



EFFICACY OF TWO POTENT SOILBORNE *STREPTOMYCES* SPP. IN CONTROLLING WILT AND ROOT ROT DISEASES OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL) UNDER GREENHOUSE CONDITIONS

AHMED I. S. AHMED

**Plant Pathology Unit, Plant Protection Department, Desert Research Center, Cairo, Egypt.*

ABSTRACT

Seventy two isolates of *Actinomycetes* were isolated from various new reclaimed areas "North Sinai, South Sinai, Northern Coastal Zone and Siwa Oasis" Egypt, from 2013-2015. All isolates were tested for their antagonistic ability against some plant pathogenic "fungi *Alternaria alternata*, *Alternaria solani*, *Cladosporium cladosporioides*, *Curvularia* spp., *Fusarium oxysporum*, *Fusarium solani*, *Fusarium moniliforme*, *Macrophomina phaseolina*, *Pythium ultimum*, and *Rhizoctonia solani*" and exhibited different antifungal effects under *in vitro* culture conditions. Twelve isolates were found to be antagonist against the most of the tested fungi, while two isolates (Act5 and Act11) exhibited antagonistic effect against all tested phytopathogenic fungi, which were identified as *Streptomyces* spp. The obtained results showed that Act5 inhibited the mycelial growth of the pathogens *Rhizoctonia solani* and *Fusarium oxysporum* f.sp. *lycopersici* by 82, 75% while the inhibition rates were 71, 78%, by Act11. The activities of two isolates of *Streptomyces* spp. were investigated on tomato plants against *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani* under greenhouse conditions. Act5 reduced disease incidence by 61.1% and 57.3%, when applied before and after artificial soil infestation, while Act11 reduced the diseases incidence by 50.8% and 42.7%, before and after artificial soil infestation, respectively compared with the control. Moreover, Act5 and Act11 have positive effects on different growth traits (shoot/root length, shoot/root fresh weight). The present study indicates that Act5 and Act11 could be potential biological control of the tested pathogens.

KEYWORDS: *biopesticide; antifungal activity; plant growth; biological control.*



AHMED I. S. AHMED

Plant Pathology Unit, Plant Protection Department, Desert Research Center, Cairo, Egypt.

Received on : 05-05-2017

Revised and Accepted on : 31-05-2017

DOI: <http://dx.doi.org/10.22376/ijpbs.2017.8.3.b394-403>

INTRODUCTION

Fungal plant diseases are considered serious problems with a wide host range and globally distribution. *Rhizoctonia solani* and *Fusarium oxysporum* are responsible for some plant diseases such as root rot and wilt in various crops¹. Excessive use of chemical pesticides causes many environmental and health problems as well as the development of pathogen resistance to fungicide². Biological control provides environmentally friendly products which have potential effects against plant pathogens as alternative to chemical control³. The increasing interest for environmental protection and organic farming leads research towards alternative control tools, such as the use of natural antagonists to control of plant pathogens⁴. Fungal plant disease control using natural fungicides such as non-pathogenic microorganisms is very interesting because the biocontrol agents are safe to the environment and human⁵⁻⁶. *Actinomycetes* are the most widely distributed group of microorganisms in nature. They are found abundantly in cultivated and uncultivated soils in various regions worldwide⁷. Sanglier *et al.*⁸ mentioned that over one thousand secondary metabolites from *Actinomycetes* were reported during the years 1988-1992, and approximately 75% of these compounds were produced by *Streptomyces*. Besides that, the *Actinomycetes* play an important role as biocontrol agents against extensive plant pathogens⁹. Several researchers have studied the control of plant pathogens by using various *Actinomycetes* agents¹⁰⁻¹¹. Many species of *Actinomycetes*, especially those belonging to the genus *Streptomyces*, are well known as antifungal agents that inhibit several pathogenic fungi. *Streptomyces* have significant role in control of some phytopathogens due to its ability to produce different types of antibiotics¹². In relation to number and variety of identified species, *Streptomyces* represents one of the largest taxonomic characters of recognized *Actinomycetes*¹³. *Actinomycetes* produce 70- 80% of bioactive secondary metabolites, where approximately 60% of antibiotics developed for agricultural use are isolated from *Streptomyces* spp.¹⁴. *Streptomyces* have been found to control plant diseases through their potential effects such as source of bioactive compounds, plant growth promoter and biocontrol of plant diseases. The antifungal mechanisms may include physical contact, toxic compounds or antibiotics, effect of hydrolytic enzymes, and competition^{11, 15}. *Actinomycetes* have the capability to produce different types of active metabolites such as antibiotics, pesticides, and enzymes like cellulase, proteinase and chitinase. Several researchers have reported that *Actinomycetes* are a promising group of fungus-antagonistic¹⁶. More than 50% of microorganisms isolated from soil showed antagonistic properties. Among them *Actinomycetes* are predominant, mainly from the genera *Streptomyces* and *Micromonospora*¹⁷. The soilborne tomato root-infecting pathogens *Fusarium oxysporum* and *Rhizoctonia solani* are particularly difficult to control using standard cultural and chemical methods due to appearance of new races of the

pathogens and instability of resistance to pathogens¹⁸⁻¹⁹. Growing awareness of the potential hazards in using agrochemicals has led to increasing interests on alternative methods for effective disease control²⁰. The present study aims to isolate antagonistic *Streptomyces* from soil to investigate its potential effects on the growth of plant pathogenic fungi *Alternaria alternata*, *Alternaria solani*, *Cladosporium cladosporioides*, *Curvularia* spp., *Fusarium oxysporum*, *Fusarium solani*, *Fusarium moniliforme*, *Macrophomina phaseolina*, *Pythium ultimum*, and *Rhizoctonia solani* under *in vitro* conditions. The effective isolates were evaluated to control of *Rhizoctonia solani* and *Fusarium oxysporum* f.sp. *lycopersici* on tomato plants under greenhouse conditions, while some biochemical and physiological properties were also investigated.

