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Biocontrol of Damping-Off Disease Caused by *Rhizoctonia Solani* in Some Medicinal Plants Using Local Strain of *Streptomyces Pactum*

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Abstract: A number of 187 *Streptomyces* isolates were isolated from different Egyptian soil samples and screened for their antifungal activities (mm inhibition zone) by agar diffusion method, only 5 isolates representing 2.67% produced antifungal metabolites. Starch nitrate agar was the most suitable medium for growth and production of antifungal metabolites by different *Streptomyces* isolates. In addition, *Streptomyces* isolate *S131* was the most efficient isolate in antifungal activities (mm) which was identified as a strain of *Streptomyces pactum*. *S131* .Furthermore, the most sensitive test organism for antifungal metabolites produced by *S. pactum* strain *S131*was *Aspergillus niger* followed by *Fusarium oxysporum, Candida albicans, Aspergillus flavus, Fusarium moniliforme*, *Rhizoctonia solani* and *Pythium sp* which resulted 20, 17, 16, 15,15, 14 and 13 mm of inhibition zone around agar (6 mm) culture disks of *Streptomyces pactum- S131*, respectively. Application of cultural filtrate in biocontrol against damping-off caused by *Rhizoctonia solani* in fennel and coriander seedlings were studied, the results revealed that there were significantly differences among all soil treatments in the percentage of total survival fennel plants from damping-off disease. The maximum percentage of total survival fennel plants from damping-off disease was recorded at uninfested soil treatment followed by soil infested with *R. solani* + rhizolex, soil infested with *R. solani* + culture filtrate and soil infested with *R. solani*, which resulted 82.35, 69.41, 55.50 and 22.28%, respectively. In addition, there were no significantly differences between uninfested soil and soil infested with *R. solani* + rhizolex in the percentage of total survival coriander plants from damping-off disease, which gave 91.67 and 90.67%, respectively**.** But there were significantly differences between soil infested with *R. solani* + culture filtrate of *Streptomyces pactum* strain *S131* and soil infested with *R. solani* only in the percentage of total survival coriander plants from damping-off disease, which gave 58.34 and 11.09%, respectively.

Key words: *Streptomyces pactum*, antifungal, coriander, biocontrol**,** fennel, damping-off

INTRODUCTION

Actinomycetes are diverse group of heterotrophic prokaryotes forming hyphae at some stages of their growth, hence referred as filamentous prokaryotes. They are a unique group of bacteria having different morphological, cultural, biochemical and physiological characters that occur in a multiplicity of natural and man-made environments (Goodfellow &Williams, 1983). The populations of actinomycetes were found in soil containing an abundance of organic matter than in poorer soil. The most abundant actinomycetes in soil were *Streptomyces*, that genus was dominated in soil over *Nocardia, Micromonospora & Streptosporangium* and it has been isolated from various types of soils, including rice paddy, water and mud of lakes, deciduous and tropical forests, wasteland, and cave soils (Kim *et al.,* 1998).The genus *Streptomyces* is represented in nature by the largest number of species and varieties, producing the majority of known antibiotics among the family actinomycetaceae. Members of the genus *Streptomyces* are well known as source of antibiotics and other important novel metabolites, including antifungal, antitumor, antihelminthic and herbicide agents (Konishi *et al*., 1991; Sanglier *et al.,* 1993; Lee *et al.,* 2003 and Thakur *et al*., 2007). Discovery of new antibiotics produced by *Streptomyces* still continues, for example, mediomycins (A&B), clethramycin, analogue of bleomycin and new benzoxazole antibiotic caboxamycin were isolated from fermentation broth of *S. mediocidicus* ATCC23936, *S. mediocidicus* NC0604, *S. verticillus var. pingyangensis* and *Streptomyces* sp. NTK 937, respectively (Bordoloi *et al*., 2001& 2002; Cai *et al*., 2007 and Hohmann *et al*., 2009).

