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Research Article

Biological Control of *Sclerotinia sclerotiorum* the causal agent of Lettuce Rot Disease by use of soil *Streptomycetes*

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ABSTRACT: *Sclerotinia sclerotiorum* (Lib) De Bary, the causal agent of lettuce rot, is asoil borne fungal pathogen which can cause extensive damage to infected plants or crop and occurs world over. It is difficult to control this pathogen by normal cultural and chemical practices. Our studies have shown that the pathogen can be managed by by use of bacteria belonging to the actinomycetes group. Preliminary screening of 40 soil isolates of actinomycetes from Kerman province of Iran was carried out for testing their ability to inhibit *Sclerotinia sclerotiorum* by using *in vitro* agar disk and dual culture evaluations. Among the tested isolates, Actinomycetes isolates B, F and S showed reasonable inhibitory capabilities in dual culture. These isolates were positive forchitinase, Amylase, Protease and Lipase activities but none of them produced HCN. Minimal inhibitory concentration of the active crude extracts varied from1.25 to 0.312 mg/ml and the thermal inactivation point was determined between 90-120°C. All three isolates had fungicidal activity and only isolate F retained its antifungal activity after exposure to chloroform. Significantly, all three isolates reduced the severity of the disease in greenhouse evaluations.

KEY WORDS: Actinomycetes, Biological control, *Sclerotinia sclerotiorum*, Lettuce rot, Basal drop disease

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INTRODUCTION

Sclerotinia sclerotiorum (Lib.) De Bary is a soil borne fungal pathogen which can cause extensive damage to infected plants or crop and occurs world over (Boland and Hall, 1994; Purdy, 1979). Basal drop disease or lettuce rot caused by either *Sclerotinia minor* or *S. sclerotiorum* (Budge *et al*., 1991; Subbarao, 1998) is one of the most common and serious diseases of lettuce (*Lactuca sativa*) worldwide, causing major yield losses to growers (Patterson & Grogan, 1985; Subbarao, 1998). Early symptoms of disease include wilting and yellowing of lower leaves. The disease is characterized by a soft, watery rot of the crown and stem of lettuce (Abawi *et al*., 1985; Patterson and Grogan, 1985) and can occur at any stage of crop development. Infected plant parts may show cottony mycelial growth, at later stages dark colored sclerotia will be seen on the surface (Osoofe *ei al*., 2005). Due to non-availability of proper management practices research has focused on use of bioagents belonging to the actinomyces group. Studies show that most actinomycetes species belong to the genus of *Streptomyces* and this group have shown potential to suppress several phytopathogenic fungi (Saadoun and Al-Momani, 1997; Saadoun *et al.,* 2000; El-Tarabily *et al.,* 2000). In the present study experiments were carried out to select strains from the actinomycetes group that have potential for effective biological control of *Sclerotinia sclerotiorum*.

MATERIALS AND METHODS

Culture media

Case in Glycerol (or starch) Agar (CGA) was applied for screening and isolating actinomycetes and composed of: glycerol or starch, 10g ; casein, 0.3g; KNO_3, 2g; NaCl, 2g; K_2HPO_4, 2g; $MgSO_4.7H_2O$, 0.05 g; $CaCO_3$, 0.02g; $FeSO_4.7H_2O$, 0.01g and agar 18g in 1L of distilled H_2O (pH 7.2) (Dhingra and Sinclair, 1995). The fungus was grown on potato dextrose agar (PDA, Difco, 39 g PDA per liter of distilled H_2O, pH of 7.2) at 25°C.

The minimal chitin agar medium (MCA) (colloidal chitin 0.4%, KH_2PO_4 1.72 mM, K_2HPO_4 5.14 mM, $MgSO_4 \cdot 7H_2O$ 2 mM, $FeSO_4 \cdot 7H^2O$ 0.035 mM, $ZnSO_4 \cdot 7H_2O$ 3.4 µM , $MnCl_2 \cdot 4H_2O$ 5 µM and agar 1.5 %, pH was adjusted to 8 to 8.5) was used to evaluate chitinase activity of *Streptomyces* strains (Hsu and Lockwood,1975). Proteolytic activity was studied on medium containing glucose 1g, casein 3g, $CaCl_2$ 2g and agar 15g in 1 L of distilled H_2O (pH 7.0)

(Dunne *et al.,* 2000). Lypolytic activity was assessedusinga media containing peptone 10 g, NaCl 5g, $CaCl_2$ 0.1g, agar 15 g and Tween-80 10 mlin 1 L of distilled H_2O (pH 7.0) (Sierra, 1957). The amilase activity was assayed on starch agar medium containing 0.2 % soluble starch plus suitable nutrient agar basal medium (Society of American Bacteriologists, 1951).

