ANTAGONISTIC AND INSECTICIDAL ACTIVITIES OF SOME *STREPTOMYCES* ISOLATES

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 and subjected to screening studies to evaluate their efficiency as antagonistic agents against some phytopathogenic fungi such as, *Rhizopus stolonifer*, *Aspergillus niger*, *Fusarium oxysporum*, *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 supernatants were then tested against the five applied phytopathogenic fungi. Results revealed that most of the isolates were varied in their antagonistic activities. Isolate S08 was active against *R. stolonifer*, *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. stolonifer* and *A. niger*. On the other hand, no antifungal activity was found against *Helminthisporum gramenium*. 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 of use such *Streptomyces* isolates as effective biopesticide agents.

Key words: *Streptomyces*, Antagonistic and insecticidal activity, Fungi, Cotton leaf worm, Toxicity, Mortality.

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 *Spodoptra 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.

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Xiong *et al.* (2004) isolated some *Streptomyces* strains from seawater and sea sediments from Beidiahe and Dagang of the east coast of China. These strains were 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*.

Recently, **Shiomi *et al.* (2005)** isolated a new antimycin group antibiotic, antimycin A9 from a cultured broth of *Streptomyces* sp. K01-0031 together with antimycins A3a, A3b, A4, and 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 *Caenorhabditis elegans* and *Artemia salina*, respectively. It inhibited bovine heart NADH oxidase at nanomolar level like other known antimycins.

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 worldwide Therefore, 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 of this standard inoculum (containing 1.5 ml spores/ml) was transferred aseptically to 50 ml of a broth medium (Not shown data) modified from starch nitrate broth medium **Waksman and Lechvalier (1961)** in a 250 ml conical flask. Inoculated flasks were incubated at 28±2°C for 6 days on a rotary shaker (160 rpm/min). Thereafter, growth was centrifuged at 10000 rpm at 4°C for 5 minutes. Then the supernatants and pellets were separately subjected to evaluation for 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 stolonifer*, *Aspergillus niger*, *Fusarium oxysporum* and *Helminthisporum gramenium*, kindly provided by Cairo MIRCEN, Faculty of Agriculture, Ain Shams University. Test was carried out as described by **Mohamed *et al.* (2001).**

Source of insect:

Sets of the cotton leaf worm (*S. littoralis*) insect belonging to family *Noctuidae* were kindly 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 Novon, 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 1X phosphate buffer saline (PBS). The crude proteins prepared by 40, 60 and 80% ammonium sulfate treatments were subjected to 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, anthelmintic, antitumour and immunosuppressive activities **(Hopwood, 1997; Katz, 1997** and **Leadlay, 1997)**.

In this study, a set of fifteen soil-streptomycete isolates from Egypt were used in the present study 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. stolonifer*, *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. stolonifer* and *A. niger*. On the other hand, no antifungal activity was found against *Helminthisporum gramenium*. 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 percentage 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, Streptosporangium, 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)** which 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 40 KDa 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 (**Ouyang *et al.*, 1984**) produces at least two kinds of insecticidal antibiotics (**Ouyang *et al.*, 1993**), first resembles dianemycin and second resembles milbemycin (**Takahashi *et al.*, 1993**). Both meilingmycin and nanchangmycin are very active against a broad spectrum of harmful nematodes and insects, and 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. stolonifer	*A. niger*	*F. oxysporum*	*H. gramenium*
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 (%)	
	Supernatant	Pellets	Supernatant	Pellets
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**