MATERIALS AND METHODS

Isolation, purification and identification of fungal pathogens

The fungal pathogens were primarily isolated from several naturally diseased plants collected from different cultivated newly reclaimed locations North Sinai, South Sinai, Northern Coastal Zone and Siwa Oasis, Egypt. All plant samples were cut into small pieces and were sterilized for 2 min in sodium hypochlorite (2%) then washed by sterilized distilled water, and dried between two layers of sterilized filter paper. All plant pieces were cultured on potato dextrose agar (PDA) medium and incubated at 28°C for 7 days. The fungal cultures were picked up and purified by hyphal tip or single spore technique. Then the fungi were identified at Plant Protection Dept., Desert Research Center, Egypt, according to their cultural, morphological and microscopical characteristics. The fungal isolates were maintained on PDA slants and kept at 4°C for further studies¹⁰.

Isolation and purification of Actinomycetes isolates

Random rhizosphere soil samples were collected from different locations North Sinai, South Sinai, North Coastal Zone and Siwa Oasis then stored in sterile plastic bags, labeled in the field and stored at 4°C until use. Soil samples (300 g) were carefully taken with spatula down to 10-20 cm below the soil surface (around the plant roots), a weight of 10 g of air-dried soil sample were immersed in 90 ml of sterile distilled water, and shake well then allowed to stand for 10 min, serial dilutions were carried out from 10⁻¹ to 10⁻⁶. Only 0.1 ml of different aqueous dilutions, 10⁻³, 10⁻⁵ and 10⁻⁶ were transferred to the growth medium in petri dishes and spread under aseptic conditions, then incubated for 6-7 days at 28±2°C for isolation²¹. Starch-casein agar, starch-nitrate agar, and King's B media were used for *Actinomycetes* isolation. The pH media was adjusted to 8.6 by 1 N NaOH solutions. *Actinomycetes* colonies were picked based on their morphological characteristics then purified and transferred into slants of starch nitrate/ NaCl. The pure culture slants were stocked at 4°C until further experiments²².

Maintenance and preservation of Actinomycetes isolates

The obtained *Actinomycetes* isolates were maintained on starch-nitrate agar slants and sub cultured periodically then kept in refrigerator. Also isolates were preserved by freezing at -20°C on starch-nitrate agar media containing 20% glycerol²³.

In vitro screening and assessment of antifungal effects of Actinomycetes against phytopathogenic fungi

The antagonistic activity of all *Actinomycetes* isolates were examined against fungal pathogens. Each *Actinomycetes* isolate was smeared on starch nitrate agar medium (SNA) as a net streaks after incubation at 28 ±2°C for 7 days. Then the antagonist was inoculated into PDA plates. After 4 days, fungal pathogen disks (5 mm) from fresh lawn cultures were added to the plates near the edges. Plates were incubated at 28 ±2°C for 6 days. Antifungal activity was studied where the rate of inhibition was calculated. Inhibiting of fungal growth was detected as the size of the clear zone, defined as the distance between the leading edge of fungal growth and the closest edge of the *Actinomycetes* growth, and the strains were ranked accordingly, inhibition was expressed relative to a control strain spotted on the same plate. Four replicates were done for each treatment. The isolates showed the greatest inhibition were selected as potential antagonistic *Actinomycetes*²⁴. To confirm previous results, Act5 and Act11 isolates were *in vitro* re-evaluated against pathogens. One disk of each fungal pathogen was placed on PDA medium at the two sides of plates and the *Actinomycetes* added as streak in the middle. The cultures were incubated at 28 °C. The radial growth of fungi was recorded after 7 days. The inhibition rate was calculated by the formula²⁵;

$$\text{Inh.} = (C-T/C) \times 100$$

Where, Inh. is the present inhibition; C, the colony diameter in control plate and T, the colony diameter in treated plate.

Characteristics of most active Actinomycetes isolates

Microscopic examination was conducted for the mature sporulated aerial mycelium color; the spore bearing hyphae and spore chain (spore chain and surface morphology for two weeks old) according to Holt *et al*²².

Biochemical and physiological characteristics of most active Actinomycetes isolates

For biochemical characterization, activity of selected isolates to utilize different nitrogen and carbon sources (glucose, xylose, galactose, lactose, sucrose, fructose, mannitol and starch) and nitrogen sources (ammonium chloride, sodium nitrate, ammonium sulphate, ammonium acetate, peptone, beef, lysin, phenyl alanine) were tested according to Vimal *et al*²⁶. Physiological characteristics such as hydrolysis of protein, starch, cellulose, pectin and protein were studied according to Venkata and Divakar²⁷. Also the thermo tolerability was tested for selected isolates,

growth at (35, 50, 60 °C) as well as under saline conditions 1 and 5% NaCl.

Light Microscopy study of Actinomycetes and fungal pathogen interaction

The interaction of potential antagonistic isolates and fungal pathogen was investigated using light microscopy. Samples were obtained from the pathogen and antagonist interaction zones in the plate assay and observed under the light microscope according to Sowndhararajan²⁸.

Effectiveness evaluation for selected Actinomycetes against tested fungi under greenhouse conditions

Pot experiment was carried out in the greenhouse of Plant Protection Department, Desert Research Center, Egypt. The experiment was designed to study the antifungal activity of two *Actinomycetes* against tested pathogenic fungi. Based on the results of the *in vitro* assays, two *Actinomycetes* isolates (Act5 and Act11) were used to evaluate their effectiveness against *F. oxysporum* f.sp. *lycopersici* and *Rhizoctonia solani* on tomato plants. Artificial inoculation was done in pots one week before transplanting. The surface layer of soil was removed, and the soils were infested with 10 ml of each pathogen suspension diluted in 90 ml distilled water. The pots were covered with plastic film and incubated for one week to promote pathogen growth. *R. Solani* and *F. oxysporum* density in the infested soils was evaluated at 10⁴ CFU g⁻¹ according to Larkin and Fravel²⁹. Tomato seedlings (Castle-Rock cultivar) were transplanted in pots. The biomass and culture filtrate mixture of each selected *Actinomycetes* were obtained after growing on starch nitrate broth media for 7 days at 28°C under continues shaking conditions, where 100 ml of *Actinomycetes* culture suspensions (10⁸-10⁹ CFU/ml) were poured into the soil twice as one week before and after inoculation, (100 ml distilled water for control)³⁰. For each treatment, six tomato seedlings were sown per pot, with ten replicates per treatment using a fully randomized complete block. After 30 days, seedlings were carefully removed from the soil and washed with tap water. Plants were drenched fungal pathogen only served as inoculated control. Plants were observed to record symptoms and disease incidence. Number of symptomatic leaves and dead plants were recorded for foliar wilt development ratings on plant and calculated by the following formula³¹

$$\text{Disease incidence (DI)} = \frac{\text{Total No. of infected plants}}{\text{Total No. of plants observed}} \times 100$$

After 50 days, plants were uprooted and plant height, plant fresh weight were recorded.