Preparation of fungal isolate

Pure culture of *sclerotiorum* was obtained from Mycology Lab, Dep. of Plant Pathology, College of Agriculture, Shahid Bahonar University of Kerman, Iran. All cultures stored at 4°C and sub-cultured as needed.

Preparation of *Sclerotinia sclerotiorum* inocula

A millet (*Panicummiliaceum*) seed-based inoculum was prepared by adding 50 g of seeds to 40 ml of distilled water in 500 ml conical flasks. Autoclaving of the flasks was done at 121°C for 30 min for two consecutive days. Ten agar plugs (6 mmdia) of actively growing culture of *S. sclerotiorum* were aseptically inoculated into the seed inoculum flasks and incubated at 25± 2 °C for 14 days. Periodic shaking of the flasks were carried out so as to enable uniform growth and cover of the pathogen. The control flasks had no pathogen..Autoclaved but uninoculated millet seeds were served as the control. The viability and purity of the prepared inoculum was tested by culturing of pathogen from colonized seeds on PDA plates before inoculation (Tahtamouni *et al*., 2009).

Greenhouse pathogenicity test

To assure pathogenicity of the fungus to be used in further evaluations, fungal colonized millet seeds –were mixed with the upper one third part of sterile soils in plastic pots at the ratio of 1% w:w. Lettuce seedlings (5-6 weeks old) were transplanted in infested soil. For healthy controls only sterile millet were seeds mixed into the soil at the similar ratio. All pots were maintained in a glasshouse at 22± 3°C and watered regularly as needed. The pathogenicity test performed in triplicates. The fungus was reisolated from all inoculated plants, confirming Koch's postulates.

Soil sampling and isolation of Actinomycetes

The methodology was as per Saadoun *et al*., 1999 and Saadoun and Gharaibeh, 2001.Random soil sampling was carried out in grasslands and vegetable fields located in the province of Kerman, Iran. Samples were collected at a depth of 10-20cm from surface by use of an open-end soil borer (20 cm in depth, 2.5 cm in diameter) (Lee and Hwang (2002). The soil samples were air dried at ambient temperature for 7 to 10 days and later sieved using 0.8 mm mesh. The samples were kept at room temperature in polyethylene bags.100 ml by adding sterile distilled water was added to 10g soil and vigorously agitated for 1 h and allowed to settle for 1 h. 1ml of the suspension was used for serial dilution and 1ml aliquots from 10^{-3} -10^{-6} soil dilutions were spread plated onto CGA. Three replicates were maintained and the plates were incubated at 28°C for 7 days. Actinomycetes colonies were purified onto CGA slants and used for further studies.

***In vitro* inhibition studies**

Toassess the inhibitory potential of isolated *Streptomyces* spp inhibitory experiments were done using the agar disk method (Aghighi, *et al*., 2004). Inhibition zone around *Streptomyces* agar disks were measured (Lee and Hwang, 2002; El-Tarabily *et al*., 2000).

Agar-disk method

From the refrigerated stocks, each Actinomycete isolate was freshly cultured on CGA medium (28± 2°C for 4- 6 days) as a single streak. Disks of the grown colonies (6mm dia obtained using sterile cork borer) was transferred to PDA plates containing fresh growing culture of *S. sclerotiorum*. The PDA plates were incubated at 28± 2°C for 6 days and the Diameter of Inhibition Zones, (DIZ mm) was measured and analyzed (Shahidi Bonjar and Karimi, 2004).