Damping-off of seedlings caused by *Rhizoctonia solani* is responsible for considerable yield losses in a variety of crop plants. Effective and economical methods of control of this disease depends on the use of broad spectrum fungicides, but such measures establish imbalance in the microbial community which render it unfavorable for the activity of beneficial organisms (Lifshitz *et al.,* 1986).Control of soil-borne plant pathogens, many strategies have been tested. One of them, which has become more important in recent years, is biological control. This is now considered as being the most beneficial for the environment, since it minimizes the use of chemical pesticides in plant disease management (Emmert and Handelsman 1999; Raaijmakers *et al*. 2002). The

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role of actinomycetes in the biological control of soil-born plant pathogens has been demonstrated against different pathogens, such as *Fusarium* spp. , *Phytophthora* spp. (Valois *et al*. 1996), *Pythium* spp. (El-Tarabily *et al*. 1997), *Rhizoctonia* spp. (Trejo-Estrada *et al*. 1998), and *Verticillium* spp. (Entry *et al*. 2000). Actinomycetes have also been used as commercially formulated biocontrol agents of plant diseases. In addition to their ability to inhibit plant pathogens, appears to be of great importance among the microbial flora of the rhizosphere. They have the capacity to decompose organic matter in the soil that gives them an important beneficial role in plant growth (Kennedy 1999). In the present work, we identify the most efficient *Streptomyces* isolate based on antifungal activity and study its culture filtrate in biocontrol of damping-off disease caused by *Rhizoctonia solani* in fennel and coriander plants.

MATERIALS AND METHODS

Isolation, Purification and Screening of Actinomycete Isolates:

Soil samples were collected from different locations in Arab Republic of Egypt, taken from 10-20 cm depth, air dried at room temperature for 7 days, mixed with $CaCO_3$ (10%) and incubated at 28 ±2 ^oC for 7 days under saturation condition to reduce the bacterial flora with no harms to the growth of actinomycetes (Tsao *et al*., 1960 and Kutzner, 1981). After incubation, 10 grams of incubated soil were suspended with 100ml sterile distilled water, shake vigorously for 1 hour, then one ml of soil suspension was subsequently diluted with sterile distilled water to 10^{-6}. One ml of final diluted soil suspension was spreading on the surface of sterile plate of starch nitrate agar and incubated at 28 ±2 ^oC for 7 days (Aghighi *et al.,* 2004). Actinomycete colonies obtained after incubation were picked up and re-cultivated several times (by streaking technique) under the same previous conditions of isolation for purity. Then, the purified actinomycete isolates were grouped according to their color of aerial mycelium and maintained on sterile slants of starch nitrate agar medium in a refrigerator at 4°C until used (Tresner & Backus, 1963 and Kutzner, 1972). Sub-culturing was usually carried out every two months, using starch nitrate agar medium. The purified actinomycete isolates were identified up to genus according to their cultural and morphological characteristics (Bergey's Manual, 1974).The purified actinomycete isolates were streaked on sterile agar plates media of starch nitrate (Waksman, 1961), yeast malt extract, oat-meal extract, inorganic salts starch, glycerol asparagine (Shirling & Gottlieb, 1966), and fish meal extract (Krassilnikov, 1970). The inoculated plates were incubated at 28 ±2 ^oC for 15 days. After growth, antagonistic activity of each actinomycete cultures were tested against different test fungi by diffusion plate method as follow: the cultural agar disks of each purified actinomycete isolate were cut with sterile cork borer 6 mm and placed into sabouraud glucose agar plates medium(Defico) separately seeded by tested fungi : Aspergillus niger, A. flavus, Candida albicans, *Fusarium oxysporum*, *F. moniliforme*, *Rhizoctonia solani* and *Pythium* sp, then the plates were incubated at 28 ±2 ^oC for 3 days. After incubation, the antagonistic activities were recorded by measuring the inhibition zone (mm) around the cultural discs (Dhingra & Sinclair, 1995).