STATISTICAL ANALYSIS

Data were statistically analyzed using the method described by Gomez and Gomez³². The values were compared by Duncan's multiple Range Test at 0.05 levels of probabilities also LSD values were used in factorial experiment for comparing all treatments.

RESULTS AND DISCUSSION

In vitro antifungal activity of Actinomycetes

The continuous search for new antifungal agents from the rhizosphere samples led to select different *Actinomycetes*. Three hundred fifty soil samples were collected from various new reclaimed areas, North Sinai, South Sinai, Northern Coastal Zone and Siwa Oasis, during 2013-2015. It was observed difference in the proportion of isolates obtained from each region, where the Siwa Oasis is the highest in terms of the proportion of isolates with 20% followed by North Sinai by 15-17% while the lowest location was Northern Coastal Zone with 5-10% of the number of soil samples containing *Actinomycetes*. Seventy two isolates of *Actinomycetes* were isolated. Among these obtained isolates twenty two showed antagonistic ability against some tested fungi. Some researchers have notified similar antifungal effectiveness of *Actinomycetes* on plant fungal pathogens. Prapagdee *et al.*³³ found that only ten isolates were effective out of 146 isolates of *Actinomycetes* isolated from rhizosphere. Khamna *et al.*³⁴ found that 27 isolates only showed antifungal activities from 396 *Streptomyces* isolates. As prescreening step, twelve *Actinomycetes* isolates from 22 isolates have the ability to inhibit the growth of most tested fungi, and had the strongest antagonistic activity against tested fungi (*Alternaria alternata*, *Alternaria solani*, *Cladosporium cladosporioides*, *Curvularia* spp., *Fusarium oxysporum*,

Fusarium moniliforme, *Macrophomina phaseolina*, *Pythium ultimum*, and *Rhizoctonia solani*). The morphological characteristics and the cell wall type explained the probability that most of these 12 isolates belonged to the genus *Streptomyces*. Five isolates designated Act3, Act4, Act5, Act9 and Act11 belonged to the genus *Streptomyces* and showed maximal antagonistic activity against most of the tested pathogens (Table I). Among the tested fungi, *F. oxysporum* f.sp. *lycopersici* and *R. solani*, were highly affected by two *Actinomycetes* isolates (Act5, and Act11) based on data obtained from preliminary screening (Fig I). The antagonistic test *in vitro* was used to assure the previous antagonistic effects of Act5 and Act11 against *F. oxysporum* f.sp. *lycopersici* and *R. solani*. The inhibition rates were observed and the results showed that the highest growth inhibition rates provided with Act5 and Act11. The most effective isolate was Act5 where inhibited the growth of *R. solani* and *F. oxysporum* f.sp. *lycopersici* by 82% and 75% respectively, while the inhibition rates by Act3, Act4 and Act9 were 33.4, 38.2, 52.7% against *F. oxysporum* f.sp. *lycopersici* and 37.5, 45, 48.2% respectively as inhibition growth of *R. solani* (Fig. II). These results were in line with preliminary screening results, Act5 and Act11 isolates have the highest antifungal effects against tested fungi. Since the highest destructive effect of these isolates revealed against fungal pathogens growth, further investigations were carried out to find out their efficacy for plant protection and disease control.

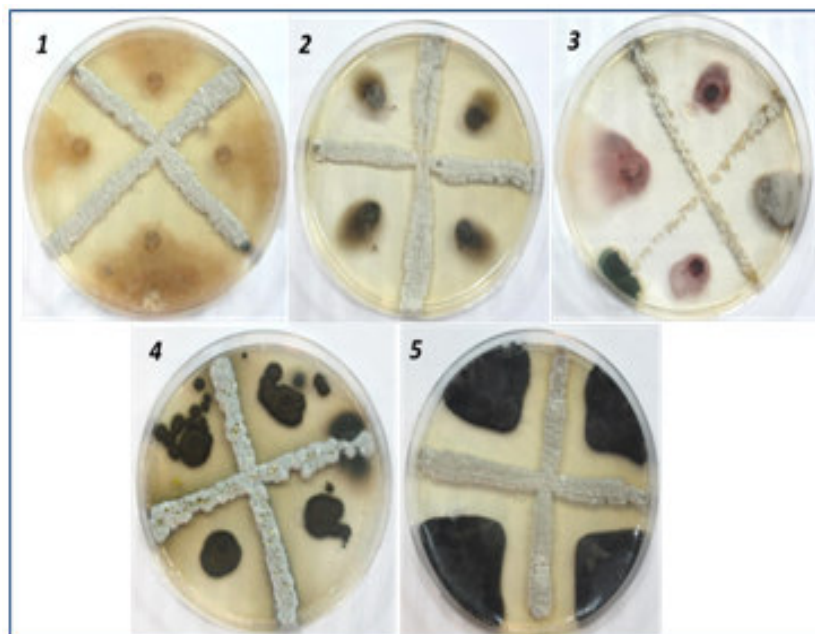


Figure I

Pre-screening of *Actinomycetes* isolates against tested pathogens under in-vitro conditions.