Dual culture bioassay

Mycelial disc (6 mm diameter) from the peripheral region of 4-6 day old culture of *S. sclerotiorum* were placed in the center of the fresh PDA plates. Six mm discs of 5- 7 day old cultures of *Streptomyces* isolates were placed at 3 cm distance from pathogen disc. Sterile agar discs were used as control at similar positions. All the plates were incubated at 28± 2°C for 8 days (Dhingra and Sinclair, 1995). The intensity of inhibition at dual cultures was calculated by subtracting the distance (mm) of fungal growth in the direction of an antagonist colony (γ) from the fungal growth radius (γ°) of a control culture to express rating as Δγ = 5-9 mm, + (weak inhibition); Δγ= 10-19 mm, ++ (moderate inhibition); and Δγ >20 mm, +++ (strong inhibition) (Lee and Hwang, 2002; El-Tarabily *et al*., 2000).

Well diffusion method

In order to evaluate the antifungal activity of aqueous samples, wells (6×4 mm, 2 cm apart) were punctured by sterile cork borer in fresh PDA lawn or at 30 mm distance from plugs of *S. sclerotiorum*. Specific concentrations of Actinomycetes crude extracts were prepared in dimethyl sulfoxide:methanol (1/1: v/v) solvent (DM solvent) and then filled into each well. Plates were incubated at 28± 2° C for 4- 6 days for lawn culture method and 14 days in dual culture method. Disk-plug bioactivity was determined

by measuring means of inhibitory zones (mm) from three replicates in comparison with controls which included DM solvent without test compounds (Aghighi *et al*., 2004).

SEM studies: Morphological characters of spores and mycelium of the isolated *Streptomyces* spp. were studied using scanning electron microscope (SEM) model Lutz 100A. 7-8 day old *Streptomyces* cultures grown on CGA were used for the study. Culture specimen was deposited on aluminum stubs and held by double stick scotch tape. Coating using the sputter was carried out for 2 min. Viewing was done at 6000-20000X and digital prints obtained.

Chloroform assay

Spores from pure cultured *Streptomyces* isolates were suspended in sterile water @ 10^8 spores/ml. Drop dotting of these suspensions was done onto starch casein agar plates (5 dots/plate). The dotted plates were incubated for 3 days at 28± 2° C. The dotted cultures were inactivated by inverting open plates over watch glass containing 4mL of chloroform for 1h.The plates were then aerated in a fume hood to remove chloroform fumes. 10 ml of 1% water agar was overlaid onto each dot cultured plate and kept for 24h. *S. sclerotiorum* was spread plated onto each plate and incubated at 28± 2° C for 4- 5 days. Inhibtion zones around dotted culture were scored as positive (Davelos *et al.*, 2004).

Submerged culture and crude culture extract

Streptomyces isolates were cultured in CG broth by using an incubator shaker at 28°C with 130 rpm. Maximum antifungal activity of crude extract was measured by the well diffusion-method where in small aliquots of the culture were drawn every 24h for 28 days and tested. Crude extract was prepared by filtering out spores and mycelia with double layered cheese cloth. Extracts were prepared by taking into account the maximum activity time (Aghighi *et al*., 2004).

Estimation of minimum inhibitory concentrations (MIC)

MIC was measured by making two-fold dilutions of , 5, 2.5, 1.25, 0.625, 0.312, 0.156 mg/mL in DM solvent and tested by using the well-diffusion technique. The MIC will indicate the minimum concentration needed for fungal inhibition (Shahidi Bonjar and Karimi, 2004).

Estimation of Thermal Inactivation Point (TIP)

The crude extract obtained from different *Streptomyces* isolates were exposed to different temperatures and tested for bioactivity. Soluble crude extract samples (20 mg mL^{-1}) were exposed to a range of temperatures 40, 50, 60, 70, 80, 90 and 100° C for 10 min and cooled with ice. Bioactivity of exposed extracts were evaluated using the well diffusion method. Control included incubation of an untreated sample at 25°C (Shafii Baftii *et al*, 2005).

Evaluation for fungicidal and/or fungistatic activity

Agar plugs (1 mm^3) were taken from inhibition zones of *S. sclerotiorum* and transferred onto fresh PDA plates and incubated at 28°C for 7 days. Visual as well as microscopic observations for growth and rejuvenated plugs were labelled as fungistatic and non rejuvenation represented fungicidal properties of the antagonist (Shafii Bafta *et al*., 2005).

