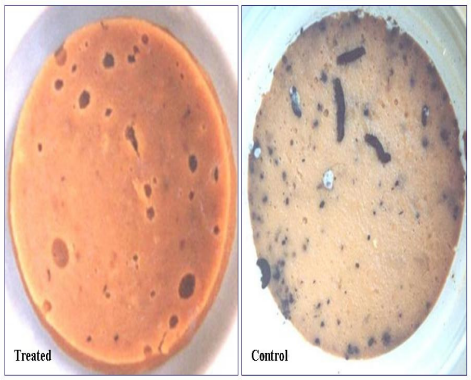

Figure -1: Insecticidal activities of *Streptomyces* isolate S08 under investigation against 1st instar larvae of cotton leaf worm (*S. littoralis*).

Table -3: Insecticidal activities of purified protein of *Streptomyces* isolate S08 against four instars larvae of cotton leaf worm (*S. littoralis*) post 72 hours from treatment.

Larvae instars	Treatment	Insecticidal activities			
		Alive		Dead	
		No.	%	No.	%
1st	Treated	00	00	30	100
	Control	27	90	03	10
2nd	Treated	00	00	30	100
	Control	28	93.3	02	6.7
3rd	Treated	00	00	30	100
	Control	28	93.3	02	6.7
4th	Treated	00	00	30	100
	Control	27	90	03	10

Thirty larvae of cotton leaf worm in three replicates were used for treatment as well as control.

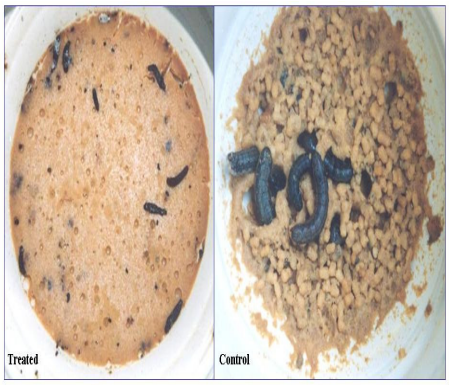

Figure-2. Insecticidal activities of purified protein of *Streptomyces* isolate S08 against 3rd instar larvae of cotton leaf worm (*S. littoralis*). Note, the control larvae of 3rd instar grew up to 6th instar.

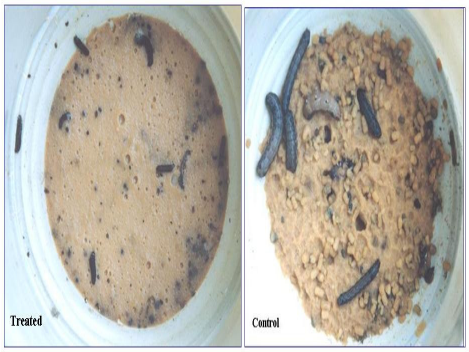

Figure-3: Insecticidal activities of purified protein of *Streptomyces* isolate S08 against 4th instar larvae of cotton leaf worm (*S. littoralis*). Note, the control larvae of 4th instar grew up to 5th and 6th instars.

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