- 1) *Rhizoctonia solani*, 2) *Alternaria solani*, 3) *Fusarium oxysporum*, 4) *Cladosporium cladosporioides*, 5) *Macrophomina phaseolina*

Light Microscope examination to determine the *Streptomyces* effect on tested pathogens

To determine the *Streptomyces* activity against fungal growth of *F. oxysporum* f. sp. *lycopersici* and *R. solani*, light microscopic examination on fungal mycelial growth near the zone of inhibition showed significant changes in hyphal morphology compared to normal growth in control. There were considerable interactions between the *Streptomyces* isolates and fungi. Microscopic examination showed significant abnormalities in the growth of fungi as morphological changes of the fungal mycelium. In case of *R. solani*, the hyphal growing tip was stunted with a lot of branches; also the hyphae became thinner in addition to the presence of voids in mycelium, this appearances and effects agreed with Sowndhararajan²⁸. With *Fusarium* the mycelium was cracking and wrapping of threaded around each other, the shape of treated culture became darker than control; also stimulate the sporulation of fungus faster than the normal period, abnormalities were seen on the peripheral cells to the spores, also the spores were thinner compared to control. Many *Actinomycetes* belong to the *Streptomyces* genus are known as biocontrol agents against various fungal plant pathogens³⁴. The antagonistic effect of *Streptomyces* against fungi is related to the production of antifungal compounds¹², and/or extracellular hydrolytic enzymes^{10, 33}.

Morphological characteristics of efficient *Streptomyces* isolates

Visual observations of morphological and microscopic characteristics were carried out using light microscopy. Most of the colonies that grew on starch-casein agar (SCA) plates belonged to the genus *Streptomyces* since the colonies were slow growing, aerobic, chalky, heaped, folded and with aerial mycelia of semi different colors with earthy odor (Fig. III). The Act5 also produced antibiotics as reflected by zones of growth more than Act11. Colonies of Act5 are pale brown and wrinkled while Act 11 was brownish white cream on SCA. In the observation with light microscopy, the number of the spores was higher in Act5 than Act11 (Figure IV-A), spore-bearing hyphae of the strain Act11 appeared as spiral chains (Figure IV-B). The taxonomic criteria of the Act5 and Act11, according to morphological examination showed that the isolates belong to the genus *Streptomyces*. The mycelia of the isolate *Streptomyces* sp. (Act5) grew actively on all tested media compared to Act11.

Physiological, Biochemical of the selected *Streptomyces* isolates

Physiological and biochemical Characterization have been conducted for each of the most efficient isolates.

As physiological criteria, the results showed ability of both isolates to hydrolysis of (protein, starch and pectin) while the Act 5 showed its ability to cellulose hydrolysis compared to Act11. In terms of the chemical characterization, both of two *Streptomyces* isolates were similar in all tested characters where the results were positive in carbon source utilization such as D-Glucose, D-Xylose, D-Galactose, lactose, sucrose, D-fructose, Mannitol and starch (Table II). These properties associated with Act5 and Act11 might explain their ability to control and suppress the growth of some fungal pathogens, the antibiosis against plant pathogens, the synthesis of particular extracellular proteins, and the degradation of phytotoxins³⁵. Also may be these two strains produced antibiotics lethal to pathogenic fungi of *F. oxysporum* f. sp. *lycopersici* and *R. solani* in antibiosis tests. Both strains significantly decreased the fungal growth compared to control treatment. These results agreed with the interpretations of Gangwar *et al.*³⁶.

Evaluation of selected *Streptomyces* against tested fungal pathogens under greenhouse conditions

In this study, some *Actinomycetes* isolates were isolated from rhizosphere soil samples showed *in vitro* production of anti-fungal activities with high inhibition effects for *R. solani* and *F. oxysporum* f.sp. *lycopersici* mycelium growth. Their abilities to reduce the wilt and root rot diseases incidence as well as promote the growth of tomato seedlings in green house experiments were investigated. Two promising *Actinomycetes* isolates were used in greenhouse conditions. The disease incidence recorded 22.4% and 26.2% of *F. oxysporum* f. sp. *Lycopersici* before and after the inoculation by Act5, while Act11 recorded 32.7% and 40.8% compared to the control (83.5%). Also the antifungal activities of Act5 reduced the disease incidence of *R. solani* from 78.4% in control to 38.3% and 41.5% as pre and post treatments respectively (Table III). Several studies mentioned the use of *Streptomyces* strains in controlling of *R. solani* root rot³⁷, also in controlling of *F. oxysporum*³⁸. The effects of selected *Streptomyces* on shoot length, root length, fresh weight of shoots and roots of tomato plants were determined 50 days after inoculation. Data in table III showed that, Act5 increased length of shoot (40.5 and 38.2 cm) with *F. oxysporum* f.sp. *lycopersici*, and by 43 and 40.4 with *R. solani* as pre and post treatment respectively. While the Act11 increased shoot length (35.7cm and 32.5 cm) with *F. oxysporum* and (33.5cm and 31.3 cm) with *R. solani* as pre and post treatments respectively compared to control (28 and 26.6 cm).

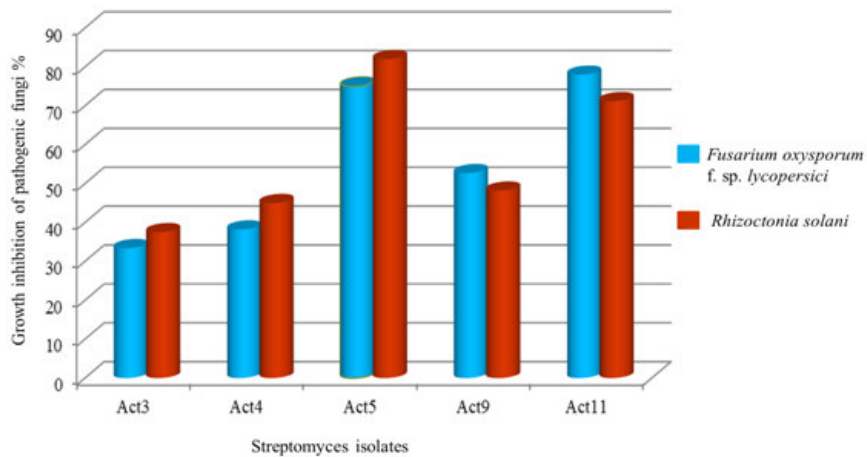


Figure II
Growth inhibition of *F. oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani* by the most effective *Streptomyces* isolates (Act 3, 4, 5, 9 and Act11) under in vitro conditions