Chitinase assay

Plain disks of *Streptomyces* isolates colony were inoculated to MCA plates and incubated at 28°C for two weeks. Then, chitinase activity was evaluated by observing the clear zones representative of enzymatic digestion of chitin in the vicinity of colonies of *Streptomyces* isolates (Sadeghi *et al.*, 2006).

Proteolytic and Lypolytic activity tests

Specific culture media were prepared for testing proteolytic and Lypolytic activity of *Streptomyces* isolates. After 4-5 days incubation at 28°C, development of clear zone was positive for protease activity and and halo of white precipitate around the colonies indicated lipase activity. (Sierra, 1957; Dunne *et al.,* 2000)**.**

Amylase assay

The *Streptomyces* isolates were inoculated on starch agar plate and incubate at 28°C for 3 day. Then Lugol's solution **(**1% iodine in 2% potassium iodide**)** was added to the plates. The colonies which secretes amylase, produced zone of clearance or decolorization against the blue color background (Selvam *et al.*, 2011).

Hydrocyanic acid (HCN) production

Streptomyces isolates were grown on CGA. One sterilized sheet of filter paper was soaked in reagent solution (0.5% picric acid in 2% sodium carbonate) for 1 min and attached to the undersurface of Petri dish lid. The lids were replaced and sealed with parafilm. Incubated for 4 days at 28°C. Development of cream, light brown or reddish brown color on the filter paper indicated positive for HCN production (Lork,1948).

Greenhouse studies

To evaluate *in vivo* activity of *Streptomyces* isolates, soil treatments were conducted under greenhouse condition. The treatments included: 1) control, 2) *S. sclerotiorum*,

3) *Streptomyces* isolate B, 4) *Streptomyces* isolate F, 5) *Streptomyces* isolate S, 6) *S. sclerotiorum* plus *Streptomyces* isolate B, 7) *S. sclerotiorum* plus *Streptomyces* isolate F and 8) *S. sclerotiorum* plus *Streptomyces* isolate S. Pathogen inocula was prepared and used as described previously in section of pathogenicity test. Antagonist inocula consisted of spore suspensions with concentration of 10^8 CFU ml^{-1}in distilled sterile water from which 10 ml thoroughly mixed with each Kg of soil in pot mix. Controls received neither pathogen nor antagonist. All treatments kept under greenhouse conditions and watered regularly. Disease symptoms recorded 14 days afterwards.

To compare the relative growth performance of the plants in the treatments, at the 14th day, plants were carefully uprooted, adhering soil removed and rinsed in tap water. Then shoots and roots cut apart and dried at 60°C for 72h and weighed with accuracy.

Data analysis

Antagonistic effects of *Streptomyces* isolates against *S. sclerotiorum* were statistically analyzed incomplete randomized design (CRD) analysis was done using SAS9.1.3 software and means were compared by Duncan's multiple range test at the 0.01 level of confidence.

RESULTS AND DISCUSSION

Screening and bioassays

In screening for actinomycetes with antifungal activity, 40 isolates were screened from which 3 isolates B, F and S showed strong antifungal activity against *S. sclerotiorum* (Fig. 1).

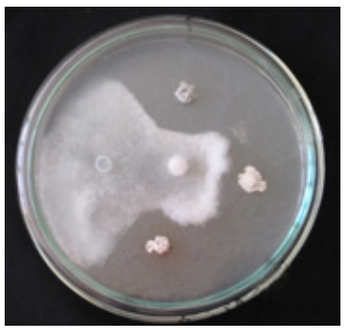

Fig. 1. Bioassay results of *Streptomyces* isolates against *Sclerotinia. sclerotiorum.* Clockwise from top: Isolates B, F, S and blank agar disk (control). Center disk is of *S. sclerotiorum* agar inoculum disk.

Scanning electron microscope studies

Scanning electron micrographs of spore chains of active *Streptomyces* isolates are shown in Fig 2.