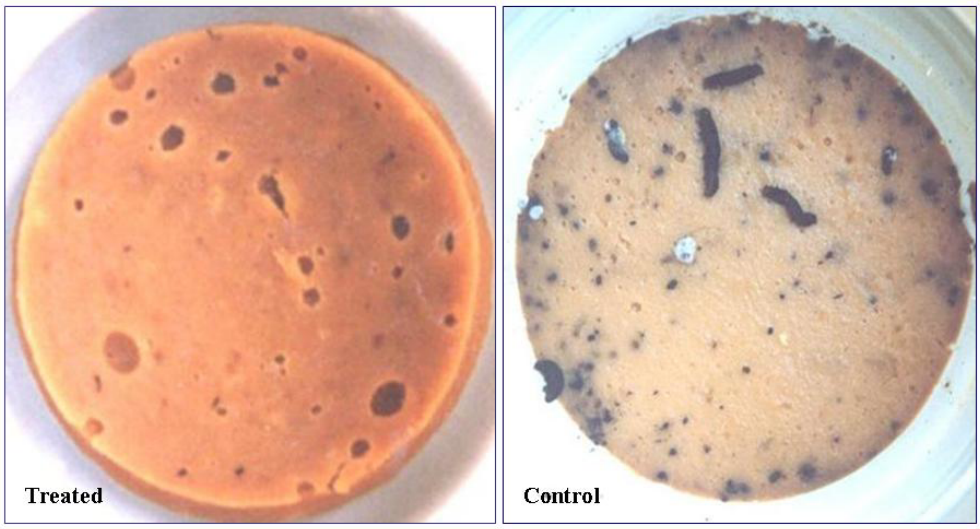

Figure (1): Insecticidal activities of *Streptomyces* isolate S08 under investigation against 1st instar larvae of cotton leaf worm (*S. littoralis*).

Table (3): Insecticidal activities of purified protein (40 KDa) 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 (40 KDa) 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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نشاط تضادي ونشاط كمبيد حشري لبعض عزلات من الأستربتوميستات

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في هذه الدراسة تم استخدام ١٥ عزلة من الأستربتوميسس وهي S01-S02-S3-S04-S05-S06-S07-S08-S09-S10-S11-S12-S13-S14-S15 معزولة من تربه مختلفة ومناطق جغرافية في مصر. وبعد إكثار العزلات خضعت لحصر لتقييم فعاليتها كعوامل ذات نشاط تضادي ضد بعض الفطريات الممرضة للنبات مثل *Rhizopus stolonifer*، *Aspergillus niger*، *Fusarium oxysporum*، *Helminthisporum gramenium*. وافه حشرية هي دودة ورق القطن المعروفة بـ *Spodoptera littoralis* . وللوصول الي هذا الغرض تم تنميه العزلات علي بيئة مرق النشا تحت ظروف رج علي درجة حرارة ٢٨ درجة مئوية لمدة ٦ أيام. ثم تبع ذلك اختبار القدرة التضادية ضد الفطريات سالفة الذكر للراشح المتحصل عليه من العزلات. وقد أوضحت الدراسة أن معظم العزلات قد تباينت في قدرتها التضادية. وكانت العزلة S08 نشطه ضد *R. nigricans*، *A. niger* ، *F. oxysporum* بينما كانت العزلات S01- S05- S11- S14 نشطة ضد *A. niger*، *F. oxysporum* والعزلات S04- S09- S13- كانت نشطه ضد *R. nigricans*، *A. niger* . وعلي الجانب الآخر لم تلاحظ أية قدرة تضادية ضد فطر الـ *Helminthisporum* sp. وبالنسبة للنشاط التضادي ضد الحشرة فقد تم استخدام الراشح والخلايا المترسبه للعزلات. وقد ثبت أن الخلايا لبعض العزلات كانت أكثر نشاطا ضد حشرة دودة ورق القطن مقارنة بالراشح. وبصفة عامة فإن العزلات S15 - S10 - S08 -S05 أوضحت ٨٠، ١٠٠، ٧٠، ٨٠% نسبة موت ضد الحشرة علي التوالي. كما تم تنقية البروتين من خلايا العزلة S08 باستخدام تركيزات كبريتات الأمونيوم هي ٤٠، ٦٠، ٨٠ % ثم تبع ذلك تقدير الوزن الجزيئي للبروتين بواسطة التفريد الكهربائي SDS-PAGE وقد ثبت تواجد بروتين ذو وزن جزيئي ٤٠ كليودالتون ذو فعالية وصلت الي درجة ١٠٠% ضد حشرة دودة ورق القطن في أربعة أطوار حشرية من الأول الي الرابع مما يوضح إمكانية استخدام الأستربتوميسيس في الزراعة كمبيد حيوي.