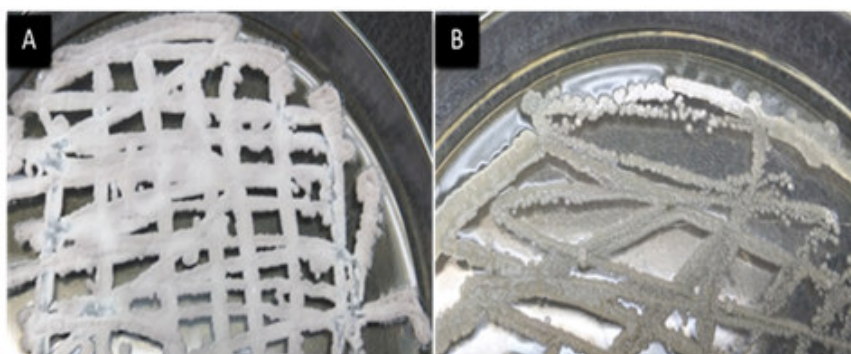


Figure III
Culture morphology of selected *Streptomyces* isolates A) Act5 and B) Act11; where the spore and aerial mycelium are different.

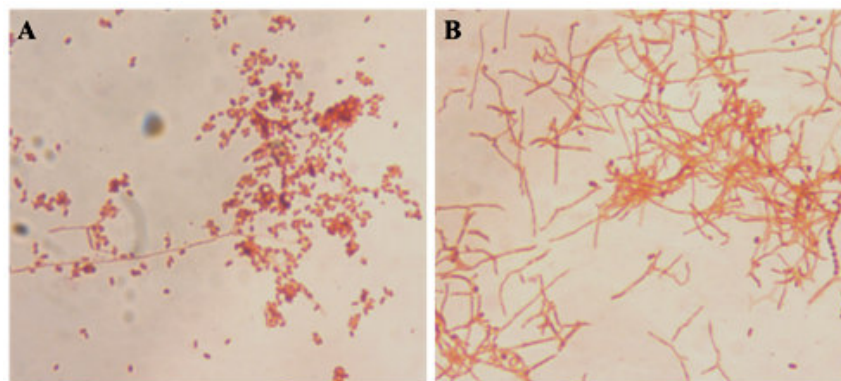


Figure IV
Morphological structures of A) Act5 and B) Act11; using image microscope at 1000X; abundance of spores, aerial mycelium, chain of spores are different.

The length of root recorded (18.3 and 16.5 cm) by Act5, while recorded (13.5 and 13) by Act11 compared to control (12.7 cm) in *F. oxysporum* f. sp. *lycopersici* while root length were increased (11.8 and 11 cm) with Act 11 compared to control (9.5 cm). The results were in the same direction in cases of shoot fresh weight, root fresh weight where increased by *Streptomyces* treatments

compared to control (Table III). The results of the present study demonstrate that selected *Streptomyces* have significant effects on the growth parameters of tomato. In other studies, the efficacy of *Streptomyces* on different soil borne fungi pathogen of tomato had also been observed^{30,39}. The presented data are in line with other investigations where the potential effects of

Streptomyces to control of various plant pathogens⁴⁰. *Streptomyces* isolates Act5 and Act11 showed significant antagonistic effects on mycelial growth and structure of pathogens as well as suppressive effects on pathogens in pot experiments. The effect of both strains proved to be highest with Act5 followed by Act11 in most of studied parameters. Determination of the necessary time for application of *Streptomyces* and defense responses before and after application was a point of interest, where the analyzed results showed higher induction when the biocontrol agents applied before inoculation of pathogens. The positive effects are probably due to the volatile and non-volatile compounds produced by *Streptomyces*, where the interaction

between other *Streptomyces* and soil pathogens such as *F. oxysporum* and *R. solani* were confirmed by El-Tarabily⁴¹, also the antifungal effects of biological agents on growth parameters of tomato plants could be due to several reasons such as production of plant growth regulators such as inorganic phosphate solubilization, production of gibberellic acid and indol-3-acetic acid⁴², therefore inhibitory effects against pathogens development structures at the early stages of infection reducing the pathogen ability to infect the plant tissues. It indicates that these *Streptomyces* might have influential antagonistic activities against soil-borne fungi and play a role in promoting the growth of their original plant hosts.

Table I
Antagonistic activity of effective actinomycetes isolates against tested pathogens under in vitro conditions.

Action-code	<i>Alternaria alternata</i>	<i>Alternaria solani</i>	<i>Cladosporium cladosporioides</i>	<i>Curvularia spp.</i>	<i>Fusarium oxysporum</i>	<i>Fusarium solani</i>	<i>Fusarium moniliforme</i>	<i>Macrophomina phaseolina</i>	<i>Pythium ultimum</i>	<i>Rhizoctonia solani</i>
Act1	+	-	+	+	+	++	+	-	+	-
Act2	+	+	-	+	+	+	+	+	+	+
Act3	+	-	-	-	++	++	++	++	++	++
Act4	±	-	+	±	++	++	++	++	++	++
Act5	+++	+++	+++	++	+++	+++	++	++	+++	+++
Act6	+	+	±	+	+	+	±	+	-	-
Act7	+	+	+	-	+	+	+	-	+	-
Act8	-	±	+	-	+	+	+	+	-	+
Act9	+++	+	++	+	++	++	++	-	+	+
Act10	++	++	+	++	+	+	+	-	++	±
Act11	++	++	+++	++	+++	++	++	++	++	+++
Act12	±	±	+	+	+	-	-	±	-	+

***, strong inhibition; **, moderate inhibition; +, partial inhibition; ±, weak inhibition; -, no inhibition.

Table II
cultural, physiological, biochemical and morphological characteristics of selected actinomycetes strains.