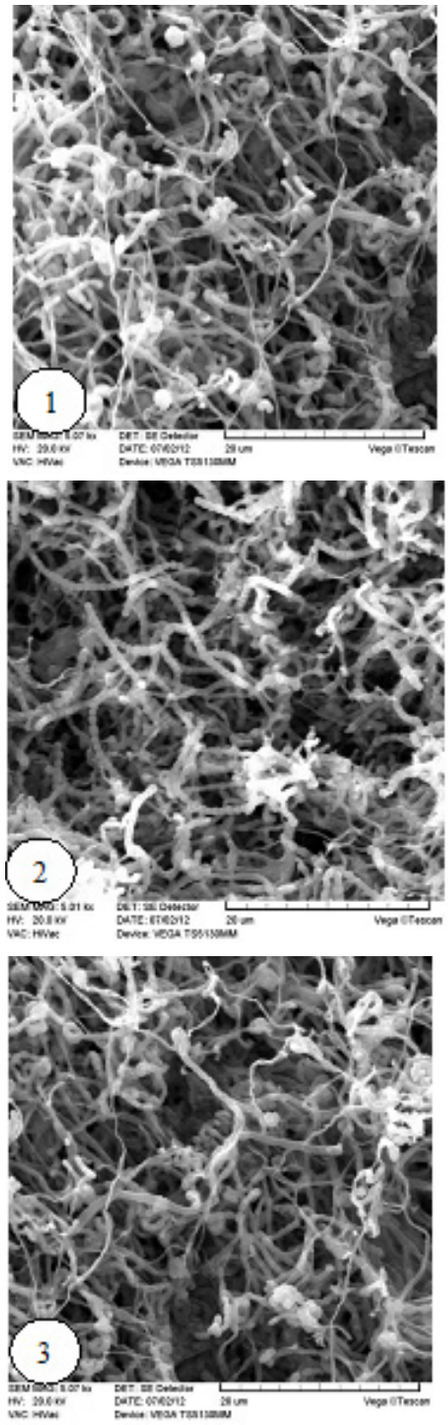

Fig. 2. Digital STM electron micrograph of sporechains of active *Streptomyces* isolates. From top to bottom: Isolates B(1), F(2) and S (3).

Chloroform assay

Among effective *Streptomyces* isolates, only isolate F retained its antifungal activity after exposure to chloroform and produced inhibition zone (Fig. 3).

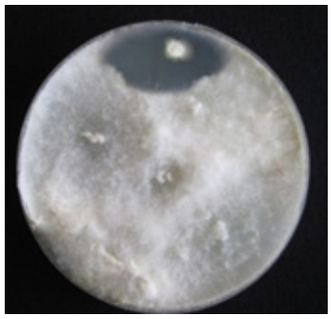

Fig. 3. Chloroform assay results: only isolate F (top) was able to inhibit fungal growth after exposure to chloroform.

Antifungal activity of submerged culture

Post seeding time versus activity for 3 isolates is indicated in Fig 4. In isolates F, S and B, activity reached the maximum at 4th, 8th and 15th days after inoculation respectively. Based on this result, crude extract were prepared at the day of maximum activity.

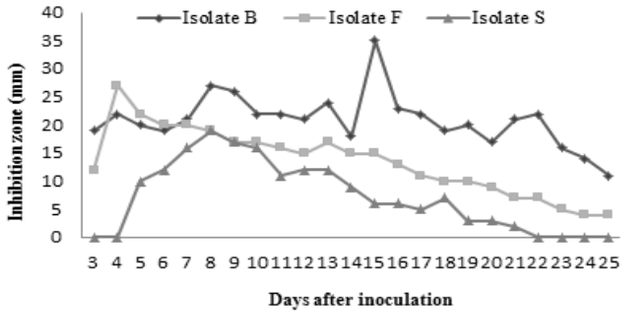

Fig. 4. Activity versus post seeding time in submerged cultures of *Streptomyces* isolates B, F and S monitored by well diffusion-method against *Sclerotinia sclerotiorum*.

Determination of MIC

In well diffusion-method, MIC of the crudes of *Streptomyces* isolates B and F against *S. sclerotiorum* was determined to be1.25 mg/ml and MIC of isolate S determined as 0.312 mg/ml.

Determination of TIP

Bioactivity of isolate B and S diminished to zero at 90°C and isolate F was inactivated at 100°C.

Fungicidal and/or fungistatic activity

Transferred blocks from inhibitory zones of lawn cultures of *S. sclerotiorum* (from dual cultures) did not regrow in fresh PDA plates which indicated the fungicidal activity of all three *Streptomyces* isolates.