Identification of the Most Efficient Streptomyces Isolate:

The most efficient *Streptomyces* isolate in antagonistic properties was identified, based on their cultural, morphological and physiological characteristics according to the standard methods adopted by Shirling & Gottlieb (1966). The key's proposed by Bergey's Manual (1974) were consulted. The description of *Streptomyces* species of the International *Streptomyces* Project (ISP) introduced by Shirling & Gottlieb ($1968_{a,b}$ and 1972) were also used.

Cultural and Morphological Characteristics:

The color of the aerial mycelium, substrate mycelium and those of the soluble pigments were observed by the naked eye after 7,14 and 21 days of incubation on 4 standard media: oat-meal agar, yeast- malt extract agar, glycerol-asparagine agar and inorganic salts starch agar (Shirling & Gottlieb, 1966). Spore chain morphology and spore surface ornamentation were examined by light and electron microscopes, respectively on inorganic salts starch agar medium after 14 days of incubation at 28±2°C.

Physiological Characteristics:

The selected *Streptomyces* isolate was investigated for its ability to produce melanoid pigment on tyrosine agar and peptone–yeast extract iron agar media (Shirling & Gottlieb, 1966) after 2 and 4 days of incubation at 28 ±2°C and for its ability to grow on Czapek´s agar medium (Prauser & Folta, 1968). Tolerance to different concentrations of sodium chloride (4, 7, 10, and 13%) was tested on inorganic salts starch agar medium. Sensitivity of the selected *Streptomyces* isolate to streptomycin sulphate (100 µg ml^{-1}) was tested in Bennet's agar medium (Jones, 1949) by using filter paper disc method according to the method as described by British Pharmacopoeia (2000). The ability of the selected *Streptomyces* isolates to use 11 different carbon compounds as sole carbon sources i.e., (D-glucose, D-xylose L-arabinose, L-rhamnose, D-fructose, D-galactose, raffinose, D-manitol, i -inositol, salicin and sucrose) was examined on inorganic salts agar medium. The carbon

compounds were separately sterilized by ethyl ether (acetone free), which added to cover the carbon compound. The ether was allowed to evaporate at room temperature over night, each sterilized carbon source was added to the sterilized medium after cooling the medium to 60 ^{o}C with shaking well and the final concentration of each carbon source was added to the medium at the rate of 1% w/v. Each of the selected *Streptomyces* isolate was inoculated by streaking on the surface of the plate medium (containing one of the carbon sources) and incubated at 28±2 ^{o}C for 14 days on medium containing D-glucose which served as positive control, while the same medium without carbon sources served as negative control (Shirling & Gottlieb, $1968_{a,b}$ and 1972).

Application of Antifungal Metabolite Produced by Streptomyces-S131 Against Damping-Off of Some Medicinal Plants:

The experiment was conducted during April, 2009, at Plant Pathology Department**,** Faculty of Agricultural, Ain Shams University.

Isolation of Rhizoctonia From Infected Medicinal Plants:

The infected roots samples of sweet fennel (*Foeniculum vulgare*) and coriander (*Coriandrum sativum*) were collected from the Center of Medicinal Plants Studies - National Organization for Drug Control& Research. The samples were thoroughly washed with tap water, cut into small pieces, surface sterilized with 1% sodium hypochlorite for 2 min followed by washing in sterilized water and dried between 2 fold of sterile filter paper. Pieces were surfed on sterile plates of Sabouraud's agar medium (Difco) and incubated at room temperature (about 26°C) for 48-72 h. The fungal isolates were purified by taken the hyphal tips from developed colony and separately transferred on Sabouraud's medium((Difco) and incubated at the same previous conditions. The growing fungal culture on Sabouraud's dextrose agar slants, stored in a refrigerator until used. Identification of the fungal was carried out by using the morphological characteristic of mycelia as described by Sneh *et al.* (1992).