Characteristics	ACT5	ACT11
Physiological Characteristics		
Hydrolysis of Protein	+	+
Hydrolysis of starch	+	+
Hydrolysis of cellulose	+	-
Hydrolysis of pectin	+	+
Biochemical Characteristics		
Carbon source utilization	+	+
D-Glucose	+	+
D-Xylose	+	+
D-Galactose	+	+
Lactose	+	+
Sucrose	+	+
D-fructose	+	+
Mannitol	+	+
Starch	+	+
Utilization of:		
Urea test	+	-
Nitrate reduction	+	+
Gas information from nitrate	+	-
Catalase test	+	+
Nitrogen source utilization		
Ammonium Chloride	+	+
Sodium nitrate	+	+
Ammonium sulphate	+	+
Ammonium acetate	+	weak
Peptone	+	+
Beef	+	+
L-lysine	+	weak
L-Phenyle alanine	+	+
Growth at		

35 C	+	+
50 C	-	-
60 C	-	-
Nacl 1%	+	+

Actinomyces isolate (A)	Application Time (T)	<i>F. oxysporum f. sp. lycopersici</i>					<i>Rhizoctonia solani</i>				
		Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Disease incidence % (DI)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Disease incidence% (DI)
Act5	Pre	40.5	18.3	26.4	6.2	22.4	43	19.5	25.5	6.6	38.3
	Post	38.2	16.5	27.3	6.8	26.2	40.4	15.8	28	5.4	41.5
Act11	Pre	35.7	13.5	23.3	5.2	32.7	33.5	11.8	22.6	5.2	46.1
	Post	32.5	13	22.5	5	40.8	31.3	11	20.2	5	50.8
Cont.	Pre	28	12.7	11.5	2.8	83.5	26.6	9.5	14.5	1.9	78.4
	Act. (A)	2.36	1.38	1.36	0.76	2.03	2.13	1.74	0.51	2.19	1.91
LSD 5%	Time (T)	2.04	0.85	0.98	1.61	1.02	4.95	2.56	0.56	1.33	2.15
	A×T	3.59	1.94	1.93	1.07	2.85	2.70	3.03	3.10	0.72	3.26
		Nacl 5%				+	weak				

Table III

Effect of Actinomyces isolates on different growth parameters in tomato and suppression of wilt disease caused by *F. oxysporum f. sp. lycopersici* and *Rhizoctonia solani* under greenhouse conditions.

CONCLUSIONS

The potential antagonistic activity of the *Streptomyces* found in this study against plant pathogenic fungi highlights their potential antifungal effects. The selected *Streptomyces* could be able to suppress of *R. solani* and *F. oxysporum f.sp. lycopersici* on tomato plants under greenhouse conditions. From the present study, it could be demonstrated that Egyptian desert soils provided a rich source of effective *Actinomyces* as biological control agents. The biochemical, physiological and morphological characteristics of two potent isolates studied clearly demonstrated that the present isolates Act5 and Act11 are belongs to the genus *Streptomyces*

which had the strongest inhibitory effects on in vitro growth of various plant pathogens and suppressive impact on root rot and wilt diseases caused by *R. solani* and *F. oxysporum* as well as plant growth promoter of tomato seedlings. It could be suggested that the selected *Streptomyces* might have significant antagonistic activities against soil-borne fungi. Molecular and formulation studies on selected *Streptomyces* are under progress to evaluate their potential effects under field conditions.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

- Singh PK and Kumar V. Fusarium Wilt of Chrysanthemum – Problems and Prospects. Plant Pathol Quarant. 2014.4 (1): 33–42. http://www.plantpathologyquarantine.org/pdf/PPQ_4_1_5.pdf
- Duniway JM.. Status of chemical alternatives to methyl bromide for pre-plant fumigation of soil. Phytopathology, 2002, 92(12): 1337-1343.<http://apsjournals.apsnet.org/doi/pdf/10.1094/PHYTO.2002.92.12.1337>
- Jenkins DJA, Wolever TMS, Leeds AR, Gassull MA, Haisman P, Dilawari J, Goff DV, Meta GL, Albert KGMM. Dietary fibres, fibre analogues and glucose tolerance: importance of viscosity. Br Med J. 1978;1:1392–1394. <https://www.ncbi.nlm.nih.gov/pubmed/647304>
- Hernandez-Suarez, M., Hernandez-Castillo, F.D., Gallegos-Morales, G. Hugo., Lira-Saldivar, R., Rodriguez-Herrera, R. and Aguilar, C.N. Biocontrol of soil fungi in tomato with microencapsulates containing *Bacillus subtilis*. Am J Agric Biol Sci, 2011, 6:189–195.Doi:10.1111/jam.12138.
- Moëne - Loccoz Y, Tichy HV, O'Donnell A, Simon R and O'Gara F. Impact of 2,4-diacetylphloroglucinol-producing biocontrol strain *Pseudomonas fluorescens* F113 on intraspecific diversity of resident culturable fluorescent pseudomonads associated with the roots of field-grown sugar beet seedlings. Applied and Environmental Microbiology, 2001, 67:3418-3425.DOI:10.1128/AEM.67.8.3418-3425.2001
- Chadha N, Prasad R, and Varma A. Plant promoting activities of fungal endophytes associated with tomato roots from central Himalaya, India and their interaction with *Piriformospora indica*. Int J Pharma. Bio. Sci. , 2015, 6(1): 333-343.http://www.ijpbs.net/download.php?download_file=cms/php/upload/3950_pdf.pdf&did=3950
- Goodfellow M and Williams ST. Ecology of actinomyces. Ann Rev Microbiol, 1983, 37: 189-216. <http://www.annualreviews.org/doi/pdf/10.1146/annurev.mi.37.100183.001201>