Chitinase activity

After 8-10 days of incubation on minimal chitin-agar media at 28° C, clear zones was observed around all the three *Streptomyces* isolate colonies which were representative of their chitinase activity (Fig. 5).

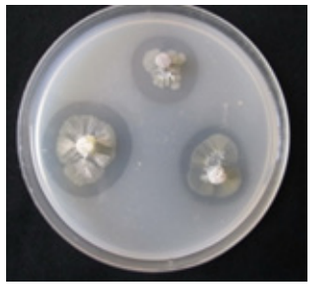

Fig. 5. Chitinase activity of *Streptomyces* isolates on minimal chitin-agar media. Clockwise from top: *Streptomyces* isolates B, S and F.

Proteolytic and Lypolytic activities

All three *Streptomyces* isolates exposed lipase activity by precipitating halo around the colonies which are representative of Tween-80 hydrolysis. Isolates B and F could also produce proteolytic enzymes as indicated by digesting protein of the medium and proded transparent zone around the their colonies (Fig.6).

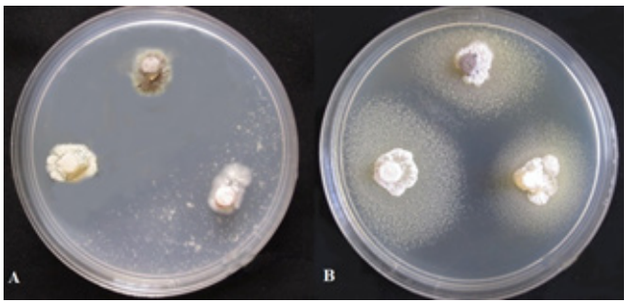

Fig. 6. Lipase (A) and Protease (B) enzyme activities of *Streptomyces* isolates. Clockwise from top in both plates: *Streptomyces* isolates B, S and F.

Amylase activity

All tested isolates could produce amylase enzyme as indicated by the transparent zone around their colonies after addition of iodine (Fig. 7).

Fig. 7. Amylase production by *Streptomyces* isolates in starch agarmedium. Clockwise from top: *Streptomyces* isolates B, S and F.

HCN production by the *Streptomyces* isolates

None of the three isolates were able to produce Hydrocyanic acid. The results are shown in Fig 8.

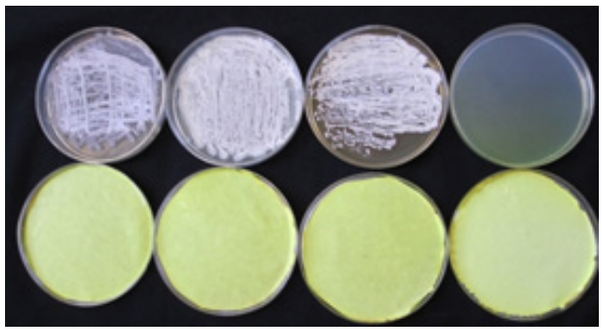

Fig. 8. HCN production assay results. Top row from right to left : control, *Streptomyces* isolates S, F and B. Lack of color change of filter papers impregnated with reagent (bottom row) is indicative of no HCN production.

In vivo greenhouse studies

Plants in soils not infested by the pathogen (Treatment 1) did not show any signs of the disease. Application of the antagonists in combination with pathogen (Treatment 6, 7 and 8), significantly (P= 0.01) reduced the incidence of lettuce rot disease compared with the treatment containing the pathogen alone (Treatment 2). In the absence of the pathogen, the *Streptomyces* isolates did not have any harmful effects on plant growth (Treatments 3, 4 and 5) and even seedlings inoculated with isolate F showed enhancement of plant growth recording significant increase in root and shoot height and shoot biomass compared with untreated controls (Figs. 9, 10 and 11). Regarding biocontrol action of *Streptomyces* isolates B, F and S against *S. sclerotiorum* significant difference in disease onset and symptom development was noticeable. In this regard, high level of antagonistic effect of *Streptomyces* isolates B, F and S against *S. sclerotiorum* was noticeable. Performance of the treated plants at three weeks period for all treatments are indicated in Table 1.