Preparation of Rhizoctonia Inoculum:

Corn-meal and sand (20:80 w/w) were mixed. Water was added until the mixture was damp. Three hundred ml of prepared mixture was then placed into bottles and autoclaved twice, for one hour. Ten bottles contain the sterile previous mixture used for preparation of *Rhizoctonia* inoculum. Under sterile condition, Five mycelial disc (6 mm) from a four days old culture of *R. solani* on Sabouraud's dextrose agar medium were used to inoculate each of 300 ml sterile prepared mixture and they were incubated at room temperature (about 26±2°C) for 3 weeks. After incubation, the cultures were air-dried for using (Kazempour, 2004).

Soil Infestation:

Clay soil was mixed with sand (sand washed three times with distilled water) at the ratio of (1:1) and sterilized by addition of adequate formalin solution 2% to the soil and mixed very well and covered with gunny bag for 7 days, afterwards when the trace of formalin smell has gone the soil is worked thoroughly, dried up and used for sowing. Five replicates of plastic pots (10x15cm) sowed with 60 seeds (12 seed /pot) were prepared for each treatment and the pots were filled with 650 g of previous prepared soils. The soil was grouped into four categories; uninfested soil, soil infested with *R. solani* (1% w/w), soil infested with *R. solani* (1% w/w) + rhizolex (50 ml /pot at the rate of 1.5 g L^{-1}) and soil infested with *R. solani* (1% w/w) + culture filtrate (50 ml/ pot).

Treatment of Medicinal Plant Seeds with Cultural Filtrate of Streptomyces:

Seed samples of sweet fennel (*Foeniculum vulgare*) and coriander (*Coriandrum sativum*) were obtained from Agricultural Research Center, Giza. Egypt, surface disinfested in 500ml beakers by immersion in 2% aqueous solution of sodium hypochlorite for 5 min through rinsing 5 times with sterilized distilled water and dried at room temperature. The culture filtrate of *Streptomyces* strain *S131* obtained under optimized conditions was filtered through sterilized filter paper to remove the pellet of *Streptomyces*. The appropriate number of each medicinal plant seeds were immersed in culture filtrate of *Streptomyces*, sterile distilled water (as negative control) for 3 hours and mixed with rhizolex (positive control) carefully before sowed pots. Each medicinal plant seeds was sowed in 4 categories of pots: uninfested soil, soil infested with *R. solani* (1% w/w), soil infested with *R. solani* (1% w/w) + rhizolex (50 ml /pot at the rate of 1.5 g L^{-1}) and soil infested with *R. solani* (1% w/w) + culture filtrate (50 ml /pot). All pots were watered and placed in a greenhouse that received 12 hours sunlight each day with fluctuating day and night temperature. Five pots were used as replicates for particular treatment. Percentages of pre- and post-emergence damping-off as well as total survival seedling were evaluated after 15 and 30 days from planting seeds (Suh, 2001).

Statistical Analysis:

Pots experiment was arranged as completely randomized design with 5 replications per treatment. Data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS institute Inc., 1996) at 5% level of significance and means values were compared using a least significant difference (LSD) test according to Gomez &Gomez (1984).

RESULTS AND DISCUSSION

One hundred and eighty seven actinomycete isolates were isolated from different Egyptian soil samples. Results revealed that they belonged to the genus *Streptomyces* as they form well developed branching, non-septate, non-fragmented aerial mycelia bearing a long spore chains and non-motile spores which not borne in verticillate sporophores (Bergey's Manual ,1986).

Data in Table (1) reveal that, *Streptomyces* isolates belonged to 6 groups according to their color of aerial mycelium.The majority number of *Streptomyces* isolates was grayish color , which attained to 110 isolates representing 58.82% of total *Streptomyces* isolates followed by whitish , brownish , reddish, yellowish and bluish color , which attained to 30 (16.04%) , 25 (13.37%), 12 (6.42%), 6 (3.21%) and 4 (2.14%) isolates, respectively. Furthermore, out of 187 *Streptomyces* isolates were screened for their antifungal activities, only 5 isolates (grayish color) have antifungal activity, representing 2.67% of total *Streptomyces* isolates. Therefore, *Streptomyces* isolates *S8, S18, S10, S131 and S181* was used for subsequent studies to screen the most efficient isolate in antifungal activity.