8. Sanglier, JJ, Haag H, Juck TA and Fehr T. Novel bioactive compounds from actinomycetes: a short review (1988-1992). Res. Microbiol. 1993, 144: 633-642. <https://www.ncbi.nlm.nih.gov/pubmed/7908142>
9. Dávila MMD, Gallegos Morales G, Hernández Castillo FD, Ochoa Fuente YM, Flores Olivás A and Cepeda Siller M. Antagonist actinomycetes metabolites against plant pathogens fungi of agricultural importance. J Sci Res Stud, 2016, 3(11): 224-230. <http://www.modernrespub.org/jsrs/pdf/2016/November/Medina%20et%20al.pdf>
10. Al-Askar AA, Rashad YM and Abdulkhair WM. Antagonistic activity of an endemic isolate of *Streptomyces tendae* RDS16 against phytopathogenic fungi. Afr J Microbiol Res, 2012, 7(6): 509-516. DOI: 10.5897/AJMR12.1792
11. Omar AM and Ahmed AIS. Antagonistic and inhibitory effect of some plant rhizo-bacteria against different *Fusarium* isolates on *Salvia officinalis*. Amer-Eurasian J Agri Environ Sciences, 2014, 14 (12): 1437-1446. DOI: 10.5829/idosi.ajeaes.2014.14.12.12488
12. Baz M, Lahbabi D, Samri S, Val F, Hamelin G, Madore I, Bouarab K, Beaulieu C, Ennaji MM and Barakate M. Control of potato soft rot caused by *Pectobacterium carotovorum* and *Pectobacterium atrosepticum* by Moroccan actinobacteria isolates. World J Microbiol Biotechnol, 2012, 28 (1):303-311. DOI: 10.1007/s11274-011-0820-5.
13. Sharma P, Das R, Kalita MC and Thakur D. Investigation of extracellular antifungal proteinaceous compound produced by *Streptomyces* sp. 5K10. Afr J Microbiol Res, 2014, 8(10): 986-993. DOI: 10.5897/AJMR2013.6264
14. Ilic, S.B., Konstantinovic, S. S., Todorovic, Z.B., Lazic, M.L., Veljkovic, V.B., Jokovic, N. and Radovanovic, B.C. Characterization and antimicrobial activity of the bioactive metabolites in streptomycete isolates. Microbiology, 2007, 76, 421-428. Doi:10.1134/S0026261707040066
15. Abdallah, M.E., S.A. Haroun, A.A. Gomah, N.E. El-Naggar, H.H. Badr. Application of actinomycetes as biocontrol agents in the management of onion bacterial rot diseases Arch. Phytopathol. Plant Prot., 2013, 46 (15), pp. 1797–1808. Doi.org/10.1080/03235408.2013.778451
16. El-Tarabily K and Sivasithamparam K. Non-streptomycete actinomycetes as biocontrol agents of soilborne fungal plant pathogens and as plant growth promoters. Soil Biol Biochem, 2006, 38, 1505-1520. Doi:10.1016/j.soilbio.2005.12.017
17. Shirokikh IG, Zenova GM, Zvyaginstev DG. Actinomycetes in the rhizosphere of barley grown on acid soddy podzolic soil. Microbiology (Moscow), 2002, 71: 455–459. <http://connection.ebscohost.com/c/articles/16296173/actinomycetes-rhizosphere-barley-grown-acid-soddy-podzolic-soil>
18. Chandel S, Eunice JA and Woodward S. Biological Control of *Fusarium oxysporum* f.sp. *lycopersici* on Tomato by *Brevibacillus brevis*. J Phytopathol, 2010, 158:470–478. DOI: 10.1111/j.1439-0434.2009.01635.x
19. Szczechura W, Staniaszek M and Habdas H. *Fusarium oxysporum* f. sp. *radicis-lycopersici* – the cause of *Fusarium* crown and root rot in tomato cultivation. Journal of Plant Protection Res, 2013, 53(2):172-176. [http://www.plantprotection.pl/PDF/53\(2\)/JPPR_53\(2\)_12_Szczechura.pdf](http://www.plantprotection.pl/PDF/53(2)/JPPR_53(2)_12_Szczechura.pdf)
20. Mishra M, Prasad R and Varma A. Endophytic fungi: Biodiversity and functions. International Journal of Pharma and BioSciences, 2015, 6(1): 18-46. http://www.ijpbs.net/cms/php/upload/3913_pdf.pdf
21. Oskay M, Tamer AU and Azeri C. Antibacterial activity of some Actinomycetes isolated from farming soils of Turkey. Afr J Biotechnol, 2004, 3 (9): 441-446. <http://www.ajol.info/index.php/ajb/article/view/14994/58902>
22. Holt JG, Krieg NR, Sneath PHA, Staley JT and Williams ST. Bergey's Manual of Determinative Bacteriology, 9th ed. Williams and Wilkins, Baltimore, MD. 1994. <http://www.worldcat.org/title/bergeys-manual-of-determinative-bacteriology/oclc/28183643>
23. Sivanandhini T, Subbaiya R, Gopinath M, Mahavinod JKV, Kabilan T and Selvam M M. An Investigation on orphological Characterization Of Actinomycetes Isolated From Marine Sediments. Res J Pharma Bio Chem Sci, 6 (2): 1234-1243. [http://www.rjpbcs.com/pdf/2015_6\(2\)/\[182\].pdf](http://www.rjpbcs.com/pdf/2015_6(2)/[182].pdf)
24. Ara I, Bukhari NA, Perveen K and Bakir MA. Antifungal activity of some actinomycetes isolated from Riyadh soil, Saudi Arabia: An evaluation for their ability to control *Alternaria* caused tomato blight in green house pot trial. African Journal of Agricultural Research, 2012, 7(13), pp. 2042-2050. DOI: 10.5897/AJAR12.398
25. Bekker TF, Kaiser C, Merwe PVD, and Labuschagne N. In-vitro inhibition of mycelial growth of several phytopathogenic fungi by soluble potassium silicate. S. Afr. J. Plant Soil, 2006, 23(3): 169-172. DOI:10.1080/02571862.2006.10634750
26. Vimal V., Rajan B.M., Kannabiran K. Antimicrobial activity of marine actinomycete, *Nocardioopsis* sp. VITSVK 5 (FJ973467). Asian J Med Sci. 2009; 1(2): 57-63. [http://maxwellsci.com/print/ajms/\(2\)57-63.pdf](http://maxwellsci.com/print/ajms/(2)57-63.pdf)
27. Venkata EN and Divakar G.. Screening and Isolation of Pectinase Producing Bacteria from Various Regions in Bangalore. Int. j. pharm. biomed. res., 2013, 4 (1). ijrbsonline.com/files/24-4144.pdf
28. Sowndhararajan K and Kang SC. *In vitro* antagonistic potential of *Streptomyces* sp. AM-S1 against plant and human pathogens. J Agric