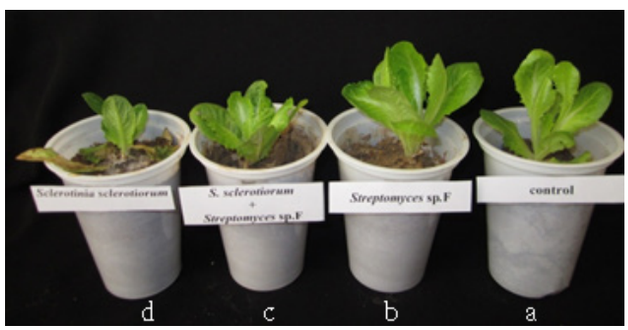

Fig. 9. *In vivo* greenhouse results in lettuce seedlings (a): In Untreated control plants, (b): Plants inoculated with *Streptomyces* isolate F alone, (c): plants inoculated with both *S. sclerotiorum* and the antagonist *Streptomyces* isolate F and (d): plants inoculated with the pathogen alone.

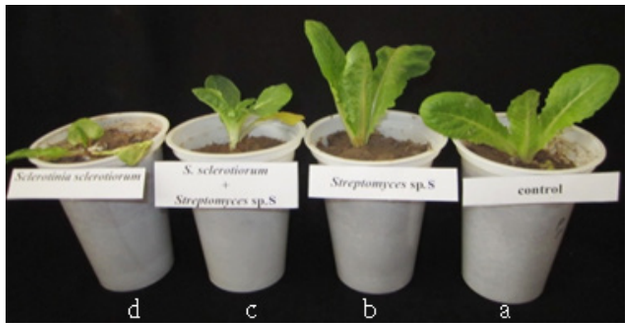

Fig. 10. *In vivo* greenhouse results in lettuce seedlings (a): In Untreated control plants, (b): Plants inoculated with *Streptomyces* isolate S alone, (c): plants inoculated with both *S. sclerotiorum* and the antagonist *Streptomyces* isolate S and (d): plants inoculated with *Streptomyces* alone.

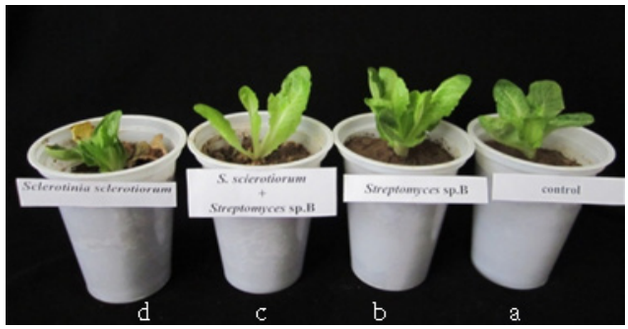

Fig. 11. *In vivo* greenhouse results in lettuce seedlings (a): In Untreated control plants, (b): Plants inoculated with *Streptomyces* isolate B alone, (c): plants inoculated with both *S. sclerotiorum* and the antagonist *Streptomyces* isolate B and (d): plants inoculated with the pathogen alone.

The deleterious effects of fungicides on the environment has made biological control as an effective alternative to control fungal pathogens particularly, soil-borne pathogens. Many species of fungi and bacteria have been known as antagonists against *S. sclerotiorum* (Adams and Ayers, 1979) but most reports are based on laboratory observations and little information is available on *in vivo* and field conditions. Although some of them such as parasitic fungus *Coniothyrium minitans* has been presented commercially and is available as Contans® (Vrije *et al.*, 2001). Actinomycetes have received considerable attention as biocontrol agents of soil-borne fungal plant pathogens as well plant growth promoters. Our findings represent the presence of potential antifungal behavior in *Streptomyces* isolates B, F and S. Since isolate B and S did not retain their antifungal activity after exposing to chloroform. Davelos *et al.,* (2004) reported that antifungal activity is not from antibiotic characteristic of these *Streptomyces* isolates. Apparently isolate F has non-enzymatic antifungal behavior. The main components in antifungal activity are lytic enzymes such as chitinases, proteases, amylases or glucanases and

Table 1. Effect of Streptomyces isolates on disease incidence and severity caused by S. sclerotiorum on lettuce seedlings under glasshouse conditions. Means within columns followed by different letters (a, b, c, d) have significant difference (P = 0.01) according to Duncan's multiple-range test

Treatment	Root height (cm)	Shoot height (cm)	Root fresh weight (gr)	Shoot fresh weigh (gr)	Root dry weigh (gr)	Shoot dry weigh (gr)
Control	5.38 b	7.176 b	3.9 ab	15.05b	3.6 ab	13.65 bc
S. sclerotiorum	4.839 c	6.797 d	1.6 c	0.65 d	0.6 d	0.2 e
Streptomyces sp. B	5.283 b	7.079 c	3.82 b	12.25 b	2.8 bc	10 c
S. sclerotiorum + B	5.268 b	7.068 c	3.7 b	6.2 c	2.1 c	5.95 d
Streptomyces sp. F	5.776 a	7.553 a	4.37 a	19.87 a	4.1 a	18.4 a
S. sclerotiorum + F	5.504 b	7.266 b	4.37 a	18.97 a	4 a	17.85 a
Streptomyces sp. S	5.413 b	7.225 b	4.38 a	17.75 ab	4.2 a	16.2 ab
S. sclerotiorum + S	5.428 b	7.126 bc	3.92 ab	14.8 b	3.81 ab	13.15 bc

they help in the in the degradation of fungal cell walls (Fernando *et al.*, 2006). Tahtamouni *et al.,* 2009) reported *in vitro* antifungal activity of some of the chitinolytic *Streptomyces* against *S. sclerotiorum.* Actinomycetes are Also, protease-producers. Apart from their antagonistic activity actinomycetes have been known to be actively involved in the decomposition of organic matter, mineralization of nutrients and plant growth promotion. The proteolytic enzymes produced by actinomycetes have been shown to degrade the cell walls of as *Phytophthora* and *Pythium* spp. (Lima *et al.*, 1998). Hence the enzymes produced by the antagonistic actinomycetes B and S are playing key role in inhibition of the tested fungal pathogen. Also reports show that amylase and lipase enzymes are produced by actinomycetes in large quantities and hence used industrially in food, biotechnological, oleochemical, pharmaceutical and detergent industries (Pandey *et al*., 2000; Schmid and Verger,1998). It is well known that HCN plays an role in disease suppression (Wei *et al*., 1991). Haas *et al*., (1991) reported that HCN produced by certain strains of *Pseudomonas fluorescens* aided in inhibition of black root rot of tobacco. Keeland Défago (1996) also reported that HCN can inhibit *Pythiumultimum* on cucumber. However, none of our isolates were able to produce HCN. In green house study, similar to *in vitro* evaluations, all three isolates were effective in controlling *S. sclerotiorum* and the percent reduction in infection and mortality were significant on the lettuce seedlings. All three *Streptomyces* isolates enhanced growth factors such as biomass, height of root and shoot of treated plants. Nassar *et al.,* (2003) showed infestation of soil with *Streptomyces* griseoluteus promoted the growth of bean plants by production of polyamines such as putrescine, spermidine, spermine and endogenous plant growth regulators (PGRs) (indole acetic acid, IAA, and gibberellic acid). Hamdali *et al.,* (2008) reported that *Streptomyces griseus*-related strain (BH7) stimulated aerial growth of wheat. Plant growth promotion by actinomycetes have also been reported on rice (Gopalakrishnan *et al.*, 2013). Our findings are in agreement with aforesaid researches. Actinomycetes may promote plant growth either by direct stimulation such as phosphate solubilization, phytohormones (IAA and gibberellic acid) production and siderophore(El-Tarabily *et al*., 2008; Hamdali *et al*., 2008) or by indirect stimulation such as production of b-1, 3 glucanase, chitinase, protease, hydrocyanic acid, antibiotics and polyamine that suppress pathogens and induce resistance in host plants (Glick,1995; Nassar *et al*., 2003; Gopalakrishnan *et al.*, 2013). The wide range of antifungal physiological activities of the *Streptomyces* strains used in this investigation establishes the fact that several mechanisms (antibiosis, HCN, cell wall-degrading enzymes etc.) are involved in disease suppression. The identified actinomycetes species from this study could be potential candidates for preparation and development of novel formulatons containing the secondary metabolites expressed by them.. Determination of the exact mechanisms of action of these biocontrol agents can help in further understanding the behavior of these eco-friendly antifungal agents.

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