Table 1: Antifungal activity of *Streptomyces* isolates.

Color of aerial mycelium	Isolates		Isolates* producing antifungal antibiotics	
	Total	%	Isolate No	%
Gray	110	58.82	*S8* *S18* *S10* *S131* *S181*	2.67
White	30	16.04	-	-
Brown	25	13.37	-	-
Red	12	6.42	-	-
Yellow	6	3.21	-	-
Blue	4	2.14	-	-
Total	187	100	5	2.67

*Growing on starch nitrate agar medium for 15 days at 28±2 ^{o}C.

Data in Table (2) show that, the degree of antagonistic activity (mm of inhibition zone) varied according to *Streptomyces* isolate, tested organism and medium composition. In addition, the most suitable medium for production of antifungal metabolites by different *Streptomyces* isolates was starch nitrate agar medium followed by oat meat extract, fish meal extract, yeast malt extract, inorganic salts starch and glycerol asparagines medium.. Furthermore, *Streptomyces* isolate *S131* grown on starch nitrate agar was the most efficient isolate for its antifungal activities against all tested fungi and yeast, resulting in 20, 17, 16, 15, 15, 14 and 13 mm inhibition zone against Aspergillus niger, Fusarium oxysporum, Candida albicans, Aspergillus flavus, Fusarium moniliforme, *Rhizoctonia solani* and Pythium sp,

The present results are in agreement with those of many investigators, that antifungal activity of different *Streptomyces* cultures are not a fixed property but can be greatly increased or completely lost under different conditions of nutrition. Therefore, the medium constitution together with the metabolic capacity of the producing organism greatly influences antibiotic biosynthesis. Changes in the nature and type of carbon, nitrogen or phosphate sources and trace elements have been reported to influence antibiotic biosynthesis in *Streptomyces* strains (Aghighi *et al.*, 2004; Al-Zahrani, 2007 and Atta, 2009). In addition, El-Abyad *et al.* (1996) found that, starch nitrate medium was the most suitable medium for maximal antifungal activities by *S. pulcher* or *S. citreofluorescens* against various plant pathogenic fungi and bacteria. They also found that the most sensitive organism for metabolites produced by some *Streptomyces* species grown on starch nitrate was *Verticillium alboatrum*. On the other hand Al-Zahrani (2007) found that, yeast malt extract agar medium was the most suitable medium for antifungal activity by *Streptomyces* isolate J12 against *Candida albicans.*

Keys of identifigation proposed by Shirling & Gottlieb (1968$_b$ and 1972) and Bergey' s Manual (1974) were used for complete identification of *Streptomyces* isolate *S131*, data in Table (3) and Figs. (1&2) show that the this isolate appeared to resemble *S. pactum* and *S. finlayi*. While, some physiological characteristics of the this isolate were found to be slight differences from *S. finlayi*. However the latter species could utilize D-xylose, L-arabinose and sucrose. On the other hand, the various properties of the morphological, cultural and physiological characteristics of the *S131* isolate appeared to be confirmed to those of *S. pactum* with slight

difference in sensitivity to streptomycin (50 µg ml^{-1}). Therefore, the experimental isolate *S131* was identified as a strain of *S. pactum*.

Table 2: Antifungal activity of the selected *Streptomyces* isolates against some pathogenic fungi.

Streptomyces isolates No.	Target fungi	Inhibition zone (mm) of *Streptomyces* agar cultures*					
		S. N.	Y. M. E.	I. S. S.	O. E.	G. A.	F. M. E.
S8	*Aspergillus niger*	13	0	0	12	13	12
	Aspergillus flavus	0	0	0	0	0	11
	Fusarium moniliforme	12	0	0	13	13	12
	Fusarium oxysporum	11	0	0	0	0	0
	Pythium sp	0	12	12	0	0	0
	Rhizoctonia solani	0	0	0	0	0	0
	Candida albicans	0	0	0	0	0	0
S10	*Aspergillus niger*	12	12	0	11	0	11
	Aspergillus flavus	12	12	0	0	0	0
	Fusarium moniliforme	11	12	0	0	0	0
	Fusarium oxysporum	13	12	0	0	0	11
	Pythium sp	0	0	0	0	0	0
	Rhizoctonia solani	0	0	0	0	0	0
	Candida albicans	0	11	0	0	0	0
S18	*Aspergillus niger*	13	14	0	16	0	15
	Aspergillus flavus	11	12	0	12	0	12
	Fusarium moniliforme	0	12	0	12	0	10
	Fusarium oxysporum	0	0	0	0	0	0
	Pythium sp	0	0	0	0	0	0
	Rhizoctonia solani	0	0	0	0	0	0
	Candida albicans	13	12	0	14	0	12
S131	*Aspergillus niger*	20	0	18	0	0	0
	Aspergillus flavus	15	0	13	0	0	0
	Fusarium moniliforme	15	0	12	0	0	0
	Fusarium oxysporum	17	0	13	0	0	0
	Pythium sp	13	0	12	0	0	0
	Rhizoctonia solani	14	0	12	0	0	0
	Candida albicans	16	0	12	0	0	0
S181	*Aspergillus niger*	13	0	0	12	0	0
	Aspergillus flavus	13	0	0	12	0	0
	Fusarium moniliforme	0	0	0	0	0	0
	Fusarium oxysporum	11	0	0	0	0	0
	Pythium sp	0	0	0	13	0	0
	Rhizoctonia solani	0	0	0	0	0	0
	Candida albicans	14	0	0	13	0	0

S.N.: starch nitrate (Waksman, 1961), Y.M.E.: yeast malt extract; I.S.S.: inorganic salts starch, O.E.: oat-meal extract, G.A.: glycerol asparagine (Shirling and Gottlieb, 1966), F.M.E: fish-meal extract (Krassilnikov, 1970).
*Incubation for 15 days at 28±2 ºC.

Efficiency of *Streptomyces pactum* strain - *S131* antifungal metabolite on controling of damping-off disease

Data presented in Table (4) indicate that the percentage of infected fennel seedlings with pre and post emergence damping-off were significantly increased at soil infested with *Rhizoctonia solani* treatment as compared to other soil treatments, which resulted 44.42 and 33.30%, respectively. Results also showed that there were significant differences in pre-emergence damping-off among uninfested soil, soil infested with *R. solani* + rhizolex and soil infested with *R. solani* + culture filtrate treatments in the percentage of infected fennel seedlings with pre-emergence damping-off, which resulted 5.85, 16.67 and 24.25%, respectively (Table,4). In contrast, there were no significant differences between uninfested soil treatment and soil infested with *R. solani* + rhizolex treatment in the percentage of infected fennel seedlings with post-emergence damping-off, which gave 11.80 and 13.92%, respectively. However, there were significant differences between soil infested with *R. solani* + culture filtrate as compared to other soil treatments in the percentage of infected fennel seedlings with post-emergence damping-off, which gave 20.25% (Table,4). Furthermore, there were significant differences among various soil treatments in the percentage of total survival fennel plants. The maximal percentage of total survival fennel plants from damping-off disease was recorded at uninfested soil followed by soil infested with *R. solani* + rhizolex, soil infested with *R. solani* + culture filtrate and soil infested with *R. solani*, which resulted 82.35, 69.41, 55.50 and 22.28%, respectively (Table,4).

Table 3: Identification of *Streptomyces* isolate *S131* up to species.

Characteristics	*S131*	*S. pactum*[1]	*S. finlayi*[2]
(1)Cultural characteristics			
Color of aerial mycelium	Gray	Gray	Gray
Color of substrate mycelium	Colorless	Colorless	Colorless
Diffusible pigments	Colorless	Colorless	Colorless
(2)Morphological characteristics Spore surface ornamentation			
spore chain morphology	Hairy Spiral	Hairy Spiral	Hairy Spiral
(3)Physiological characteristics			
Melanoid pigment produced	-	-	-
Growth on Czapek`s medium	±	±	ND
Sodium chloride tolerance	≤10	≤10	>7[3]
Sensitivity to streptomycin (50 µg ml^{-1})	Not sensitive	Sensitive	Sensitive
Antifungal activity[4]	Positive	Positive	Positive[3]
Antibacterial activity[5]	Weakly	Positive	Positive
Utilization of different carbon sources			
No Carbon	-	-	-
D-Glucose	+	+	+
D-Xylose	-	-	(+)
L-Arabinose	-	-	(+)
L-Rhamnose	+	+	+
D-Fructose	+	+	ND
Galactose	-	-	-
Raffinose	-	-	-
D-Mannitol	-	-	-
Inositol	-	-	-
Salicin	-	-	ND
Sucrose	-	-	(+)

1-Shirling and Gottlieb (1972) and Bergey's Manual (1974), 2- Shirling and Gottlieb (1968_b) and Bergey's Manual (1974), 3- Williams *et al.* (1983) , 4- against (*Aspergillus niger, A. flavus, Fusarium oxysporum, F. moniliforme*, *Candida albicans* , *Rhizoctonia solani* and *Pythium* sp), 5- against (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella* sp.), ND: Not determine.

Table 4: The efficiency of *Streptomyces pactum* strain *S131* culture filtrate on suppression of fennel damping-off caused by *Rhizoctonia solani*.

Soil Treatments[1]	Damping-off (%)		Survival plants[3] (%)
	Pre-emergence[2]	Post-emergence[3]	
Uninfested soil	5.85[a]	11.80[a]	82.35[d]
Soil infested with *R. solani*[4]	44.42[d]	33.30[c]	22.28[a]
Soil infested with *R. solani*[4] + rhizolex[5]	16.67[b]	13.92[a]	69.41[c]
Soil infested with *R. solani*[4]+ culture filtrate[6]	24.25[c]	20.25[b]	55.50[b]

(1): Five replications for each treatment, (2): Data were recorded after 15 days of cultivation, (3): Data were recorded after 30 days of cultivation, (4): Soil infested with *R. solani* (1% w/w), (5): Rhizolex (50 ml /pot at a rate of 1.5 g L^{-1}), (6): culture filtrate (50ml/ pot). Values in the same column followed by same letter are not significantly different according to ANOVA (L.S.D. $P \leq 0.05$).

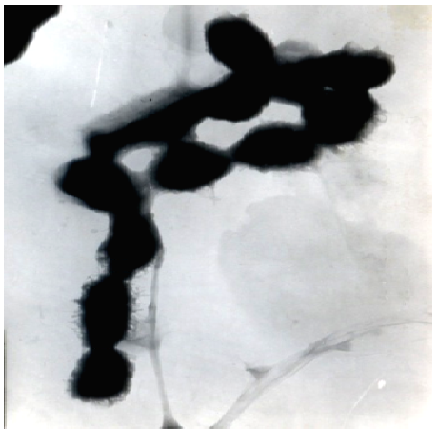

Fig. 1: Microphotograph of spore chains morphology of *Streptomyces* isolate S131 (X 13000).

Data recorded in Table (5) reveal that there were significant differences among soil infested with *R. solani* as compared to other soil treatments in the percentage of infected coriander seedlings with pre-emergence damping-off, which gave 18.33%. In contrast, there were no significant differences among uninfested soil, soil infested with *R. solani* + rhizolex and soil infested with *R. solani* + culture filtrate treatments in the percentage

of infected coriander seedlings with pre-emergence damping-off, which gave 2.75, 3.55 and 9.08%, respectively.

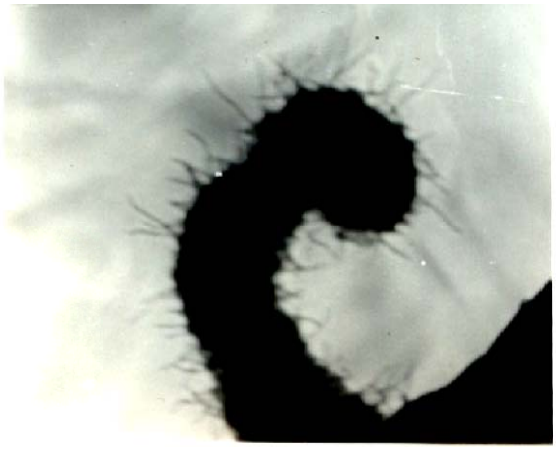

Fig. 2: Electron micrograph of spore surface ornamentation of *Streptomyces* isolate S131(X 25000).

Table 5: The efficiency of *Streptomyces pactum* strain *S131* culture filtrate on suppression of coriander damping-off caused by *Rhizoctonia solani*

Soil Treatments[1]	Damping-off (%)		Survival plants[3] (%)
	Pre-emergence[2]	Post-emergence[3]	
Uninfested soil	2.75[a]	5.58[a]	91.67[c]
Soil infested with *R. solani*[4]	18.33[b]	70.58[c]	11.09[a]
Soil infested with *R. solani*[4] + rhizolex[5]	3.55[a]	5.78[a]	90.67[c]
Soil infested with *R. solani*[4]+ culture filtrate[6]	9.08[a]	32.58[b]	58.34[b]

(1): Five replications for each treatment, (2): Data were recorded after 15 days of cultivation, (3): Data were recorded after 30 days of cultivation, (4): Soil infested with *R. solani* (1% w/w), (5): Rhizolex (50 ml /pot at a rate of 1.5 g L^{-1}), (6): culture filtrate (50ml /pot). Values in the same column followed by same letter are not significantly different according to ANOVA (L.S.D. $P \leq 0.05$).

The current data revealed that there were no significantly differences between uninfested soil and soil infested with *R. solani* + rhizolex treatments in the percentage of infected coriander seedlings with post-emergence damping-off which resulted 5.58 and 5.78 %, respectively, beside that there were significant differences among both treatments and other soil treatment in the percentages of infected coriander seedlings with post-emergence damping-off (Table, 5).

On the other hand, the percentage of infected coriander seedlings in post-emergence in soil infested with *R. solani* + culture filtrate treatment was significant differences as compared to other soil treatments, which gave 32.58%. In addition, the percentage of infected coriander seedlings with post-emergence damping-off in soil infested with *R. solani* treatment was significant higher as compared to other soil treatments, which gave 70.58% (Table, 5).

Furthermore, there were no significant differences between uninfested soil and soil infested with *R. solani* + rhizolex in the percentage of total survival coriander plants from damping-off disease, which gave 91.67 and 90.67%, respectively. In contrast there were significant differences among both previous treatments and other soil treatments. Also there were significant differences between soil infested with *R. solani* + culture filtrate of *Streptomyces pactum* strain *S131* and soil infested with *R. solani* only in the percentage of total survival coriander plants from damping-off disease, which gave 58.34 and 11.09%, respectively. In contrast there were significant differences among both previous treatments and the other soil treatments. Also there were significant differences between soil infested with *R. solani* + culture filtrate and soil infested with *R. solani* in the percentage of total survival coriander plants from damping-off disease, which gave 58.34 and 11.09%, respectively (Table, 5).

The abovementioned data were in agreement with those found by many investigators, who reported that the antifungal produced by some microorganisms had significant effect on decreasing the percentage of damping-off disease in tested plants (Trejo-Estrada *et al*., 1998 and Errakhi *et al*., 2007).

On the light of abovementioned results, it would be concluded that the application of antifungal antibiotic produced by *Streptomyces pactum* strain *S131* against damping-off disease in fennel seedling was more efficiently in decreasing of infected seedling percentage as compared to coriander seedling percentage. Also the

efficiency of application of antifungal against damping-off disease of coriander seedlings was lower than fungicide but the first can be contributed to decrease the environmental chemical pollutions. Therefore, the suggestion study was using the cultural filtrate of *Streptomyces pactum* strain *S131* in biological control of fennel and coriander damping-off caused by *Rhizoctonia solani*.

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