- Chem Environ, 2012, 1 (1): 41-47.<http://dx.doi.org/10.4236/jacen.2012.11007>
29. Larkin RP and Fravel DR. Efficacy of Various Fungal and Bacterial Biocontrol Organisms for Control of Fusarium Wilt of Tomato. *Plant Disease*, 1998, 82(9): 1022-1028.<http://apsjournals.apsnet.org/doi/pdf/10.1094/PDIS.1998.82.9.1022>
 30. Dhanasekaran D, Sivamani P, Panneerselvam A, Thajuddin N, Rajakumar G and Selva-mani S. Biological control of tomato seedling damping off with *Streptomyces* sp. *PI Pathol J*, 2005, 4:91-95.DOI: 10.3923/ppj.2005.91.95
 31. Sahu G.K. and S.S. Sindhu, 2011. Disease Control and Plant Growth Promotion of Green Gram by Siderophore Producing *Pseudomonas* sp.. *Res J Microbiol*, 6: 735-749.DOI: 10.3923/jm.2011.735.749
 32. Gomez KA and Gomez AA. 1984. *Statistical Procedures for Agricultural Research*. 2nd Ed., John Wiley and Sons, New York, USA.http://pdf.usaid.gov/pdf_docs/PNAAR208.pdf
 33. Prapagdee B, Kuekulvong C and Mongkolsuk S. 2008. Antifungal Potential of Extracellular Metabolites Produced by *Streptomyces hygrosopicus* against Phytopathogenic Fungi. *Int J Boil Sci*, 4(5): 330-337. DOI:10.7150/ijbs.4.330.
 34. Khamna S, Yokota A, Peberdy JF and Lumyong S. Antifungal activity of *Streptomyces* spp. isolated from rhizosphere of Thai medicinal plants. *Int J Integrat Biol*, 2009, 6(3): 143-147.<http://repository.cmu.ac.th/handle/665394383/2/5775>
 35. Barakate MY, Ouhdouch OY and Beaulieu C. Characterization of rhizospheric soil streptomycetes from Moroccan habitats and their antimicrobial activities. *World J Microbiol Biotechnol*, 2002, 18 : 49-54.DOI: 10.1023/A:1013966407890
 36. Gangwar M, Dogra S and Sharma N. Antagonistic Bioactivity of Endophytic Actinomycetes Isolated from Medicinal Plants. *J Adv Lab Res Biol*, 2011, 2 (4): 154-157.<http://www.nsays.in/journals/ViewArticle.php?id=93783177>
 37. Patil HJ, Srivastava AK, Kumar S, Chaudhari BL and Arora DK. Selective isolation, evaluation and characterization of antagonistic actinomycetes against *Rhizoctonia solani*. *World J of Microbiol Biotechnol* 2010, 26:2163-70.DOI: 10.5604/17331331.1185232.
 38. Kamal R and Sharma AK. Control of Fusarium wilt using biological agent *Streptomyces* sp.CPP-53 isolated from compost with plant growth promoting effect on tomato under greenhouse condition. *J Microbiol Antimicrob*, 2014, 6(6):97-103.DOI: 10.5897/JMA2014.0318
 39. Kunova A, Bonaldi M, Saracchi M, Pizzatti C, Chen X and Cortesi P. Selection of *Streptomyces* against soil borne fungal pathogens by a standardized dual culture assay and evaluation of their effects on seed germination and plant growth. *BMC Microbiology*. 2016, 16:272.Doi:10.1186/s12866-016-0886-1.
 40. Goudjal Y, Toumatia O, Sabaou N, Barakate M, Mathieu F and Zitouni A. Endophytic actinomycetes from spontaneous plants of Algerian Sahara: indole-3-acetic acid production and tomato plants growth promoting activity. *World J Microbiol Biotechnol*, 2013.DOI 10.1007/s11274-013-1344-y.
 41. El-Tarabily KA, Nasser AH, Hardy GE and Sivaithamparam K. Plant growth promotion and biological control of *Pythium aphanidermatum*, a pathogen of cucumber, by endophytic actinomycetes. *J Appl Microbiol*, 2008, 106:13-26. DOI:10.1111/j.1365-2672.2008.03926.x
 42. Anitha A, and Rabeeth M. Control of Fusarium Wilt of Tomato by Bioformulation of *Streptomyces griseus* in Green House Condition. *Afr J Basic ApplSciences*, 2009, 1 (1-2): 9-14.DOI: 10.5897/JMA2014.0318

Reviewers of this article



Prof. Dr. Ram Prasad

Assistant Professor
Amity Institute of Microbial Technology
Amity University, Uttar Pradesh, INDIA



**Prof. Dr. M.Ranga Priya, M.Pharm., Ph.D.,
R.Ph.**

Professor, Dept of Pharmaceutics, Sun
Institute Of Pharmaceutical Education &
Research, Kakupall, Nellore Rural, Nellore,
Andhra Pradesh 524346

Dr. Soosamma Mathews.

Professor and HOD, Dept. of Botany, Mount
Carmel College, 58, Palace Road, Bangalore - 560
052, India.



Prof. Dr. K. Suriaprabha

Asst. Editor, International Journal
of Pharma and Bio sciences.



Prof. P. Muthuprasanna

Managing Editor, International
Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript